

**ADVANCED TOPICS  
IN SCIENCE AND TECHNOLOGY IN CHINA**

## **ADVANCED TOPICS IN SCIENCE AND TECHNOLOGY IN CHINA**

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Ping Wang  
Qingjun Liu

# Biomedical Sensors and Measurement

With 215 figures



ZHEJIANG UNIVERSITY PRESS  
浙江大学出版社



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## **Foreword**

This book introduces the basic fundamentals of biomedical sensors and the measurement technology as well as the recent advancements in this field in recent years. There are five chapters in this book which can be subdivided into two major parts. The first part places emphasis on the fundamentals and the development of modern biomedical sensors and the measurement technology including their basic features and special requirement in application. This part also provides essential information on the basic sensitive reaction mechanisms, characteristics and processing approaches of the biomedical sensors. The second part introduces the typical sensors including the physical, chemical and biological sensors as well as the discussion of their measurement techniques. The practical applications of each of these sensors are also described in detail. There are two unique features in this book: (1) The combination of the discussion on the biomedical sensor technologies and their required measurement techniques which include the fundamentals and the practical applications of the biomedical sensors; (2) It The description of the rationale and the needs of the integration of discrete sensing elements into a meaningful and practical sensor array which can become an intelligent sensing system. The authors have given a very persuasive and sound approach in this important scientific and practical endeavor. It also should be acknowledged that the authors have systematically provided a clear roadmap for the development of various sensors by first introducing macro-size sensors for the detection of physical properties and then leading to the advancement of micro-size chemical and biological sensing systems.

The advancement of the micro-size for chemical, biological and biomedical sensors or sensor micro-systems requires multi-disciplinary skills and expertise. This includes the understanding and expertise in microfabrication and micromachining processing, electronic and ionic conductive materials, sensor operational principles, electronic transduction interface technologies and many others. In this book, the authors have logically and systematically discussed and analyzed the interwoven relationship of these techniques and their applications to the development of scientifically and commercially sound practical chemical, biological and biomedical sensors.

The authors, Professor Ping Wang and Associate Professor Qingjun Liu have been engaging, for many years, in the research and teaching of sensor technology, particularly in the field of biomedical sensors. They have been involved in this

research effort more than a decade and their appreciation of the multi-disciplinary nature of the sensor research and the unique requirements for the advancement of the biomedical sensors and their measurement techniques can be well recognized throughout this book. As mentioned, this book is derived from the research work on biomedical sensors and measurement, in recent years, at Zhejiang University, Hangzhou, China. Thus, this book will serve as an excellent reference source for researchers in biomedical sensor and measurement. It's intended for scientists, engineers, and manufacturers involved in the development, design, and application of biomedical sensors and measurement. The reference list given in each chapter is very thorough and relevant, this book will also be a very good book for the senior undergraduate and graduate students who wish to pursue a professional career in this field.

Biomedical sensors and the measurement are scientifically and commercially important in numerous applications at this juncture, and this book will be most welcome for researchers and students who wish to understand this field further and to make a meaningful contribution to this important endeavor.



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January, 2011

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## Preface

In the 20th century, technological innovation has had such a rapid development that science and technology permeates all aspects of our lives, especially in the biomedical field which attracts a large number of professional scientists and engineers. Biomedical engineering is a combination of two developing areas: biomedical and information technology. It promotes the biomedical sensor design and development, as well as applications in clinical diagnosis and treatment technology. Biomedical engineering covers many research areas: bio-mechanics, bio-materials, physiological modeling and sensoring technology, detection technology, signal and image processing. One important research field is biomedical sensoring and detection technology, which obtains original information from primitive organisms (especially human body), one of the most crucial procedures.

In the 1960s, scientists and engineers paid more attention to sensors for they met many of the practical requirements. The development of chemical and biological sensors creates selective sensors, which makes possible the direct detection of a variety of ions and molecules. Micro-sensors and micro-electrodes quickly replaced traditional large-size sensors and were applied to biological and medical fields.

At present, the quick clinical digital thermometer, blood pressure monitors, and wearable home-used blood glucose meter have been widely used. CT (computed tomography scanning) and ultrasound technology are recognized as common advanced diagnostic tools. However, many have omitted that sophisticated sensors play an important role in these instruments. Sensors have brought about revolutionary changes in the field of biomedical diagnostics and application of medical instrumentation, and they will have a positive effect on human life quality in the 21st century. Sensors have the following applications:

- Digital medical image tools like CT, ultrasound, etc.;
- For the traditional image tools such as X-ray machines, it improves and gets more information and reduces the amount of radiation;
- Portable clinical multi-parameter monitoring equipment;
- Portable home-use monitoring and diagnostic equipment;
- Implantable, self-calibration equipment which will be widely used in the future;
- Intelligent systems of sensors can replace our sense system, such as sight, taste, smell, touch, etc.;

**■ Rapid diagnosis tools based on immunization and DNA-chip technology.**

Although biomedical sensors are being applied more and more, in many cases, the theory is not entirely clear. It's controversial in the expression of stimulus signal theory, signal extraction and measurement. The development of new biomedical sensors indicates a great fundamental research work, which is the key part at present.

Biomedical sensors convert biological signals into easy-to-measure electrical or optical signals. It's the interface between organisms and electronic systems. Meanwhile, effective detection technology, including low-noise and anti-jamming circuits, and data processing techniques are essential during the conversion from bio-medical to electronic signals, as well as for further processing. This book adds the detection technology with sensoring technology according to the actual teaching requirement so that students and other researchers may systematically learn and comprehend the relevant knowledge in this field.

This book can be used as a reference book for researchers and senior undergraduates and graduate students. This book combines detection technology with sensoring technology and strengthens the links between them. In addition, the authors have added an introduction of regular physical sensors and chemical sensors and reorganized and reviewed the latest international development trends of chemical sensors, biological sensors and their intelligent systems, such as electronic nose, electronic tongue, microfluidic chips and micro-nano biosensors and their applications.

Biomedical sensoring and detection techniques require synthesizing the interdiscipline of physics, electronics, materials, chemistry, biology, and medical, etc. The authors are trying to meet this requirement through a detailed description of working principle, sensitive technology, and detection circuit and identification system theory of sensors or devices. We believe that this book will be of great value for those academics, engineers, graduates and senior undergraduates in the biomedical and relevant fields.

The book is composed of five chapters. Chapter 1 introduces the development of biomedical sensoring and detection technology; Chapter 2 describes fundamental knowledge of modern sensoring and detection technologies; Chapter 3 describes the physical sensor and its detection technology; Chapter 4 describes chemical sensors and detection techniques; Chapter 5 describes the biosensors and detection technology. Some content in this book belongs to the international research frontier. Biomedical sensoring and detection technology promotes the reorganization of biomedical information transmission, processing and perception, as well as the development of biomedical engineering and the interdisciplinary field.

The book is the result of several years of study, research and development of the faculties, PhD candidates and many other affiliated to the Biosensor National Special Laboratory of Zhejiang University. We would like to give particular thanks to Jun Wang, Wei Cai, Qi Dong, Gong Cheng, Di Wang, Jun Zhou, Cong Zhao, Lin Wang, Liang Hu, Wei Zhang, Lidan Xiao, Hui Yu, Kai Yu, Wen Zhang, Huixin Zhao, Chunsheng Wu, Liping Du, Ning Hu, Weiwei Ye and Yishan Wang. We sincerely thank them all for their contributions.

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As biomedical sensors and measurement involved in wide interdisciplinary areas, and in consideration of the limit of authors' knowledge and experiences, errors of judgment are, of course, inevitable; comments and suggestions will, therefore, be appreciated.

*Ping Wang and Qingjun Liu  
Hangzhou, China  
January, 2011*



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# **Chapter 1**

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## **Introduction**

### **1.1 Definition and Classification of Biomedical Sensors**

The sensors are devices that can transform non-electrical signals into electrical signals. The biomedical sensor is a very important kind of sensors. First we will introduce some basic knowledge about biomedical sensors including a definition and classification.

#### ***1.1.1 Basic Concept of Sensors***

Sensor or transducer is a device which can respond to a measured object and transform it into signals which can be detected. A sensor is usually composed of a sensitive component which directly responds to a measured object, a conversion component and related electronic circuits. Along with the development of modern electronic technology, micro-electronic technology and communication technology, which represent some various useful signals, electrical signals are most convenient for processing, transporting, displaying, and recording.

Sensors often provide information about the physical, chemical or biological state of a system. Measurement is defined as an operation that aims to obtain the measured value of the quantity. Therefore, sensors are defined as devices that can transform non-electrical signals into electrical signals. Sensors often provide information about the physical, chemical or biological state of a system. Measurement is defined as operations that aim to obtain the measured value of the quantity. Therefore, sensor detection technology is one that uses sensors to transform measured quantities into physical quantities which are easy for communication and processing, and then goes on with transformation, communication, displaying, recording and analysing.

Biomedical sensors are special electronic devices which can transfer various non-electrical quantities in biomedical fields into easily detectable electrical

quantities. For this reason, they are incorporated into health care analysis. They expand the sensing function of the human sensing organ while the key parts consist of various diagnostic medical analysis instruments and equipment. Biomedical sensing technology is the key to collecting human physiological and pathological information and is also an important disciplinary branch.

### ***1.1.2 Classification of Biomedical Sensors***

Biomedical sensors can be classified in the following categories according to their detection quantities. Classified by working principle, sensors include physical sensors, chemical sensors, and biological sensors.

*Physical sensors:* It refers to the sensor made according to physical nature and effect. This kind of sensors is mostly represented by sensors such as metal resistance strain sensors, semiconductor piezoresistive sensors, piezoelectric sensors, photoelectric sensors, etc.

*Chemical sensors:* It refers to the sensor made according to chemical nature and effect. This kind of sensors usually uses ion-selective sensitive film to transform non-electricity such as a chemical component, content, density, etc. to related electric quantity, such as various ion sensitive electrodes, ion sensitive tubes, humidity sensors and, etc.

*Biological sensors or biosensors:* It refers to the sensor using biological active material as a molecule recognition system. This kind of sensors usually uses enzyme to catalyze some biochemical reaction or exams the type and content of large molecule organic substances through some specific combination. It is a newly developed sensor in the second half of the century, and examples include enzyme sensors, microorganism sensors, immunity sensors, tissue sensors, DNA sensors and, etc.

Classified by detection type, there are displacement sensors, flow sensors, temperature sensors, speed sensors, pressure sensors, etc. As for pressure sensors, there are metal strain foil pressure sensors, semiconductor pressure sensors, capacity pressure sensors and other sensors that can detect pressure. As for temperature sensors, it includes thermal resistance sensors, thermocouple sensors, PN junction temperature sensors and other sensors that can detect temperature.

This is also the method that classifies sensors according to the human sense organ that each sensor can replace, such as vision sensors, including various optical sensors and other sensors that can replace the visual function; hearing sensors, including sound pick-up, piezoelectric sensors, capacity sensors and other sensors that can replace the hearing function; olfaction sensors, including various gas sensors and other sensors that can replace the smelling function (Harsányi, 2000). This kind of classification is good for the development of simulation sensors.

In many situations, these classification methods are used together. For example, the strain gauge pressure sensor, conductance cardiac sounds sensor,

thermoelectric glucose sensor and so on. The classification has met problems as a result of the diverse development of sensing technology. Therefore the classification methods have their advantages and disadvantages. Any standard classification methods don't exists so far.

## 1.2 Biomedical Measurement Technology

Biomedical signals are usually weak, random with strong noise and interference, allow dynamic change and exhibit significant individual differences. Therefore, biomedical measurement technologies are more complex and rigid than common industrial detection technology.

Biomedical measurement is a guiding technology in the acquisition and processing of biomedical information, and is directly related to the research of biomedical sensing technology, biomedical measurement methods, electronics and measuring systems. Therefore, the innovative research and development in biomedical measurement has a direct effect on the design and application of sensors and medical instruments.

Biomedical measurement technology involves the detection of physical, chemical and biological signals in different levels of organisms. For example, ECG, EEG, EMG are electrical physiological signals; blood pressure, body temperature, breath, blood flow and pulse are non-electrical physiological signals; blood and urine are chemical or biological signals; enzymes, proteins, antibodies and antigens are biological signals. Similarly, the biomedical measurement systems demands particular reliability and security.

Nowadays the measurement of physical signals has been popularized and many measurements of chemical signals have practical applications. The measurement of biological signals is mostly at the laboratory research stage. With a greater combination of microelectronics, optoelectronics, quantum chemistry and molecular biology with traditional sensing technology, the measurement methods and system for detecting complex organisms will enjoy a bright future. Biomedical measurement technology will also develop into mini-type, multiple parameter and practical applications. The advancements of electronics, IC technology, computer technology and advanced signal processing and intelligent algorithms will promote the application of biomedical measurement.

### 1.2.1 Bioelectrical Signal Detection

The detection of physiological quantities in the circulatory system and nervous system develops relatively early and rapidly and its importance always leads to a large amount of research reports. Take ECG as an example. Many researchers are

still working on automatic extraction and discriminating arrhythmia information from ECG under strong interference. In addition, the detection of the P wave, the ST segment in ECG, research on obtaining an ECG of a fetus from a mother's body surface, on high frequency ECG, on body surface real-time detection and late potential detection have been improved to different extents. ECG detection is mainly applied in diagnosing heart disease and preventing sudden cardiac death. Moreover, it could also aid surgical investigational procedures (Tigaran et al., 2009). Although these research achievements are not mature enough to be put into clinical use, they improve the function of ECG diagnosis and monitoring devices.

### ***1.2.2 Biomagnetic Signal Detection***

The biomagnetic field comes from the *in vivo* human body with biological electrical activities, such as MCG, MEG, MMG, etc. In addition, it also includes the magnetic field caused by the magnetic medium in the tissue when affected by an external magnetic field. An invasive strong magnetic mass can also cause an internal biomagnetic field. At present we can detect these magnetic fields in the laboratory. However, commonly a biological magnetic field is very weak. For example, MCG intensity is about  $10^{-10}$  T and MEG is about  $10^{-12}$  T. Therefore SQUID (superconducting quantum interference device) in the liquid nitrogen container is used to detect the biological magnetic field and the measurement system should be placed in a special shielding environment.

In contrast to the detection of bioelectricity, the detection of a biomagnetic field has many features. Take the measurement of MCG as an example. The detection system does not directly come in contact with the organism, which means that the detection uses a detecting coil rather than an electrode to pick up the biological signal. Therefore it receives no effect from the surface of the objects and does not cause an electrode artifact, which is electrically safe. Besides, the detecting signals come from a certain spot or place rather than the difference between two points. Therefore, a location measurement can take place. The magnetoconductivity in tissue is well-distributed which means that biomagnetic signals will not distort when spreading in the body. As a result, research on biomagnetic detecting methods has become one of the pioneering and hot topics and has good application prospects. With the development of room temperature superconductor technology, biomagnetic detection will reach the clinical application stage.

### ***1.2.3 Other Physiological and Biochemical Parameter Detection***

It has become a common practice to use sensors non-invasively to detect non-invasive blood pressure, blood flow, breath, pulse, body temperature and cardiac sounds, which lead to wide applications in clinical examinations and other

monitoring techniques. The trend is to develop new non-invasive or slightly invasive detecting methods and use one sensor once to detect multiple physiological parameters. For example, use the photoelectric method to detect the pulse as well as other information such as the heart rate, blood pressure, oxygen saturation; use electromagnetic coupling or optical coupling to detect intracranial pressure, and pressure in the mouth. Non-contact and long-distance detection also lead current development trends.

Biochemical parameter detection usually uses blood and body fluid as a sample to conduct the measurement. Therefore, most of the methods are invasive and cannot measure the changes in the parameters over a long-time and in real-time. At present, non-invasive and slightly invasive biochemical parameter detecting methods have received great attention. For example, researchers have detected phenacetin in the saliva and compared it with the results of blood plasma tests; researchers extract lixivium by exerting a small amount of negative pressure on the skin and then use ion field effect transistor sensors to detect blood sugar; dielectric spectroscopy (DS) has been applied to monitor changes in the glucose level by combining electromagnetic and optical sensors (Talary et al., 2007).

### 1.3 Characteristics of Biomedical Sensors and Measurement

Biomedical measurement has specificity when used for human signal detection and is a non-invasive, safe and reliable measurement. It has become an important research project in recent years. Non-invasive detection, which causes no wound or a slight wound, is easily administered to people. It helps to keep the physiological status of objects, and long-time or real-time monitoring can take place. Therefore it is convenient for clinical examination, monitoring and recovery evaluation. Non-invasive detection has become an important part of biomedical measurement technology.

Biomedical measurement research involve some special measurement methods, e.g., low-noise and anti-interference technology, picking up signals, analyzing and processing technology and measurement system, analog and digital circuits, computer hardware and software and even BCI (brain-computer interface) technology, etc. It also depends on the development of the life sciences (such as cytophysiology, neurophysiology, biochemistry, etc.). The diversity of research objects in biomedical detecting technology makes the research projects dispersive in this field (Wang and Ye, 2003). However, any promotion of detection methods in physiological quantities and biochemical quantities will greatly compel the advancement of the whole of life science as well as the invention of new diagnosis and treatment devices.

### 1.3.1 Features of Biomedical Sensors and Measurement

*Interdisciplinary Research:* Biomedical sensor technology, as an active discipline, combines electronic science and biomedicine. Biomedical sensor technology meets the requirement of early diagnosis, quick diagnosis, bedside monitoring, monitoring *in vivo* and more advanced health care; provides indispensable support for gene probes, molecular recognition, monitoring of neurotransmitters and neuromodulators and more advanced scientific study. The developing disciplines such as microelectronics technology, biological technology, molecular biology and photonics technology lay the foundation for biomedical sensor technology. With such a background, biomedical sensor technology has made significant and rapid progress.

*Basic research and technological innovation:* In the 1970s, sensors were involved in the technological and scientific field and focused on new product development. The basic research paid more attention to the advanced and high-level product exploration process. The primary targets were a description of the molecular recognition mechanism, which is the basis of improving SNR, and mastery of the interface process, the key to shortened response times. To put results into products, all kinds of processing technology including precision machining, semiconductor technology, chemical etching and biotechnology should be applied to technological innovation.

*Sensitive materials and film formation technology:* The core components of a sensor-sensitive membrane consist of sensitive materials combined with the matrix material. As to popular film formation technology, semiconductor thin-film, thick-film and molecular beam extension are used in physical sensors, physical adsorption and embedded technology, chemical cross-linking and molecular assembly for chemical sensors, and multi-enzyme system membranes, monoclonal antibody films, conductor films and LB film for biochemistry sensitive membranes.

*Knowledge-intensive:* Many disciplines are involved in sensor design, production and utilization. Take a chemical sensor for example. A knowledge of quantum chemistry is necessary for sensitive materials design, super-molecular chemistry, host-slave chemistry and biotechnology for materials synthesis; interface chemistry, physical interface and molecular assembly technology for film formation technology; microelectronic technology, photonics technology and precision machining for transfer devices.

*High reliability:* For a biosensor to be in direct contact with the human body, it must have high reliability. Sensors should be controlled strictly by the FDA in America and put on the market only if proved to be safe for the human body in the long term and to provide reliable monitoring data. Sensors detecting body fluids should be corrosion resistant and be easy to clean; embedded or implanted sensors should withstand rejection by the human body.

*Fine technology:* Fine technology is necessary for high-precise sensors. A matrix sensor, in the operation of integration technology, needs special implantable technology to reject leaking or deformation when soaked for a long time; coupling

of a sensitive membrane and fiber cross-section requires fine technology; although a glass microelectrode can be stretched by certain machines. Precision machining is the combination of machining and chemical technology. Sensors are not only a product but also a fine artwork.

### ***1.3.2 Special Requirement of Biomedical Sensors and Measurement***

For biomedical measurement, it has specificity when used for human signal detection: it is a non-invasive, safe and reliable measurement. It has become an important research project in recent years. Non-invasive detection, which causes no wound or a slight wound, is easily received by people. It helps to keep the physiological status of objects, and long-time or real-time monitoring can take place. Therefore it is convenient for clinical examination, monitoring and recovering evaluation. Non-invasive detection has become an important part of biomedical measurement technology.

Biomedical measurement research involve some special measurement method, e.g., low-noise and anti-interference technology, picking up signals, analyzing and processing technology and measurement systems, analog and digital circuits, computer hardware and software and even BCI (brain-computer interface) technology, etc. It also depends on the development of the life sciences (such as cytophysiology, neurophysiology, biochemistry, etc.). The diversity of research objects in biomedical detecting technology make the research projects dispersive in this field. However, any promotion of detection methods in physiological quantities and biochemical quantities will greatly compel the advancement of the whole of life science as well as the invention of new diagnosis and treatment devices.

The most different aspect in designing biomedical sensors from other sensors is the consideration of biocompatibility. Because this type of sensor directly makes contact with tissue or blood, the sensor design should include hemocompatibility and histocompatibility.

The first and the most important issue in manufacturing sensors is the material selection. The metallic materials used in sensors have to be inert metals such as stainless steels, titanium alloys. The polymers have to be degradable materials, such as PMMA, silicones. All the materials used for sensor structure should be strictly selected to avoid serious host response and function normally after inserting into the animal body. Material's rigidness and flexibility should also meet the requirement since the implanted sensors need to adjust to anatomical structures of the measured objects.

Secondly, a series of experiments on animals and clinical trials should be carried out before clinical application. Even though choosing inert and least harmful materials at present, we still have to do full sequence tests for biocompatibility because the implanted sensors are under a different physiological environment.

Finally, we apply biological methods to evaluate the host response. The *in vivo* biocompatibility can be evaluated by analyzing the cell population present, measuring the mediator and metabolite cells excreted, and analyzing the morphologic characteristics of the tissue and the capsule thickness around the implant.

Besides, some biological samples such as enzymes, proteins, cells and tissues have to be analyzed externally. An appropriate immobilization on the sensor surface is required for maintaining biological viability and activity. Hence, the biocompatibility for *in vitro* biomedical sensors should also be taken into account in sensor design.

## 1.4 Development of Biomedical Sensors and Measurement

Biomedical sensors and measurements have been developing rapidly over the past 30 years, and the development is represented in various aspects. The development of the medical sensor has basically changed the traditional mode, forming the development trend of smart, micro, multi-parameter, remote-control and non-invasive, and achieved some technical breakthroughs. Other new types of sensors such as DNA sensors, fiber sensors, and biological tissue sensors are also being developed. The revolution of medical sensor technology will help promote the development of modern medicine.

### 1.4.1 *Invasive and Non-invasive Detection*

Miniaturization of sensors makes direct and continuous monitoring of vascular parameters (such as blood pressure, temperature, and flow rate) possible, which has become a new clinical diagnosis tool. Although commercial products have been practically used, their potential has not been fully developed. Chemical and biological sensor technology plays an important role in the field of public health by supporting those who focus on rapid detection, high sensitivity and expertise. Clinical doctors also need a way to monitor patients with key metabolite concentrations of various diseases. A lot of effort is being made in this respect, using chemical and biological sensors to expand into non-traditional clinical chemistry analysis.

In addition, non-invasive detection of body fluids is being developed. Traditional body fluids (blood, urine, myeloid fluid, saliva, sweat, ascites, semen, etc.) are required to be extracted from patients, and most are invasive or *in vitro* measurements. In order to be able to carry out continuous measurement, it is necessary to develop different types of non-invasive or minimally invasive detection of body fluids.

Non-invasive detection means no or nearly no invasion during detection.

Non-invasive detection is not only more acceptable by receivers, but also has little effect on the human body, and is more reliable, easy-to-operate, and easy for sterilization and results in the possibility of less infection. Non-invasive detecting sensors should have a higher sensibility, accuracy, anti-interference and signal-to-noise rate.

Nondestructive monitoring is the most receptive monitoring method of the patient and has received widespread attention. At present, progress has been made in percutaneous blood gas sensors which can monitor blood gas non-invasively ( $Po_2$ ,  $Pco_2$ ) and the use of non-blood measuring to monitor blood glucose, urea, etc.

International biomedical research sensor technology is synchronous with advances in the development of biomedicine. A major issue is how to improve the clinical technology and develop biomedical research.

Biosensors are bioanalytical devices that use biological materials such as proteins, cells and tissues as sensitive elements to be integrated with various physicochemical transducers for sensing the desired signals. With continuous research in biomedicine and physics, chemistry, electronics, and the discovery of materials and inventions, this has quickly led to important applications in the area of biosensors, such as micro-structure and the integration of biomedical sensors, biochips, nanotechnology sensors, etc.

#### **1.4.2 Multi-parameters Detection**

In clinical medicine, physiological parameters and multiple sensors are usually needed to support clinical operations. Multi-parameter sensor are detection system with small dimension and multiple function, using a single sensor system to measure multiple parameters simultaneously to obtain the functions of multiple sensors. Multi-parameter sensor integrate various sensitive components on one chip. Since the working condition is the same, it is easy to compensate and correct the system differences. Compared with using multiple sensors, it has higher accuracy, good stability, smaller dimension, less weight and lower cost.

Serial operation is hard to overcome when discrete sensors are used for monitoring different parameters, which have low efficiency and cannot meet simultaneous requirements in terms of time and space. Integrated technology creates conditions for multi-parameter sensors. At the beginning of the 1980s, British researchers invented an integrated blood electrolyte sensor which monitors 5 parameters ( $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ ,  $Cl^-$  and pH). At the end of the 1980s, some researchers designed LAPS for monitoring multiple biochemical parameters (Wu et al., 2001).

Nowadays, the improvement of living standards requires continued advancement in diagnosis and therapy methods. Physical sensors will have more significant applications in the biomedical field, especially with the development of MEMS

technology for developing more precise and down sized sensors and novel measuring technology. It is apparent that physical sensors will become more miniaturized, with higher precision and integration, and also more multifunctionalized.

### **1.4.3 In vitro and in vivo Detection**

*In vivo* detection is to detect the structure and function of living humans or animals while *in vitro* detection is to detect the blood, urine, living tissues or pathological specimens *in vitro* (Gründler, 2007). These detecting technologies are very important in clinical laboratory tests. *In vitro* analysis and detection of tissue slices and blood or gas samples aim to quantitatively analyze the composition and quantity of those substances and evaluate whether they are normal or whether there are some pathological microorganisms. *In vitro* detection requires high detection accuracy and precision and quick response. Because of the variety of detection categories, multiple kinds of automatic detection are required to make the most of the samples and testing reagents. Based on the requirements above, many detection methods have been developed and new chemical and biological sensors have been invented. In addition to the update of conventional clinical analysis detection, the following new technological fields have made great improvements (Wang and Liu, 2009a):

- Minim and tracing element detection;
- Super minim hormone detection;
- Molecular level and cellular level detecting technology;
- Biosensor micro system development and applications;
- Cancer cells self-recognition;
- Chromosome automatic classification;
- DNA automatic analysis;
- Detection of olfactory and gustatory quantities.

As with an increase in the importance of clinical biochemical analysis and the amount of analyzing samples and contents, *in vitro* detection is becoming multi-functional, continuous and automatic. All different kinds of automatic biochemical detecting devices, using the methods of optical analysis and electrochemical analysis, will rapidly improve with the development of computer automated recognition and analysis technology.

*In vitro* detection mostly belongs to the biochemical quantity detection. This detection involves many fields including gene engineering, protein engineering, LB film techniques, biosensor techniques, image analysis process and automatic measurement.

Monitoring *in vivo* can be done by observing physiological and pathological processes in real-time, from a fixed-point over a long period of time (Hauser and Fhrs, 2009). *In vivo* monitoring provides important information which cannot be obtained in other ways. Along with the progress in emerging sensor technology,

there are also wide ranges of monitoring technologies: implanted sensors can send information from inside to outside of the body; and the catheter sensor can continuously detect gas/ion in intravascular blood or the heart. The main problem of *in vivo* monitoring is how to improve the compatibility between the organs and the issues (Vo-Dinh and Cullum, 2000).

Brain-computer interface (BCI), sometimes called a brain-machine interface, is a direct communication pathway between a brain and an external device. BCIs are often aimed at assisting, augmenting or repairing human cognitive or sensory-motor functions. Neuroprosthetics is an area of neuroscience concerned with neural prostheses, using artificial devices to replace the function of impaired nervous systems or sensory organs. Neuroprosthetics typically connect the nervous system to a device, whereas BCIs usually connect the brain (or nervous system) with a computer system. Practical neuroprosthetics can be linked to any part of the nervous system—for example, peripheral nerves, while the term “BCI” usually designates a narrower class of systems which interface with the central nervous system.

Moreover, the sensor in a molecular system can identify proteins. The processor can ascertain the structure of the gene and the actuator can cut or unite the gene, which is the molecular system that can control and modify the gene and affect the life course. The design and synthesis of molecular systems is a new task for medicine and pharmacology. The research into anti-cancer drugs is moving in this direction.

The research has made achievements in two aspects: the first is the property of all magnetic waves and infrared light passing through skin and human tissue; the second is the coupling method of internal and external information. The common method of internal and external information exchange is an echo response which implants the energy needed for *in vivo* detection and the controlling device from an *in vitro* body sends the detected signal *in vivo* to outside of the body for later processing (Ricci, 2010). Another form is to send a stimulus or program controlled signal into the body and pick up the signal outside the body using a coupling coil. Take an implantable temperature detecting device as an example. A quartz crystal should be implanted *in vivo* in the body to measure the temperature and a magnetic coupling coil should be placed externally. Linear FM signals are supplied *in vitro* and the temperature is measured by using the linear relation between the crystal resonance frequency and temperature. Measurement error can be controlled under 0.1 °C and the method is quite stable over a long time.

#### 1.4.4 Intelligent Artificial Viscera

The invention of an intelligent artificial pancreas provides a reference to intelligent artificial organs. There are a lot of relationships between the viscera and other organs. Actual artificial organs just have one function, so they cut down all the

connections to other organs. Intelligent artificial viscera, which are equipped with a sensor system, microsystems or a molecular system, are intended to have all the functions of normal organs. Xenotransplantation is faced with problems of insurmountable rejection, so it will be an effective way to equip an anti-rejection molecular system on the transplanted organs (Nakamura and Terano, 2008).

Detecting is one of the most important development trends. Usually the whole process, including sampling, submitting and reporting, takes over half an hour, it is highly disadvantageous in saving time and doing good surgery. To solve the problem, bedside monitoring sensors have been developed. Bedside monitoring sensors should be simple, durable, lightweight and be in a continuous or semi-continuous operation for the convenience of medical professionals (Ricci et al., 2009).

At the same time, wearable devices developed quickly in recent years. Smart textiles using fabric-based sensors have been utilized in biomedical applications, such as monitoring gesture, posture or respiration. Most of fabric-based sensors were fabricated by either coating piezo-resistive materials on a fabric or directly knitting conductive fibers into fabrics. The sensors used are generally physical sensors like resistance sensors, capacitance sensors and inductance sensors in bio-monitoring, rehabilitation, and telemedicine.

#### ***1.4.5 Micro-nano Systems***

Modern sensors have changed from traditional structure design and manufacturing technique to micromotion. Micro-sensors are made by micro-mechanic technology, including photoengraving and corrosion. Its sensitive component is micron-level small.

Micro-sensors can enter a part of the human body such as the inside of the viscera and disease focus that is unreachable for traditional sensors to get information. In addition, since the micro-sensor is very small, it largely reduces the impedance and effect to normal physiological activity, which makes the measured value more genuine and reliable.

Using silicon technology, it is possible to integrate a CPU and a miniature sensor on the same silicon chip, which promotes intellectualized micro-sensor technology. The microsystem is a silicon chip integrated micro-sensor, microprocessor and micro-actuator. Now comes the molecular biomedical era, at the level of the system, organs, tissues, cells and macromolecules, sensor detection types are changing with developments in life science research, from mechanical sensors, physical sensors, chemical sensors, and biosensors to molecular sensors (Table 1.1).

On a technological level, sensor miniaturization and electronic devices miniaturization are being carried out at the same pace. A nanode, whose tip-diameter is of nanometer size, already exists. Moreover, in-nucleus detection has been on the agenda for some time.

**Table 1.1** Development history of sensors

Time	Electronic technology	Sensor technology
1960s	Vacuum tube	Normal sensor (cm)
1970s	Transistor	Small sensor (mm)
1980s	Microelectronics	Micro-sensor ( $\mu\text{m}$ )
1990s	Cell-molecular electronics	Cell-molecular sensor ( $\mu\text{m}$ , nm)
2000s	Nano-molecular electronics	Nano-sensor (nm)
2010s	Nano electro-mechanical system	Nano-sensor (nm)

Nano-technology involves many disciplines across advanced technologies that study the structure and property of substances at the level of 1 – 100 nm. The key to this technology is the study of how to make molecules produce substances and how to control the process, which is also called the molecular production process. Therefore, nano-technology brings opportunities to functionally sensitive materials in biosensors and compatible nano-devices. It also provides hope for molecular detection and diagnoses (Kricka, 2001).

Because of the specificity of its structure, nano-material has some specific effects, mainly the micro-size effect and interface effect. Nano-biosensors will play an important role in sensing and detection technology as it is different from typical sensors and has specific biological effects.

#### 1.4.6 Biochips and Microfluidics

Current biochemical analyzers in the laboratory departments of domestic hospitals in China are large in size and expensive (thousands of dollars). According to the aim of developing economical biomedical engineering, both at home and abroad, low-input and high-output detecting devices must be emphasized (Mohanty and Koulianou, 2006; Whitesides, 2006). This has several advantages such as economic, easy to operate, etc. Therefore the performance price ratio is much higher than large precision instruments of the same kind.

Early diagnoses should not depend too much on imaging apparatus. Biochemical changes take place earlier than organic changes and immune sensors can quickly detect a-FT.

The gene controls cell activities and the processes of men's life. Gene detection is viewed as one of the core techniques in modern life science. Gene detection uses traditional biochemical methods and gene probes at present. The disadvantages of these methods are complicated operations and low efficiency and an effective solution is to develop a DNA/RNA sensor. These researches are actively proceeding.

The cell is the basic unit of the human body, and is where the main human physiological and biochemical processes take place in. It is a hot topic in life sciences to monitor ion incidents and molecular events in cells. Ion-selective microelectrode technology which is used for monitoring ion incidents is becoming more and more mature. And ion-selective microelectrode technology which is

used to monitor molecular events is being researched.

### 1.4.7 Biomimetic Sensors

There are various sensors in the human body, which have good features such as high sensitivity, good selectivity, and high density. The development of biomimetic sensors is an important direction in biomedical sensor technology. There are many kinds of receptor sensors, nerve cell sensors, and biomimetic nerve cell sensors. The main problem with directly using biomaterials is that when the sensors leave their original environment they will lose their activity. The main solution is to use biomimetic chemistry to modify or synthesize sensitive materials (Zeravik et al., 2009; Wang and Liu, 2009b).

As two of the basic senses of human beings, olfactory and taste play a very important role in daily life. These two types of chemical sensors are important for recognizing environmental conditions. Electronic nose (e-Nose) and electronic tongue (e-Tongue), which mimics animals' smell and taste to detect odors and chemical components, have been carried out due to their potential commercial applications for biomedicine, food industry and environmental protection. The biomimetic artificial nose and tongue will be presented. Firstly, the smell and taste sensors mimicking the mammalian olfaction and gustation will be described, and then, some mimetic application with the signal processing methods for odorants and tastants detecting will be developed. Finally, olfactory and gustatory biosensors are presented as the developing trends of this field.

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## **Chapter 2**

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### **Basics of Sensors and Measurement**

This chapter introduces basic sensor microfabrication technology, characteristics of sensors and measurement technology as well as common measuring methods and systems. Moreover, the serious concern about devices that comes from direct contact with the human body, which termed as “biocompatibility”, will be discussed here.

#### **2.1 Introduction**

Biomedical sensing and measurement involves sensitive technology and measurement techniques. Different sensors are adopted to detect different types of measuring objects. Due to the diversity of the measurements, there are different sensor types, working principles and sensor structures. Despite the difference of various sensors, the evaluation methods of sensor characteristics are almost the same. Sensors working under certain environment will become less sensitive, which is termed as “lifetime”; sensors produce uniform response to the same input, which is termed as “repeatability” and so on. Such kinds of features are categorized into static characteristics and dynamic characteristics, which provide unified criteria for evaluation of different sensors. The important and most commonly mentioned property of sensors is “4s”: selectivity, sensitivity, stability, and safety. Safety should be entirely considered in design and operation. Likewise, the measurement technology for different sensors is similar. A sensor detection system usually consists of sensors, measuring circuits and an output system. For different measuring purposes, circuits are designed differently such as parameter converting circuits, computing circuits and so on. The measurement methods can be classified by the measuring object’s features, such as direct and indirect, active and passive, invasive and non-invasive, wired and wireless. For some implantable biomedical sensors in particular biological measurement environment, the compatibility between biological tissues and biomedical sensors is one of fundamental problems. This issue has been extended to sensor detection *in vitro*.

Therefore, this chapter tries to make a concrete introduction to the common parts of the biomedical sensors: the basic microfabrication technology, basic sensor characteristics and measuring methods and systems. We also intend to emphasize the biocompatibility design of biomedical sensors because of its unique and important status. The following chapters will utilize the basic concept and basic knowledge of biomedical sensors mentioned in this chapter.

## 2.2 Sensor Characteristics and Terminology

Since a sensor influences the characteristics of the whole measurement system, it is important to describe its performance. The characteristics of a sensor may be classified as being either static or dynamic, and these parameters are essential in describing its behavior. Static characteristics can be measured after all transient effects have stabilized to their final or steady state, while dynamic characteristics describe the sensor's transient properties, and they can be measured in the sensor's responses to the time-varying inputs.

### 2.2.1 *Static Characteristics*

Static characteristics are measured under the standard static condition, which means no acceleration, no vibration or shock (unless shock is the measurand), and that the temperature is  $25\pm 5$  °C, the relative humidity is lower than 85%, and the atmospheric pressure is  $101.3\pm 8$  kPa.

Under the standard static condition, using instruments with a higher accuracy to measure the sensor's output repeatedly, and then the static calibration curve can be plotted depending on the measured data. The static characteristics can be obtained from the static calibration curve.

Because of the biomedical sensors' special features, some static characteristics (such as lifetime, sensitivity, selectivity, etc.) are particularly important for them, and these characteristics are highlighted in this section.

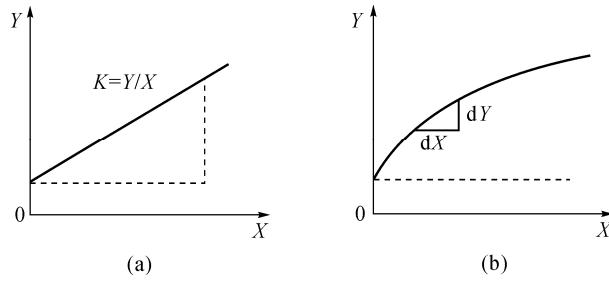
*Lifetime* is the length of time that sensors remain sensitive under normal operational conditions. As biomedical sensors may sometimes be implanted into a body, they should be able to withstand the internal environment of the human body and also maintain the normal functions.

*Selectivity* is a sensor's ability to measure a single component in the presence of others. For example, a calcium ion-selective microelectrode that does not show a response to other ions such as  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Mg}^{2+}$ , may be considered as selective.

*Sensitivity* is the ratio of incremental change in the output of the sensor to its incremental change of the measurand in input. For example, if a gas sensor's output voltage increases by 1 V when the oxygen concentration increases by 1,000 ppm,

then the sensitivity would be 1 mV/ppm. In many cases, the signals measured by biomedical sensors are relatively weak. Therefore, sensitivity is a very meaningful parameter for biomedical sensors, which is especially significant in the measurement of electrophysiological signals.

A linear sensor's sensitivity is a constant, while for a nonlinear one, its sensitivity changes with inputs (Fig. 2.1). The static sensitivity can be obtained from the static calibration line, and for a linear sensor, its static sensitivity is the slope of the static calibration line.



**Fig. 2.1.** Static sensitivity curves: (a) Linear sensor; (b) Nonlinear sensor

*Detection limit* is the minimum detectable signal that can be extracted in a sensing system when noise is taken into account. If the noise is large relative to the input, it is difficult to extract a clear signal from the surrounding noise. As the human body is a very complicated system, various signals impact each other, so for biomedical sensors, how to extract target signals from the complex noise background is an important issue.

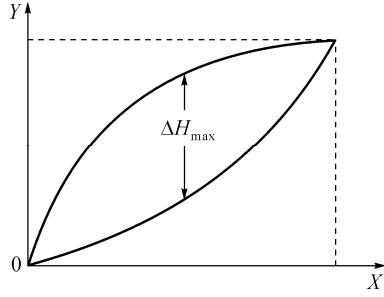
Apart from the points described above, there are also many other static characteristics extremely important for all sensors.

*Linearity* is the closeness of a sensor's calibration curve to a specified straight line, and the specified straight line usually is the sensor's theoretical behavior or its least-squares fit. Linearity is expressed as a percent of the full-scale output ( $Y_{F.S.}$ ), which is the maximum deviation ( $\Delta_{max}$ ) of any calibration point from the corresponding point on the specified straight line, and it is usually denoted as  $\varepsilon$ :

$$\varepsilon = \frac{\pm \Delta_{max}}{Y_{F.S.}} \times 100\% \quad (2.1)$$

*Hysteresis* is the maximum difference in output  $\Delta H_{max}$  at any measured value within the specified range, when the value is approached first with an increasing and then a decreasing measured, which is shown in Fig. 2.2. It is also given in percent of  $Y_{F.S.}$ :

$$\delta_H = \pm \Delta H_{max} / Y_{F.S.} \times 100\% \quad (2.2)$$



**Fig. 2.2.** Hysteresis curve

*Repeatability* is the sensor's ability to produce the same response for successive measurements of the same input, when all operating and environmental conditions remain constant.

*Error* is defined as the difference between the measured result and the true value, which is always inevitable in any measurement. But it is true that for all measurements, errors must be reduced by improvements in techniques and ideas, and then the validity of results can be estimated.

*Accuracy* defines how correctly the sensor output compares to the true value, and it is related to the bias of a set of measurements. Accuracy is measured by the absolute and relative errors, which are calculated by:

$$\text{absolute error} = \text{result} - \text{true value} \quad (2.3)$$

$$\text{relative error} = \frac{\text{absolute error}}{\text{true value}} \quad (2.4)$$

In order to assess the accuracy of a sensor, either the measurement should be calibrated against a standard measurand or the output should be compared with a measurement system with a known accuracy (Wilson, 2005).

*Precision* signifies the number of decimal places to which a measurand can be reliably measured. It relates to how carefully the final measurement can be read, not how accurate the measurement is. And it implies the agreement between successive readings, not the closeness to the true value.

*Resolution* signifies the smallest incremental change in the measurand that will result in a detectable increment in the output signal. It is the minimal change of the input necessary to produce a detectable change at the output.

*Drift* is the gradual change in the sensor's response while the measurand remains constant. Drift is the undesired and unexpected change that is unrelated to the input. It may be attributed to aging, temperature instability, contamination, material degradation, etc. (Kalantar-zadeh and Fry, 2008).

*Dynamic range or span* is the range of input signals that will result in a meaningful output for the sensor. All sensors are designed to perform over a specified range. Signals outside of this range may cause large inaccuracies, and

may even result in damage to the sensor.

### 2.2.2 Dynamic Characteristics

Dynamic characteristics are the response characteristics to time-varying inputs. The response comprises two parts, the transient one and the steady-state one. Transient response is the process from an initial state to a final state. And steady-state response is the output state when time is moving to infinity. Since the majority of physiological signals detected by biomedical sensors are functions of time, in order to obtain the real body information, the biomedical sensors should possess both good static and dynamic characteristics, which means the sensor's output curves should be the same or similar to the measurand curves during the same time period.

It is advantageous to use the linear differential equations with constant coefficients for sensing systems. Such representations have been widely studied and used, and they are easy to extract information from and give an overall vision about the sensing systems. The relationship between the input and output of any linear time invariant measuring system can be written as:

$$\begin{aligned} & a_n \frac{d^n y(t)}{dt^n} + a_{n-1} \frac{d^{n-1} y(t)}{dt^{n-1}} + \dots + a_1 \frac{dy(t)}{dt} + a_0 y(t) \\ & = b_m \frac{d^m x(t)}{dt^m} + b_{m-1} \frac{d^{m-1} x(t)}{dt^{m-1}} + \dots + b_1 \frac{dx(t)}{dt} + b_0 x(t) \end{aligned} \quad (2.5)$$

where  $x(t)$  is the measured quantity (input signal) and  $y(t)$  is the output reading and  $a_0, \dots, a_n, b_0, \dots, b_m$  are constants.

According to the order of Eq. (2.5), there are three basic types of sensing systems: zero-order, first-order and second-order systems, which not only because these three basic types can describe most common biomedical sensing systems, but the more complex ones can also be approximately represented with these three types. In analysis of their dynamic characteristics, the step and sinusoidal signals are often regarded as typical experimental signals.

#### **Zero-order sensors**

The differential equation of zero-order system is:

$$a_0 y(t) = b_0 x(t) \quad (2.6)$$

and the transfer function is:

$$H(s) = b_0 / a_0 = K \quad (2.7)$$

where  $K$  is a constant and is defined as the static sensitivity. A zero-order sensor sensing system has ideal dynamic characteristics.

### **First-order sensors**

They can be represented by such differential equations:

$$a_1 \frac{dy(t)}{dt} + a_0 y(t) = b_0 x(t) \quad (2.8)$$

$$\text{or } \tau \frac{dy(t)}{dt} + y(t) = Kx(t) \quad (2.9)$$

and the transfer function is:

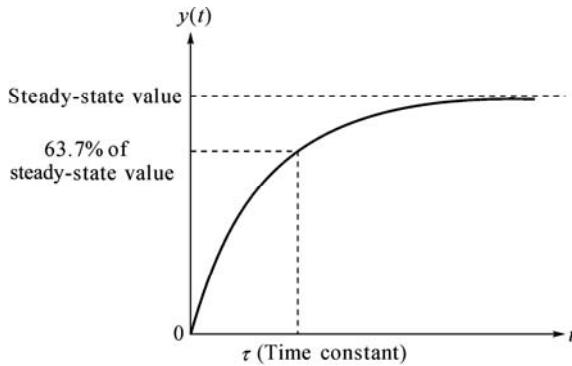
$$H(s) = K / (1 + \tau s) \quad (2.10)$$

where  $K = b_0/a_0$  is the static sensitivity, and  $\tau = a_1/a_0$  is the time constant.

The unit step response of a first-order sensor is:

$$y(t) = K(1 - e^{-t/\tau}) \quad (2.11)$$

which is shown in Fig. 2.3, and  $\tau$  is the time constant when the output value reaches 63.7% of its steady state value.



**Fig. 2.3.** Unit step response curve of a first-order sensor

### **Second-order sensors**

On the other hand, the representation of a second-order system can be defined either as

$$a_2 \frac{d^2 y(t)}{dt^2} + a_1 \frac{dy(t)}{dt} + a_0 y(t) = b_0 x(t) \quad (2.12)$$

$$\text{or} \quad \frac{1}{\omega_0^2} \frac{d^2y(t)}{dt^2} + \frac{2\xi}{\omega_0} \frac{dy(t)}{dt} + y(t) = Kx(t) \quad (2.13)$$

where  $w_0 = \sqrt{a_0 / a_2}$  is defined as natural frequency,  $\xi = a_1 / (2\sqrt{a_0 a_2})$  is damping ratio of the sensor, and  $K = b_0 / a_0$  is static sensitivity of the sensor. And the transfer function is:

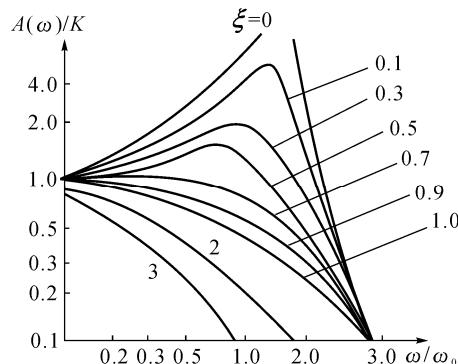
$$H(s) = \frac{\omega_0^2 K}{s^2 + 2\xi\omega_0 s + \omega_0^2} \quad (2.14)$$

From the transfer function presented above, its amplitude-frequency and phase-frequency characteristics can be written as:

$$A(\omega) = \frac{K}{\sqrt{[1 - (\omega/\omega_0)^2]^2 + 4\xi^2(\omega/\omega_0)^2}} \quad (2.15)$$

$$\text{and} \quad \Phi(\omega) = -\arctan \left[ \frac{2\xi(\omega/\omega_0)}{1 - (\omega/\omega_0)^2} \right] \quad (2.16)$$

Figs. 2.4 and 2.5 show the amplitude-frequency and phase-frequency characteristics of second-order sensors respectively, in which the damping ratio  $\xi$  has a great influence on the frequency characteristics. When  $\xi > 1$ , the amplitude-frequency curve will be decreasing without any peak. Thus the width of the amplitude-frequency curve's flat part is closely related to  $\xi$ , and when  $\xi = 0.707$  around, the flat part has the largest width. On the other hand, an under-damped system ( $0 < \xi < 1$ ) is faster to reach the steady state than a critical damped one ( $\xi = 1$ ), while the over-damped system ( $\xi > 1$ ) is slow to respond. Therefore, under-damped systems are generally used, and the value of  $\xi$  usually is 0.6 – 0.8.



**Fig. 2.4.** Amplitude-frequency characteristics

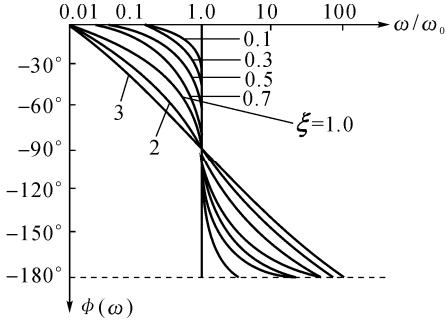


Fig. 2.5. Phase-frequency characteristics

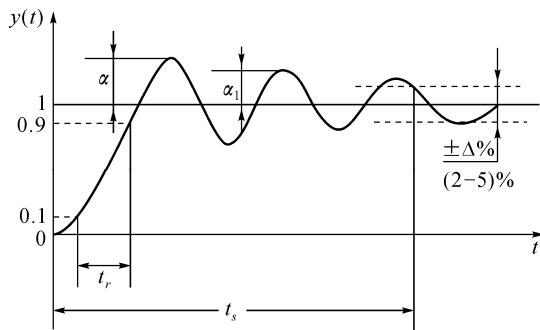


Fig. 2.6. Unit step response curve of a high-order sensor

Fig. 2.6 shows the response of a high-order system to the unit step input, from which some other parameters in time-domain can be defined as following:

**Rise time  $t_r$ :** the required time that the steady-state value rises from 10% to 90%.

**Response time  $t_s$ :** the required time that the output response maintains stability in the  $\pm \Delta\%$  error tolerances.

**Overshoot  $\alpha$ :** the value that the maximum output exceeds the steady-state value.

**Delay time  $t_o$ :** the required time that the output first reaches 50% of steady-state value.

**Attenuation  $\psi$ :** the percentage of height decline between two adjacent peaks (or troughs),  $\psi = (\alpha - \alpha_1)/\alpha \times 100\%$ .

Among the performance parameters in time-domain above,  $\tau$ ,  $t_s$  and  $t_r$  reflect the response rate of a system, but  $\psi$  and  $\alpha$  indicate the relative stability of a system. And it should be noted that these performance parameters are not all used under any case; for example, in a damping system, attenuation and overshoot are not necessarily utilized.

## 2.3 Sensor Measurement Technology

The sensor measurement technology is used to detect a signal. According to different classifications, the measurement methods can be classified as direct and indirect, active and passive, invasive and non-invasive, wired and wireless. To detect signals, a proper sensor system is needed. A sensor system should include the sensor interface, signal preprocessing circuits, as well as other computer-aided Digital Signal Processing hardware and software and so on. Sometimes, in some specific environments, it is difficult for a sensor system to detect signals. In this case, some other methods are implemented to improve the performance of the sensor system.

### 2.3.1 Measurement Methods

In this section we will introduce the following measurement methods: direct and indirect measurement, active and passive measurement, invasive and non-invasive measurement, and wired and wireless measurement.

#### 2.3.1.1 Direct and Indirect Measurement

The direct measurement method uses the outputs of instruments as the results. For example, the measurement of a circuit by an electromagnetic current meter, and the measurement of pressure by a bourdon tube pressure gauge are both direct measurements.

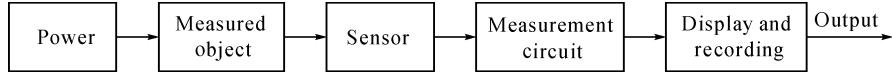
The indirect measurement method uses the calculated results of some measured parameters as the final ones. For example, the measurement of inductance by measuring the resonant frequency of the circuit is an indirect measurement. This method has more steps and requires a longer time, and generally, it is only used when direct measurement is inconvenient.

#### 2.3.1.2 Active and Passive Measurement

According to the power supply of the measurement system, measurement is divided into active measurement and passive measurement.

##### *Active measurement*

The structure of the active measurement system is shown in Fig. 2.7. It provides power to the measured object. For example, when measuring the impedance, we need to supply an electrical voltage perturbation to measured sample.

**Fig. 2.7.** Active measurement system

### **Passive measurement**

The structure of the passive measurement system is shown in Fig. 2.8. The passive measurement system does not need power supplies from outside.

**Fig. 2.8.** Passive measurement system

#### **2.3.1.3 Invasive and Non-invasive Measurement**

Invasive measurement uses methods that will influence or even injure the objects while the non-invasive measurement will have tiny or no influences on the objects. For example, there are many methods to detect cancers, including gene detection (Aaroe et al., 2010), blood detection (Oono et al., 2010), PET-CT (Fischer et al., 2009), e-Nose (Wang D. et al., 2009) and so on. Among these methods, only the electronic nose is non-invasive as it just collects the patients' exhaled without any injuries. This is thought to be the great advantage of the electronic nose compared with other methods.

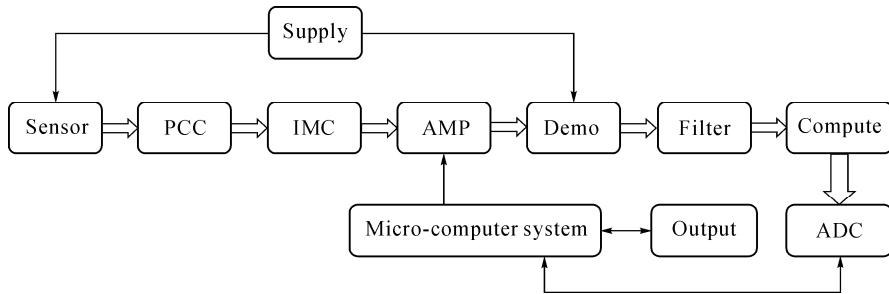
#### **2.3.1.4 Wired and Wireless Measurement**

In recent years, wireless measurement has developed very fast and is being used in more and more fields such as communication technology, biomedical fields and so on. For example, an RFID-Based Closed-Loop Wireless Power Transmission System has been reported in use in biomedical field, especially for inductively powering implantable biomedical devices. In this system, the transmitter and receiver coils are in a wireless power transmission. Any changes in the distance and misalignment will cause a significant change in the received power. As it is a closed loop, this change will get detected. This system can be used on the implanted chips to better detect diseases (Mehdi and Maysam, 2010).

### **2.3.2 Sensor Measurement System**

With the development of micro-processing technology, the composition of the sensor detecting system has also developed significantly. Smart sensor detecting

systems with a microprocessor is the direction for modern sensor detecting technology. The basic components are shown in Fig. 2.9.



**Fig. 2.9.** Fundamental composition of a sensor detecting system

The detecting system is generally composed of sensors, measuring circuits (sensor interface and signal pre-processing circuit) and output system. The sensors detect the measured signals in the measurement environment, and convert them into electric signals. Sensors are generally called the primary instrument, while the following measuring circuits and output circuits are called the secondary instrument.

In the process of measurement, the measuring circuits can be divided into the sensor interface circuit and the pre-processing circuit. The sensor interface circuit connects the sensor and the pre-processing circuits. It is usually composed of the parameter conversion (basic conversion circuit), sensor output signal modulation and impedance matching circuit. The sensor interface circuit extracts the measured signal. The signal pre-processing circuit is usually composed of operations, demodulation, filtering, A/D and D/A. It detects the measured signal, and conducts the discrete signal processing if necessary. Intelligent sensing detection systems can not only detect and process signals, but also self-diagnose and self-recover.

#### **Parameter converting circuits**

The measured signal makes the sensors' electrical parameters change, such as resistance, inductance and capacitance. In general, these changes are converted into voltage or frequency signals that are proportional to them. Therefore, there must be parameter converting circuits (PCC).

The commonly used parametric conversion circuits include: the bridge circuit (converting resistance, inductance or capacitance into voltage); current-voltage (IV) converting circuit; resistance, inductance, capacitance (RLC) oscillation circuit (converting resistors, inductors, capacitors into voltage or frequency digital signal), etc.

#### **Impedance converting circuits**

Sensors can be regarded as a signal source with certain output impedance, while

the following interface circuit has certain input impedance. In order to decrease the effects of the input impedance on the output signal, the impedance transformation is used in the design of the sensor interface circuit. By taking high input impedance amplifiers, the sensors' high output impedance is converted into low output impedance. For example, in the design of measuring circuits of the voltage equivalent circuit using piezoelectric sensor, voltage amplifier has the effects of impedance conversion and signal amplification.

### ***Computing circuits***

In the measurement circuits, operation circuits mainly consist of ratio circuits, addition and subtraction circuits, integral circuits, differential circuits, logarithmic circuits, exponential circuits, multiplication and division circuits, and so on.

*Ratio computing:* The outputs of the sensor are generally relatively small, usually in millivolt level which should be amplified. The integrated operational amplifier can be used as a signal amplification circuit device. According to different ways of op amp feedback connection, the integrated operational amplifier can be divided into the non-inverting proportional circuit (constituting voltage amplifier) and inverting proportional circuit (constituting current-voltage conversion amplifier).

*Addition and subtraction circuits:* To achieve the addition and subtraction of different signals and adjust the amplitude of the signal bias. Differential amplifier circuit is one of the basic types of such circuits.

*Logarithmic and exponential circuits:* To achieve the non-linear operation in the circuits. For example, logarithmic circuits can linearize the output of the exponential signals.

### ***Analogue filter circuit***

In the measurement system, the analog filter in the signal pre-processing circuit is very important as it can select the needed frequency components. Filtering circuits can pass specific frequency components but greatly decrease other frequency components, so as to filter the noise. Filters are generally divided into four categories based on the amplitude-frequency characteristics: low pass, high pass, band pass and band stop. Allowing for the components, filters can be divided into RC, LC, and crystal resonator filters. Also, filters can be classified as active and passive according to the components. Finally, based on the characteristics of amplitude-frequency and phase-frequency near the cut-off frequency, filters are divided into Butterworth filters, Chebyshev filters, Bessel filters, and so on.

### ***DAC and ADC interface circuits***

Both the signals from the sensor to the sensor interface and those the pre-processing circuits involve are analog signals. In order to adopt DSP in the measurement system, analog signals must be converted to digital signals so that computers can handle them. A/D converters can convert analog signals to digital

signals. The high frequency of sampling and the required accuracy of the measurements are the main technical requirements to choose the suitable A/D converter and D/A converter. A/D interface, D/A interface and the computer constitute an intelligent closed-loop control system.

### **Digital Signal Processing**

The use of computer and Digital Signal Processing technology greatly advances the measurement system. Computers can analyze, judge the signals, and display the results. For example, in the measurement and control system, the zero point error correction will be very simple under the microchip's control. In a system without a microchip, the only choice to reduce the zero-point error caused by temperature drift is to choose components with low drift coefficients and to design a temperature compensation circuit.

Digital signal processing has the advantages of stability and good repeatability, compared with analogue signal processing. To improve detection accuracy and reduce the cost of the hardware circuit, sensor linearization software is very applicable in engineering practices. With the development of advanced high-speed computer and the increase of storage capacity, technologies such as the multi-sensor detection have been widely used.

### **2.3.3 Signal Modulation and Demodulation**

If the sensor's output values are relatively small, the noise voltage of the amplifier, the DC amplification temperature drift of the measuring circuits, zero-point drift and inter-stage coupling will result in serious measuring errors. In order to improve the anti-interference capability, the sensor's output signal is modulated in a parametric conversion circuit so that the sensor would output an AC signal, with the change of the signal corresponding to signal amplitude, frequency or phase changes.

In the measuring circuit, narrow-band filter demodulation and the relevant demodulation can both be used to detect the measured signal. Generally the slow-varying signals which control the high-frequency oscillation is called modulation wave (measured signal); the high-frequency oscillatory signals containing the variables are called the carrier (the excitation signal in the parameters conversion circuit); and modulated high-frequency oscillatory waves are called the modulated wave (the sensor output signal). Demodulation processes the modulated wave to gain the slowly changed measured signal.

#### **2.3.3.1 Signal Modulation**

Generally a high-frequency sinusoidal signal is used as the carrier signal. A sinusoidal signal has three parameters: amplitude, frequency, and phase, which can

be modulated, and be referred to as amplitude modulation (AM), frequency modulation (FM) and phase modulation (PM) respectively. Pulse signals can also be used as the carrier signal. Different features of the pulse signal can be modulated. The most commonly used method is the pulse width modulation, called PWM.

#### ***Amplitude modulation***

Amplitude modulation (AM) uses the modulation signal to control the amplitude of the high-frequency carrier signal. A commonly used method is the linear amplitude modulation, that is, the amplitude of the AM signal changes with the modulation signal linearly. The process of AM is as follows: The high-frequency carrier signal is multiplied by the modulation signal, thus forming the AM signal. In this case, the amplitude of the modulation signal is contained in the AM signal.

#### ***Frequency modulation***

Frequency modulation (FM) uses the modulation signal to control the frequency of the high-frequency carrier signal. A commonly used method is linear FM, that is, the frequency of the FM signal changes linearly with the modulation signals. Thus, in the demodulation process, by measuring the change of the frequency of the FM signal, the modulation signal is detected.

#### ***Phase modulation***

Phase modulation uses the modulation signal to control the phase of the high-frequency carrier signal. A commonly used method is the linear phase modulation, that is, the phase of the PM signal changes linearly with the modulation signal. In the demodulation process, the modulation signal will be detected by measuring the phase change of the PM signal.

#### ***Pulse-width-modulation***

Pulse-width-modulation (PWM) is widely used in pulse modulation. PWM uses the modulation signal to control the width of the high-frequency pulse signal. The most commonly used method is the linear PWM, that is, the width of the PWM signal is linear with the amplitude of the modulation signal. When the amplitude of the modulation signal gets larger, the width of the PWM signal becomes larger accordingly, and vice versa.

##### **2.3.3.2 Signal Demodulation or Detection**

The process of extracting the measured signal from the modulated signal is called demodulation. Amplitude modulation makes the amplitude of the AM signal change with that of the modulation signal, so that the envelope shape is consistent

with that of the modulation signal. Demodulation is achieved as long as the envelope curve can be detected. This method is called envelop detection.

#### ***Envelope detection***

Envelope detection can demodulate the modulation signal by detecting the envelope. By low-pass filter, the high-frequency signal is filtered, so that the low-frequency modulation one is obtained. Envelope detection is based on the principle of rectification.

#### ***Full-wave linear detection***

As diodes and transistors have a certain dead zone voltage  $V$ , that is, only after diode forward voltage and the transistor launch junction voltage exceed a certain value, they can be turned on, and the characteristics are curved. The characteristics of diodes and transistors  $V$  deviate from the ideal characteristics, which will result in errors. In order to improve the detection accuracy, a sophisticated detection circuit is adopted, which is called the linear detection circuit.

#### ***Phase-sensitive detection***

A phase-sensitive detection circuit can identify the phase of the modulation signal. There are two issues around envelope detection.

First, the main process of demodulation is the half-wave or full-wave rectification of the AM signal, in which the phase of the modulation signal cannot be detected from the output of the detector. For example, when an inductive transducer is used to detect the displacement of work pieces, when the core moves up and down for the same amount by its equilibrium position, the amplitudes of the sensor's output signal are the same, while the phase difference is  $180^\circ$ . From the output of the envelope detection circuit it cannot be determined whether the core moves up or down.

Second, the envelope detector circuit itself cannot differentiate signals with different carrier frequencies. The envelope detection circuit rectifies carrier signals with different frequencies in the same way to restore the modulation signal, that is to say, it is not able to identify signals. In this case, a phase-sensitive detection circuit is adopted, so that the detection circuit can distinguish phases and frequencies of signals.

#### ***2.3.4 Improvement of Sensor Measurement System***

The overall errors in the sensor detecting system are integrated by the errors of three basic parts. Thus, to improve the detection performance of the system, the improvement of the measurement precision is one of the main tasks.

The sensor is the first input of a detection system, whose errors will be passed

throughout the entire system. The relative errors caused by the sensor itself can be seen as an input to the following parts. Therefore, improving the accuracy of the sensor is of vital importance.

The design of sensors is the integration of mechanics, electricity, chemistry, biology, materials science and many other aspects. Thus, it is difficult to manufacture a sensor with good performance in all these aspects. However, in the actual application there may be some aspects that do not have influences on a particular signal. Therefore, in the design and choice of sensors, the characteristics of the measured signals which determine sensor performance should first be considered. It is not necessary to pursue the perfect performance. For example in a static test, there is no need to pursue the dynamic performance. The following focuses on how to improve the sensor's performance:

#### ***Choosing proper sensors***

When designing and choosing sensors, the following requirements should be considered: the detecting environment, the characteristics of the detected signals, and the requirements for the test accuracy. With all the above aspects fulfilled, the type, structure, material, size, weight and life expectancy of the sensors should also be considered.

#### ***Stabilization technology***

The various materials and components that constitute the sensor will change with time and environment. Then the performance of the sensor will change, resulting in instability. In this case, it is necessary to stabilize materials, components or even the overall sensors (also known as the aging processing). Electrical materials, such as magnetic materials, conductive materials, insulation materials, are first stored and even exposed to a certain voltage for a period of time to stabilize their performance and then the good ones among them are chosen.

#### ***Linearization technology***

The non-linearity of the sensor may be caused by the non-linearity of the input-output model or a poor sensor manufacture process. Thus, in the practical application, the input and output of the sensor is hardly linear. To improve the linearity of the sensor, generally some methods such as the differential design and the sensor linearization correction circuits (this method is related to the design of sensor interface circuit) are used.

#### ***Averaging technique***

The averaging technique uses a certain number of sensor units to measure the signal at the same time, whose outputs are the addition of these sensor units. The outputs are composed of the signal and the noise. According to the probability theory, the noise of each sensor can be considered to be unrelated and isolated, so

the addition of the outputs of the certain number sensor units will eliminate the noise and keep the signal at the same time.

## 2.4 Biocompatibility Design of Sensors

The term “biocompatibility” is used extensively within biomaterial science. Sensors used for biological detection and medical diagnosis must seriously consider biocompatibility in order to avoid hemo- or histo- problems with direct contact. This section introduces the concept, the classification and the evaluation of biocompatibility. The topic of biocompatibility related to the sensor design focuses on two aspects—*invasive sensors for implanting and monitoring physiological environment *in vivo* and non-invasive sensors for monitoring cell or tissue *in vitro*.*

### 2.4.1 Concept and Principle of Biocompatibility

The following content will include the concept of biocompatibility, classification of biocompatibility and evaluation, which provide essential rules for biomedical sensor fabrication.

#### 2.4.1.1 Biocompatibility

Biomedical sensors implanted in animal or human bodies or in direct contact with tissue *in vitro* must be non-toxic, non-allergenic, non-irritating, non-genetic toxic and non-carcinogenic. Besides, they are not supposed to make the animal or human tissues, blood and immune systems produce any adverse reaction. Sensors should be compatible with the chemical composition in organisms. Therefore, the biocompatibility of the material is a priority to be considered. David (2008) enhances the list of events that has to be avoided. He believes that the key to understanding biocompatibility is the determination of which chemical, biochemical, physiological, physical or other mechanisms become operative, under the highly specific conditions associated with contact between biomaterials and the tissues of the body, and what are the consequences of these interactions. Accordingly, not only the mechanisms by which materials and human tissues respond to each other but also the particular natural processes in different applications have to be carefully evaluated.

Biocompatibility is a serious problem, especially when it comes to biomedical sensors for practical clinic use. There is a large battery of *in vitro* tests that are used in accordance with ISO 10993 to determine if certain material is biocompatible. These tests constitute an important step towards animal testing and

finally towards clinical trials by implanting medical devices. Indeed, since the immune response and repair functions in the body are so complicated, it is not adequate to describe the biocompatibility of a single material in relation to a single cell type or tissue. Generally speaking, in the design of implantable biomedical sensors, the following factors should be considered:

- Sensors are not supposed to be corroded or toxic. In this way, they must be compatible with the chemical composition in organism.
- The shape, size and structure of sensors implanted in the human body should adjust to the anatomical structures of the measuring object. What is more, sensors should never injure the tissue.
- Sensors should be solid enough. When implanted, the sensor should not be damaged.
- Sensors must be electrically insulated. Once the sensor is damaged in a human body, it should be certain that the voltage imposed on the human body is under the safe security value.
- Sensors should neither place physical activities a burden, nor interfere with the normal physiological function.
- The *in vivo* sensors used for long term implantation should not cause any vegetation.
- The structure of the sensor should be easily disinfected.

The biomedical materials used as mediators between sensors and measuring objects should also be investigated. The major interaction between biomedical materials and the human body are listed in Table 2.1. The material changes including physical and chemical changes, both of which cause the corresponding changes in organisms by mechanical or physical or chemical interaction. Some of these are the major determinants of the host response, while others are of greater importance in device functioning.

**Table 2.1** The interaction between biological materials and the human body

	Biomedical materials changes	Response and changes in organisms
Physical changes	Size, shape, flexibility, rigid plasticity, brittleness, relative density, melting point, conductivity, and thermal conductivity	Acute systemic reaction, fever, paralysis Chronic systemic toxicity, teratogenesis, immune response, dysfunction
Biochemical changes	Hydrophilic, hydrophobic, pH value, adsorption, dissolution, permeability	Acute local reactions Chronic local reaction Inflammation, thrombosis, necrosis, rejection Carcinogenic, calcification, inflammation, ulceration
Interaction between biomedical materials and organisms: (1) mechanical interaction: friction, impact, bending; (2) physical and chemical interaction: dissolution, absorption, permeability, degradation; (3) chemical interactions: decomposition, modification		

Factors causing biomedical materials change:

- The dynamic mechanics of bones, joints and muscles in physical activities;
- Cellular bioelectricity, magnetic fields, electrolysis and oxidation in cells;

- Biochemistry and enzyme-catalyzed reaction in metabolic procedure;
- Adhesion and phagocytosis of cells;
- Biodegradation by various enzymes, cytokines, proteins, amino acids, peptides, and free radicals in body fluids.

Factors causing organism reactions:

- The residual toxic low-molecular-weight substances in the materials;
- The residual toxic, irritating monomer in polymerization process;
- The adsorbed chemical agents and the split products caused by high temperature in the sterilization process;
- The shape, size, surface smooth level of the materials and products;
- The pH value of the materials.

#### 2.4.1.2 Classification of Biocompatibility

##### ***Blood compatibility***

Biomedical sensors used in the cardiovascular system are in direct contact with blood. Thus, the reactions between them should be carefully considered. Implanted devices need to coexist with blood; in that case, they can function under the intended purpose without reactions to blood and tissue. Many reactions can be triggered by catheter insertion, which will cause sensor failure or tube plug due to the accumulation of fiber and blood clots. In addition, the existence of devices may put patients at risk, which may be due to toxic products being released into the blood or body fluids, or the increasing thrombosis caused by the sensor's charge or the external leakage current of the sensor. Blood clotting time is the most obvious indicator to show biological incompatibility of materials exposed to blood, which has been widely used in biomedical sensor technology. PTFE, which was considered to be the most inert and most compatible biomedical polymer, has a blood clotting time of 11 – 13 min, which therefore is regarded as 100%. While other ordinary inorganic silicon and glasses have very short blood clotting time, usually less than 10 min. This is why the procedure composition of sensors and the catheter surface, as well as surface modifying become critical issues in invasive sensor research and development.

##### ***Histocompatibility***

Biomedical sensors implanted out of cardiovascular system mainly focuses on the interaction between the materials and the tissues or organs. The immune system at first makes antibodies for all sorts of antigens, including those it has never been exposed to, but stops making them to antigens present in the body. If the body is exposed to foreign antigens, it attacks the foreign materials. Therefore, the materials in sensors should be histocompatible to avoid immune system attack.

### **2.4.1.3 Evaluation of Biocompatibility**

The quality and biocompatibility of biomedical materials and all kinds of medical equipment made by them are directly related to the safety of patients. Thus, authorities should set a standard registration and approval procedure for such products like ISO 10993. Biomaterials and medical equipment must pass the biological evaluation in either research or manufacture to ensure their safety. Generally speaking, biomedical materials safety includes the following four aspects: physical properties, chemical properties, biological properties and clinical researches. While in a narrow sense, biomaterials and medical device safety evaluation refers to the biological assessment.

Biological assessment generally contains the following aspects:

- Contact tissues: body surface and body tissues, bones, teeth, blood;
- Contact means: direct contact and indirect contact;
- Contact time: Temporary contact is less than 24 h, short and medium-term exposure is longer than 24 h but shorter than 30 d, and long-term exposure is more than 30 d;
- Purpose: general function, reproductive and embryonic development, biodegradation.

For example, silk fibroin materials used for constructing artificial nerve grafts is biocompatiblely evaluated with tissues and cells (Yang et al., 2007). The physical and biological characterization of the materials was studied. Biological assessment directly contacting with cells was also investigated. The rat dorsal root ganglia cells' outgrowth was observed by light and electron microscopy coupled with immunocytochemistry. Schwann cells' morphology and proliferation was tested by MTT test and cell cycle analysis.

### **2.4.2 Biocompatibility for Implantable Biomedical Sensors**

The state-of-the-art implantable biomedical sensors fall into the category of biophysical sensors for bioelectric detection and biochemical sensors for certain metabolite detection. For example, sensors for detecting electric signals in the brain (Schneider and Stieglitz, 2004) and sensors for monitoring bio-analytes in the brain (Hu and Wilson, 2002).

For the sake of causing less chemical reactions in metallic systems, the corrosive plain carbon and vanadium steels were replaced by stainless steels, then by the strongly passivated cobalt-chromium alloys, titanium alloys and platinum group metals. In the case of cardiac pacemakers and implantable cardioverter defibrillators, which help to manage a wide variety of cardiac rhythm disorders, the active components are encapsulated in sealed titanium can. Leads transmit pulses for both sensing and delivery purposes from the can to the electrode placed at the relevant site on the heart. The electrode, which requires the delivery of the electrical impulse, should be concentrated on high electrical conductivity, and

fatigue resistant and corrosion resistant alloys such as the platinum group metal alloys or the cobalt-chromium group (Crossley, 2000).

With polymer, the readily available and versatile nylons and polyesters were replaced by the more degradation resistant PTFE, PMMA, polyethylene and silicones. However, with the development of degradable implantable materials and systems, the concept of biocompatibility enriches its content. The degrading material performs a function before or during a process with acceptable host response. For example, the drug delivery system utilizes degradable polymers such as polylactide and polyglycolides' (Shive and Anderon, 1997) or poly (methylidene malonate) (Fournier et al., 2006) to form microspheres.

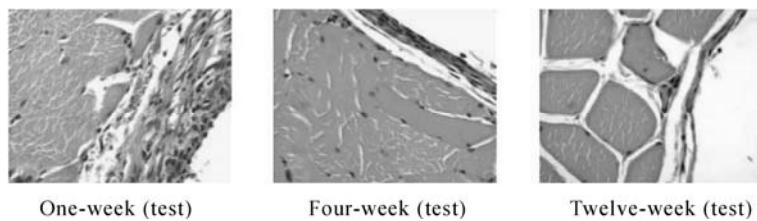
The biocompatibility design for biomedical sensors *in vivo* involves several problems: surface materials, device morphology, infection, toxicology and host response affects toward the responses of the device (Reach et al., 1994). The chemically modified working electrode must have a compatible surface to permeate the desired solute as well as reject interferent species. The structure and properties of the sensor's outer membrane, which is in direct contact with the host tissue or blood, is essential to consider carefully. The membrane should provide high transport selectivity and rate for analytes to be measured and to avoid leakage of potentially toxic components. The morphological characteristics also influence the sensitivity of the sensor. If the membrane is subject to mechanical damage or with stripping from the underlying enzyme layer, the sensor will operate abnormally. The adherence of bacteria to the surface of the sensor (foreign body) and toxicity to the host are the problems to overcome. Recently, progress has been made in the development of clinically important chemical sensors, such as catheter-type sensors placed within the radial artery of hospitalized patients for blood-gas measurements such as pH,  $P_{O_2}$ , and  $P_{CO_2}$ . This type of sensor is substantially replaced by the non-invasive pulse-oximeters, which make the same measurement through the skin (Collison and Meyerhoff, 1990). The needle-type glucose sensor is constructed by multiple membrane coating and electrodeposition of the glucose oxidase layer (Robert et al., 1989). Moreover, ion-selective electrodes and ion-selective fields effect transistors for monitoring electrolytes such as  $Na^+$ ,  $K^+$  and  $Ca^{2+}$  is invented (Kimura, 2001).

How to evaluate the biocompatibility of the sensor? Yang et al. (2003) developed an optic micropressure sensor and evaluated its biocompatibility using the International Organization for Standardization (ISO) test standard 10993-6, "Biological Evaluation of Medical Devices Part 6: Tests for local effects after implantation." The sensor consists of an optical fiber and a diaphragm. The optical fiber is made of inert silica glass and the diaphragm is made from polyimide. The sensor is a flat-shaped cylinder, 10 mm in length and 360  $\mu m$  in diameter (Fig. 2.10). The *in vivo* biocompatibility of the material can be evaluated by analyzing the cell population present, measuring the mediator and metabolite cells excreted, and analyzing the morphologic characteristics of the tissue and the capsule thickness around the implant. Here, the capsule thickness was analyzed to evaluate the host response. Twelve healthy white rabbits were divided into three groups for

different implantation periods (1, 4 and 12 weeks), with four animals tested during each time period. After the surgical procedure, the sensor was implanted in the paravertebral muscles on one side of the spine about 2 – 5 cm from the midline and parallel to the spinal column. The section orientation in relation to the implant dimensions and implant orientation was recorded. The biological response parameters assessed and recorded included: (a) the extent of fibrous capsule and inflammation; (b) the degeneration determined by changes in tissue morphology; (c) the number and distribution of inflammatory cells types, namely macrophages, polymorphonuclear leukocytes, lymphocytes, plasma cells, giant cells, as a function of distance from the material/tissue interface; (d) the presence of necrosis determined by nuclear debris and/or capillary wall breakdown; (e) the other parameters such as material debris, fatty infiltration and presence of granulomata. The one-week group results show that the inflammatory reaction was mild (Fig. 2.11). The capsule wall was composed of three layers. The inner layer was mostly composed of macrophages and monocytes. Eosinophils were located in the middle layer. The outer layer was mainly composed of fibroblasts. The four-week group results show that the capsule thickness decreased sharply. From the four-week to twelve-week groups, the capsule thickness decreased slightly. In conclusion, the biocompatibility of implanted sensors requires histologic and morphologic *in vivo* examinations, even when inert materials are selected.



**Fig. 2.10.** An optic micropressure sensor structure



**Fig. 2.11.** View of implanted site at 1, 4 and 12 weeks after implantation (reprinted from (Yang et al., 2003), Copyright 1995, with permission from Elsevier Science B.V.)

#### 2.4.3 Biocompatibility for *in vitro* Biomedical Sensors

Biocompatibility has traditionally been concerned with implantable devices that

have been intended to remain within an individual for a long period of time. Biochemistry reaction in the organism is mysterious and complex. It is a tough way to approach the principle and mechanism of these reactions through direct invasive measurements. And it is a big challenge to analyze the signals collected from the complex environment inside the organism. As a result, biomedical samples such as proteins, cells and tissues are often extracted from the primary source and studied *in vitro*. Biomedical sensors are a good choice for biological samples in research of *in vitro*. At first, biocompatibility of the biomedical materials on sensor surfaces must be enhanced to maintain the activity and viability of these samples.

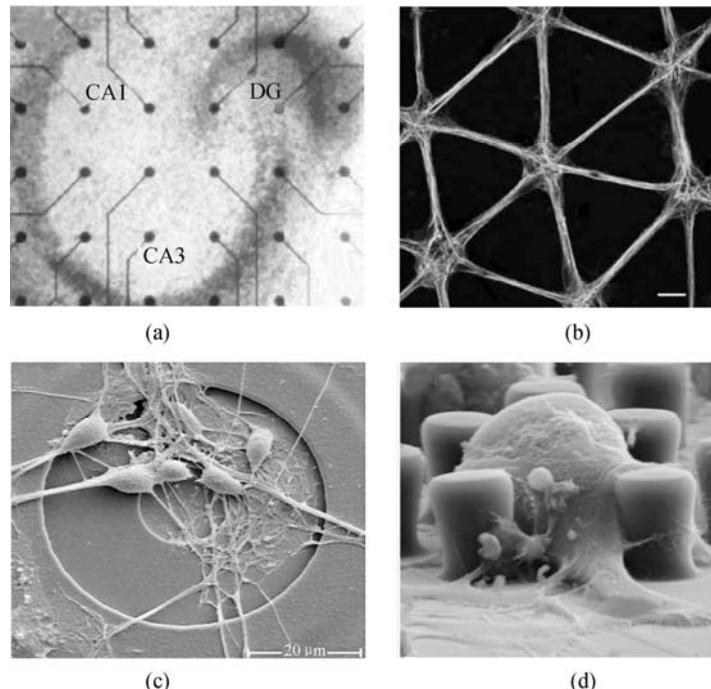
The biocompatibility for biomedical sensors *in vitro* is an essential part for the whole biological sample hybrid system, which should be evaluated completely in biomedical sensor design. The biocompatibility of sensor surface materials affects the biological sample's viability and activity. Furthermore, it is one of the crucial factors of the final output of the system to control the quality of sample immobilization. It is an important concept but there still exist many uncertain methods and mechanisms within biomedical sensor design. The surface material is selected on the basis of non-toxic, non-immunogenic, non-thrombogenic, non-carcinogenic, non-irritant and so on, which must be tested *in vitro* according to biocompatibility requirements in biological evaluation of medical devices in ISO 10993.

Surface materials used in biomedical sensors are usually of two types: passivated materials (e.g., silicon, glass, polymers and photoresist) or metal and metal oxide materials. These materials are quite different from the environment inside biological organism in physical and chemical properties. Therefore, some methods are employed to improve the surface characteristics in hydrophilicity improving, roughness modifying and surface chemical coating. Nowadays, more and more innovative applications are involved in this area.

Functional proteins such as enzyme, antigen and antibody play an important role as receptors in biomedical sensor systems. Receptor parts contain the biologically active components that are capable for specific chemical reactions with the analytes. Various physical and chemical methods are utilized to immobilize these proteins onto sensor substrate surfaces, which enhance the biocompatibility of the surface and maintain the samples good activity and stability. Various approaches are used to modify the characteristics in electrostatics, physical adsorption, and surface chemical group immobilization of the biomedical sensor surfaces. Besides, these samples are very sensitive to temperature and pH value changes.

Isolated cells and tissue can still provide physiological responses, which can be detected by biomedical sensors. Hydroxyl ion implantation into the silicon surface can significantly affect the structural properties of the surface. It is well known that a surface with better hydrophilicity facilitates cell and tissue adherence and proliferation (Fan et al., 2000). Studies have shown that neurons can adhere and spread on the silicon surface with appropriate roughness. Silicon chips are dipped into hydrofluoric acid, which efficiently enhances the roughness to 25 nm

from the original 3 nm (Fan et al., 2002). To coat a biocompatible material on the whole surface is the most convenient way to enhance the functional groups on the biomedical sensor surface. As shown in Fig. 2.12, cells and tissues are respectively immobilized on glass, silicon, and metal substrates (Bergen et al., 2003; Jungblut et al., 2009; Krause et al., 2006; Zeck and Fromherz, 2001).

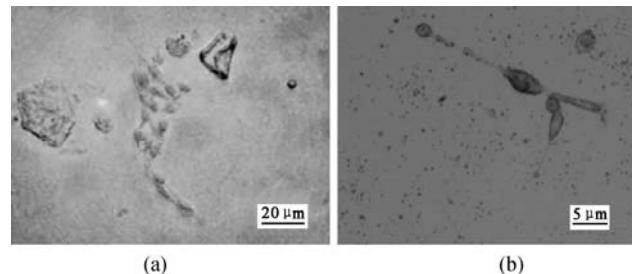


**Fig. 2.12.** Cells and tissues immobilized on different substrates: (a) Slice of hippocampal tissue immobilized on sensor, including CA1, CA3 and DG regions (reprinted from (Bergen et al., 2003), Copyright 2003, with permission from Elsevier Science B.V.); (b) Artificial triangular patterned neuron network formation on glass. Scale bar: 10  $\mu\text{m}$  (reprinted from (Jungblut et al., 2009), Copyright 2009, with permission from Springer); (c) SEM image of passive palladium electrodes with cultured neuronal cells after 3 d *in vitro* with a developing glial carpet (reprinted from (Krause et al., 2006), Copyright 2005, with permission from Elsevier B.V.); (d) A single neuron trapped on silicon chip surface (reprinted from (Zeck and Fromherz, 2001), Copyright 2001, with permission from the National Academy of Sciences)

Cell- and tissue-based biosensors, which treat living cells and tissue as sensing elements, can reflect the functional information of bioactive analytes. Cells and tissue can be extracted from primary sources and successfully cultured *in vitro*. The special activities relating to cellular functions can be detected by biomedical sensors (Gross et al., 1995; Maher et al., 1999; Kovacs 2003; Stett et al., 2003).

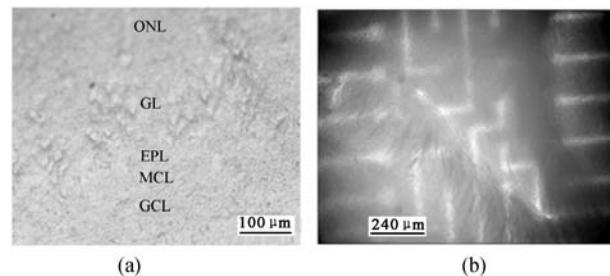
The biosensor composed of light-addressable potentiometric sensors (LAPS), taste or olfactory neurons can detect the extracellular potentials, and is sensitive to tastant or odorous change. In the manner of invasive and light addressable, cell

response to living environment changing was monitored. Taste and olfactory cells are difficult to immobilize onto sensors. For the uniform surface of a LAPS chip, using chemical coating was one of the most efficient methods. Before cell culture, a thin layer of poly-L-lysine and laminin was deposited on the LAPS chip, which makes better biocompatibility for cell immobilization (Zhang et al., 2008; Wu et al., 2009). The cell immobilization result is shown in Fig. 2.13.



**Fig. 2.13.** Taste and olfactory cells immobilized on LAPS: (a) Taste receptor cells were cultured on LAPS chip surface. (reprinted from (Zhang et al., 2008), Copyright 2007, with permission from Elsevier B.V.); (b) Olfactory cells were immobilized on LAPS chip surface (reprinted from (Wu et al., 2009), Copyright 2008, with permission from Elsevier B.V.)

Microelectrode array (MEA) can record the multisite potentials simultaneously and allow for the ability of long-term recording of the firing of neural networks *in vitro*. In the present study, we managed to combine the intact olfactory tissue slice with MEA (Liu et al., 2006). Compared to the cultured olfactory cells, the olfactory tissue can be obtained conveniently with the primary cell structure well preserved. Mimicking the *in-vivo* process of gas sensing, it is a good candidate for the biological elements of bioelectronic nose. MEA can record the extracellular potentials of the olfactory receptor neurons in the tissue slice. The multi-channel signal analysis may reveal some spatial and temporal information of olfactory information processes in the olfactory bulb. The olfactory bulb slice morphology structure is well reserved, along with tissue immobilization on the MEA chip shown in Fig. 2.14.



**Fig. 2.14.** Olfactory tissue immobilized on MEA chip: (a) Olfactory bulb tissue morphology structure; (b) Olfactory bulb tissue slice immobilized on MEA chip

And various methods in biofunctionalized polymer surfaces analysis are well considered in surface characterization evaluation, including spectral methods (X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy, Atomic Force Microscopy (AFM), and others) as well as non-spectral methods (water contact angle, dye assays, biological assays, and zeta potential).

Besides the factors of the materials mentioned above, environment control is another important aspect for *in vitro* cell and tissue culture on biomedical sensors. It is essential to keep the activity of the living cell and tissue during real-time analysis. Relative parameters including temperature, medium components, pH, osmotic pressure, gas pressure, etc. are required to be well controlled.

## 2.5 Microfabrication of Biomedical Sensors

The biomedical sensors are strictly required to be compatible to the objected bio-components and cause lower invasive injury in *in-vivo* measurements. Additionally, the development of microfabrication technology excites the potential of biomedical sensors with characteristics of low cost, low consumption of samples, high-throughput performance and the ability be easily integrated into systems. Thus in this section, the fundamental microfabrication including lithography, film forming and etching is introduced and an example of fabrication procedure of a typical sensor is illustrated.

### 2.5.1 Lithography

Lithography in MEMS is typically the transfer of a pattern to photosensitive material. The transfer of the designed image onto a resist-coated wafer is very critical in sensor fabrication. Two main processes of lithography used in sensor fabrication are the photolithography and electron beam lithography.

*Photolithography* is the most widely used form of lithography. In photolithography, a photo mask is required, which is usually a nearly optically flat glass or quartz plate with a metal absorber pattern. The photo mask is placed into direct contact with photoresist surface coating. When the wafer is exposed to the ultraviolet radiation, the absorber pattern on the photo mask is opaque to ultraviolet light and the pattern is transferred to the photoresist based on its polarity. Photolithography has matured rapidly and has become better and better at resolving smaller and smaller features. For sensor fabrication, this continued improvement in resolution makes it very suitable to constructing micro sensors in the dimension of several microns.

*Electron beam lithography* can overcome the limit of the resolution by light diffraction in photolithography, because the quantum mechanical wavelengths of

high energy electrons are exceedingly small. In electron beam lithography, a photo mask is not required and direct writing is processed on the resist-coated surface. The major advantages of electron beam lithography are the ability to register accurately over small areas of a wafer, lower defect densities, and a large depth of focus because of continuous focusing over topography. Meanwhile, electron beam lithography has some disadvantages. Electrons scatter quickly in solids, limiting practical resolution to dimensions greater than 10 nm. The resolution of electron beam lithography tools is not simply the spot size of the focused beam. Serious variations of exposure over the patterns occur when pattern geometries fall into the micrometer and sub-micrometer ranges. Electrons also need to be held in a vacuum, making the apparatus more complex and expensive than that for photolithography. Besides, the difficulties with scanning electron-beam lithography include the relatively slow exposure speed and high system cost, which keep it away from wider use.

### 2.5.2 Film Formation

Film formation process in micro fabrication is the growth and deposition of layers with different materials. To form films of various materials, different film formation processes are involved, such as silicon oxidation, physical vapor deposition (PVD), and chemical vapor deposition (CVD).

*Silicon oxidation* is a primary process in sensor fabrication, since silicon is a normally used base material in sensor fabrication. Silicon dioxide growth involves the heating of a Si wafer in a stream of steam at 1 atm wet or dry oxygen/nitrogen mixtures at elevated temperatures (600 – 1,250 °C). The high temperature aids diffusion of oxidant through the surface oxide layer to the silicon interface to form thick oxides in a short amount of time. In fabrication of most sensors based on silicon structure such as light addressable potentiometric sensor (LAPS) (Yu et al., 2009), silicon oxidation is usually a necessary procedure.

*Gas phase deposition* is a key technology in film formation. Two major categories of gas phase deposition can be distinguished as PVD and CVD.

*Physical vapor deposition (PVD)* is a direct line-of-site impingement type deposition. PVD reactors may use a solid, liquid, or vapor raw material in a variety of source configurations. Evaporation and sputtering are two major methods of PVD. Thermal evaporation represents one of the oldest thin film deposition techniques. Material is heated and boiled off onto a substrate in a vacuum. Evaporators emit material from a point source, resulting in shadowing and sometimes causing problems with metal deposition, especially on very small structures. Meanwhile, during the sputtering process, the target (a disc of the material to be deposited, i.e., Au), at a high negative potential, is bombarded with positive argon ions (other inert gases such as Xe can be used as well) created in a plasma (also glow discharge). Sputtering is preferred over evaporation in many applications due to a wider choice of materials to work with, better step coverage,

and better adhesion to the substrate. Actually, sputtering is employed in laboratories and production settings, whereas evaporation mainly remains a laboratory technique (Madou, 2002).

*Chemical vapor deposition (CVD)* is a diffusive-convective mass transfer technique. There are several different CVD processes with different operational pressures and temperatures. In plasma-enhanced chemical vapor deposition (PECVD), an RF-induced plasma transfers energy into the reactant gases allowing the substrate to remain at lower temperature. Besides, all of the dry etching equipment can be used for PECVD as well. The most significant application of PECVD is probably the deposition of  $\text{SiO}_2$  or  $\text{Si}_3\text{N}_4$  over metal lines. In low pressure CVD (LPCVD) at below 10 Pa, large numbers of wafers can be coated simultaneously without detrimental effects to the film uniformity. However, LPCVD suffers from a low deposition rate and the relatively high operating temperatures.

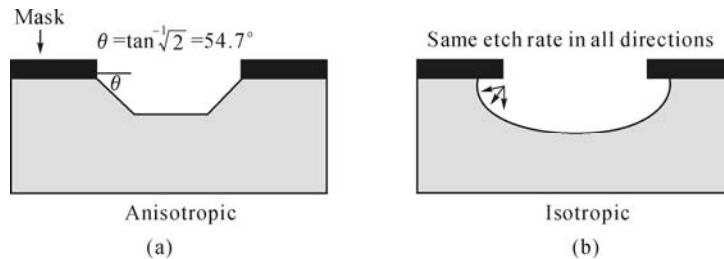
*Screen printing* is essential in low cost disposable sensor fabrication. Low cost disposable sensors are an important developing trend of biosensor applications. In screen printing, a paste of ink is pressed onto a substrate through openings in the emulsion on a stainless steel screen. The paste consists of a mixture of the material of interest, an organic binder and a solvent. The lithographic pattern in the screen emulsion is transferred onto a substrate by forcing the paste through the mask opening with a squeegee. In a first step, paste is put down on the screen, and then the squeegee lowers and pushes the screen onto the substrate forcing the paste through opening in the screen during its horizontal motion. During the last step, the screen snaps back, the thick-film paste which adheres between the screening frame and the substrate shears, and the printed pattern is formed on the substrate. The resolution of the process depends on the openings in the screen and the nature of the pastes.

### 2.5.3 Etching

Etching is a process to remove material from the unprotected area for forming functional structure on a wafer. It usually follows after the process of lithography to present an accurate pattern matching with the photomask. In the MEMS fabrication, two major types of etching are wet etching and dry etching.

*Wet etching* is performed as a chemical reaction to selectively dissolve the film by etchant. The process usually includes three steps: reactive species diffuse from liquid bulk to the surface of the film; reaction happens at the surface to form solvable species; reaction products are diffused away from the surface of the film to liquid bulk. The etch rate is related with temperature, specific reaction and liquid concentration, which results in isotropic etching and anisotropic etching by the relative etch rates in different directions. In isotropic etching (Fig. 2.15b), etching progresses at the same speed in all directions and the surface of etched grooves can be atomically smooth. For the material with crystal structure such as silicon, it exhibits anisotropic etching because of the different etch rates in

different directions (Fig. 2.15a). In wet etching, a good selectivity can often be obtained because the etch rate of target material is considerably higher than that of the mask material.

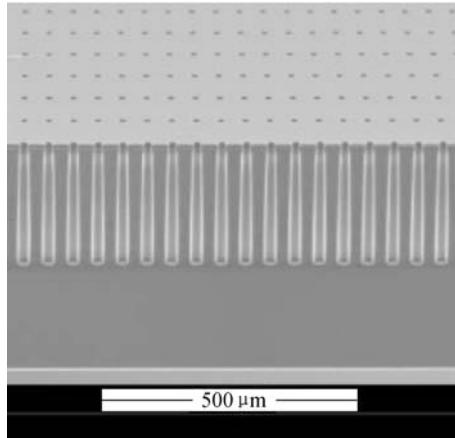


**Fig. 2.15.** Anisotropic and isotropic etch: (a) An anisotropic etch on a silicon wafer creates a cavity in the cross-section. The sides of cavity are  $<111>$  planes with an angle of 54.7°; (b) In isotropic etch, it has the same etch rate in different directions

*Dry etching* is commonly used due to its ability to better control the etching process and eliminate handling of dangerous acids and solvents in wet drying. It effectively etches targeted film through: (1) Chemical reactions with reactive gases or plasma; (2) Physical bombardment of atoms; (3) The combination of both physical removal and chemical reactions.

Plasma etching is a purely chemical dry etching technique. In a plasma system, it ionizes and generates reactive species by using RF excitations and they diffuse to the surface of the wafer. After adsorption, the chemical reactions occur between the reactive species and the material being etched. The generated byproducts in reaction are desorbed and diffused into the bulk of the gas. Sputtering is a physical dry etching technique that removes the targeted material by bombarding it with highly energetic but chemically inert species or ions. Compared with chemical methods, such a physical removal process is non-selective and it will hit the mask layer covering the targeted layer through the bombarding species. Thus it has never become popular as a dry etching technique for MEMS fabrication.

Reactive ion etching (RIE) has been a fundamental process in semiconductor fabrication. It combines chemical and physical techniques to accelerate the chemically reactive ions to bombard and etch away the targeted material. In comparison to plasma etching, RIE has a higher probability to have movement in the direction given by the electric field and owns an advantage in terms of anisotropy. In addition, the etch rate of RIE is highly controllable, selectivity to the material is reasonable and the endpoint of the etching process is optical. RIE can etch deeply and has been developed as a subclass, the deep RIE. It is based on the “Bosch process” and there are two different gas compositions alternating in the reactor. The etch depth of hundreds of micrometers can be achieved with almost vertical sidewalls. Thus a lot of patterns in biochips are formed by RIE or deep RIE in MEMS development. Fig. 2.16 shows an example of a biochip fabricated by deep RIE techniques from SINTEF.



**Fig. 2.16.** DRIE for through wafer holes: Average etch rate was 12  $\mu\text{m}/\text{min}$  and each hole was 400  $\mu\text{m}$  deep with diameter of 50  $\mu\text{m}$  holes

#### 2.5.4 Design of the Biomedical Sensors

Generally, the procedure in the development of a biosensor includes several steps: design, fabrication, package, performance test and application. Here we take an example of microelectrode array (MEA) to describe the fabrication process.

MEA, a typical type of cell-based biosensors, is an array of metallic film disks used to detect the extracellular field potential of electrogenic components such as cardiac cells and neuronal cells (Mayer et al., 2004; Gross et al., 1995). It is generally fabricated on wafers of glass or silicon grown with silicon oxide. Before fabrication, two masks are prepared for lithography to form the patterns of metallic film layer and the passivation layer, respectively. In Fig. 2.17, it shows the procedure of fabrication based on the MEMS techniques such as lithography, film formation and etching. In Fig. 2.17, it applies the glass as the substrate of MEA because of its low dielectric constant.

- A wafer of glass is cleaned and then the Cr (30 nm) and the Au (300 nm) are sequentially sputtered or evaporated onto it. The Cr layer is used to enhance the adhesion of the Au layer onto the substrate (Figs. 2.17a and 2.17b).
- The photoresist is then spin coated on the metallic layer and protects the pattern of electrodes and traces by standard photolithographic (Fig. 2.17b). Here the first mask is used to pattern the photoresist.
- A composited metallic layer (Cr/Au) is chemically etched away. After that the photoresist stay on the electrodes and traces are developed (Fig. 2.17c). The electrodes and interconnects are present.
- The alternative  $\text{SiO}_2/\text{Si}_3\text{N}_4/\text{SiO}_2$  as a passivation layer is deposited onto

- the surface of the chip by PECVD (Fig. 2.17d).
- The photoresist is spin coated on the chip again and exposes the sensory sites by photolithography with a second mask (Fig. 2.17e). Then a three-step chemically etches the exposed passivation layer (Fig. 2.17f).
- Usually, the electrodes are in a diameter of 10 – 50  $\mu\text{m}$  and its high impedance causes a high noise in the signal. It needs to increase the roughness of the electrode surface to lower down the noise and stabilize the baseline. In this step, we take the TiN as the surface treatment option. It is reactive sputtered onto the chip in a nitrogen/argon atmosphere and then it removes the TiN on photoresist by lift-off process. In this way, only the sensory sites are treated with TiN (Figs. 2.17g and 2.17h).

If the surface is treated by platinum black, the plating process can be performed after removing the photoresist on the sensory sites and applying a constant current of 5 nA/mm<sup>2</sup> onto the electrodes in electrolyte of chloroplatinic acid for about 30 s.

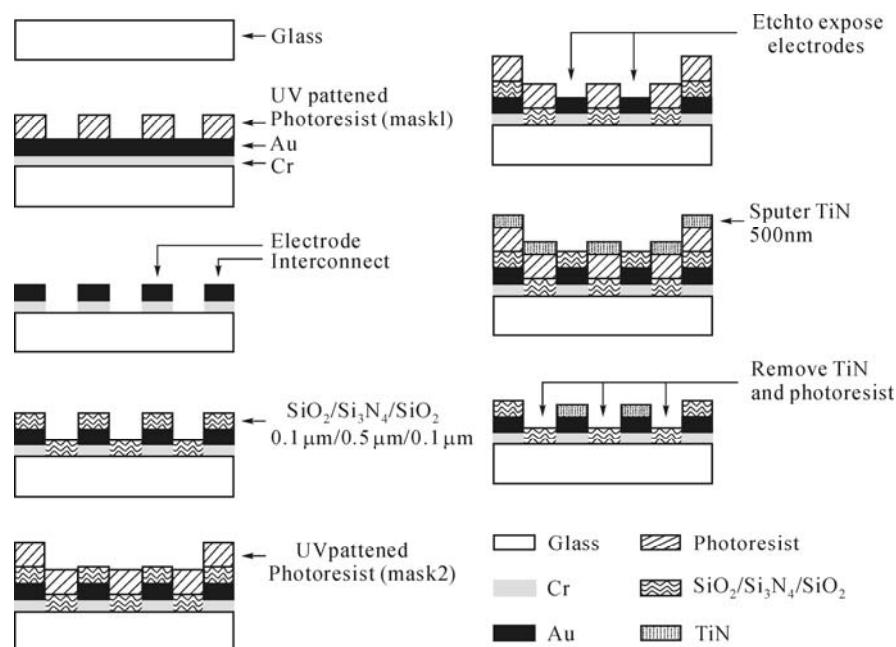
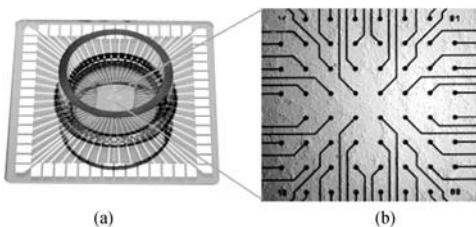


Fig. 2.17. The fabrication procedure of microelectrode array

After fabrication, a group of chips on the same wafer, usually with a diameter of 4 inches, are scribed into individual ones. For the chips with a small size of several millimeters, they are usually fixed onto a PCB board and then pads of chip are bonded with pads of PCB board by gold wire. The signals detected by electrodes on chips are conducted into external setup through the circuits on the

PCB. For the chips with a size of about two centimeters, the larger pads on the chips are pressed with spring tips to conduct the signal to the external setup. Finally, a chamber is fixed onto a chip to form a room for the cell culture. The chip in Fig. 2.18a is a packaged commercial MEA (Multi Channel Systems MCS, GmbH) whose pads contact with the spring tips to create output signals. The electrode arrays are located in the center of the chamber to help to effectively concentrate the cells onto the surface of the electrodes. Fig. 2.18b shows that the rat hippocampal cells are cultured on the MEA and they have shown good biocompatibility and have formed a communicated network on the chip. By detecting the extracellular field potential in different sites in parallel, MEA can provide valuable information about the spike patterns and communication in the neural network.



**Fig. 2.18** Microelectrode array: (a) The picture of packaged MEA; (b) Rat hippocampal cells are culture on the MEA for 12 d

In summary, a series of sensors can be fabricated by combining different fundamental techniques. The development of fabrication helps to further explore chips with refined structures and small sizes in nanometers. Thus various sensors with multichannels and good compatibility can be designed to study molecules, receptors, cells and organs.

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## **Chapter 3**

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### **Physical Sensors and Measurement**

Physical sensors have been widely used in the biomedical field. The commonly used sensors include resistance sensors, inductive sensors, capacitive sensors, piezoelectric sensors, electromagnetic sensors, photoelectric sensors, and thermoelectric sensors. Physical sensors will have more significant applications in biomedicine, especially with the development of MEMS technology for developing more precise and compact sensors, along with the development of the novel measuring technology.

#### **3.1 Introduction**

The nature of physical phenomena includes mechanical, thermal, electrical, magnetic, atomic and nuclear, each having the properties of bodies or physical systems. Based on these natural properties, some physical effects become part of the fundamentals of physical sensors for measuring physical quantities and converting them into signals which can be read by an observer or an instrument. For example, a thermocouple converts temperature to an output voltage which can be read by a voltmeter.

Taking into account that the output signal is definitely determined by the input signal, and basing our criteria on the differences of measuring objects in the biomedicine field, physical sensors can be classified as pressure sensors, displacement sensors, speed sensors, acceleration sensors, flow sensors and temperature sensors. Whereas another criterion based on different physical effects is also significant. In this case, there are resistance sensors, inductive sensors, capacitive sensors, piezoelectric sensors, electromagnetic sensors, photoelectric sensors, and thermoelectric sensors. It is a well-known fact that customers of sensors will choose a sensor in connection with the physical nature of information to be obtained about a phenomenon or a physical system.

As the most widely used measuring device, physical sensors have already made a great contribution to the development of industry, agriculture, military and

aerospace, and they are playing an important role even in our daily life. Along with the rapid development of biomedicine in the latter half of the 20th century, physical sensors have become a measurement instrument of paramount importance in medical diagnosis and therapy. Combining the good features of materials like optical fiber, superconductor or nanophasse materials and the semiconductor micro fabrication technology, the possibility of multifunction, high precision, and integration for physical sensors is guaranteed.

## 3.2 Resistance Sensors and Measurement

As a primary kind of resistance sensor, the resistance strain sensor is capable of converting strain into a resistance variation. Another type of resistance sensor, known as the piezoresistive sensor, is based on the piezoresistive effect and has the advantages of high sensitivity, good resolution and smaller size. They are both widely used in the measurement of blood pressure, pulse and intraocular pressure, intracranial pressure, and eyelid pressure (Shaw et al., 2009) in the biomedical field.

### 3.2.1 Resistance Strain Sensors

It is a basic phenomenon that deformations of elastic elements bring about resistance change of strain sensitive materials under functions of tested physical parameters. The most commonly used sensing element is resistance strain gage.

#### 3.2.1.1 Strain Effect and Characteristics

##### *Strain effect*

As the working principle of resistance strain gage, strain effect means resistance value changes with mechanical deformation of elastic elements. As shown in Fig. 3.1, metal resistance wire will elongate along the axial direction and shorten along the radial direction when subjected to force in its elastic range.



**Fig. 3.1.** The schematic diagram of strain effect:  $L$  is the initial length of resistance wire,  $dL$  is the increment of length,  $r$  is the radius of cross-section,  $dr$  is the increment of radius, and  $F$  is force

The relative variation of resistance value can be calculated as follows:

$$\frac{\Delta R}{R} = (1 + 2\mu)\varepsilon + \frac{\Delta\rho}{\rho} \quad (3.1)$$

where  $\mu$  is the Poisson ratio of resistance wire and  $\varepsilon$  is the strain. Generally, the variation of resistance value caused by unit strain is called sensitivity coefficient ( $K$ ) of the resistance wire.  $K$  is constant in the stretch limit of the resistance wire.

### ***Categories of resistance strain gage***

Among the great varieties of forms, strain gages can be generally classified as bonded foil/non-foil types, thin film types and semiconductor types.

Bonded foil types are made from alloy foil like Constantan or Karma with a thin polyamide or cast epoxy backing. Bonded non-foil types are very similar to bonded foil types except metal wire is used as the gage element.

Thin film types, made by sputtering insulation and gage elements directly on a polished sensing area, have eliminated instability caused by adhesive bonds. With merits of greater sensitivity coefficient, greater allowable current density, and a large working range, they are widely used.

Semiconductor strain gage can be formed as bonded, unbonded, or integrated strain gage units. They have a high gage factor and are suitable for dynamic measurements. However, they are more temperature-sensitive and inherently more nonlinear than metal strain gages.

New materials are designed to improve security and insure innocuousness for human beings, either in rehabilitation or health monitoring. Elastic resistance strain gages with flexible conductive elastomers (such as electrically conductive liquid silicon rubber) have been used. They are easy to deform, and have good mechanical, electrical, ageing, fast vulcanization properties (Martinez et al., 2009) and biocompatibility, and have great potential in biomedical measurement.

### ***Temperature error***

Temperature error of strain gage refers to the additional error brought by the temperature change in the measuring circumstance. The cause for temperature error includes:

- Affection of the resistance temperature coefficient. Resistance of the sensing element changes with temperature.
- Affection of different linear expansion coefficients of tested pieces and resistance sensing materials. Once temperature changes, distortion will happen, thus additional resistance is produced.

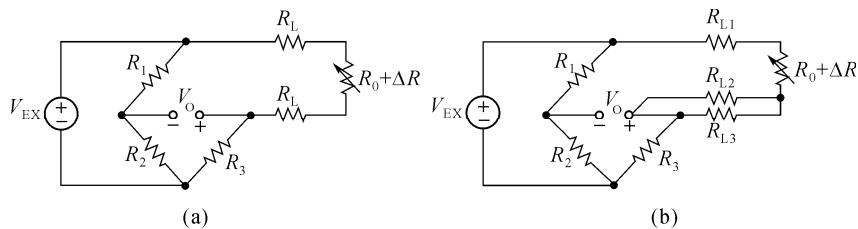
#### **3.2.1.2 Measurement**

Resistance change, caused by an extremely small strain, needs to be measured out,

and the relative resistance change needs to be converted into a variation of voltage or current. As mechanical strain is generally small, strain gages are almost always used in a bridge configuration, usually a direct current (DC) bridge and alternating current (AC) bridge, with a voltage excitation source.

The general Wheatstone bridge consists of four resistor arms with an excitation voltage, which is applied across the bridge. The bridge is balanced since the product of two opposite arms is equal to that of the left, which means the voltage output is zero. If one arm is replaced with a strain gage (quarter bridge circuit), any changes in the strain gage resistance will unbalance the bridge and result in a nonzero output voltage. Thus, strain can be calculated from the output voltage. Note that the temperature error needs to be minimized, two strain gages are generally used in a bridge, where one gage is active and the other is placed transverse to the applied strain, which is similar to circuitry temperature compensation. By making both gages active (one mounted in tension, the other in compression) in a half-bridge configuration, the sensitivity of the bridge and the output voltage can be doubled. Similarly, a full-bridge configuration with four of the arms replaced with active strain gages can further increase the sensitivity of the bridge.

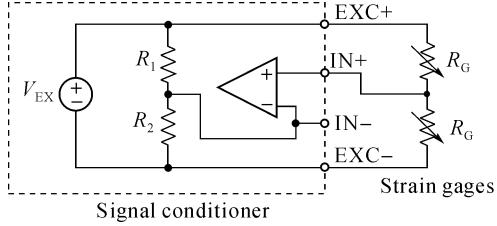
In practice, lead resistance can not be ignored to acquire higher precision. As shown in Fig. 3.2a, lead adds  $2R_L$  of resistance to the strain gage arm and desensitizes the output of the bridge. This error can be compensated by measuring the lead resistance  $R_L$  and accounting for it in the strain calculations. Another problem worthy of mentioning is that the lead resistance changes due to temperature fluctuations, which means a slight change in temperature, can generate a measurement error of several microstrains. Using a 3-wire connection (Fig. 3.2b) (<http://zone.ni.com/devzone/cda/tut/p/id/3642>) can eliminate the effects of variable lead resistance since it only affects adjacent legs of the bridge.



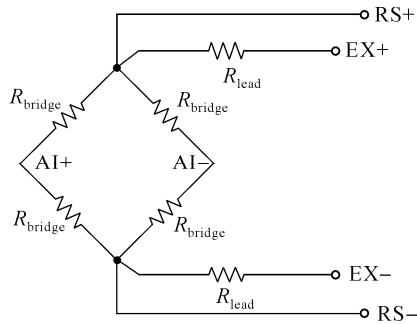
**Fig. 3.2.** Connections of quarter-bridge circuit: (a) 2-wire connection; (b) 3-wire connection

Besides, signal conditioning is indispensable for reliable measurements. A signal conditioner is always needed to complete the bridge with reference resistors as long as it is not a full-bridge, especially for a half-bridge configuration, as shown in Fig. 3.3. Since the signal measured in a biomedical application is always feeble, amplifiers are usually used in signal conditioners to boost the signal level to increase measurement resolution and improve signal-to-noise ratios. Sometimes telemedicine may raise problems concerning remote sensing. Once the measuring circuit is located a distance away from the signal conditioner, a possible source of

error can be voltage drop. Therefore, some signal conditioners include an extra voltage to compensate for this error. As shown in Fig. 3.4, extra wires serve to regulate the excitation supply through negative feedback amplifiers to compensate for lead losses and deliver the needed voltage at the bridge.



**Fig. 3.3.** Connection of half-bridge completion,  $R_1$  and  $R_2$  are reference resistors



**Fig. 3.4.** Compensation for remote sensing error: EX is an excitation voltage for compensation for lead losses, RS is reference voltage at the bridge

However, the most common error will be temperature error, which can be extremely severe in some cases. Accordingly, the methods of temperature compensation include circuitry compensation and self-compensation.

In measurement of strain, a working strain gage is bonded on the surface of the tested piece. Circuitry compensation means the bonding of a compensation gage onto the compensation piece which has absolutely the same material with the tested piece and serves as the adjacent bridge arm. Note that only the tested piece bears strain. Circuitry compensation has good effect and is commonly used. It should be pointed out that four conditions must be met to realize complete compensation:

- The other two arms with the same resistance must be guaranteed.
- Working gage and the compensation one must have the same resistance temperature coefficient, linear expansion coefficient, strain sensitivity coefficient and initial resistance.
- The materials of pasting compensation gage and working gage must be the same, and with the same linear expansion coefficient.

- The two strain gages must be in the same temperature field.

It is known that some strain gages have functions of self-compensation. The following equation is worth mentioning:

$$\alpha_0 = -K_0(\beta_g - \beta_s) \quad (3.2)$$

where  $\alpha_0$  is the temperature coefficient,  $K_0$  the sensitivity coefficient,  $\beta_g$  and  $\beta_s$  are the linear expansion coefficient of tested piece and sensing element, respectively. If the sensing materials (consider parameters like  $K_0$ ,  $\alpha_0$ ,  $\beta_s$ ) is chosen reasonably to make Eq. (3.2) true, temperature self-compensation can be easily realized.

### 3.2.1.3 Biomedical Applications

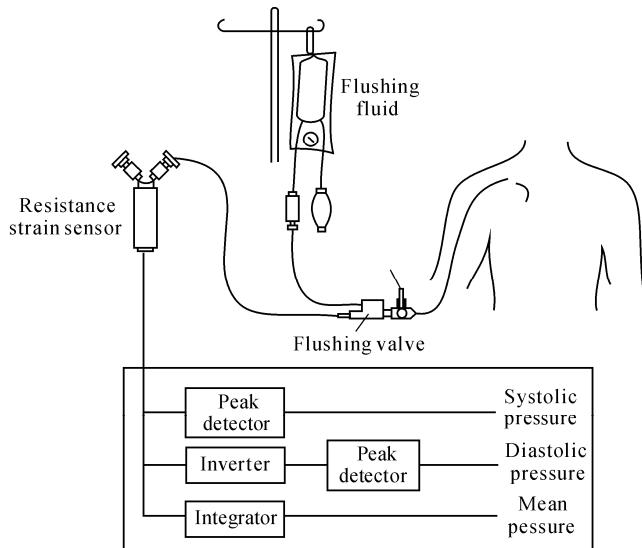
#### *Blood pressure measurement*

As a primary indicator of physiological distress, blood pressure is very important in determination of the functional integrity of the cardiovascular system.

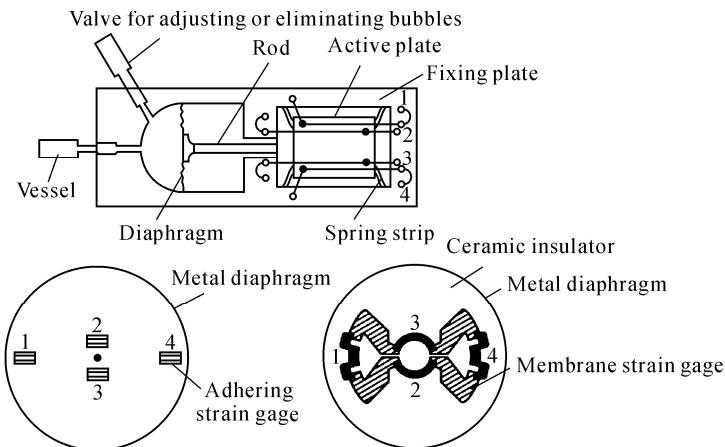
Non-invasive blood pressure measurement (Zhang and Wu, 2003), like cuff-based blood pressure measurement, which has the advantages of facility, safety, painlessness and more acceptance, is generally used in home health monitoring (Jobbágy et al., 2007) and conventional physical examination. As “normal” blood pressure varies during the day, with age, state of health and clinical situation, and also has beat-to-beat variations, sometimes the non-invasive blood pressure monitoring is not possible or likely to be inaccurate.

Continuous, invasive blood pressure monitoring (Hu and Wang, 2008) is the gold standard of blood pressure measurement giving accurate beat-to-beat information. As it allows repetitive and convenient sampling for blood gases analysis, it is also used when long-term measurement in critically ill patients is required, avoiding the problem of repeated cuff inflation, which will cause localized tissue damage.

A typical method for invasive blood pressure measurement uses the extravascular system (Peng, 2000), as shown in Fig. 3.5. A catheter is placed in the artery or vein and is connected to a 3-way stopcock and the pressure sensor. In this system a catheter couples a flush solution (heparinized saline) through a disposable pressure sensor with an integral flush device to the sensor port. The 3-way stopcock is used to take blood samples and zero the pressure sensor. The catheter must be flushed frequently (every few minutes) to prevent blood clotting at the catheter tip. The catheter is inserted by a surgical cut-down or by a percutaneous insertion (surgical needle and a guide wire). Blood pressure is transmitted via the catheter to the sensor’s diaphragm. The strain gage sensor has several types, as shown in Fig. 3.6.



**Fig. 3.5.** Invasive blood pressure measurement system



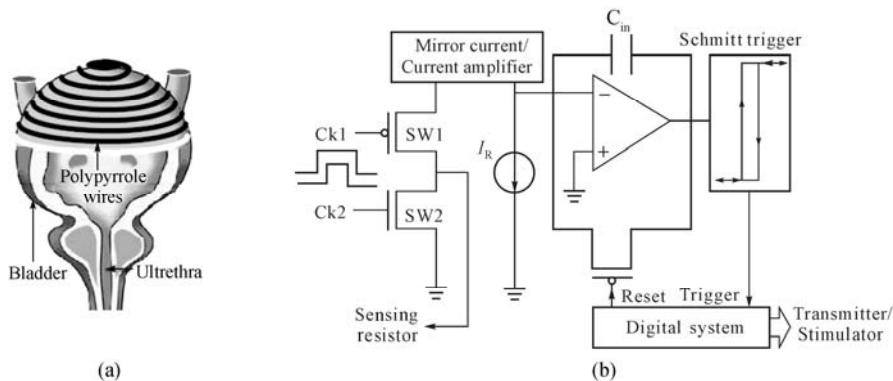
**Fig. 3.6.** Resistance strain sensor applied in invasive blood pressure measurement system:  
 (a) Four wire type strain gages with the same resistance are connected into a balance bridge;  
 (b) Schematic diagram of adhering strain gage to metal diaphragm, the adhering position is determined via mechanical analysis for the site of generating the maximum strain, and the four strain gages are connected to a balance bridge; (c) Another way (vacuum deposition) of depositing membrane with strain effect directly on the surface of metal diaphragm

#### ***Bladder volume measurement in patients with urinary dysfunction***

Millions of people have been persecuted by urinary bladder dysfunction, which leads to loss of voluntary control over the bladder muscles and cuts off sensorial

feedback to the central nervous system (Gaunt and Prochazka, 2006). The prevalent therapeutic method in clinical practice is stimulating the sacral root at the base of the spine to produce microstimulation. In the past few years, direct sacral nerve stimulation, using a dual implantable stimulator (Ba and Sawan, 2003), has proved to be clinically feasible. The stimulation can be permanent, selective, or involve conversion between the two types. But the best choice is the process which creates the ability to trigger emptying of the bladder in response to maximal bladder volume, which is similar to the automatic sensorial feedback. So bladder volume detection becomes the key concern.

There are some traditional ways to measure bladder volume, such as using a pressure sensor, ultrasound measurements and bioelectric impedance measurements. However, they are not entirely satisfactory because of some potential defects or unwanted interference. A new method reported by Rajagopalan et al. (2008) is employing an implantable polypyrrole-based strain sensor, using a conductive polymer as the sensing device. The conducting polymer-polypyrrole (PPY) is coated on a flexible fabric and inserted over the upper portion of the bladder (Fig. 3.7a).



**Fig. 3.7.** Bladder volume measurement: (a) Illustration of bladder covered by strip with strip lines of PPY; (b) Interface circuit (reprinted from (Rajagopalan et al., 2008), Copyright 2008, with permission from MDPI Publishing)

Like most soft tissues in the body, the urinary bladder wall is non-linear, viscoelastic and anisotropic. The collagen fibers are kinked and coiled when the bladder is relaxed and begins to stretch during filling. Correspondingly, the collagen fibers allow for high strain, which means that the urinary bladder can cater for a volume of up to 11 times its resting volume.

Fig. 3.7b shows the implantable measuring circuit, which can read out the changes in resistance. The sensing current proportional to the sensing resistor is extracted through the clocking system (SW1, SW2) which is amplified in the current mirror block and then integrated using a capacitor ( $C_{in}$ ). The output of the Schmitt trigger block drives the digital counter which outputs a value proportional to the input resistance value. The circuit can provide continuous resistance outputs

for a given input voltage. This resistance reading can then be transmitted wirelessly to a wearable display positioned just outside the body.

### 3.2.2 Piezoresistive Sensors

Piezoresistive sensors are based on piezoresistive effect of monocrystalline silicon and are made up by using an integrated circuit technique. They are generally used in measuring pressure and some other physical parameters that can be converted into pressure. Microfabrication and MEMS technology have made the miniaturization of piezoresistive sensors possible and applicable in practice.

#### 3.2.2.1 Piezoresistive Effect

The piezoresistive effect describes the changing electrical resistance of a material due to applied mechanical stress. It only causes a change in resistance and does not produce an electric potential. If a mechanical stress  $\sigma$  is applied on a resistor, the resistivity change  $\Delta\rho/\rho$  can be calculated as:

$$\Delta\rho/\rho = \Pi\cdot\sigma \quad (3.3)$$

where  $\Pi$  is the piezoresistivity coefficient. The sensitivity of piezoresistive devices is characterized by the gage factor:

$$K = (\Delta R/R)/\varepsilon \quad (3.4)$$

where  $\Delta R$  is the change in resistance due to deformation,  $R$  is the undeformed resistance and  $\varepsilon$  is the strain.

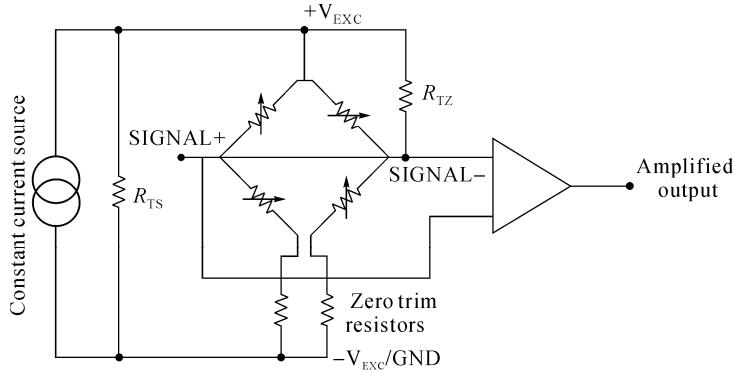
As shown in Eq. (3.1), the resistance of silicon changes is affected not only by the stress dependent change of geometry, but also by the stress dependent resistivity of the material. The latter is the dominant factor, which results in sensitivity to orders of magnitudes larger than those observed in metals. This effect is present in materials like germanium, polycrystalline silicon, amorphous silicon, silicon carbide, and single crystal silicon, which are among the several types of semiconductor materials. Since silicon is today the material of choice for integrated digital and analog circuits, the use of piezoresistive silicon devices has been of great interest. Many commercial devices such as pressure sensors and acceleration sensors employ the piezoresistive effect in silicon.

#### 3.2.2.2 Measurement

The measuring circuits for piezoresistive sensors are similar to strain sensor measurement. A wheatstone bridge is primarily used in piezoresistive sensor

devices, but with different signal conditioners. As mentioned in Subsection 3.2.1.1, semiconductor materials are more temperature-sensitve and nonlinear than metal materials. Piezoresistive pressure sensors are usable only after corrections have been made for offset and compensation for temperature.

For medium-accuracy sensors, a resistor network can compensate for offset, offset drift, and FSO (full-scale output) drift (Fig. 3.8). Zero trim resistors adjust for initial offset. But the bridge resistors have a positive temperature coefficient that causes the bridge voltage to rise with temperature, so resistor  $R_{TS}$  is used to stabilize the sensitivity by shunting an increasing amount of excitation current as temperature rises. Besides, resistor  $R_{TZ}$  works against the change of offset with temperature.



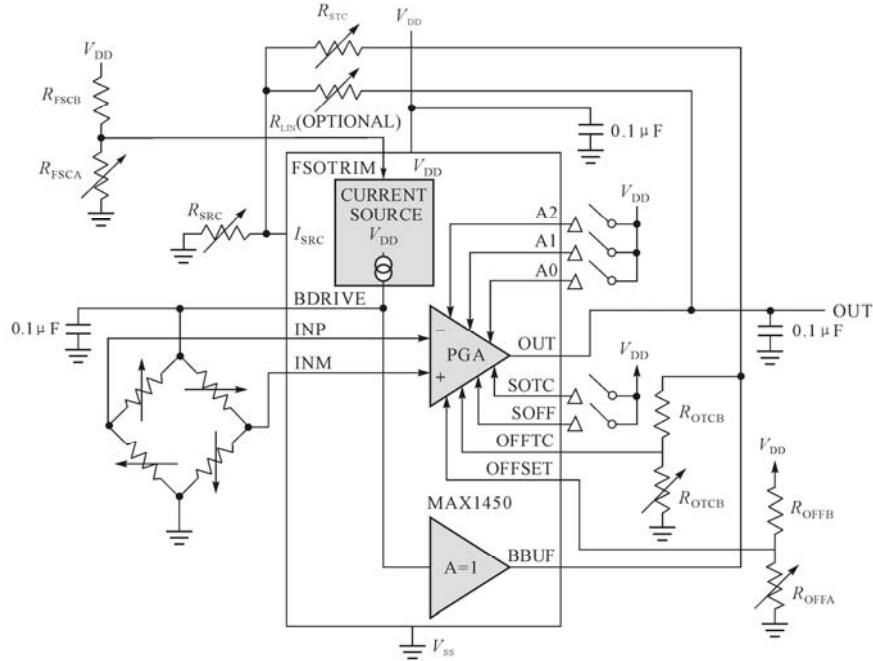
**Fig. 3.8.** Interaction of the three compensation mechanisms in a conventional resistive compensation circuit ( $R_{TS}$  for sensitivity drift,  $R_{TZ}$  for offset drift, and zero trim resistors)

But biomedical detection needs better precision. Fig. 3.9 shows an intergrated compensation circuit which includes two main functional blocks: a controlled current source for driving the sensor, and a programmable-gain amplifier (PGA, implemented in switched-capacitor technology and virtually free of offset). The numerous external resistors and voltage dividers are commonly realized with hybrid technology and adjusted with laser trimming. The temperature drift is adjusted by feeding back the sensor's drive voltage (from the BDRIVE pin) to the ISRC pin. The circuit's initial sensitivity is adjusted at the FSOTRIM pin of MAX1450. Compensation of offset and offset drift is accomplished at the PGA and decoupled from the sensitivity compensation. The key function, however, is the controlled current source, which implements a unique algorithm for compensating the sensitivity drift.

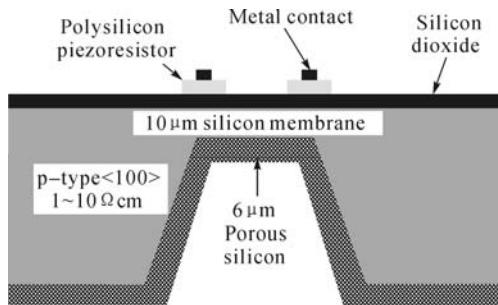
### 3.2.2.3 Biomedical Applications

Piezoresistive sensors are widely used to measure pressure in biomedicine. To reduce the unavoidable nonlinearity increase with sensitivity, Marco et al. (1996)

used thin structured membranes in piezoresistive pressure sensors to obtain high-performance. A generally used Si/Porous-Si membrane is shown in Fig. 3.10. Pramanik and Saha (2006) also used various diaphragms to realize low pressure measurement in biomedicine, such as respirators, ventilators and spiroimeters. Among the diaphragms, nanocrystalline silicon, which is a three-phase mixture of silicon, silicon oxide, and voids and formed by electrochemical etching of silicon, increases the sensitivity almost three times to that of conventional piezoresistive pressure sensors of similar dimensions. Besides, silicon nanowire can be used to enhance sensitivity of piezoresistive sensors (Kim et al., 2009).



**Fig. 3.9.** Laser-trimmed resistor dividers in the MAX1450 signal conditioner provide better than 1% compensation full scale over temperature

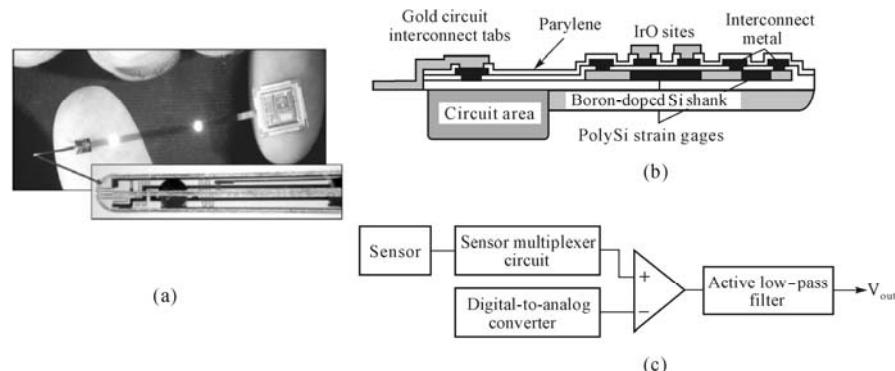


**Fig. 3.10.** Pressure sensor with Si/Porous-Si composite membrane

With the development of MEMS technology, ultra-miniaturized piezoresistive pressure sensors (Gowrishetty et al., 2008) are developed to monitor intra-cranial pressure during neurosurgery, air pressure for respiratory disease, blood pressure during surgery/intensive care, intra-uterine for obstetrics, and abdominal/urinary pressure for the diagnosis of respective disorders. According to Gowrishetty, the dimensions of the fabricated sensor are  $650 \mu\text{m} \times 230 \mu\text{m} \times 150 \mu\text{m}$  (length, width, thickness) with  $2.5 \mu\text{m}$  thick diaphragms, and sensitivity of the sensors with half Wheatstone bridge configuration is determined to be  $27 - 31 \mu\text{V/V/mmHg}$ .

Apart from pressure sensing, a piezoresistive sensor is also applied in a position-sensing system for a MEMS-based cochlear implant (Wang et al., 2005). Fig. 3.11a shows the prototype of a fully-implantable thin-film cochlear prosthesis (Kensall et al., 2008). A polysilicon piezoresistive position-sensing system is monolithically integrated into the electrode arrays in order to provide real-time visualization of array position for the surgeon. The sensing array of the cochlear microsystem consists of the electrode array, flip-chip bonded to a signal-processing chip. As shown in Fig. 3.11b, the position sensors with each formed using two strain-sensing resistors in a half-bridge configuration are implemented underneath electrode sites using piezoresistive polysilicon strain gages and extra passivating dielectrics (Kensall et al., 2008). Polysilicon wall-contact and strain gages are buried under IrO sites distributed along the shank.

A microprocessor gives instructions to the circuit chip to select the addressed sensor, and the addressed sensor bridge is connected to the positive input of an instrumentation amplifier (Wang et al., 2005), which is referenced to a voltage generated by the DAC (Fig. 3.11c). Then the bridge output signal is amplified and filtered to determine local bending.



**Fig. 3.11.** MEMS-based cochlear implant: (a) The prototype of a fully-implantable thin-film cochlear prosthesis; (b) Cross-sectional diagram of an electrode array with position sensors; (c) Selected sensor output is read out using an instrumentation amplifier and a LP filter (reprinted from (Kensall et al., 2008), Copyright 2008, with permission from Elsevier)

Another development of piezoresistive sensors is as an application in wearable devices with smart textiles to monitor gesture, posture, or respiration. The piezoresistive sensors can be yarn-based (Huang et al., 2008), and can measure

respiratory rate even in the rapid running motion (Jeong et al., 2009).

### 3.3 Inductive Sensors and Measurement

Based on the electromagnetic induction principle, non-electric quantities, such as displacement, stress, flux, vibration, can be converted into variations of self-inductance  $L$  or mutual inductance  $M$  of the coil, which will be finally output as voltage or current through a measuring circuit. This kind of device is called an inductance sensor, which has reliable performance and high measurement accuracy. The main shortcoming is that its sensitivity, linearity and measurement range restrict each other, and the frequency response does not apply to rapid dynamic measurement.

#### 3.3.1 Basics

Based on the conversion mode from non-electric parameters to voltage, inductive sensors can be classified as self-inductance sensors and mutual inductance ones.

##### 3.3.1.1 Self-inductance Sensors

###### *Variable reluctance sensor*

A variable reluctance sensor is a typical self-inductance sensor. It consists of a coil, core and armature. The coil and armatures are made of permeable magnetic materials like silicon steel sheet and permalloy. There exists an air-gap with a thickness  $\delta$  between them. Once the core moves, the thickness  $\delta$  will change, which results in the change of reluctance in the magnetic circuit, and the inductance value of the inductance coil. If the coil is placed in an AC circuit, the change of inductance can be used to change the voltage drop across the inductor or it can be used in an oscillator circuit to change the frequency of the circuit.

###### *Eddy current sensor*

According to Faraday's law of electromagnetic induction, when a massive metal conductor is placed in a mutative magnetic field or moves in a magnetic field cutting magnetic lines, induced eddy-electric current will be produced in the conductor, which is called eddy current. The eddy current effect has relations with not only resistivity, magnetic permeability and geometric shape, but also geometric parameters, exciting current frequency in coil and distance between coil and conductor. The eddy current sensor is made up of sensor coils and a tested conductor. It can continuously and through non-contacting, measure displacement,

thickness and surface temperature.

### **3.3.1.2 Differential Transformer Sensors**

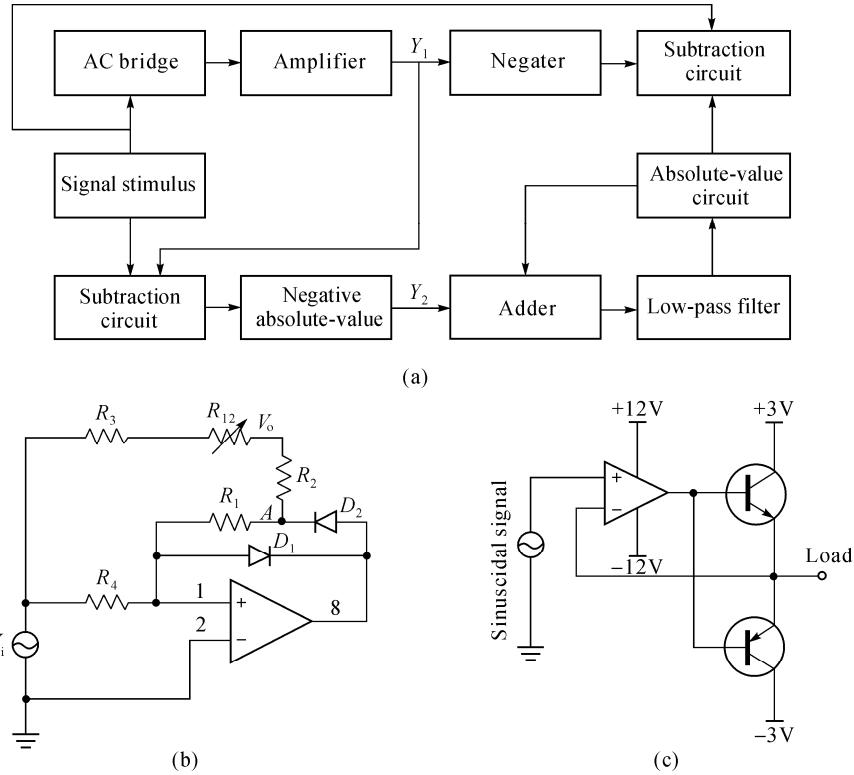
A mutual inductance sensor, which is made up based on the basic principle of a transformer, has the capacity for converting non-electrical variation into change of mutual inductance. As the secondary coil is connected in differential forms, it is also called a differential transformer sensor.

A differential transformer sensor has a variety of structures, including variable gap type, variable area type and solenoid type. Their work principle is similar. In non-electrical quantity testing, solenoid type is the most widely used. It consists of a primary coil, two secondary coils and an inserted columniform core in the middle of the coil and can measure the mechanical displacement within a scope of 1 – 100 mm with high accuracy, high sensitivity, simple structure, and reliable performance.

### **3.3.1.3 Measurement**

The measuring circuit of an inductance sensor mainly includes an AC bridge and resonant circuit. An AC bridge converts inductance change to output voltage, which then will be amplified, go through phase-sensitive detection circuit, and be filtered. However, a noise proof circuit is indispensable for detection of weak signal when using AC bridge circuits. In resonant circuit measurement, inductive sensors become a part of the oscillation bridge, where the frequency change indicates the inductance change.

In infinitesimal displacement measurement, the high precision of the results is important. However, stabilization of signal stimulus, precision of phase-sensitive detection circuit, and amplification and filtering of signal all together influence the quality of the result to a large extent. A method for improving the precision was developed a few years ago (Ding et al., 2008), which used a crystal oscillator with a stable frequency as the signal stimulus. MAX293 is an eight-level ellipse filter. It can convert square waves into high-precision sine wave signals, which then will be inputted into a bandpass filter to generate an excellent sinusoidal carrier wave signal. Meanwhile a follower circuit (Fig. 3.12c) with a combination of an operational amplifier and triode is used to drive the load to guarantee the stabilization of the stimulus signal. The phase-sensitive detection circuit, which applies operational amplifier AD620 to realize differential amplification, is shown in Fig. 3.12a. To obtain high-precision amplitude-modulated signals, a secondary amplifier is also used. The absolute value circuit (Fig. 3.12b) is the key part of a phase-sensitive detection circuit. It uses unilateral electric devices like diodes to convert bipolar signals into unipolar signals to realize rectification. Since a diode reduces the voltage, an operational amplifier absolute circuit is used to compensate for the voltage drop.



**Fig. 3.12.** Method for improving the precision of inductance sensor: (a) Block diagram of phase-sensitive detection circuit; (b) Schematic diagram of single operational amplifier absolute value circuit; (c) Follower circuit

### 3.3.2 Applications in Biomedicine

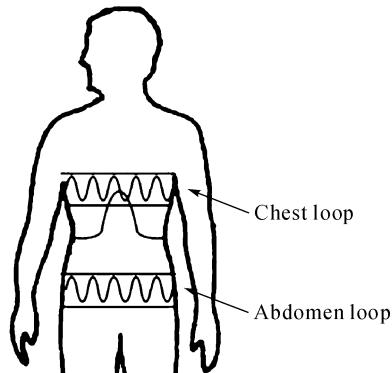
Because of the high sensitivity of an inductive sensor, which has a maximum resolution of  $0.01 \mu\text{m}$ , an inductive sensor is mainly used to measure slight displacement in biomedical engineering. A typical application is respiratory inductance plethysmography (RIP) (Mazeika and Swanson, 2007), which is probably the most commonly accepted method for quantitative and qualitative non-invasive respiratory measurements in infants and adults. Respiratory measurements, such as, respiratory rate and tidal volume, are important indicators showing a person's health condition, therefore are of great significance in first aid for the family. With the help of some techniques proposed for calibration (Poole et al., 2000), RIP may be used quantitatively, which makes respiration measurement more effective.

Since both the thoracic and abdominal area change reflects the value of minute

volume, the long time measurement of a respiratory movement can be realized through measuring the variation of the cross-sectional area. In RIP, two elastic belts, into which a zigzagging (coiled) wire (for expansion and contraction) is sewn, are essential, with one worn around the chest, and the other worn around the abdomen, resembling two inductance loops (Fig. 3.13). Based on the principle of Faraday's Law, an alternating current applied through a loop of wire with high frequency and low amplitude generates a magnetic field normal to the orientation of the loop. According to Lenz's Law, a change in the area enclosed by the loop, which causes a variation in the self-inductance coefficient, creates an opposing current within the loop directly proportional to the change in the area. The frequency of the alternating current is set to be more than twice the typical respiratory rate in order to achieve adequate sampling of the respiratory effort waveform. In measurement, the breathing activity changes the cross-sectional area of the patient's body, and thus changes the shape of the magnetic field generated by the belt, "inducing" an opposing current that can be measured. The variation of minute volume  $\Delta V$  can be calculated as follows:

$$\Delta V = K_1 \Delta L_R + K_2 \Delta L_A \quad (3.5)$$

where  $\Delta L_R$  is the output inductance change of thoracic belt,  $\Delta L_A$  is that of abdominal belt,  $K_1$  and  $K_2$  are volume coefficient of chest and abdomen, respectively.



**Fig. 3.13.** Diagrammatic sketch of RIP

With RIP, no electrical current passes through the body. Even though a weak magnetic field is present, it does not affect the patient or any surrounding equipment. Otherwise, the worry about measurement being interfered with by the surrounding environment is unnecessary. The signal produced is linear and is a fairly accurate representation of the change in cross-sectional area. Actually RIP is reliable in measuring respiratory movement, with advantages of convenience, non-invasive and ambulatory monitoring. In addition, RIP has superiority in displaying respiratory frequency, evaluating coordination of chest and abdomen respiratory movement, with no heart interference.

Over all, RIP is more widely used in sleep respiratory monitor apparatus to record chest and abdomen respiratory movement, as well as in diagnosing sleep apnea syndrome. Besides, Moreau-Gaudry et al. (2006) have demonstrated that RIP has potential in a swallowing monitor to analyze swallowing disorders and putting in place medical supervision of swallowing for individuals who might aspirate, especially in the elderly.

### 3.4 Capacitive Sensors and Measurement

Capacitive sensors electronically measure the capacitance between two or more conductors in a dielectric environment, usually air or a liquid. They are built with conductive sensing electrodes in a dielectric, with excitation voltages and detection circuits which turn a capacitance variation into a voltage, frequency, or pulse width variation.

The first reference to capacitive sensors is found in Nature, 1907, but the penetration today is only a small percentage of all sensor types. This is surprising, with the technology's low cost and stability and its simple conditioning circuits—often, the offset and gain adjustments needed for most sensor types are not required, as the raw output span of the signal on the capacitive sensors can be nearly to the supply rails. These advantages are attracting many converts.

#### 3.4.1 Principle and Configuration

The simplest electrode configuration is two close-spaced parallel plates. The capacitance, neglecting a small fringe effect, can be calculated by

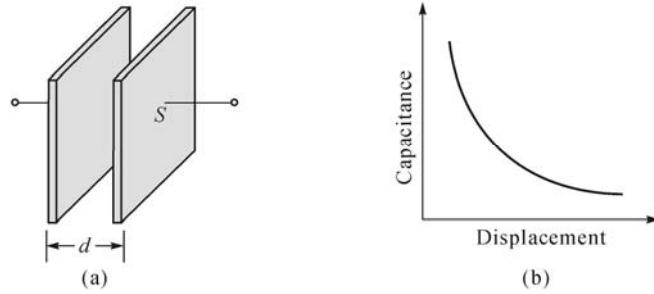
$$C = \frac{\epsilon A}{d} \quad (3.6)$$

where  $\epsilon$  is the permittivity,  $\epsilon = \epsilon_0 * \epsilon_r$ ,  $\epsilon_0$  is the vacuum permittivity and  $\epsilon_r$  is the relative permittivity of media;  $A$  is the area of the parallel plates,  $d$  is the distance between the parallel plates.

Fluctuations in any of the parameters caused by the variables being detected will be revealed in the variation of the capacitance, which can be converted into electric output by the detection circuits. Thus, capacitive sensors can be divided into three types: space-variant capacitive sensors, area-variant capacitive sensors, and permittivity-variant capacitive sensors.

### 3.4.1.1 Space-variant Capacitive Sensors

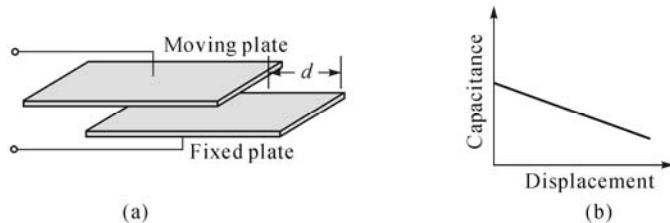
Space-variant capacitive sensors (Fig. 3.14) are often used for motion detection if the spacing change is far less than the electrode size. The parallel plate capacitance formula shows that capacitance has an approximate inverse ratio to spacing, but it does often require signal conditioning which can compensate for the parabolic capacitance-displacement relationship. In actual practice, differential configuration and media with high permittivity are usually employed.



**Fig. 3.14.** The principle of space-variant capacitive sensor: (a) Schematic structure; (b) Relationship between capacitance and plates space

### 3.4.1.2 Area-variant Capacitive Sensor

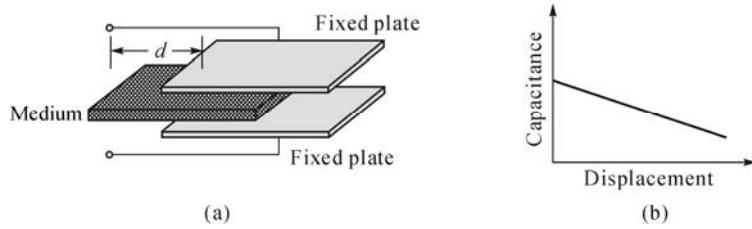
For transverse motion, whose area varies with constant spacing (Fig. 3.15), capacitance will change linearly with motion. Quite long excursions are possible with good linearity, but the gap needs to be small and well-controlled. As with spacing variation, overlap is needed so that unwanted sensitivities are minimized. Area-variant capacitive sensors have some other forms, just as the rotary motion sensors.



**Fig. 3.15.** The principle of area-variant capacitive sensor: (a) Schematic structure; (b) Relationship between capacitance and plates space

### 3.4.1.3 Medium-variant Capacitive Sensors

The schematical representation of the medium-variant capacitive sensor is given in Fig. 3.16. As the medium slides transversely, capacitance changes linearly with motion. Several methods help with sensitivity, such as using high permittivity media.



**Fig. 3.16.** The principle of medium-variant capacitive sensor: (a) Schematic structure; (b) Relationship between capacitance and plates space

### 3.4.2 Measurement Circuits

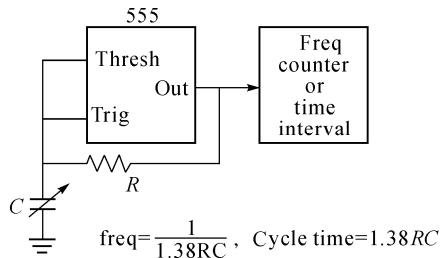
Measurement circuits convert capacitance variations into a voltage, frequency, or pulse width modulation. Very simple circuits can be used, but simple circuits may be affected by leakage or stray capacitance, and may not be suitable for applications with very small capacitance sense electrodes.

#### Pulse operation

A single pulse can be used to sample a variable capacitor, like a microcomputer read pulse, or a train of pulses can be used. This method can result in simpler electronics but will have higher noise.

#### Oscillator

An R-C relaxation oscillator such as the venerable 555 or its CMOS update, the 7555, converts capacitance change into a change of frequency or pulse width.

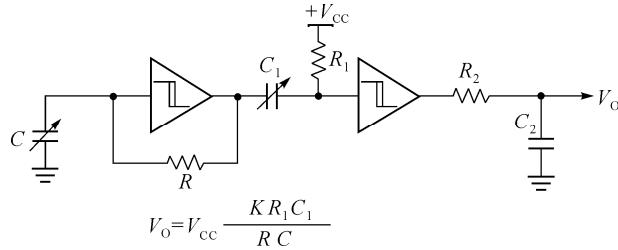


**Fig. 3.17.** Block diagram of RC oscillator circuit

The RC oscillator (Fig. 3.17) used with a spacing-variation capacitor will produce a frequency output which is linear with spacing, while an area-variation capacitor is linearized by measuring pulse width.

#### Simple circuit

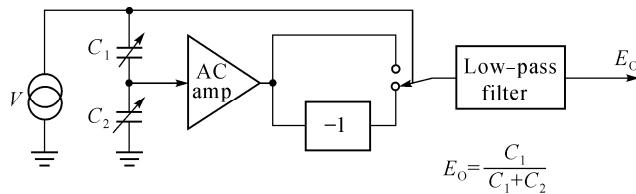
This circuit (Fig. 3.18) uses a CMOS Schmitt inverter as an  $RC$  oscillator followed by a one shot  $R_1C_1$  (with a smaller time constant) followed by low pass  $R_2C_2$  (with a larger time constant). The output can be either capacitance-linear or 1/capacitance-linear, depending on the location of the sense capacitor. Unfortunately, it is not particularly stable with temperature and power supply and it may require a floating sense capacitor.



**Fig. 3.18.** Simple circuit

#### Synchronous demodulator circuit

A square wave excitation voltage  $V$  feeds the variable capacitance ( $C_1$ ,  $C_2$ , or both may be variable) and also use a CMOS switch. A high-impedance unity-gain AC amplifier feeds the switch directly and also feeds an inverter. If the phase shift through the amplifier is low, the switch output is an accurate demodulation, probably contaminated by narrow spikes caused by switching transients. These transients are eliminated in the lowpass filter.



**Fig. 3.19.** Synchronous demodulator circuit

An advantage of the synchronous demodulator is that out-of-band signal components are eliminated in the low pass filter. This is important in applications where power line harmonics or other crosstalk contaminate the signal.

#### 3.4.3 Biomedical Applications

Capacitive sensors are mainly used to measure pressure in biomedical engineering.

### 3.4.3.1 Capacitive Pressure Sensors

Pressure sensors are required in many applications, such as biomedical systems, industrial process controls, and environmental monitoring (He et al., 2007).

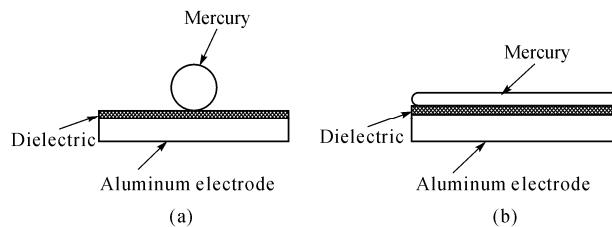
Capacitive pressure sensors are particularly noteworthy and can provide very high-pressure sensitivity, low power, low noise, large dynamic range and low temperature sensitivity. Nowadays, capacitive pressure sensors have become one of the most popular MEMS sensors. In 1980, the micro capacitive pressure sensor was first fabricated by using micro machining technology (Sander et al., 1980). With a length of 3 mm and a height of 425  $\mu\text{m}$ , the main structure of this sensor was a chamber and when pressure deformed the thin upper layer of the chamber, the capacitance was changed. From then on, more and more micro-electro-mechanical-systems capacitive pressure sensors were designed for biomedical applications.

A new capacitive pressure sensor with extremely high sensitivity ( $2.24 \mu\text{F}/\text{kPa}$ ) (Bakhoum et al., 2010) is applicable to detect external pressure of human beings, such as non-invasive blood pressure measurement (Fig. 3.20).



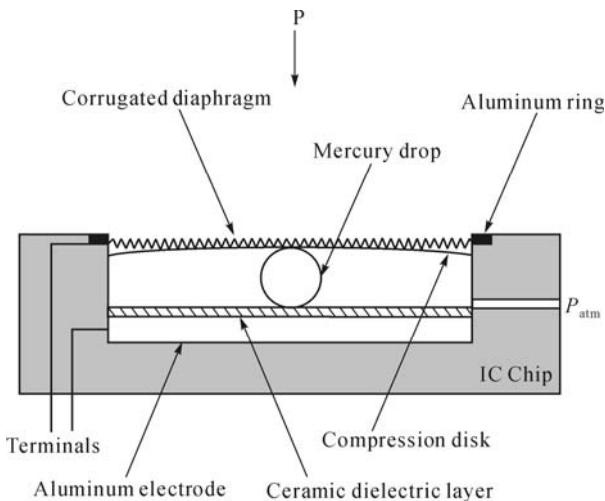
**Fig. 3.20.** Non-invasive blood pressure measurement

The basic concept of the new device is to mechanically deform a drop of mercury that is separated from a flat aluminum electrode by a thin layer of a dielectric material, so as to form a parallel-plate capacitor where the electrode area is variable to a high degree. This principle is illustrated in Fig. 3.21. Under zero pressure, the mercury drop remains at its nearly-spherical shape. With the pressure increasing, the mercury drop is flattened against the aluminum electrode. A parallel-plate capacitor with one liquid electrode is formed.



**Fig. 3.21.** The change in capacitance between the two configurations, which is proportional to the change in the contact area of the liquid electrode, can be several hundred fold: (a) A drop of mercury is flattened against an aluminum electrode that is covered with a layer of a dielectric material. A parallel-plate capacitor with one liquid electrode is formed; (b) Under zero pressure, the mercury drop returns to its nearly-spherical shape. The change in capacitance between the two configurations, which is proportional to the change in the contact area of the liquid electrode, can be several hundred folds

As shown in Fig. 3.22, a drop of mercury with a diameter of 3 mm is placed on top of a flat aluminum electrode that is covered with a 1- $\mu\text{m}$ -thick layer of a ceramic material with a very high permittivity (specifically, BaSrTiO<sub>3</sub>, with a permittivity of 12,000 – 15,000). This ceramic material was deposited on the surface of aluminum electrode by using the electrophoretic deposition technique. The drop is held in place by means of an aluminum disk that serves as the compression mechanism. The compression disk, in turn, is acted upon by means of a corrugated stainless-steel diaphragm. The compression disk is slightly curved, such that the spacing between the disk and the ceramic layer is exactly 3 mm at the center but less than 3 mm everywhere else. In this manner, the mercury drop will be forced to the center each time the stainless steel diaphragm retracts. The diaphragm is held in place by a thin aluminum ring. The entire assembly is mounted inside an open-cavity, 24-pin DIP IC package.

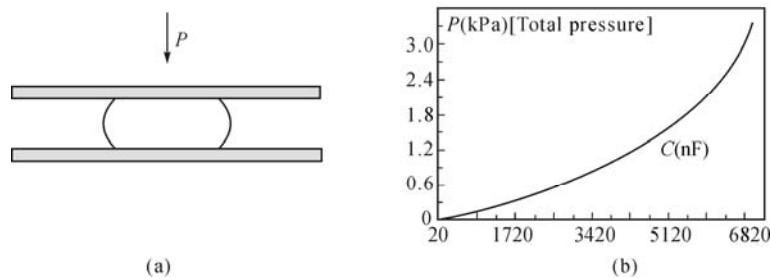


**Fig. 3.22.** A sensor with a drop of mercury

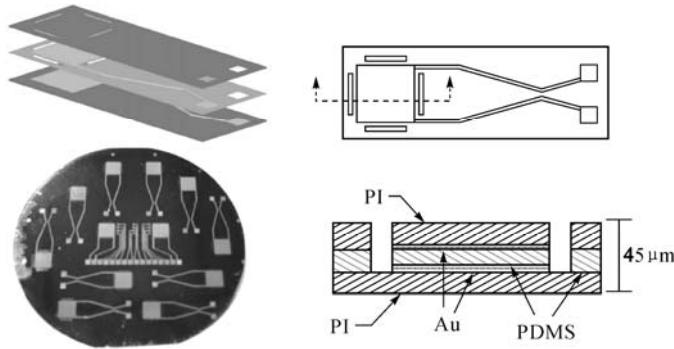
Since the air that surrounds the mercury droplet must be allowed to exit from the sensor and reenter as the sensor is pressurized/depressurized, an atmospheric pressure relief conduit is drilled in the IC package. In most applications, that conduit will be connected to an atmospheric pressure environment via, for example, an external tube to be connected to the sensor.

As shown in Fig. 3.23a, the vertical pressure  $P$  changes the geometry of the drop of mercury, which leads to the variation of surface area of the electrode. Capacitance-pressure relationship in Fig. 3.23b can be obtained from theoretical arithmetic and experiments.

Capacitive pressure sensors are also used to detect internal pressure. The micro capacitive pressure sensor shown in Fig. 3.24 was developed to be embedded into the cuff electrode for *in situ* monitoring of the interface pressure between implanted cuff and nerve tissue (Chiang et al., 2007).



**Fig. 3.23.** A drop of mercury: (a) Pressures and geometry in the deformation; (b) Total pressure acting on the sensor as a function of the measured capacitance



**Fig. 3.24.** Structure of the flexible capacitive pressure sensor (reprinted from (Chiang et al., 2007), Copyright 2007, with permission from Elsevier)

Cuff electrode (Fig. 3.25a) is an indispensable component of a neural prosthesis system. It is often employed to apply electrical stimuli on motor nerve fibers that innervate muscles or alternatively to record neural signals from the peripheral nerves. It is reported that a pressure over 20 mmHg is harmful for the nerve trunk. Therefore, measuring the interface pressure between the cuff and a nerve trunk provides a means to monitor the health of the nerve tissue.

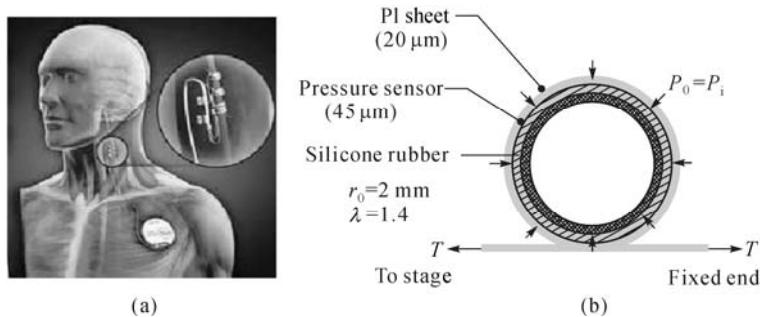
The structure of the capacitive pressure sensor consists of two parallel electrical sensing plates, one dielectric layer sandwiched between the two sensing plates, and two outer insulating layers (Fig. 3.24). Polyimide (PI, Durimide 7320) is chosen as the material of the insulating layers because of its biocompatibility and insulating capability. The polydimethylsiloxane (PDMS, Sylgard 184) serves as the material of the dielectric layer. The dielectric constant of PDMS is 2.65. It is greater than the dielectric constant of air so that a larger initial capacitance and higher capacitance change can be obtained. Left part of Fig. 3.24 also shows the fabricated array of capacitive pressure sensors before the lifting.

For biomedical applications, animal experiments will be conducted to test performance of the capacitive pressure sensor. In the *in-vitro* test, a calibration system developed in our previous work was employed to measure the pressure between the outer surface of a silicone rubber tube and the inner surface of a cuff

made by a PI sheet. The closed silicone rubber tube was filled with water, the flexible sensor was wrapped tightly on the silicone rubber tube, and the capacitive pressure sensor was further encircled by a circular loop made of PI sheet to simulate the spiral cuff electrode (Fig. 3.25b). The PI sheet has a thickness of 20  $\mu\text{m}$  and width of 8 mm except 12 mm at center and the circular loop was formed by pulling one end of the sheet through a pre-cut small slit at the center. The other end of the circular loop is fixed on a platform and the movable end is fastened to a translation stage which can move forward to pull the PI sheet. A load cell fixed on the stage was utilized to measure the tension,  $T$ , on the PI sheet. The pressure,  $P_0$ , between the PI sheet and the silicon rubber can be formulated by

$$P_0 = \frac{T}{(1+2\pi\lambda)r_0} \quad (3.7)$$

where  $\lambda$  is the coefficient of static friction between the PI sheet and the flexible sensor, and  $r_0$  is the outer radius of the silicon rubber. Pressure calculated from Eq. (3.7) is compared with the capacitance changed detected by the sensor. A straight line between the pressure applied by the PI sheet and change of the capacitance of the flexible can be fitted.

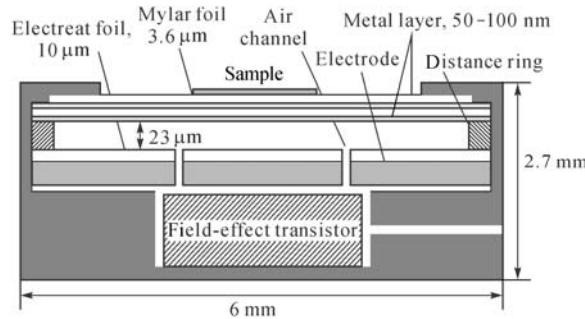


**Fig. 3.25.** The structure of the capacitive pressure sensor: (a) Cuff electrode; (b) *In vitro* circular compression test of the flexible pressure sensor

### 3.4.3.2 Electret Microphone

An electret microphone is a type of condenser microphone, which eliminates the need for a polarizing power supply by using a permanently-charged material. An electret is a stable dielectric material with a permanently-embedded static electric charge. Electrets are commonly made by first melting a suitable dielectric material such as a plastic or wax that contains polar molecules, and then allowing it to re-solidify in a powerful electrostatic field. The polar molecules of the dielectric align themselves to the direction of the electrostatic field, producing a permanent electrostatic “bias”. Electret microphones are useful in acoustic and audio applications such as hearing aid appliances.

Silicon micromachining enables the integration of mechanical parts with preamplifiers. A silicon-based microphone structure is shown in Fig. 3.26 (Schenk, 1996).



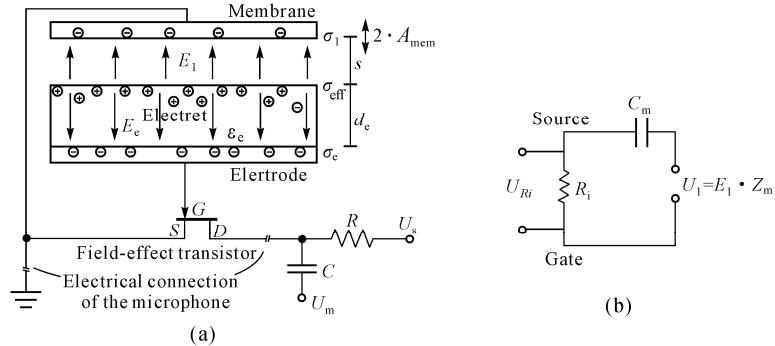
**Fig. 3.26.** Schematic cross-section and size of the silicon-based microphone (not to scale)

The membrane of the microphone consists of a 3.6  $\mu\text{m}$  thick mylar foil that is coated on both sides with a layer of about 100 nm metal. The distance between the membrane and the electrets amounts to 23  $\mu\text{m}$  and the thickness of the electrets foil is about 10  $\mu\text{m}$ . Air channels in the stationary electrode enlarge the air volume between the membrane and the electrode. Hence, the part of the counteracting force that is generated by air compression is reduced when the membrane is pressed down. To diminish acoustic interference for experiments with air, a small hole is drilled in the lower side of the closed microphone capsule. Interference is reduced to about 50% because disturbing sound waves act now simultaneously on both sides of the membrane. This modification additionally enables measurements with the microphone in a vacuum. The effect of this hole for the sensitivity and frequency response of this type of electret microphone has proven to be significant. The overall size of the electret microphones used is in the range of a few millimetres. Their diameter amounts to 6.0 mm and their height to 2.7 mm. Because of the small capacitance of the system (a few pF only) a field-effect transistor is integrated into the microphone capsule acting as an impedance converter. It amplifies the very low signals induced by the movement of the membrane and, thus, the influence of the stray capacitance is reduced.

Because the membrane and electrode are electrically shortened across the finite input impedance of the FET (about  $10^{12} \Omega$ ), the microphone cannot transduce static deflections of the membrane and, hence, no static measurement of the force is possible (Fig. 3.27a). The capacitance  $C_m$  between the membrane and the electret-electrode and the input resistance  $R_i$  of the FET forms a high pass filter with the cut-off frequency  $f_g = (2\pi R_i C_m)^{-1}$  (Fig. 3.27b). It amounts to about 0.5 Hz for the microphone used. When the membrane is deflected, the microphone signal will be zero after  $t_0 > 1/f_g$ .

Vibration of the membrane will generate the voltage  $U_{Ri}$  at the input resistance of the FET, and  $U_{Ri}$  controls, via the gate, the drain-source resistance of the FET and, hence, the microphone output voltage  $U_m$ . The drain-source resistance of the FET and the resistance  $R$  represent a voltage divider. A capacitor  $C$  decouples the

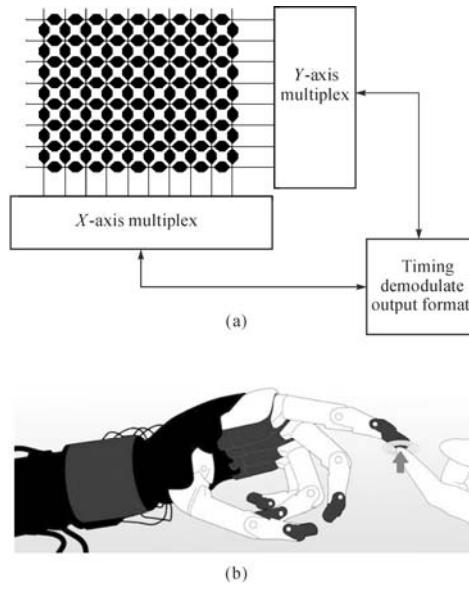
microphone signal from the supply voltage  $U_s$ .



**Fig. 3.27.** Circuit of the electret microphone: (a) Electrical circuit; (b) Equivalent circuit

### 3.4.3.3 2D Capacitive Sensors

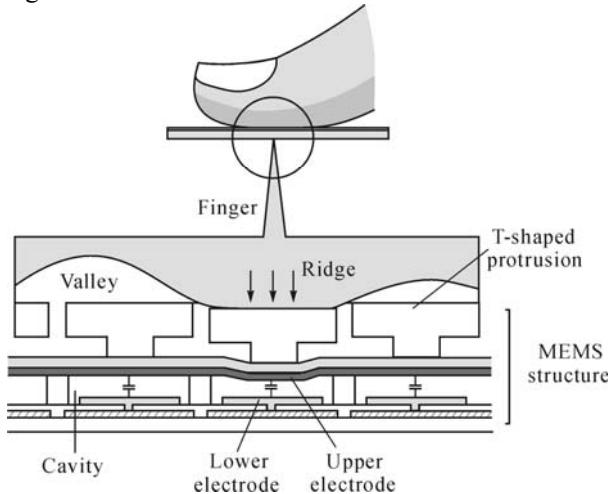
In recent years, sensor array has been one of the technology hotspots and has developed rapidly. The 2D capacitive sensor shown in Fig. 3.28 is a typical sensor array, which consists of an  $X-Y$  array of pressure sensitive cells. By detecting the capacitance change of every cell, many aspects of the force applied on the sensor can be obtained, such as magnitude, location and direction. This kind of 2D capacitive sensor is widely used in human-computer interfaces, mobile devices and robotic applications.



**Fig. 3.28.** A 2D capacitive sensor: (a) Schematic diagram; (b) Robotic applications

The use of a high density capacitive array, such as the sensitive part of the fingerprint sensor (Rey et al., 1997), is very suitable for the growing market of portable equipment for the low power consumption of capacitance detection. For this application, the density of sensitive cells is much higher than the density required for the tactile sensors used in robotic applications.

One type of these fingerprint sensors (Sato et al., 2005) has an array of about 57,000 pixels in an area of  $(11.2 \times 12.8)$  mm<sup>2</sup>. Each pixel is a capacitive pressure sensor that has a MEMS cavity structure stacked on a CMOS LSI. Integrating sensing circuits, just below the MEMS cavity structures, enables a sufficiently large number of pixels. The MEMS cavity structure consists of parallel electrodes and a protrusion with a shape of a block as shown in Fig. 3.29. When a finger touches the sensor surface filled with a lot of protrusions, ridges of the finger deform the upper electrode via the protrusions and increase the capacitance between the upper and lower parallel electrodes. The capacitance change is detected by the sensing circuits and converted into digitized signal levels in the CMOS LSI, and the detected signals from all the pixels generate one fingerprint image in a gray-scale. Thus, the MEMS fingerprint sensor obtained shows clear fingerprint images.



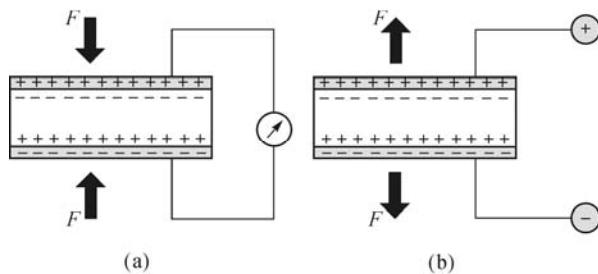
**Fig. 3.29.** A kind of the MEMS fingerprint sensor

### 3.5 Piezoelectric Sensors and Measurement

Over the past 60 years, piezoelectric sensors have proven to be a versatile tool for the measurement of various processes. Today, this type of sensor has found very wide applications, as for example in medical, aerospace and nuclear, etc. It is regarded as a mature technology with an outstanding inherent reliability and an excellent linearity over a wide measurement range, while their size remains small.

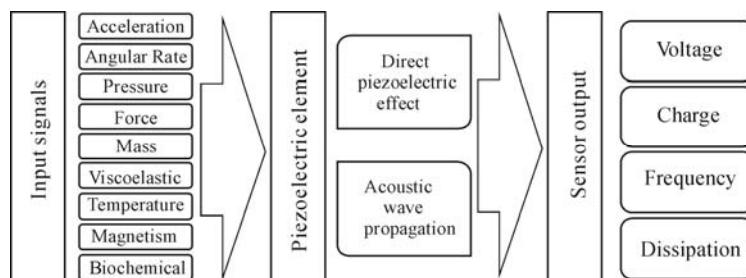
### 3.5.1 Piezoelectric Effect and Piezoelectric Materials

Piezoelectric sensors are based on the piezoelectric effect, which was discovered by the Curie brothers in the late 19th century. While investigating a number of naturally occurring materials such as tourmaline and quartz, Pierre and Jacques Curie realized that these materials had the ability to transform energy of a mechanical input into an electrical output. More specifically, when a pressure (piezo is the Greek word for pressure) is applied to a piezoelectric material, it causes a mechanical deformation and a displacement of charges. Those charges are highly proportional to the applied pressure, which is called piezoelectric effect (Fig. 3.30a). Later on, the converse piezoelectric effect (Fig. 3.30b) that an application of a voltage across the electrodes can induce mechanical deformation of the media, was observed as well.



**Fig. 3.30.** Energy conversion between mechanical and electrical forms: (a) Piezoelectric effect; (b) Converse piezoelectric effect

From the Curies' initial discovery, it took until the 1950's before the piezoelectric effect was used for industrial sensing applications. Since then, the utilization of this measuring principle has experienced a constant growth. Typically, piezoelectric sensors are configured as direct mechanical transducers or as resonators (Tadigadapa and Matet, 2009), where the observed resonance frequency and amplitude are determined by the physical dimension properties and materials comprising the device and most importantly by mechanical and interfacial inputs to the device. Fig. 3.31 schematically illustrates these configurations of piezoelectric sensors.



**Fig. 3.31.** Schematic illustration of the two modes of operation of piezoelectric sensors

Two main groups of materials are used for piezoelectric sensors: piezoelectric crystal and piezoelectric ceramic. However, the piezoelectric constitutive equations should be introduced in any discussion of piezoelectric materials, as in Eqs. (3.8) and (3.9), where  $S_j$  is the mechanical strain,  $\sigma_j$  is the mechanical stress,  $E_i$  is the electric field,  $D_i$  is the electrical displacement,  $c_{ij}$  is the elastic stiffness constant,  $s_{ij}$  is the elastic compliance coefficient, and  $\epsilon_{ii}$  is the permittivity. If a parameter has a superscript, such as  $S_j^E$ , this is the elastic compliance when the electric field is held constant. The piezoelectric coefficients,  $d_{ij}$  and  $e_{ij}$ , are the third rank tensors which in reduced tensor notation correspond to a  $3 \times 6$  matrix. In this reduced notation, the indices ( $i = 1, 2, 3$ ) define normal electric field or displacement orientation, ( $j = 1, 2, 3$ ) define normal mechanical stresses or strains and ( $j = 4, 5, 6$ ) represent shear strains or stresses:

$$D_i = d_{ij}\sigma_j + \epsilon_{ii}^T E_i \quad \text{or} \quad D_i = e_{ij}S_j + \epsilon_{ii}^S E_i \quad (3.8)$$

$$S_j = s_{ij}^E \sigma_j + d_{ij} E_i \quad \text{or} \quad T_j = c_{ij}^E S_j - e_{ij} E_i \quad (3.9)$$

These equations are useful in describing the basic relationships for the direct and converse piezoelectric effects. In the direct effect, using Eq. (3.8), a mechanical stress  $\sigma_j$  or strain  $S_j$  causes a net electrical displacement,  $D_i$ , on the  $i$  faces of the material, the magnitude of which depends on  $d_{ij}$  and  $e_{ij}$  respectively. Similarly, the converse effect expressed by Eq. (3.9) relates the induced normal and shear stress or strain to the applied electric field via the piezoelectric coefficient tensor (Schwartz et al., 2004). In general, large  $d$  piezoelectric coefficients, with units pC/N, are desired in actuator applications and large  $e$  coefficients are desired for sensor applications.

### **Quartz**

Although found naturally, most quartz in practical use is synthetic, *AT*-cut single crystal, right handed and  $\alpha$ -phase. Below the Curie temperature of 573 °C, quartz has a trigonal structure and above that temperature, it becomes  $\beta$ -quartz with a hexagonal structure (Bottom, 1982). Although the *AT*-cut is used for its near zero temperature coefficient of frequency, other cuts such as the *Y*-cut or the dual mode *SC*-cut with a high sensitivity of the resonance frequency with respect to temperature can be used accurately for temperature measurement (Goyal et al., 2005). Quartz is only useful in single crystal form and to achieve high resonance frequencies, the thickness of this crystal quartz must be minimized. Using micromachining techniques, resonators with thicknesses of less than 10  $\mu\text{m}$  and diameters of less than 100  $\mu\text{m}$  have been realized for quartz crystal microbalance (QCM) applications and chemical sensing techniques.

### **Langasite**

Another material, which has similar temperature coefficients as quartz, but has a

quality factor five times higher and a piezoelectric coupling coefficient three times higher, is langasite ( $\text{La}_3\text{Ga}_5\text{SiO}_{14}$ ). A relatively new non-ferroelectric piezoelectric material, langasite single crystals have been grown using the Czochralski method and single crystal thin films have been grown using the liquid phase epitaxy technique. Langasite does not experience phase transitions up to the melting point and has low acoustic wave propagation losses.

#### **Lithium niobate and tantalate**

Lithium niobate and lithium tantalate are well-known ferroelectric crystals discovered in 1949 and have been successfully grown into single crystals from melting by the Czochralski technique since 1965 (Xu, 1991). Both are important in surface acoustic wave (SAW) devices and high-frequency filter applications. Like quartz, these materials must be grown in bulk and have different properties based on their cuts. The cuts usually used are *Y*-cut for  $\text{LiNbO}_3$  and *X*-cut for  $\text{LiTaO}_3$ .

#### **Lead zirconate titanate (also called PZT)**

PZT are the most widely used ferroelectric materials. The higher electromechanical coefficient of PZT makes it a very attractive material for actuator and sensor applications. PZT films at the morphotropic phase boundary with a Zr/Ti ratio of 52/48 have been shown to exhibit a maximum in the piezoelectric response and are typically used in MEMS device applications.

#### **Relaxor ferroelectrics**

Lead-titanate-relaxor-based ferroelectric systems, PZN–PT, PMN–PT and PYN–PT, show extremely large electromechanical coupling coefficients and piezoelectric coefficients compared to PZT, but have integration issues that have limited their use such as a lower dielectric constant and a lower stress response. The low Curie temperatures of PMN–PT and PZN–PT limit their operating range, whereas PYN–PT has a higher Curie temperature. Research in relaxor ferroelectrics is still very promising due to the wide array of different compositions of the  $\text{PbZrO}_3\text{--PbTiO}_3\text{--Pb(B}_1\text{B}_2\text{)}\text{O}_3$  ternary system.

#### **Polyvinylidene fluoride**

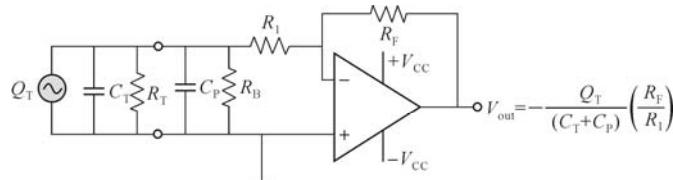
Polyvinylidene fluoride (PVDF) ( $-\text{CH}_2\text{--CF}_2-$ ), a semi-crystalline homopolymer, is technologically very important due to its piezoelectric and pyroelectric properties. These characteristics make it useful in sensor and battery applications requiring the highest purity, strength, and resistance to solvents, acids, bases and heat and low smoke generation (Jiang et al., 1997). PVDF is very flexible, exhibits good stability over time and does not depolarize when subjected to very high alternating electric fields. Like other current electroactive polymers, the application potential of PVDF can be enormously escalated if its piezoelectric properties are enhanced to produce high electromechanical coupling and large force generating capabilities.

### 3.5.2 Measurement Circuits

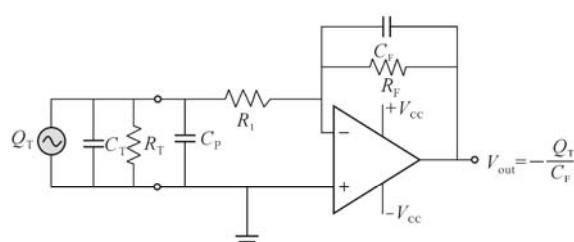
A piezoelectric sensor can be modeled as a charge source  $Q_T$  with a shunt capacitor  $C_T$  and resistor  $R_T$  or as a voltage source with a series capacitor and resistor. The charge produced depends on the piezoelectric constant of the device and the mechanical input signals. The capacitance is determined by the area, the width and the dielectric constant of the piezoelectric material. The resistance accounts for the dissipation of static charge through leakage.

Since the piezoelectric sensor has high inner resistance and low output energy, it is necessary to include the preamplifier circuit with high input resistance, so that high input resistance is replaced by low input resistance; and the weak output of sensors is amplified.

According to the output of piezoelectric sensors, the preamplifiers can be classified into voltage amplifiers and charge amplifiers. The voltage amplifier circuit shown in Fig. 3.32a is typically used when the amplifier circuit can be located very close to the transducer and when the effect of the parasitic capacitance  $C_P$  can be minimized in the performance of this circuit. The resistor  $R_B$  is typically very large and provides the required biasing for the input stage of the circuit. The charge amplifier circuit is based on the Miller integrator circuit as shown in Fig. 3.32b. The feedback resistor  $R_F$  is required to prevent the circuit from saturating due to the charge build-up on the capacitor  $C_F$ . In this circuit, the amplifier keeps the two input terminals at the same voltage, and therefore the parasitic capacitance does not affect this circuit.



(a)



(b)

**Fig. 3.32.** Typical circuits used with piezoelectric transducers. The sensor element is represented as a charge source  $Q_T$  with an equivalent capacitance  $C_T$  and leakage resistance  $R_T$  all connected in parallel: (a) Voltage amplifier circuit; (b) Charge amplifier circuit

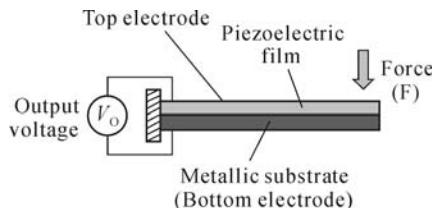
### 3.5.3 Biomedical Applications

Piezoelectric sensors are configured as direct mechanical transducers or as resonators.

#### 3.5.3.1 Sensors Based on Direct Piezoelectric Effect

A wide variety of mechanical sensors are based on the piezoelectric effect, which can be applied for detecting pressure impulse or movements.

Monitoring of breathing conditions during sleeping is one of the crucial tests for appropriate diagnosis of sleep disorders. The structure of a kind of piezoelectric flexible transducers (Yuu et al., 2008) is shown in Fig. 3.33.



**Fig. 3.33.** A schematic configuration for airflow monitoring

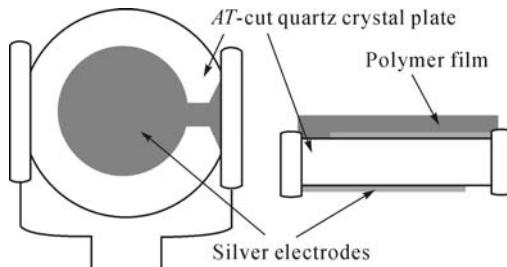
The sensor consists of a stainless steel (SS) foil substrate, piezoelectric ceramic film, top electrode and protection film. The PZT composite piezoelectric film is fabricated onto the 40  $\mu\text{m}$  thick SS foil using a sol-gel spray technique. The thickness of the piezoelectric film was 60  $\mu\text{m}$ . The top electrode is constructed using a silver paste. The SS foil serves as a bottom electrode as well as the substrate. The dimension of the active transducer area is 4 mm by 20 mm. The sensor is covered with a polymeric film that protects the sensor from moisture and scratches. Due to the porosity inside the film and the thin substrate, the sensor has high flexibility. The transducer can be used as an ultrasonic probe and unimorph-type bending sensor which can be applied in various types of industrial applications such as nondestructive testing, medical diagnosis and physiological monitoring.

#### 3.5.3.2 Sensors Based on Piezoelectric Resonators

##### *Quartz crystal microbalance*

Quartz crystal microbalance (QCM) have been in steady use for a number of years as a convenient tool to determine mass loading of material layers with layer thicknesses ranging well below the monolayer level. A QCM typically consists of a slab of thin, single-crystal, piezoelectric quartz, with very large lateral dimensions in

comparison to its thickness, which is sandwiched between two metal electrodes. Fig. 3.34 shows a schematic representation of the quartz crystal microbalance.



**Fig. 3.34.** Schematic representation of the quartz crystal microbalance

QCMs utilize the piezoelectric qualities of quartz crystals. Applying alternating current to the quartz crystal will induce oscillations. With an alternating current between the electrodes of an *AT*-cut crystal, a standing shear wave is generated. The resonance is so narrow that oscillators are highly stable and accurate in the determination of the resonance frequency. It has been shown that there is an explicit quantitative relationship between a shift in the resonant frequency and added mass on the silver electrode,  $\Delta m$  (Kurosawa et al., 2006).

$$\Delta F_N = -N \frac{2F_1^2}{A\sqrt{\mu\rho}} \Delta m \quad (3.10)$$

where  $N$  is the order of the overtone ( $N=1, 3, 5, 7, \dots$ ),  $\Delta F_N$  is the change in the oscillation frequency of a quartz crystal of  $N$ th mode,  $\Delta m$  represents the change in mass on crystal electrodes. The fundamental frequency of the quartz crystal is represented as  $F_1$ ,  $A$  is the electrode area of the quartz,  $\mu$  is the elastic modulus of the quartz, and  $\rho$  is the quartz density. The equation was examined experimentally by measuring changes in oscillation frequency when the deposit mass of polymer films affected the oscillating frequency of QCM.

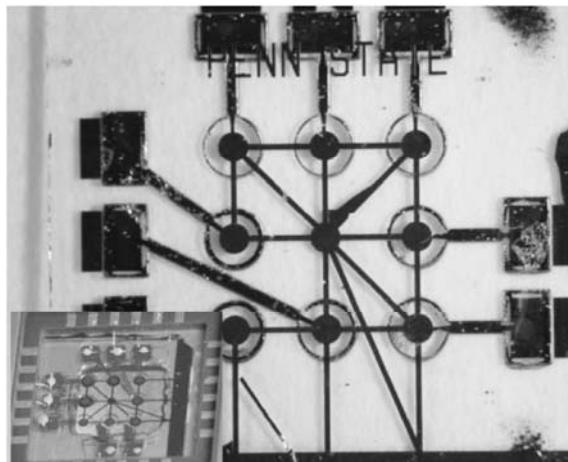
The QCM can be used under vacuum, in gas phase (King, 1964) and more recently in liquid environments (Höök, 2001). It is useful for monitoring the rate of deposition in thin film deposition systems under vacuum. In liquid, it is highly effective at determining the affinity of molecules (proteins, in particular) to surfaces functionalized with recognition sites. Larger entities such as viruses or polymers can be investigated, as well.

#### ***Infrared-sensitive resonator***

Thermal infrared detectors are broadband detectors and can be operated at room temperature without cooling. These detectors can be designed to operate near the room temperature thermodynamic noise limit arising from the thermal conductance fluctuation between the sensing element and the supporting substrate.

More recently, thickness shear mode resonators from quartz have been proposed and demonstrated as sensitive infrared detectors. The unprecedented temperature sensitivity along with the low noise performance that can be achieved in quartz crystal oscillators is the principle of operation of several thermal sensors based on acoustic waves.

A micro-machined *Y*-cut quartz-resonator-based thermal infrared detector array (Kao and Tadigadapa, 2009) is shown in Fig. 3.35. One mm diameter and 18  $\mu\text{m}$  thick (90 MHz) inverted mesa configuration quartz resonator arrays with excellent resonance characteristics have been fabricated by RIE etching of quartz. The average resonance frequency of the array was 89.65 MHz with a maximum deviation in the resonance frequency of  $\pm 0.59\%$ . The temperature sensitivity of the resonators was measured by placing the resonator array in an oven and varying the temperature in the range of 22 – 38 °C. The resonator array was allowed to equilibrate at each temperature for more than 30 min until a stable resonance frequency was achieved. As expected, a linear dependence on temperature for all the three resonators was observed and the average temperature sensitivity was measured to be 7.2 kHz/°C. Fig. 3.36 shows the measured temperature dependence of the resonance frequency.



**Fig. 3.35.** Photograph of the fabricated quartz resonator infrared sensor array with eight resonators per chip (reprinted from (Kao and Tadigadapa, 2009), Copyright 2009, with permission from Elsevier)

Modern imagers based upon thermal infrared detectors are typically implemented using micromachining techniques. Deposition of thin film ferroelectric materials has allowed the construction of better thermally isolated IR sensor structures. The microbridge structures offer better thermal isolation and lower cost products through batch fabrication techniques (Hanson et al., 2001). Fig. 3.37a shows the exploded view of the thermal infrared detectors pixel, Fig. 3.37b shows a SEM photograph of the section of a 320×240 pixel array, and thermal infrared

detectors, and Fig. 3.37c shows a thermal image obtained. Although the noise equivalent temperature difference of the detector was only  $0.21\text{ }^{\circ}\text{C}$ , the images show very high quality which has been attributed to the high modulation transfer function of these detectors.

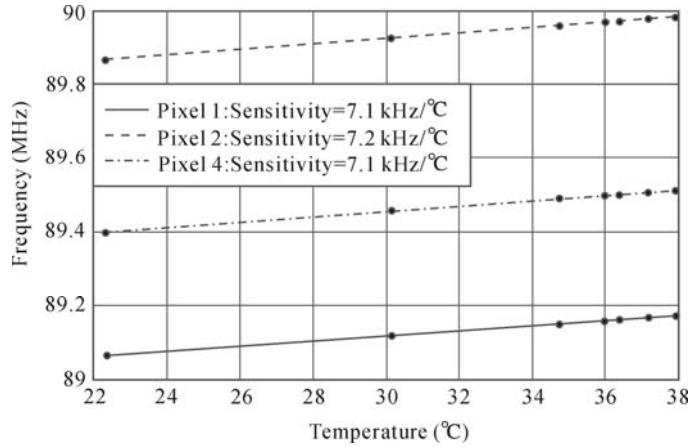


Fig. 3.36. Experimentally measured temperature sensitivity of the *Y*-cut quartz resonators

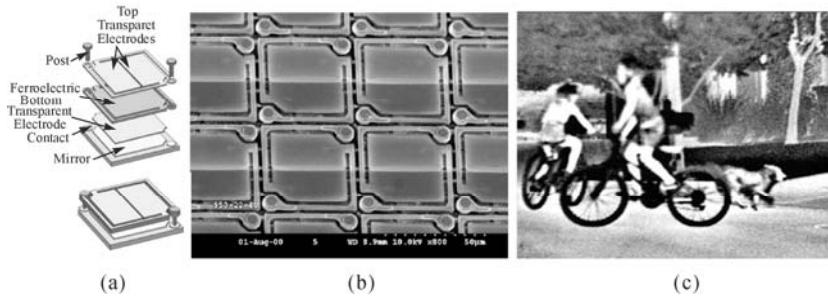


Fig. 3.37. Thermal infrared detectors: (a) Exploded view of a thermal infrared detectors pixel; (b) SEM photograph of a part of  $330 \times 240$  pixel IR imaging array; (c) An IR image obtained (reprinted from (Hanson et al., 2001), Copyright 2001, with permission from SPIE)

### 3.6 Magnetoelectric Sensors and Measurement

Magnetoelectric sensor is a kind of sensor that is able to convert displacement, velocity, acceleration and other measuring quantities to electrical signals by magnetoelectric effect. They can be divided into magnetoelectric induction sensors and hall sensors. Magnetoelectric sensors, with large output power, stable performance, as well as bandwidth working-frequency, are widely used in medical, automation, mechanical engineering and other fields.

### 3.6.1 Magnetolectric Induction Sensors

Magnetic induction sensors are active sensors which do not require auxiliary power.

#### 3.6.1.1 Working Principle

According to Faraday's law of electromagnetic induction, when a conductor is moving in a stable magnetic field perpendicularly to the magnetic field direction, the induced electromotive force of the conductor is

$$e = \frac{d\Phi}{dt} = \frac{B/dx}{dt} = Blv \quad (3.11)$$

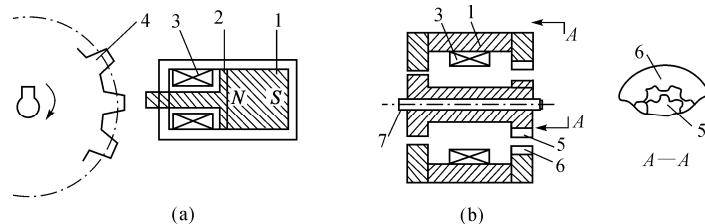
where  $B$  is the magnetic induction intensity of the stable magnetic field,  $l$  is the effective length of the conductor,  $v$  is the relative velocity of the conductor to the stable magnetic field.

When there are  $\omega$  circles in the time-varying electromagnetic field, the induced electromotive force of the induction coil is

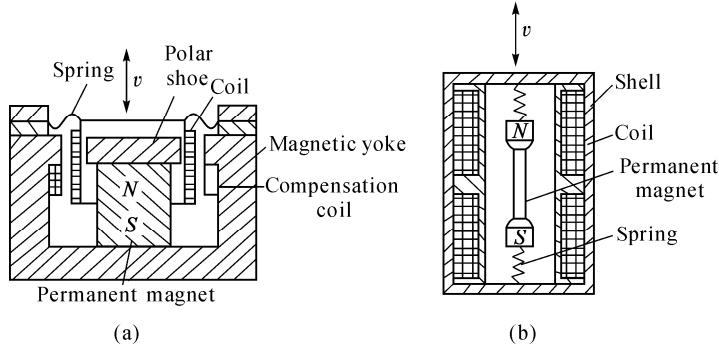
$$e = -\frac{\omega d\varphi}{dt} \quad (3.12)$$

For the different modes above, there are two different types of magnetoelectric induction sensors: variable magnetic flux mode (Fig. 3.38) and constant magnetic flux mode (Fig. 3.39).

Fig. 3.38 is a variable magnetic flux type sensor used to measure the rotation angular velocity of rotating objects. Each turning, which causes the teeth bumping, changes the magnetic flux generating electromotive force. Fig. 3.39 is the typical structure of constant flux-type magnetic sensors, which consists of a permanent magnet, coil, springs and metal skeleton. The magnetic circuit system produces a constant DC magnetic field, the work of the magnetic circuit air gap is fixed, and therefore a constant air gap of the magnetic field is constant. Its moving parts can be a coil (moving-coil) or a magnet (an iron-type). The principle of the moving-coil-type (Fig. 3.39a), and the moving-iron-type (Fig. 3.39b) are identical.



**Fig. 3.38.** Structural drawing of magnetoelectric induction sensor with variable magnetic flux: (a) Open magnetic circuit; (b) Closed magnetic circuit. 1—permanent magnet, 2—soft magnet, 3—induced coil, 4—iron gear, 5—internal gear, 6—external gear



**Fig. 3.39.** Structural drawing of magnetoelectric induction sensor with constant magnetic flux:  
(a) Moving coil; (b) Moving iron

The induced electromotive force of constant magnetic flux is:

$$e = -B_0 l W v \quad (3.13)$$

where,  $B_0$  is the magnetic induction intensity in working air gap,  $l$  is the average length of the coils,  $W$  is the numbers of the coils in working air gap, and  $v$  is the relative velocity.

### 3.6.1.2 Basic Characteristics

In Fig. 3.40 the output current of magnetoelectric induction sensor  $I_0$  is:

$$I_0 = \frac{E}{R + R_f} = \frac{B_0 l W v}{R + R_f} \quad (3.14)$$

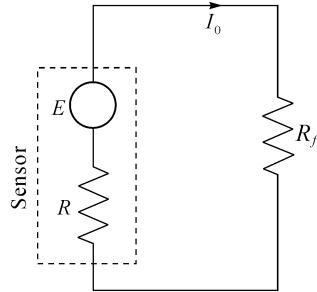
where  $R_f$  is the input resistance,  $R$  is the equivalent resistance of the coils. The current sensitivity of the sensor is:

$$S_I = \frac{I_0}{v} = \frac{B_0 l W}{R + R_f} \quad (3.15)$$

When the working temperature varies or under external magnetic field's disturbing and mechanical vibrating, the sensitivity will change, and the relative error is

$$\gamma = \frac{dS_I}{S_I} = \frac{dB}{B} + \frac{dl}{l} - \frac{dR}{R} \quad (3.16)$$

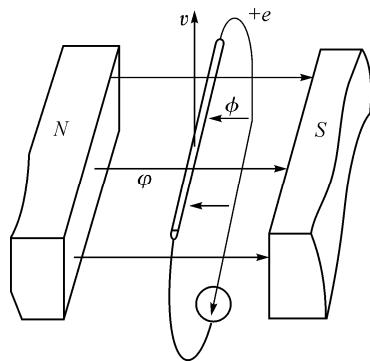
When the temperature changes, all the three items in Eq. (3.16) are nonzero values. Temperature compensation is necessary by using a thermo magnetic splitter.



**Fig. 3.40.** The simplified model of the magnetoelectric induction sensor

In addition to the above, Magnetic sensors also generate a certain degree of non-linear error. When the current  $I$  goes through the sensor's coils, the alternating magnetic flux  $\Phi_l$  exists. It will be added to the working magnetic flux of the permanent magnet, and thus causes the change of the magnetic flux in air gap.

In Fig. 3.41, when the relative velocity of the coils to the permanent magnet increases, there will be a larger induced electromotive force and larger induced current. The additional magnetic field's direction is opposite to the working magnetic field, thus weakens the working magnetic field and decreases the sensitivity. Otherwise, when the coils move in the opposite direction, the additional magnetic field's direction is the same with the working magnetic field, thus increasing the sensitivity. The higher the sensor's sensitivity, the larger the current in the coils, and thus the worse the nonlinear error. To eliminate the error, it is possible to add compensating coils to the sensor as Fig. 3.39a.

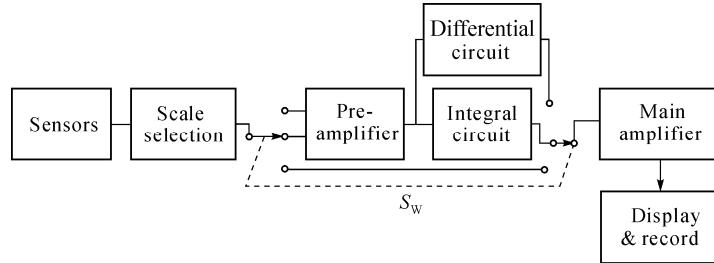


**Fig. 3.41** Magnetic effect of the sensor's current

### 3.6.1.3 Measuring Circuit for Magnetoelectric Induction Sensor

Magnetoelectric induction sensors cause the output of the induced electromotive force directly, with high sensitivity and do not require any gain amplifier. Since it is a speed sensor, integral and differential circuits are needed to get the

displacement or acceleration required to complete the measurements.



**Fig. 3.42.** Measuring circuit for magnetoelectric induction sensor

### 3.6.2 Applications in Biomedicine

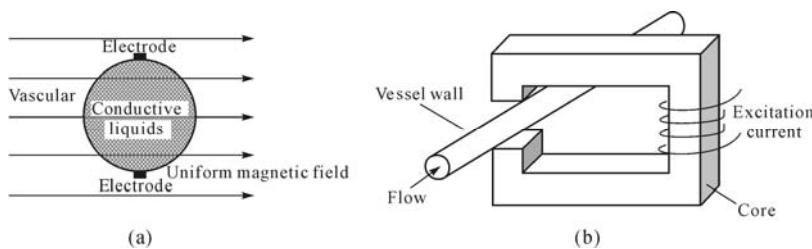
Magnetic sensors can be used in biomedical flow tests (Section 2.1.3). At present, the magnetic flow meter has been used as a standard method for the measurement of intravascular blood flow. The sensitivity of magnetic measurement for the volume flow rate has nothing to do with the velocity distribution. The magnetic method can be widely applied from the thickest blood vessels to a 1 mm diameter in humans.

According to the previous description of the electromagnetic induction law, when a conductor moves in a magnetic field while cutting the magnetic field lines, it will generate induced electromotive force. As shown in Fig. 3.43a, the uniform magnetic flux density  $B$ , the electromotive force generated from diameter  $EE'$  is equal to

$$V = 2aBv \quad (3.17)$$

where  $B$  is magnetic flux density (Gs),  $v$  is velocity (cm/s), then the type can be used as the volume flow rate  $Q$  (cm/s)

$$V = \frac{2QB}{\pi a} \quad (3.18)$$



**Fig. 3.43.** Magnetic blood flow meter: (a) Cross-section diagram; (b) Principle

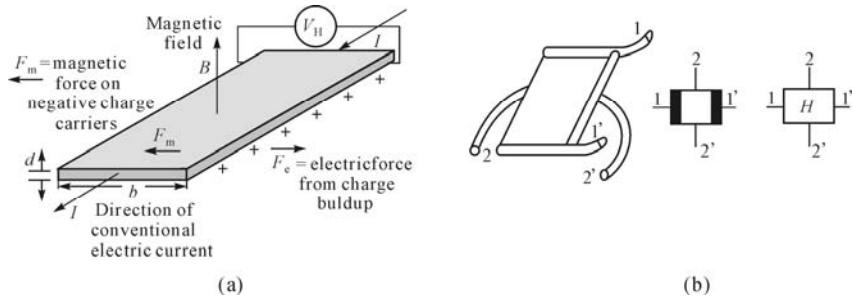
The advantage of the electromagnetic flow method is the electromotive force has nothing to do with the blood flow distribution. For a certain degree of blood vessel diameters and the magnetic induction intensity, the electromotive force only relates to the instantaneous volume flow rate.

### 3.6.3 Hall Magnetic Sensors

A hall sensor is a kind of sensor based on the Hall Effect which is a magnetic-electric effect. This phenomenon is discovered by U.S. physicist Hall (Dr. Edwin Hall, 1855 – 1938) in 1879 while studying metal conductive bodies. With the development of semiconductor technology, Hall components began to use semiconductor materials. At present, the Hall sensors are widely used in electromagnetic, pressure, acceleration, vibration and other measurements.

#### 3.6.3.1 Hall Device

Dr. Hall found, when a magnet was placed so that its field was perpendicular to one face of a thin rectangle of gold through which current was flowing, a difference in potential appeared at the opposite edges. He found that this voltage was proportional to the current flowing through the conductor, and the flux density or magnetic induction perpendicular to the conductor (Fig. 3.44a).



**Fig. 3.44.** Hall device: (a) Hall effect; (b) External structural schematic diagram, graphic symbol. 1,1'—exciting electrode, 2,2'—Hall electrode

For a simple metal where there is only one type of charge carrier (electrons) the Hall voltage  $U_H$  is given by

$$U_H = \frac{BI}{ned} \quad (3.19)$$

The Hall coefficient is defined as  $R_H=1/ne$ , so,

$$U_H = R_H \frac{IB}{d} = K_H IB \quad (3.20)$$

where  $K_H = R_H/d$  is defined as the sensitivity of the Hall device.

As can be seen from Eq. (3.20), the Hall voltage is in direct proportion to excitation and magnetic field intensity, and the sensitivity is in direct proportion to the Hall coefficient  $R_H$  and in inverse proportion to the thickness of the Hall device. To improve the sensitivity, the Hall device is usually made into thin slices. The resistance between the excitation electrodes is  $R = \rho l/(bd)$ , and  $R = U/I = El/I = vl/(\mu nevbd)$  (because  $\mu = v/E$ , where  $\mu$  is the electron mobility). So,

$$\rho l/(bd) = l/(\mu nevbd), \text{ and } R_H = 1/(ne) = \rho\mu \quad (3.21)$$

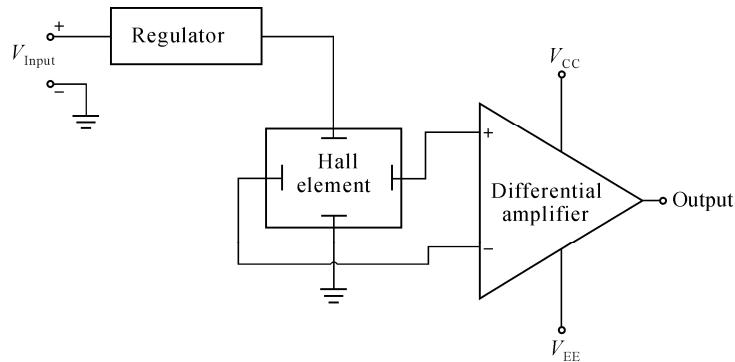
From the above equation, only the semiconductor materials are suitable for the manufacture of the Hall device, because the semiconductor has a better Hall coefficient  $R_H$ .

The structure of the Hall device is shown in Fig. 3.44b. 1,1' are connected to an electrode with exciting current or voltage (exciting electrode). 2,2' are Hall output down-lead (Hall electrode).

The Hall element is the basic magnetic field sensor. It requires signal conditioning to make the output usable for most applications. The signal conditioning electronics needed are an amplifier stage and a temperature compensation. Voltage regulation is needed when operating from an unregulated supply. Fig. 3.45 illustrates a basic Hall Effect Sensor.

If the Hall voltage is measured when no magnetic field is present, the output is zero. However, if voltage at each output terminal is measured with respect to ground, a non-zero voltage will appear. This is the common mode voltage, and is the same at each output terminal. It is the potential difference that is zero. The amplifier shown in Fig. 3.45 must be a differential amplifier so as to amplify only the potential difference—the Hall voltage.

The Hall voltage is a low-level signal on the order of 30 mV in the presence of a one-gauss magnetic field. This low-level output requires an amplifier with low noise, high input impedance and moderate gain. A differential amplifier with these characteristics can be readily integrated with the Hall element using standard bipolar transistor technology. Temperature compensation is also easily integrated.



**Fig. 3.45.** Basic Hall effect sensor

### ***Rated exciting current and maximum permitted exciting current***

When the Hall element of the excitation current is zero, if the location of the magnetic flux density components is zero, then the hall electric potential should be zero. However, in the actual cases, this value is usually not zero. Then the measured electric potential is called the allelic potential. The reasons of this phenomenon are:

- Hall electrodes asymmetry, or not in the same equipotential surface;
- Non-uniform resistivity semiconductor material causing non-uniform resistance or geometry;
- The poor contact of the Incentive electrodes causes the unevenness of the electrode current.

Allelic resistance can also state the allelic potential,

$$r_0 = \frac{U_0}{I_H} \quad (3.22)$$

where  $U_0$  is equipotential potential,  $r_0$  is Allelic resistance, and  $I_H$  is excitation current.

### ***Input resistance and output resistance***

When the external magnetic field is zero and the Hall element is input with AC excitation, there is a DC potential, called the parasitic direct potential, in addition to the potential allelic exchange. The value of parasitic DC potential is generally below 1 mV, which affects the Hall element drift through temperature. It is caused by the following reasons:

Poor contact of the Incentive electrodes and Hall electrodes which forms the contact capacitance and causes a rectification effect;

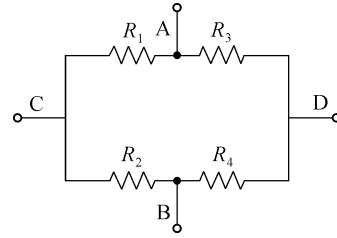
Asymmetry in the size of two Hall electrodes causes the two different heat capacity points of electrodes, so that the thermal states are different, and causes formatting of the polar temperature potential.

### ***Temperature coefficient for Hall voltage***

In certain magnetic induction intensities with an excitation current, the temperature changes 1 °C, the Hall potential percentage changes in the temperature coefficient of electrical potential, and is called the temperature coefficient for Hall voltage. Usually, the temperature is an important factor of a Hall effect sensor.

#### **3.6.3.2 Compensation for Hall Device's Unequal Electrical Potential**

In ideal situations, as shown in Fig. 3.46 A and B will be on the same equipotential surface, and unequal potential  $U_{AB}$  will be zero. However, it is not in this case in real situation.



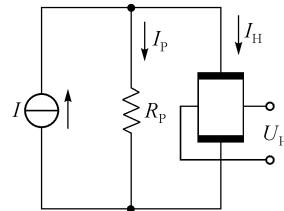
**Fig. 3.46.** Equivalent bridge of Hall device. A, B: Hall electrode; C, D: exciting electrode

### 3.6.3.3 Temperature Compensating for Hall Device

To reduce the temperature error of the Hall device, it is important to select suitable temperature coefficient devices and other constant temperature methods. For  $U_H = K_H IB$ , when choosing constant current supply, the Hall voltage will be more stable, by reducing the variation of the exciting current  $I$  caused by the variation of the input resistance as the temperature changes. The relationship between the sensitivity coefficient and temperature is:

$$K_H = K_{H0}(1 + \alpha\Delta T) \quad (3.23)$$

where  $K_{H0}$  is the changed  $K_H$  under temperature  $T$ ,  $\Delta T$  is the change of temperature,  $\alpha$  is the temperature coefficient of Hall voltage.  $\alpha$  is generally a positive value. When the temperature increase, the Hall voltage will increase by  $\alpha\Delta T$  times. If decreasing the exciting current to keep the  $K_H$  unchanged, the effect of  $K_H$ ' change can be avoided.



**Fig. 3.47.** Temperature compensating circuit

Fig. 3.47 shows the temperature compensating circuit. Suppose the initial temperature is  $T_0$ , the input resistance is  $R_{i0}$ , the sensitivity coefficient is  $K_H$ , and the shunt resistance is  $R_{p0}$ . When temperature increase to  $T$ , the parameters in the circuit will be

$$R_i = R_{i0}(1 + \delta\Delta T) \quad (3.24)$$

$$R_p = R_{p0}(1 + \beta\Delta T) \quad (3.25)$$

where  $\delta$  is the temperature coefficient of input resistance,  $\beta$  is the temperature coefficient of shunt resistance.

$$I_H = \frac{R_p I_s}{R_p + R_l} \quad (3.26)$$

By arranging the equations above and omitting the high items as  $\alpha, \beta, (\Delta T)^2$ ,

$$R_{p0} = \frac{(\delta - \beta - \alpha)R_{l0}}{\alpha} \quad (3.27)$$

When the devices are selected, the input resistance  $R_{l0}$ , temperature coefficient  $\delta$ , and the temperature coefficient of Hall voltage are set values. So the shunt resistant  $R_{p0}$  and temperature coefficient  $\beta$  can be calculated.

### 3.6.3.4 Biomedical Applications

#### *Hall whisker sensor*

Hall effect devices produce a very low signal level and thus require amplification. While suitable for laboratory instruments, the vacuum tube amplifiers available in the first half of the 20th century were too expensive, power consuming, and unreliable for everyday applications. It was only with the development of the low cost integrated circuit that the Hall effect sensor became suitable for mass applications. Many devices now sold as Hall effect sensors in fact contain both the sensor described above and a high gain integrated circuit (IC) amplifier in a single package. Recent advances have resulted in the addition of ADC (Analog to Digital) converters and I<sup>2</sup>C (Inter-integrated circuit communication protocol) for direct connection to a microcontroller's I/O port being integrated into a single package. Reed switch electrical motors using the Hall Effect IC is another application. Here is an application of the Hall sensor (Zhong et al., 2009).

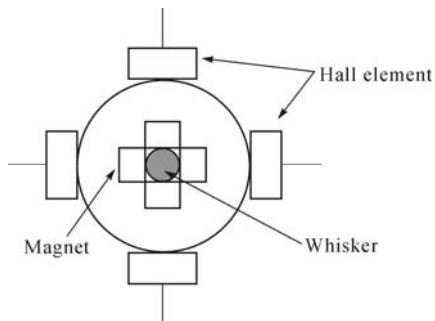


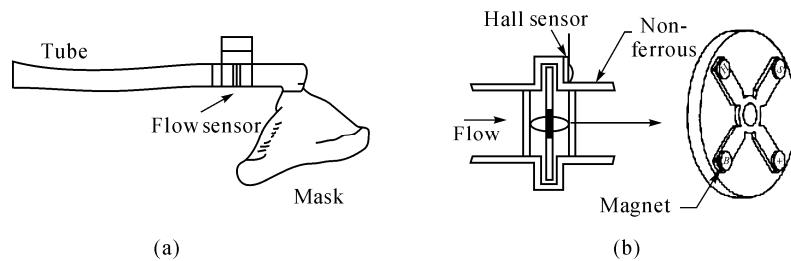
Fig. 3.48. Structure of whisker Hall sensor

The sensor is a two-tier board structure whose lower root fixes the antennae and the upper layer feels the displacement of the tentacle. The two plates are connected by a nylon column and maintain a spacing of 20 cm to 30 cm as shown in Fig. 3.48. The tentacle crosses the center of the upper hole (diameter 1,215 mm) and four small magnets fixed at the top of the tentacle at a 5 – 15 mm away from the hole. The small magnets around the hole correspond with the four Hall elements (UGN3503), these constitute a “follicle” role.

When the tentacles are bent, the magnets deviate from the original position, the change of magnetic fields cause the increases and decreases of the Hall element output voltage, and thus the signals in the  $x$  direction and  $y$  direction form a differential output respectively. The sensor has a two-dimensional awareness.

#### **Hall respiration flow sensor**

There are various methods for designing Hall flow meters, but the general principle is the same: each actuation of the sensor, by a magnet or by shunting the magnetic field, corresponds to a measured quantity of water or air. In the example shown, the magnetic field is produced by magnets mounted on the impeller blade (Fig. 3.49). The impeller blade is turned by the air flow. The sensor produces two outputs per revolution. This kind of flow rate sensor needs two conversions; firstly, the flow rate is converted to the rotation speed of the blade turn. Secondly, the Hall sensor converts rotation speed to pulse signal.



**Fig. 3.49.** Respiration flow sensor: (a) Mask of respiration flow meter; (b) Structure of the Hall respiration flow sensor

## **3.7 Photoelectric Sensors**

A photoelectric sensor is a device used to detect the distance, absence, or presence of an object by using a light transmitter and a photoelectric receiver.

### **3.7.1 Photoelectric Element**

A photoelectric element can convert the light signals into electrical signals using a

**Photoelectric Effect.** It is the primary element that constitutes the photoelectric sensor. There are many advantages such as quick response, simple structure, convenient to use, ability to detect the signal without contact, and high reliability.

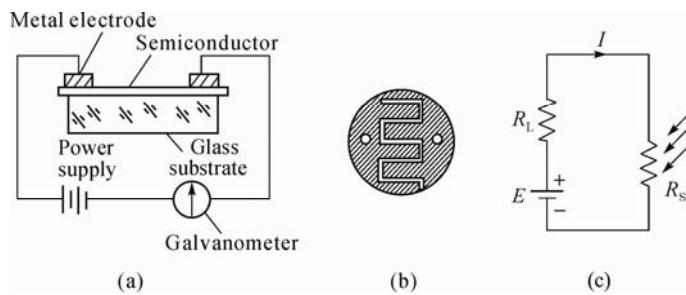
### 3.7.1.1 Photoresistor

A photoresistor or light dependent resistor or cadmium sulfide (CdS) cell is a resistor whose resistance decreases with increasing incident light intensity. It can also be referred to as a photoconductor. A photoresistor is made of a high resistance semiconductor. If light falling on the device is of enough high frequency, photons absorbed by the semiconductor give bound electrons enough energy to jump into the conduction band. The resulting free electrons (and its hole partner) conduct electricity, thereby lowering resistance.

#### *The structure and working principle of the photoresistor*

Photoresistor, known as a light tube, is an optoelectronic device made of semiconductor materials. It has no polarity, just a resistance. Without the illumination, the dark resistance is very large, and the dark current is very small. When it is exposed to the light with a certain range of wavelength, bright resistance will decrease; meanwhile the bright current will increase.

Hence, for a photoresistor, the greater the dark resistance, while the smaller the light resistance, the higher the sensitivity of the photoresistor will be. Fig. 3.50 shows the structure of photoresistor. It is applied on the glass substrate layer of semiconductor material with metal electrodes at both ends, and the metal electrode access to the circuit.



**Fig. 3.50.** The structure of the photoresistor: (a) The structure of the photoresistor; (b) The electrode of the photoresistor; (c) The wiring diagram of the photoresistor

#### *Some important parameters of photoresistor*

**Dark resistance:** the resistance value without the effect of the light. The corresponding current is called dark electricity.

**Bright resistance:** the resistance value within the effect of the light. The corresponding current is called bright electricity.

*Photocurrent:* the difference between dark resistance and the bright resistance.

#### **Basic characteristics of the photoresistor**

*Volt-ampere characteristic:* Under certain illumination, the relationship between current and resistance of the photoresistor is called volt-ampere characteristic.

*Illumination characteristic:* The illumination characteristic of the photoresistor describes the relationship between photocurrent ( $I$ ) and the illumination intensity. Most photoresistor's illumination characteristics are nonlinear.

*Spectrum characteristic:* The relationship between the relative photosensitivity of the photoresistor and the incidence wavelength is defined as the spectrum characteristics of the photoresistor or spectrum response. Different materials have different spectrum response. And even the same material will have different photosensitivity due to the change of the wavelength.

*Temperature characteristic:* Temperature change will affect the spectral response of the photoresistor, at the same time, the sensitivity of the photoresistor and dark resistance is also greatly affected by temperature.

#### **3.7.1.2 Photodiode and Transistor**

A photodiode is a type of photodetector capable of converting light into either current or voltage, depending upon the mode of operation.

In the circuit, the photodiode always works in the reverse state. Without the illumination, the reverse resistance is very large and the countercurrent (dark current) is very small; when PN junction is exposed to the light, photoelectron and photo-induced cavities will be produced near the PN junction, thus the photocurrent is formed. In other words, without the illumination, the diode is in the cut-off state, and under the light, it is in the conduct state.

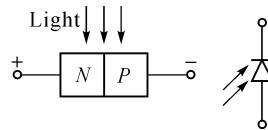


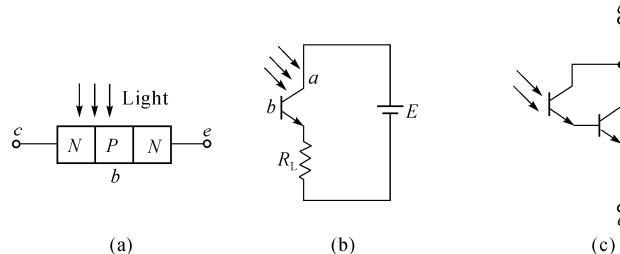
Fig. 3.51. Structure and symbol of the photodiode

#### **Structure**

Photodiodes are similar to regular semiconductor diodes except that they may be either exposed (to detect vacuum UV or X-rays) or packaged with a window or optical fiber connection to allow light to reach the sensitive part of the device.

Photosensitive transistors have two PN junctions. When the collecting electrode is connected to a higher electrical potential than the emitting electrode and leaving the base electrode unconnected, the collecting electrode will have

inverse biased potential. When light irradiates to the collecting electrode, the Electron-Hole Pair will appear. The photo-induced electron will be pulled to the collecting electrode and the hole will be in base electrode, increasing the electrical potential between base electrodes and emitting electrode. Thus more electrons will flow to collecting electrode to form output current.



**Fig. 3.52.** NPN type photosensitive transistor: (a) Structure; (b) Basic circuit; (c) Equivalent circuit of the darlington photosensitive transistor

### Basic characteristics

*Spectrum characteristic:* the peak wavelength of silicon is about  $0.9 \mu\text{m}$ , the Ge peak is about  $1.5 \mu\text{m}$ . At this moment, the sensitivity reaches the greatest point and the relative sensitivity falls when the wavelength of incident light increases or decreases. The dark current Ge tube is large, which performs poorly, therefore a silicon tube is generally used to detect objects in the visible or red hot state. However, in the detection of infrared light, the germanium tube is more appropriate.

*Volt characteristic:* Under the illumination, the countercurrent will increase if the illumination intensity increases. Under different illumination intensities, the volt-ampere characteristic curves are almost parallel which means that unless it reaches saturate, the output will never be influenced by the value of the different voltage.

*Temperature characteristic:* Temperature characteristic of a photosensitive transistor is the relationship among its dark current, light current and temperature. Temperature changes light current very little but changes dark current greatly. For this reason, measurements with electronic circuits should be carried out on the dark current temperature compensation. Temperature compensation for dark current is necessary in the electronic circuit.

### Applications

P-N photodiodes are used in similar applications to other photodetectors, such as photoconductors, charge-coupled devices, and photomultiplier tubes. Photodiodes are used in consumer electronic devices such as compact disc players, smoke detectors, and the receivers for remote controls in VCRs and televisions. In other consumer items, such as camera light meters, clock radios (the ones that dim the display when it is dark) and street lights, photoconductors are often used rather

than photodiodes, although in principle either can be used.

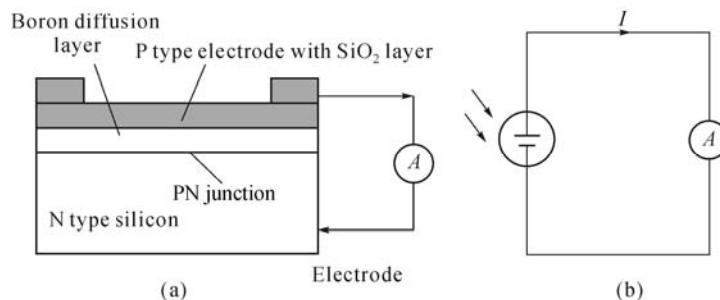
Photodiodes are often used for accurate measurement of light intensity in science and industry. They generally have a better, more linear response than photoconductors. They are also widely used in various medical applications, such as detectors for computed tomography (coupled with scintillators), instruments to analyze samples (immunoassay) and pulse oximeters.

### 3.7.1.3 Photovoltaic Sensors

The photovoltaic effect involves the creation of a voltage (or a corresponding electric current) in a material upon exposure to electro-magnetic radiation. Though the photovoltaic effect is directly related to the photoelectric effect, the two processes are different and should be distinguished. In the photoelectric effect, electrons are ejected from a material's surface upon exposure to radiation of sufficient energy. The photovoltaic effect is different, in which the generated electrons are transferred from different bands (i.e., from the valence to conduction bands) within the material, resulting in the buildup of a voltage between two electrodes.

#### *Working principle—photo-induced Volta effect*

Actually it is a large-area PN junction. When one surface of the PN junction for example the P surface, is exposed to the light, if the electron energy is larger than the prohibit bandwidth of the semiconductor material, then a pair of free electrons and a hole will be produced while the P surface absorbs a photon. Free electron-hole pairs are diffused inward from the surface quickly, and finally produce a electromotive force closely related with the illumination intensity under the effect of a junction electric field.



**Fig. 3.53.** The principle diagram of the silicon photovoltaic sensor: (a) Structure principle diagram; (b) Equivalent circuit

#### *Basic characteristics*

*Spectrum characteristic:* the sensitivity of photovoltaic changes when the wavelength of light changes. The peaks of spectral are corresponding to the

incident light wavelength. Silicon photovoltaic is near  $0.8 \mu\text{m}$  and selenium is  $0.5 \mu\text{m}$ . The spectral response of Silicon photovoltaic is from  $0.4 - 1.2 \mu\text{m}$  in the wavelength range, while the selenium is only  $0.38 - 0.75 \mu\text{m}$ . Therefore, Silicon photovoltaic cells can be applied in a wide wavelength range.

*Illumination characteristic:* In different illuminations, the photocurrent or photo-emf is different; the relationship between the illumination and the photocurrent is called the illumination characteristic. In the wide range, the short-circuit current and light intensity is a linear relationship. However, the open-circuit voltage is nonlinear while in the 2000 lx illumination, it tends to saturated. Therefore, as a measuring element, the photovoltaic must be treated as a current source and not a voltage source.

*Temperature characteristic:* Temperature characteristic of photovoltaic describes the open circuit voltage and short circuit current with temperature changing. As it relates to the temperature drift of photovoltaic, this characteristic affects the precision of measurement. The open-circuit voltage decreases fast with the temperature increasing. However, the short circuit current increases slowly. For these reasons, it is best to ensure that the temperature stays constant or to take into account the temperature compensation.

### 3.7.2 Fiber Optic Sensors

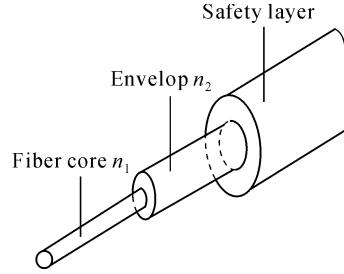
A fiber optic sensor is a sensor that uses optical fiber either as the sensing element (“intrinsic sensors”), or as a means of relaying signals from a remote sensor to the electronics that process the signals (“extrinsic sensors”).

Optical fibers can be used as sensors to measure strain, temperature, pressure and other quantities by modifying a fiber so that the quantity to be measured modulates the intensity, phase, polarization wavelength or transit time of light in the fiber. Sensors that vary the intensity of light are the simplest, since only a simple source and detector are required. A particularly useful feature of such fiber optic sensors is that they can, if required, provide distributed sensing over distances of up to one meter.

#### 3.7.2.1 Optical Fiber's Structure and Its Principle of Transmitting the Light

##### *Optical fiber structure*

As shown in Fig. 3.54, fiber core is a central cylinder, envelop is the layer outside fiber core and safety layer is the layer outside of the envelop which is used to enhance the mechanic intensity. The refractive index of the fiber core is larger than that of the envelop. The conductivity of optic fiber is based on the characteristics of the fiber core and envelop.

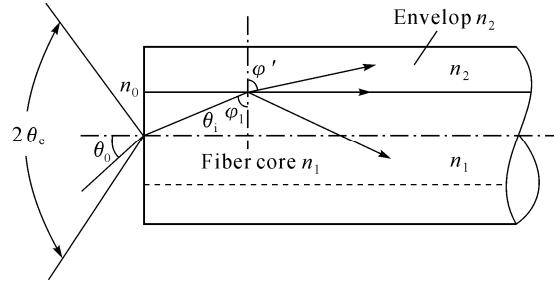
**Fig. 3.54.** Optical fiber structure***Light transmission principle of optic fiber—total reflection***

As shown in Fig. 3.55, to satisfy the total reflection in optic fiber, the angle of incidence  $\theta_i$  should satisfy

$$\theta_i \leq \theta_c = \arcsin \frac{(n_1^2 - n_2^2)^{1/2}}{n_0} \quad (3.28)$$

Generally, the optic fiber is set in atmosphere, where  $n_0 = 1$ . So the above equation can be represented by

$$\theta_i \leq \theta_c = \arcsin(n_1^2 - n_2^2)^{1/2} \quad (3.29)$$

**Fig. 3.55.** Light transmission principle of optic fiber**3.7.2.2 Basic Characteristics of Optic Fiber*****Numerical aperture (NA)***

$$NA = \arcsin(n_1^2 - n_2^2)^{1/2} \quad (3.30)$$

Numerical aperture represents the optic fiber's capability of the collecting light. It is defined as: The larger the  $NA$ , the more powerful the optic fiber will be. No matter how large the emitting power is, only in the case when the angle of incidence is smaller than  $2\theta_c$ , will the optic fiber be conductive. When the angle of incidence is too large, the light will escape from the optic fiber causing the leaking of the light.

### **Mode of optic fiber**

In order to provide light with a different angle of incidence, the amount of reflection on the interface will be different and the landscape intensity of the light interference will also be different. This will cause a different transmitting mode.

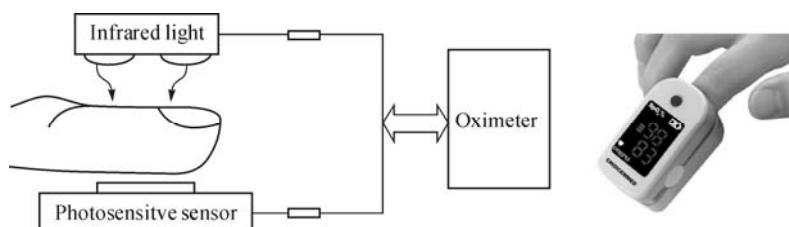
### **3.7.3 Applications of Photoelectric Sensors**

Photoelectric sensors have many applications. Here, the detection of pulse oximeter and fiber optic temperature sensor will be introduced.

#### **3.7.3.1 Detection of Pulse Oximeter**

A pulse oximeter is a medical device that indirectly measures the oxygen saturation of a patient's blood (as opposed to measuring oxygen saturation directly through a blood sample) and changes in blood volume in the skin. It is often attached to a medical monitor so staff can see a patient's oxygenation at all times. Most monitors also display the heart rate. Portable, battery-operated pulse oximeters are also available for home blood-oxygen monitoring. A blood-oxygen monitor displays the percentage of arterial hemoglobin in the oxyhemoglobin configuration. Acceptable normal ranges are from 95% to 100%, although values down to 90% are common.

A pulse oximeter is a particularly convenient non-invasive measurement instrument. Typically it has a pair of small light-emitting diodes (LEDs) facing a photodiode through a translucent part of the patient's body, usually a fingertip or an earlobe (Fig. 3.56). One LED is red, with wavelengths of 660 nm, and the other is infrared, with Wavelength of 905, 910, or 940 nm. Absorption of these wavelengths differs significantly between oxyhemoglobin and its deoxygenated form, therefore from the ratio of the absorption of the red and infrared light the oxy/deoxyhemoglobin ratio can be calculated. The absorbance of oxyhemoglobin and deoxyhemoglobin is the same (isosbestic point) for the wavelengths of 590 and 805 nm; earlier oximeters used these wavelengths for correction for hemoglobin concentration.



**Fig. 3.56.** Schematic diagram and picture of fingertip Pulse Oximeter—MD300C

### 3.7.3.2 Fiber Optic Temperature Sensor

A fiber optic sensor, because of its unique properties and widespread attention, has rapidly developed. The most commonly used one is the fiber optic temperature sensor. Fiber not only has the electric insulation, but also is very little affected by the temperature. Therefore, it is widely used in body temperature measurement.

The liquid crystal optical fiber temperature sensor is the earlier development of a fiber optic sensor. For a certain wavelength, the color or reflectivity of liquid crystal changes with temperature.

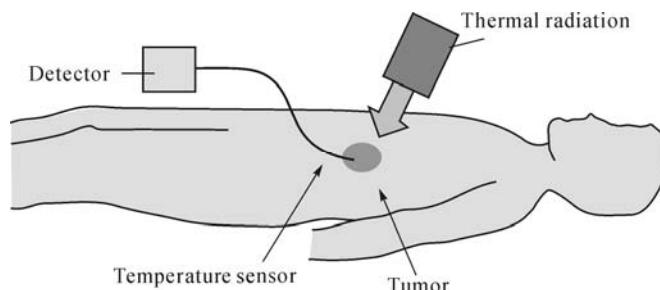
The principle of this fiber optic sensor is shown in Fig. 3.57. An incident light injects into the fiber, and after being reflected by the liquid crystal, it is transmitted by the outgoing optical fiber and then detected by the receiver. As the crystal is affected by the temperature, the reflected light intensity changes. Therefore, the reflected light intensity is a function of temperature.



**Fig. 3.57.** Optical fiber sensor structure

Fiber optic sensors are mainly characterized by high sensitivity, small size, anti-interference and no electric signal, so it can be safely used to check the life and the body, especially for heart-related measurements.

In addition, because the special feature that fiber can be curved, optical fiber temperature sensors can be used to detect the body temperature changes in certain specific locations, such as hyperthermia in tumors, the sensor travels through hypodermic needles or a catheter into the cancer patient's body, where it monitors and controls the temperature of tumorous lesion (Fig. 3.58).



**Fig. 3.58.** Optical fiber temperature sensor for monitoring the tumor tissue

## 3.8 Thermoelectric Sensors and Measurement

Temperature is one of the most important variables of the environment and the human body. Using the particular effect of thermosensitive elements or thermocouple sensors, temperature measurement (Wang and Ye, 2003) has long been a reality. The basics of temperature measurement are summarized by Fraden (Fraden, 1991). As a potent method for measuring temperature, thermoelectric sensors have been widely used not only in scientific and engineering applications, but also in the biomedical field for temporary or long-term monitoring of body temperature. To satisfy the rising demand for continuous and local measurement, integrated thermometer sensors need to be developed. Here we will introduce the basis of thermosensitive elements, thermocouple sensors and newly developed integrated temperature sensors, and how they can be used for body temperature measurement.

### 3.8.1 Thermosensitive Elements

The electrical resistivities of conductor and semiconductor materials vary with changing temperature. This phenomena is called thermoresistive effect. The high-accuracy resistance-temperature relationship of some materials can be used to sense various non-electrical quantities just as temperature, velocity, concentration and density of medium. With the development of technology, thermometers based on thermoresistive effect have been used from the triple point of equilibrium hydrogen to the freezing point of silver.

#### 3.8.1.1 Resistance Temperature Detectors

A resistance temperature detector (RTD) (Harsányi, 2000) is the commonly used term for temperature sensors, the operation of which is based on the positive temperature coefficient of metals. The resistance-temperature relationship of RTD can be derived from:

$$R(T)=R_0(1+\alpha\Delta T) \quad (3.31)$$

where  $R_0$  is the resistance at a reference temperature  $T_0$ ,  $\alpha$  is the temperature coefficient of resistance at  $T_0$ , and  $\Delta T$  is the actual temperature difference related to  $T_0$ . For the majority of metals,  $\alpha$  is the function of temperature, but can be seen as a constant within a limited range.

Resistance thermometer elements must meet four conditions as follows:

- High temperature coefficient of resistance;
- Stable chemical and physical properties;
- High electrical resistivity;
- Excellent reproducibility.

Common resistance materials for RTDs include platinum, nickel and copper. Among these materials, platinum is used widely and in fact, as an interpolation standard. The resistance of platinum via temperature characteristic can be approximated as:

$$R_t = R_0[1+At+Bt^2] \quad (0 < t < 640 \text{ } ^\circ\text{C}) \quad (3.32)$$

$$R_t = R_0[1+At+Bt^2+C(t-100)t^3] \quad (-240 < t < 0 \text{ } ^\circ\text{C}) \quad (3.33)$$

where  $A=3.96847\times 10^{-3}$ ,  $B=-5.847\times 10^{-7}$ ,  $C=-4.22\times 10^{-12}$ . Since the coefficients  $B$  and  $C$  are relatively small, the resistance changes almost linearly with the temperature.

Copper is used occasionally as an RTD element. Its low resistivity forces the element to be longer than a platinum element, but its linearity and very low cost make it an economical alternative in industrial applications. Its upper temperature limit is only about 150 °C. The resistance of copper via temperature characteristic can be approximated as

$$R_t = R_0[1+At+Bt^2+Ct^3] \quad (-50 < t < 150 \text{ } ^\circ\text{C}) \quad (3.34)$$

where  $A=4.28899\times 10^{-3}$ ,  $B=-2.133\times 10^{-7}$ ,  $C=-1.233\times 10^{-9}$ .

As shown in Fig. 3.59, these elements nearly always require insulated leads attached. At low temperatures, PVC, silicon rubber or PTFE insulators are common to 250 °C. Above this, glass fiber or ceramic are used. The measuring point and usually most of the leads require a housing or protection sleeve. This is often a metal alloy which is inert to a particular process. Often more consideration goes into selecting and designing protection sheaths than sensors as this is the layer that must withstand chemical or physical attack and also offer convenient process attachment points. In order to minimize the effects of the lead resistances, a three or four-wire configuration can be used.

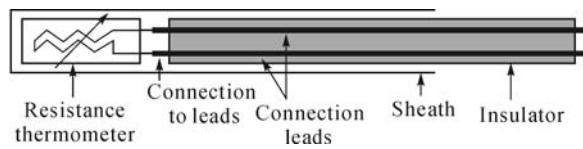


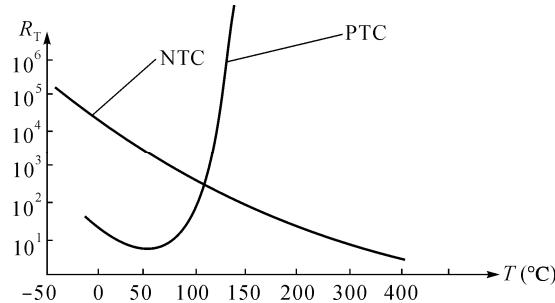
Fig. 3.59. Construction of resistance thermometer

### 3.8.1.2 Thermistors

Thermistors differ from RTDs in that the material used in a thermistor is generally a ceramic or polymer, while RTDs use pure metals. The temperature response is also different; RTDs are useful over larger temperature ranges, while thermistors typically achieve a higher precision within a limited temperature range.

Thermistors can be classified into two types, depending on the sign of

temperature coefficient of resistance. If the resistance increases with increasing temperature, the device is called a positive temperature coefficient (PTC) thermistor. If the resistance decreases with increasing temperature, the device is called a negative temperature coefficient (NTC) thermistor. Typical characteristics of two temperature-dependent thermistors are compared in Fig. 3.60.



**Fig. 3.60.** Typical characteristics of two temperature-dependent thermistors

PTC-thermistors are made of a doped polycrystalline ceramic containing barium titanate ( $\text{BaTiO}_3$ ) and other compounds. Steep increases of resistance at a certain critical temperature make them particularly useful as self-regulation heating elements, current limiting devices, etc.

NTC-thermistors are made from a pressed disc or cast chip of semiconductor oxides, such as the precisely controlled mixtures of the oxides of Mn, Co, Ni, Cu and Zn. NTC-thermistors can be used as inrush-current limiting devices in power supply circuits and are regularly used in automotive applications.

The main parameters of thermistors are:

- Nominal resistance  $R_0$  at  $25^\circ\text{C}$ ;
- Temperature coefficient of resistance  $\alpha$ , generally at  $20^\circ\text{C}$ ;
- Dissipation factor  $H$ , the measure of loss-rate of power;
- Specific heat capacity  $C$ , the measure of the heat energy required to increase the temperature of a unit quantity of a thermistor by a unit of temperature;
- Time constant  $r$ , the ratio of dissipation factor  $H$  and specific heat capacity  $C$ .

The high resistivity of the thermistor affords it a distinct measurement advantage. The four-wire resistance measurement may not be required as it is with RTDs. A measurement lead resistance of  $10\ \Omega$  produces only  $0.05\ ^\circ\text{C}$  error. This error is a factor of 500 times less than the equivalent RTD error.

### 3.8.2 Thermocouple Sensors

Thermocouple sensors are one type of temperature sensors that are easy to use and

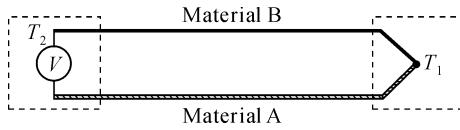
obtain. With the high sensitivity, linearity and functional long-term stability, they are widely used in industry.

### 3.8.2.1 Principle of Thermocouples

Thermocouple sensors are inexpensive and interchangeable, and can measure a wide range of temperatures. The main limitation is its accuracy: system errors of less than one Kelvin (K) are difficult to achieve.

The thermoelectric effect (also called Seebeck effect) is the theoretical basis for thermocouple sensors. The effect is that a voltage is created in the presence of a temperature difference between two different conducting (metal or semiconductor) materials, A and B. This causes a continuous current in the conductors if they form a complete loop.

As shown in Fig. 3.61, a simple thermocouple is made from a coupling or junction of two materials. In the circuit,  $T_1$  is the DUT temperature of the “hot” point and  $T_2$  is the stable reference temperature of the “cold” point.



**Fig. 3.61.** Schematic representation of the thermocouple

Charge carriers in the materials (electrons in metals, electrons and holes in semiconductors, ions in ionic conductors) will diffuse when one end of a conductor is at a different temperature than the other. Hot carriers diffuse from the hot end to the cold end, since there is a lower density of hot carriers at the cold end of the conductor. Cold carriers diffuse from the cold end to the hot end for the same reason. The voltage caused by this phenomenon can be derived from:

$$E_T = - \int_{T_0}^T (\sigma_A - \sigma_B) dt \quad (3.35)$$

where  $\delta_A$  and  $\delta_B$  denote the Thomson coefficient of the materials A and B.

Charges also diffuse in the junction. The created electrical potential difference is defined as follows:

$$E_c = k(T_1 - T_0) \ln(N_A / N_B) / e \quad (3.36)$$

where  $k$  is Boltzmann constant,  $N_A$  and  $N_B$  are the free-charge densities of the materials A and B, and  $e$  is the electronic charge. Thus the total thermoelectromotive force is

$$E_{AB}(T_1, T_0) = E_c - E_T = k(T_1 - T_0) \ln(N_A / N_B) / e - \int_{T_0}^T (\sigma_A - \sigma_B) dt \quad (3.37)$$

### 3.8.2.2 Sensitivity of Thermocouple Sensors

The thermoelectromotive force can be approximated as follows:

$$E_{AB}(T_1, T_0) = a(T_1 - T_0) + b(T_1^2 - T_0^2) \quad (3.38)$$

where  $a$  and  $b$  are constants. For small temperature differences, the sensitivity  $K$  of thermocouple sensors can be taken from derivation of Eq. (3.38):

$$K = \frac{dE_{AB}(T_1 - T_0)}{dT} = a + 2bT \quad (3.39)$$

Generally,  $K$  is between 6 and 80  $\mu\text{V/K}$ .

### 3.8.2.3 Cold Junction Compensation

Having a junction of known temperature, which is useful for laboratory calibration, is not convenient for most measurement and control applications. Instead, they incorporate an artificial cold junction using a thermally sensitive device such as a thermistor or diode to measure the temperature of the input connections at the instrument, with special care being taken to minimize any temperature gradient between terminals. Hence, the voltage from a known cold junction can be simulated, and the appropriate correction applied. This is known as cold junction compensation.

## 3.8.3 Integrated Temperature Sensors

A traditional analog temperature sensor, including thermoresistive sensors and thermocouple sensors, may have poor linearity in certain scope of temperature, which means cold junction compensation or lead wire compensation is inevitable. By contrast, integrated temperature sensors have advantages of good sensitivity, high linearity and quick response. As the name implies, an integrated temperature sensor is the integration of a driving circuit, data processing circuit and a requisite logical control circuit on a single IC. With small size and convenient usage, integrated temperature sensors have been more widely used in recent years.

### 3.8.3.1 Diode Temperature Sensor

The ordinary semiconductor diode can be used as a temperature sensor. The forward biased voltage across a diode has a temperature coefficient of about 2 mV/ $^{\circ}\text{C}$  and is reasonably linear. For an ideal diode, the diode voltage  $V_F$  can be expressed as follows:

$$V_F = [E_{g_0} - kT \ln(\alpha T \gamma / I_F)]/q \quad (3.40)$$

where  $E_{g_0}$  is the band gap of semiconductor at absolute zero,  $k$  is the Boltzmann constant,  $\alpha$  is a constant temperature-independent and related with cross-sectional area,  $\gamma$  is the constant dependent on electron mobility,  $I_F$  is the current in diode, and  $q$  is the electron charge.

With proper doping concentration, diode voltage  $V_F$  can be proportional to  $T$  within a certain temperature scope. To acquire larger linearity, two differential pair diodes with the same characteristics are applied (working currents are  $I_{F1}$  and  $I_{F2}$  respectively), and only the forward current will appear in the result:

$$\Delta V_F = kT \ln(I_{F1}/I_{F2})/q \quad (3.41)$$

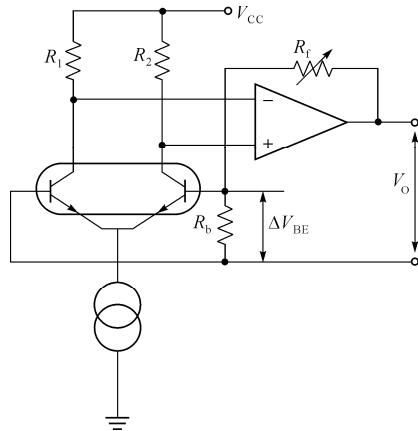
Thus, the voltage difference  $\Delta V_F$  is independent of the reverse currents, and is absolutely linear to temperature  $T$ .

### 3.8.3.2 Triode Temperature Sensor

A triode temperature sensor is based on the principle that the base-emitter voltage  $V_{BE}$  is linear to temperature  $T$  if the current in collector  $I_C$  is constant. Take the case of NPN crystal triode, the voltage  $V_{BE}$  and current  $I_C$  have the following relation:

$$V_{BE} = E_{g_0}/q - kT \ln(\alpha T \gamma / I_C)/q \quad (3.42)$$

where  $\alpha$  is a constant temperature-independent and related with junction area and base width,  $\gamma$ , the constant dependent on electron mobility,  $E_{g_0}$ , the band gap of semiconductor at absolute zero. Similar to diode temperature sensors,  $V_{BE}$  has an approximate linear relation with  $T$  if  $I_C$  remains constant. In pursuit of a higher precision of measurement, the linear compensation method, like differential pair triodes, as shown in Fig. 3.62, is necessary.



**Fig. 3.62.** Temperature measuring circuit using differential pair triodes

### 3.8.3.3 Integrated Temperature Sensor

A triode temperature sensor, together with peripheral detection circuits, will compose an integrated temperature sensor. Within a working range of  $-50 - 150$  °C, it generates current or voltage generally proportional to temperature.

Integrated temperature sensors can be divided into voltage output types and current output types. Voltage output temperature sensors such as AD22100, AD22103, LM135/235/335, and so on, provide voltage output signals with relatively low output impedance. All require an excitation power source and are essentially linear. Current output sensors act as a high-impedance, constant current regulator and require a supply voltage. AD590, AD592, TMP17 and LM134/234/334 are all very common current output sensors. Provided by ADI, AD590 is a 2-terminal integrated temperature sensor and can be used in any temperature-sensing application below 150 °C. The inherent low cost of a monolithic integrated circuit combined with the elimination of support circuitry makes the AD590 an attractive alternative for many temperature measurement situations.

As shown in Fig. 3.63, the measurement circuit for current output temperature sensors often uses proportional to absolute temperature (PTAT) and the output voltage is expressed as follows:

$$V_o = \frac{R_2}{R_1} \left( \frac{kT}{q} \right) \ln n \quad (3.43)$$

where  $n$  is area ratio of triode  $Q_1$  and  $Q_2$ ,  $k$  the Boltzmann constant,  $T$  the measurand temperature,  $q$  the electron charge.

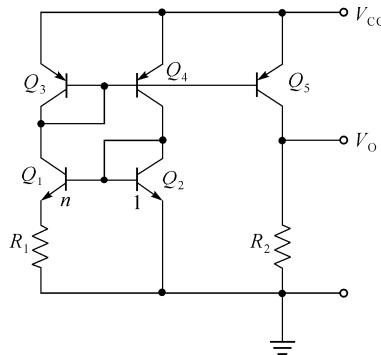


Fig. 3.63. PTAT circuit

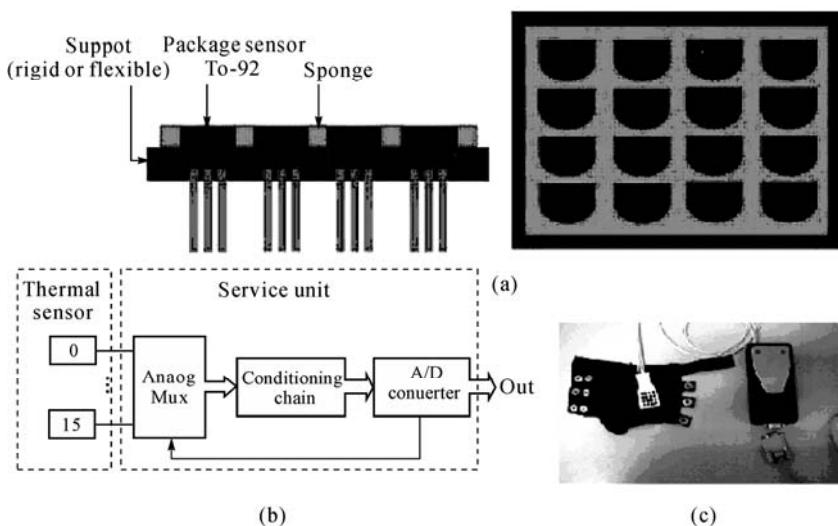
### 3.8.4 Applications in Biomedicine

#### *Thermal sensor*

Thermometers have been used in clinical testing during the past few decades.

Nowadays the demand of continuous monitoring devices is rising, especially wearable instruments (Bonato, 2003). A skin thermography testing system is no exception. In 2007, Giansanti and Maccioni (Giansanti and Maccioni, 2007) made their research on a wearable integrated thermometer sensor for skin contact thermography known to the public, which now makes continuous monitoring in breast cancer investigation possible.

The thermal sensor unit is arranged through a 4 row×4 column matrix box, where each cell corresponds with an area of 4 mm×4 mm, and is monitored by one thermometer that carries the IM335 component, as shown in Fig. 3.64a. The face of the matrix box, made of a special permeable sponge, makes contact with the skin, and carries the 16 thermal sensor packages. As shown in Fig. 3.64b, a service unit comprising the multiplexing circuit, the processing and conditioning circuit, the power supply unit with the oscillation and stabilization circuitry, and a Pentium IV PC guarantees the device's normal operation.

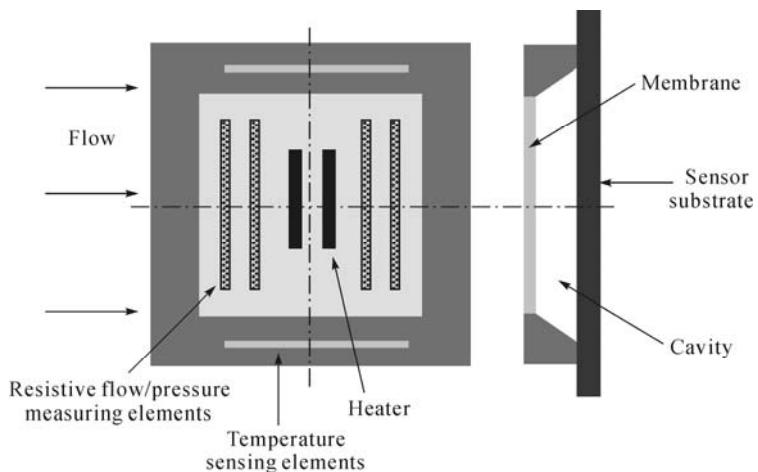


**Fig. 3.64.** A wearable integrated thermometer sensor: (a) The 3D design of the integrated thermometer; (b) Block diagram of the device; (c) Details of the integrated thermometer, the elastic bandage and the service unit (reprinted from (Giansanti and Maccioni, 2007), Copyright 2007, with permission from Elsevier)

A test was designed to measure hand skin temperature under conditions of gradual loading of physical activity, as shown in Fig. 3.64c. The result indicates a thermal resolution better than  $0.03\text{ }^{\circ}\text{C}$ , and a spatial resolution equal to  $1.6 \times 10^{-5}\text{ m}^2$ . In clinical testing, the maximum rate of thermal skin variation equals to  $3.1\text{ }^{\circ}\text{C}/0.25\text{ h}$ . The usage of this type of wearable thermometer paves the way for testing of breast cancer thermography.

### Multiple-sensor micro-system for pulmonary function diagnostics

Asthma and COPD (chronic obstructive pulmonary disease) affect 10% – 20% of the population world wide, and this number is still increasing. The development of a portable pocket sized electronic multiple-sensor micro-system for low cost, high volume equipment for improved diagnosis of pulmonary diseases and diagnostic functions in general have been implemented by van Putten et al. (2000). The microsystem can measure peak expiratory flow, relative humidity, pressure and temperature. Different operating modes allow the measurement of pressure, flow velocity and temperature with the same sensor configuration. The core part is a single chip multiple-sensor (Fig. 3.65) which is obtained by applying silicon and MEMS technology. The sensing elements adopt P-type doped resistive elements, which guarantee best reproducibility and accuracy. Two separate temperature-sensing elements have been integrated with a value of about  $60\text{ k}\Omega$  at room temperature. The temperature measurements reflect an almost linear relationship between the temperature coefficient and the doping concentration.



**Fig. 3.65.** Principle parallel etched MEMS structure for measuring pressure, flow and temperature. Not on scale. Actual sensor size is about  $4\text{ mm}^2$

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## Chapter 4

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# Chemical Sensors and Measurement

Chemical sensors have been widely used in the biomedical field. With the rapid development of microelectronics and microprocessing technology, chemical sensors have grown to be more and more miniaturized and integrated. Combined with new information processing technology, intelligent chemical sensor arrays such as e-Nose and e-Tongue have been developed. Meanwhile, microfluidic chips enable continuous monitoring of chemical substances in living organisms.

## 4.1 Introduction

This chapter introduces the principles and characteristics of some typical chemical sensors including ion sensors, gas sensors and humidity sensors. Furthermore, e-Nose, e-Tongue, microfluidic chips and wireless sensor networks are also presented.

### 4.1.1 *History*

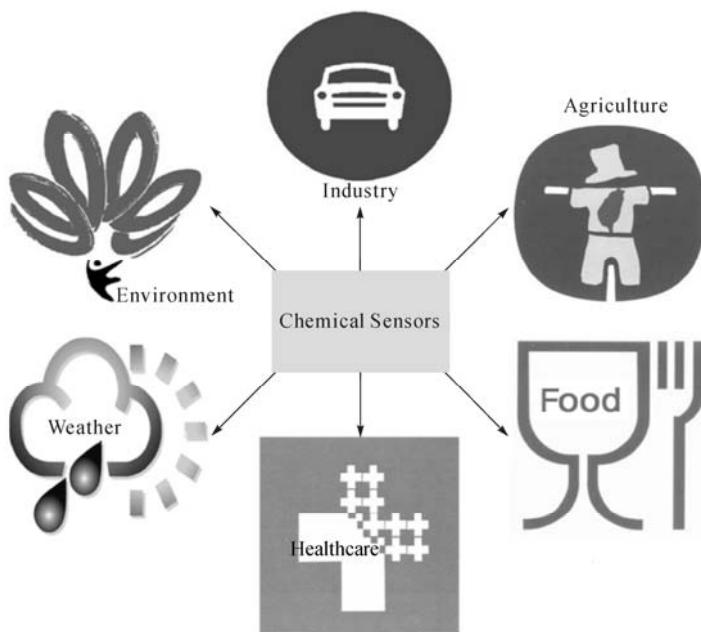
The history of chemical sensors can be traced back to 1906. Cremer, the pioneer doing research on chemical sensors, discovered the phenomenon that glass thin films respond to hydrogen ions in a solution and invented the glass electrode for measuring pH. This allowed for the development of chemical sensors. With continuous ongoing studies, glass-based thin-film pH sensors entered the practical stage in 1930. However, the research on chemical sensors progressed slowly before the 1960s during which only a study on humidity sensors employing lithium chloride was reported in 1938.

Since the 1960s, numerous phenomena, like the ion-selective response of the silver halide film and the selective response of zinc oxide to flammable gases, have been discovered. Along with the application of new materials and principles, the research on chemical sensors has entered a new era and developed very rapidly.

Chemical sensors such as pressure sensors, acoustic sensors and optical sensors were invented. Electrochemical sensors in which the ion-selective electrodes were dominant, occupied 90% of all chemical sensors during this period.

In the late 1980s, the measuring methods and fabrication technologies of chemical sensors were constantly expanded by microelectronic technology. Chemical sensors based on optical, thermal and mass signals were fully developed. They greatly contributed to the research topics and formed a large family of chemical sensors including electrochemical sensors, optical chemical sensors, mass sensors and thermochemical sensors. When electrochemical sensors lost their edge, the modern history of chemical sensors began.

Chemical sensors with advantages of high selectivity, high sensitivity, fast response, wide measuring range, etc., have caught people's attention and have served in many different fields such as environmental protection and monitoring, industrial and agricultural production, food testing, weather forecast, health care and diagnosis of diseases (Fig. 4.1). It has become one of the main development trends in contemporary analytical chemistry.



**Fig. 4.1.** Some fields that chemical sensors are applied to

The 1st International Meeting on Chemical Sensors was held in Fukuoka, Japan in 1983. Subsequently, it has been held every two years since the 3rd meeting in 1990 and there have been a total of 13 sessions to the present day. At the same time, some other international academic conferences associated with chemical sensors such as Biosensors, Eurosensors and the East Asia Conference on Chemical Sensors were held one after another. Chemical sensors also played an

important role in the Pure and Applied Chemistry International Conference. All of these show that the research and development of chemical sensors are very active and eye-catching throughout the world.

Along with the rapid development of modern science and technology and the mutual penetration between the disciplines, basic research on chemical sensors has become more and more active. The emergence of new technologies such as microprocessing, molecular imprinting, functional membrane, pattern recognition, micromachining, etc., enables chemical sensors to be functioned, arrayed and integrated with neural network and pattern recognition chips. Chemical sensor networks also show great vitality. Thus the measurement performance and remote testing capabilities of chemical sensors are significantly improved. In a word, chemical sensors will become more miniaturized, integrated, multifunctional, intelligent and network capable in the future.

### 4.1.2 Definition and Principle

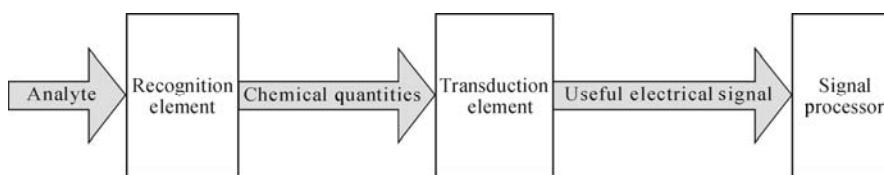
The definition of chemical sensors by Wolfbeis in 1990 is as follows:

Chemical sensors are small-sized devices comprising a recognition element, a transduction element, and a signal processor capable of continuously and reversibly reporting a chemical concentration.

The description above is pragmatic while the definition by the IUPAC (International Union of Pure and Applied Chemistry) in 1991 is general:

A chemical sensor is a device that transforms chemical information, ranging from concentration of a specific sample component to total composition analysis, into an analytically useful signal.

As a kind of analytical device, chemical sensors are so effective that they can detect the object molecules in the presence of interfering substances. This sensing principle is shown in Fig. 4.2.

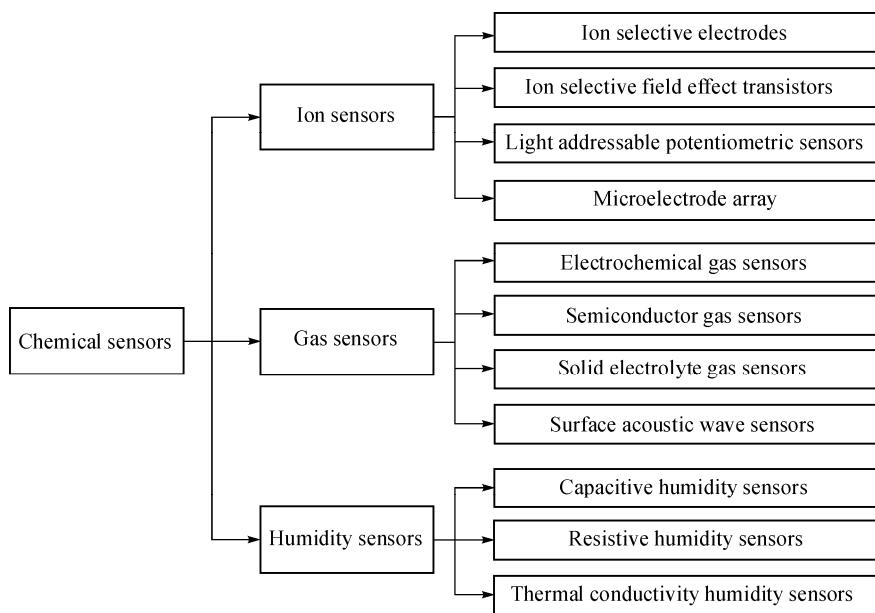


**Fig. 4.2.** Sensing principle of chemical sensors

### 4.1.3 Classification and Characteristics

There are millions of chemical substances of different compositions and properties existing in the natural world. A certain chemical can be detected by more than one

kind of chemical sensor so that the species of chemical sensors is multitudinous. Classification of chemical sensors has been accomplished in several different ways. The classification following the principles of signal transduction was made by IUPAC in 1991. In this chapter, chemical sensors are classified into ion sensors, gas sensors and humidity sensors according to the property of analytes (Fig. 4.3). In combination with computer information processing technology, intelligent chemical sensor arrays like e-Nose and e-Tongue were developed in the last several decades. And because of the demand for miniaturization, integration and portability, micro total analysis system ( $\mu$ TAS) has emerged.



**Fig. 4.3.** Classification of chemical sensors according to the property of analytes

The characteristics of chemical sensors, listed as follows, are generally accepted. Chemical sensors should:

- Transform chemical quantities into electrical signals;
- Respond rapidly;
- Maintain their activity over a long time period;
- Be small;
- Be cheap;
- Be specific, i.e., they should respond exclusively to one analyte, or at least be selective to a group of analytes.

The above list can be extended with, e.g., the postulation of a low detection limit, or a high sensitivity. This means that low concentration values should be detected (Gründler, 2006).

## 4.2 Ion Sensors

There are several sensors that can be used in the determination of ions such as ion-selective electrode sensors (ISE), ion-selective field-effect transistor sensors (ISFET), light addressable potentiometric sensors (LAPS) and microelectrode array sensors (MEA). We will describe each of these ion sensors in detail in the following sections.

### 4.2.1 Ion-Selective Electrodes

An ion-selective electrode is defined as an electro-analytical sensor with a membrane whose potential indicates the activity of the ion to be determined in the analyte. Making measurements with an ISE is therefore a form of potentiometry. An ion-selective membrane is the key component of all potentiometric ion sensors. It establishes the preference with which the sensor responds to the analyte in the presence of various interfering ions (Koryta, 1986).

#### 4.2.1.1 Principle

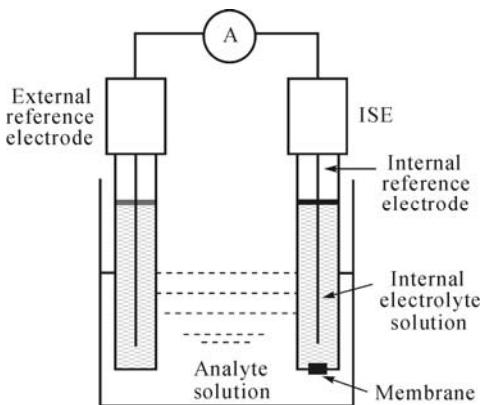
Fig. 4.4 illustrates the typical measurement schematic of an ISE. As shown in the right part of the figure, the ion-selective membrane of the ISE is between the sample solution with the ionic activity  $\alpha_x$  and the internal reference solution with the different ionic activity  $\alpha_0$  ( $\alpha_0$  is a constant). The ion-exchange and mass diffusion occurs on the membrane interface. Supposing the membrane is only permeable to the sample ion, the potential difference  $E_{\text{ISE}}$  across the membrane can be described by the Nernst equation:

$$E_{\text{ISE}} = RT / (ZF) \cdot \ln(\alpha_x / \alpha_0) = K + (2.303RT / (ZF)) \log(\alpha_x) = K + S \cdot \log(\alpha_x) \quad (4.1)$$

where  $R$  is the gas constant,  $T$  is the absolute temperature,  $Z$  is the number of electrons transferred,  $F$  is the Faraday constant,  $K$  is a constant to account for all other interfacial potentials, and  $S=59.16/Z$  (mV) at 298 K. Briefly, the measured voltage is proportional to the logarithm of the ionic activity of the sample solution. Generally the membrane potential cannot be measured directly, so it demands an external reference electrode (the left part of Fig. 4.4) to form an electrolytic cell with the ISE. When the potential of the external reference electrode is positive and the potential of the ISE is negative, the cell potential difference  $E_{\text{cell}}$  is

$$E_{\text{cell}} = E_{\text{ref}} - E_{\text{ISE}} = C - \frac{RT}{ZF} \ln(\alpha_x) \quad (4.2)$$

where  $E_{\text{ref}}$  is the potential of the external reference electrode,  $C$  is a constant.



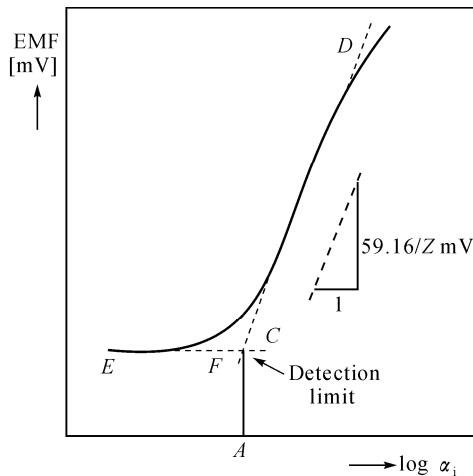
**Fig. 4.4.** The schematic illustration of an ISE measurement

#### 4.2.1.2 Characterization

The properties of an ISE are characterized by parameters like:

##### **Detection limit**

According to the IUPAC recommendation, the detection limit is defined by the cross-section of the two extrapolated linear parts on the ion-selective calibration curve. As shown in Fig. 4.5, when the activity gets smaller, the linear part of the calibration curve *CD* gradually bends into another linear part *EF*. The detection limit is the ionic activity *A* corresponding to the potential where *CD* and *EF* intersect.



**Fig. 4.5.** The schematic illustration of the calibration curve and the detection limit

### Selectivity coefficient

Selectivity is one of the most important characteristics of an electrode, as it often determines whether a reliable measurement in the sample is possible or not. However, a membrane that is truly selective for a single type of ion and completely non-selective for other ions does not exist. The influence of the presence of interfering species in a sample solution on the measured potential difference is taken into consideration in the Nikolski-Eisenman formalism:

$$E = C + \frac{RT}{ZF} \ln \left[ \alpha_A + \sum K_{FAX}^{pot} (\alpha_X) \frac{Z_A}{Z_X} \right] \quad (X = B, C, \dots) \quad (4.3)$$

where  $\alpha_A$  is the activity of the target ion,  $Z_A$  is its charge, and  $\alpha_B, \alpha_C, \dots$  are the activities of the interfering ions,  $Z_B, Z_C, \dots$  are their charges and  $K_{FAX}^{pot}$  is the selectivity coefficient. Preference for the target ion relative to the interfering ions is available when the value of  $K_{FAX}^{pot}$  is small.

### Impedance

The resistance of an ISE is determined by the electrode materials, for example the resistance of the glass membrane electrode is several hundred megohm while it is only a few kilo-ohms for the crystal membrane electrode.

In practice, we usually use the resistance of an ISE to describe the impedance of the electrolytic cell that consists of an ISE-sample solution-reference solution. We can calculate the resistance of the ISE by measuring the potential difference  $E_x$  of the electrolytic cell first, and then the potential  $V$  of a resistance  $R_e$  paralleled with the cell is obtained, so the resistance of the cell is:

$$R_x = \frac{E_x - V}{V} R_e \quad (4.4)$$

### Response time

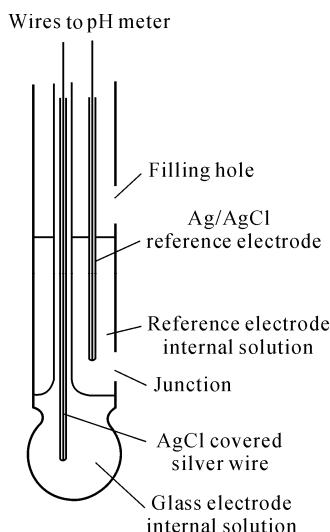
In earlier IUPAC recommendations, it was defined as the time between the instant at which the ISE and a reference electrode are dipped in the sample solution (or the time at which the ion concentration in a solution is changed on contact with ISE and a reference electrode) and the first instant at which the potential of the cell becomes equal to its steady-state value within 1 mV or has reached 90% of the final value (in certain cases also 63% or 95%). Usually the response time is less than 1 s, or even only a few milliseconds.

#### 4.2.1.3 Applications

Among various classes of chemical sensors, ISEs are one of the most frequently

used potentiometric sensors during laboratory analysis as well as in industry, process control, physiological measurements, and environmental monitoring. The most commonly used ISE is the pH glass electrode, which contains a thin glass membrane that responds to the  $\text{H}^+$  concentration in a solution. Other ions that can be measured include fluoride, bromide, cadmium and gases in solutions such as ammonia, carbon dioxide and nitrogen oxide.

As shown in Fig. 4.6, a typical commercial electrode is made of a glass tube ended with small glass bubble. Inside the electrode is usually filled with a buffered solution of chlorides (for pH probe is usually 0.1 mol/L HCl) in which silver wire covered with silver chloride is immersed. The active part of the electrode is the glass bubble with a typical wall thickness of 0.05 – 0.2 mm. When the glass membrane is exposed to the solution, a thick hydrated layer is formed (5 – 100 nm), which exhibits improved mobility of the ions.

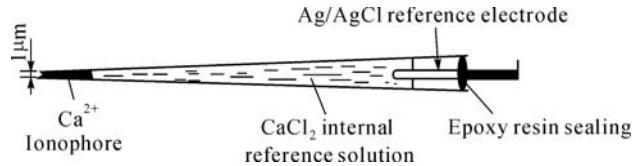


**Fig. 4.6.** Scheme of typical combination glass electrode, which is made of a glass tube ended with small glass bubble

Besides, the glass electrodes can also be applied to the detection of sodium, potassium and ammonium ions. This depends mainly on the component of the glass materials. The normal glass membrane is composed of  $\text{Na}_2\text{O}/\text{Al}_2\text{O}_3/\text{SiO}_2$ , and the selectivity for different ions is available while the proportion of these three components changes.

Nowadays intracellular environmental monitoring has been given increasing attention. It can be classified to monitoring of ions ( $\text{Ca}^{2+}$ ,  $\text{H}^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$ , etc.), small molecules ( $\text{O}_2$ ,  $\text{CO}_2$ ,  $\text{NH}_3$ , etc.) and a variety of macromolecules.  $\text{Ca}^{2+}$  is a regulator of physiological functions. It plays an important role in the nerve conduction, muscle contraction and second messenger regulation. So it is crucial to monitor the calcium ion.

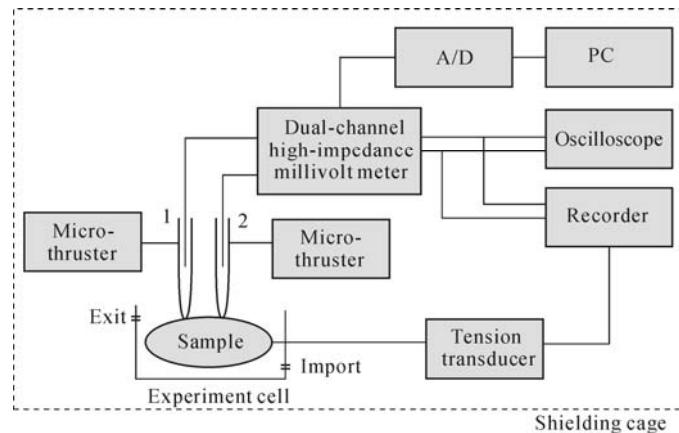
Ion selective microelectrodes can be applied to monitor the intracellular calcium ion, for example the transient releasing of extracellular  $\text{Ca}^{2+}$  stimulated by light in cardiac myocytes can be measured by microelectrodes. As the ion-selective microelectrode shown in Fig. 4.7, the diameter of the tip is less than 1  $\mu\text{m}$ , and a liquid calcium ionophore (ETHI29) is utilized as the electrode-sensitive material.



**Fig. 4.7.** The structure chart of calcium ion-selective microelectrode

The microelectrode must be calibrated before and after use. The calibration device is shown in Fig. 4.8, and it is carried out in a solution and the pCa of the standard solution is 2 – 7.

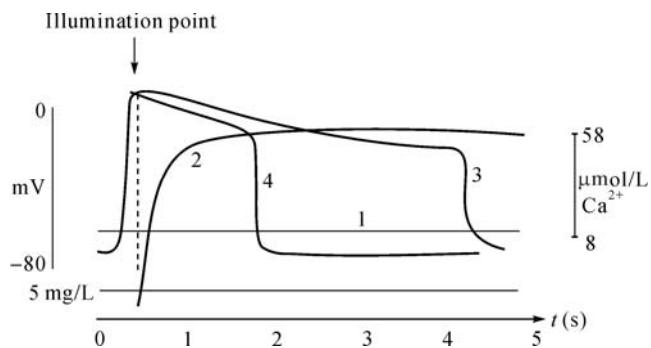
The myocardium whose diameter was 0.3 – 0.4 mm and whose length was about 0.5 mm used in the experiment was obtained from living frogs and stored in a none-calcium solution. First, the myocardium was moved into the physiological cell as shown in Fig. 4.8. And then  $\text{K}^+$  and  $\text{Ca}^{2+}$  microelectrodes were inserted into the myocardium using a micro-thruster. The signals of the two microelectrodes obtained by a high-impedance millivolt meter were shown through using an oscilloscope. An electrical pulse was used to stimulate and record the action potential signals and tension changes. At last, ultraviolet light pulse (wavelength 350 nm, pulse width 100  $\mu\text{s}$ , energy about 100 mJ) was added to the back of the experiment cell.



**Fig. 4.8.** Experimental setup of microelectrode: (1)  $\text{K}^+$  microelectrode; (2)  $\text{Ca}^{2+}$  microelectrode

In order to investigate the effect of  $\text{Ca}^{2+}$  on the myocardial action potential, DM-nitro-phenol calcium was added into the solution. The compound releases

$\text{Ca}^{2+}$  under the light pulse. Fig. 4.9 shows the results of this experiment, which briefly demonstrates the effect of extracellular  $\text{Ca}^{2+}$  on the cardiac myocytes calcium channel.



**Fig. 4.9.** The effect of  $\text{Ca}^{2+}$  on the myocardial action potential: (1)  $\text{Ca}^{2+}$  concentration before illumination; (2) The increase of  $\text{Ca}^{2+}$  concentration after illumination; (3) The action potential before illumination; (4) The action potential after illumination

#### 4.2.2 Ion-Selective Field-Effect Transistors

In 1970, Bergveld replaced the metal plate in an IGFET (insulated-gate field-effect transistor) with a glass electrode membrane and obtained the first ISFET (ion-selective field-effect transistor) (Dzyadevych et al., 2006). In this device, the drain current of the field-effect transistor, which is the measured quantity, depends on the field in the insulator ( $\text{SiO}_2$  or  $\text{Si}_3\text{N}_4$ ) separating the ion-selective membrane from the p-type silicon wafer of the transistor. The field is a function of the membrane potential. During the next 40 years, ISFETs for the determination of  $\text{H}^+$ , halide ions,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{CN}^-$  and other ions, have been reported.

##### 4.2.2.1 Characteristics

ISFETs are used to measure the ionic activity in the electrolyte solution with both electrochemical and transistor characteristics. Compared to the traditional ISE, they have the following advantages:

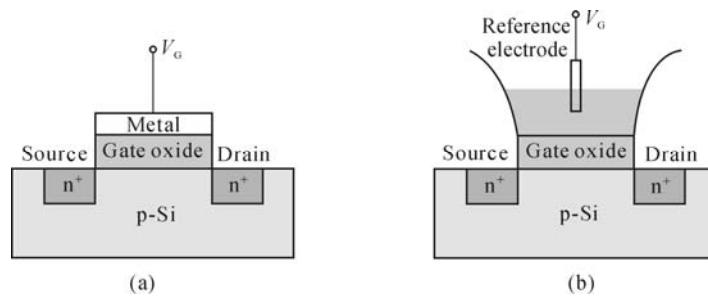
- High sensitivity, fast response time, high input impedance and low output impedance, with both impedance conversion and signal amplification functions which can be used to avoid interference from external sensors and secondary circuit.
- Small size, especially applicable for biodynamic monitoring.
- They are suitable for mass production and easy to be miniaturized and integrated by the integrated circuit technology and micro-processing

technology.

- All solid-state structure makes the high mechanical strength available.
- Easy to realize on-line control and real-time monitoring.
- The sensitive materials can be conductive or insulated.

#### 4.2.2.2 Principles

ISFET is in fact nothing more than a metal-oxide-semiconductor field-effect transistor (MOSFET) with the gate connection separated from the chip in the form of a reference electrode inserted in aqueous solution which is in contact with the gate oxide (Fig. 4.10) (Bergveld, 2003). The areas having electronic conductivity ( $n^+$ -areas, namely,  $n^+$ -source,  $n^+$ -drain) are created in the silicon substrate by hole conductivity (p-type Si). The controlling electrode is a gate separated from the substrate by the subgate dielectric (Dzyadevych et al., 2006).



**Fig. 4.10.** Schematic view of (a) MOSFET and (b) ISFET

For MOSFET, the threshold voltage can be calculated as:

$$V_T = \Phi_{MS} - \frac{Q_{ss} + Q_B}{C_{ox}} + 2\psi_F \quad (4.5)$$

where  $C_{ox}$  is the oxide capacitance per unit area,  $\Phi_{MS}$  is work function of the gate metal and silicon,  $Q_{ss}$  is the charge density at the oxide-silicon interface,  $Q_B$  is the depletion charge in the silicon, and  $\psi_F$  is the Fermi potential of the substrate material.

In the case of ISFET, the same fabrication process is used, resulting in the same constant physical part (the second part of Eq. (4.5)) of the threshold voltage.  $\Phi_{MS}$  is replaced by the work function  $\Phi_{cs}$  between the ion-selective membrane and the silicon. The potential difference between the sample solution and the membrane is:

$$E^0 = \frac{RT}{ZF} \ln \left( \alpha_i + K_{ij} \alpha_j \frac{z_i}{z_j} \right) \quad (4.6)$$

where  $E^0$  is the surface dipole potential of the solution,  $\frac{RT}{ZF} \ln \left( \alpha_i + K_{ij} \alpha_j \frac{z_i}{z_j} \right)$

is the function of pH values. The threshold voltage then becomes:

$$V'_T = E_{ref} + E^0 - \frac{RT}{ZF} \ln \left( \alpha_i + K_{ij} \alpha_j \frac{z_i}{z_j} \right) + \varPhi_{CS} - \frac{Q_{SS} + Q_B}{C_{OX}} + 2\varPsi_F \quad (4.7)$$

where  $E_{ref}$  is the potential of the reference electrode.

The gate voltage of the ISFET is:

$$V'_G = V_G + \frac{RT}{ZF} \ln \left( \alpha_i + K_{ij} \alpha_j \frac{z_i}{z_j} \right) \quad (4.8)$$

Accordingly, the drain current of ISFET in non-saturated zone is:

$$I_D = C_{OX} \mu \frac{W}{L} \left[ (V'_{GS} - V'_T) V_{DS} - \frac{1}{2} V_{DS}^2 \right] \quad (4.9)$$

where  $V_G$  is the gate voltage,  $V_{DS}$  is the drain-source voltage,  $W$  and  $L$  are the channel width and length, correspondingly,  $\mu$  is electron mobility in the channel.

Therefore, the interface charge will alter while the pH value of the solution changes, leading to the variety of membrane potential. In theory, changes of pH value and redox potential can be measured by ISFETs.

#### 4.2.2.3 Ion Sensitive Membranes

The key component of an ISFET is the sensitive membrane which is primarily fabricated by insulating material. The first ISFET gate material utilized was silicon dioxide, obtained in the conventional MOSFET technology by heating silicon up to 1,100 °C in a dry oxygen atmosphere. Accompanied with this type of structure, there are many disadvantages such as poor insulativity, low sensitivity and bad linearity. Therefore, we often use a double-gate structure (double-layer or multi-layer), such as a redeposited layer of Al<sub>2</sub>O<sub>3</sub> on the insulating layer to achieve a good response to pH values.

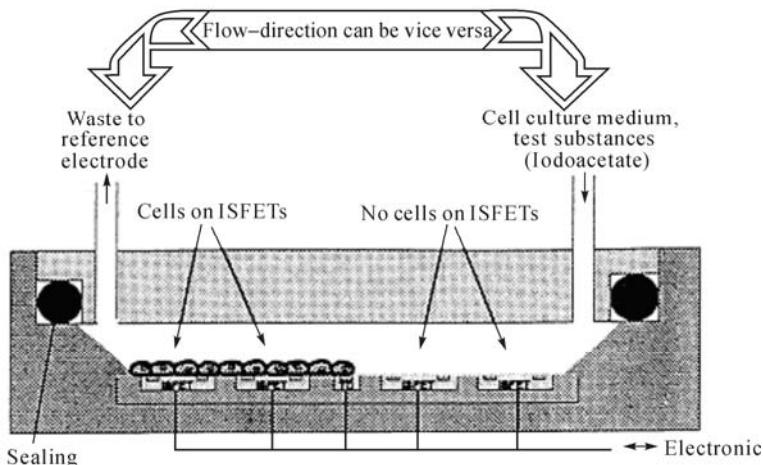
*Solid film:* This is a film with high ion selectivity. Sodium ion-sensitive film is formed by aluminosilicate or sodium silicate materials while potassium and calcium ions-sensitive film is fabricated by organic polymer membrane materials.

*Liquid film:* The polyvinyl chloride (PVC) film is commonly used by putting the ion activity solution and plasticizer together with the PVC to form a layer of liquid film.

#### 4.2.2.4 Applications

Initially ISFETs were serving as new probes for electrophysiological experiments, but this challenge has not been taken up by the field. Recently, publications paid more attention on the monitoring of cell metabolism in which electrophysiological signals are not measured but are physiological. It mainly focused on the extracellular acidification rate of a cell culture. The pH in the cellular microenvironment ( $\text{pH}_M$ ) is an important regulator of cell-to-cell and cell-to-host interactions. This is, for example, of particular importance in the field of tumor biology and in intercellular signaling. The  $\text{pH}_M$  is reduced significantly in the interstitium of solid tumors in comparison to the values of normal interstitial fluid. Additionally, the extracellular acidification rate of a cell culture is an important indicator of global cellular metabolism (Lehmann et al., 2000).

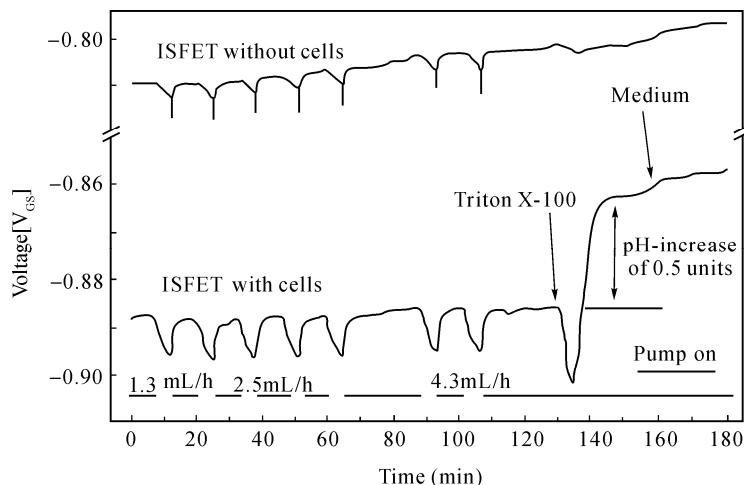
Lehmann et al. (2000) developed a method measuring the  $\text{pH}_M$  on line and in real time in the immediate vicinity (10 – 100 nm) of the cell plasma membranes. As shown in Fig. 4.11, in a flow through chamber, adherent tumor cells (LS174T) were cultured on specially developed pH-ISFET arrays to elucidate how the pH of cell-covered ISFETs differs from the pH of ISFETs in cell-free regions. The pH-sensitivity of the  $\text{Al}_2\text{O}_3$ -ISFETs is  $56.119 \pm 2.12 \text{ mV/pH}$ . The output signal of the ISFETs as the measure for the pH value is given by the source voltage  $V_{GS}$  relative to the reference potential. The perfusion rate of the cell culture medium was increasing between 1.3 and 4.3 mL/h in a stop and flow mode, and the effect of Triton X-100 on the pH of the cells was studied then.



**Fig. 4.11.** Measurement setup showing the four ISFETs, two loaded and two without cells (reprinted from (Lehmann et al., 2000), Copyright 2000, with permission from Elsevier Science B.V.)

As the results shown in Fig. 4.12, the pH of cell-covered ISFETs is less than that of the ISFETs in cell-free regions, and immediately after Triton X-100

containing medium reached the cell culture, the sensor with the cells showed a characteristic acidification peak. The sensor without cells did not show that peak. The acidification peak of the cell-ISFET was followed by an increase of 29.294 mV relative to the constant pumping signal which is equivalent to a pH-increase of 0.529 pH-units.



**Fig. 4.12.** The whole measurement showing the difference between cell-covered ISFETs and ISFETs in cell-free regions, and the effect of Triton X-100 addition (reprinted from (Lehmann et al., 2000), Copyright 2000, with permission from Elsevier Science B.V.)

### 4.2.3 Light Addressable Potentiometric Sensors

As a result of the development of semiconductors, the light addressable potentiometric sensor (LAPS) has gradually become the hot item in the late 1980s, and it is the most popular ion sensitive sensor at present.

#### 4.2.3.1 Principle

Since LAPS-based devices belong to the family of field-effect-based sensors, they have the same function with the chemical field effect transistors but with a more simple structure (Barcelo, 2006). LAPS is a class of surface potential sensitive sensors, the operational principles of LAPS are based on the effect of the solid/electrolyte interfacial potential difference, which affects the electrical field effect in the semiconductor. All responses that change the surface potential can be measured by LAPS, such as hydrogen ions on the silicon nitride surfaces and the acetone gas on a platinum surface.

LAPS consists of an electrolyte-insulator-semiconductor (EIS) structure. The

semiconductor will always be a p-type or n-type doped silicon wafer (e.g.,  $1 - 10 \Omega \text{ cm}$ ,  $350 - 400 \mu\text{m}$ ,  $<100>$ ), and the insulating layer may be a  $30 - 50 \text{ nm}$  thick oxide layer, e.g.,  $\text{SiO}_2$ , produced by dry oxidation, or  $\text{Si}_3\text{N}_4$ . The insulator depends on the later application, since it provides the sensitivity towards a specific substance. For pH sensing,  $\text{Si}_3\text{N}_4$  and  $\text{Ta}_2\text{O}_5$  are known as stable and sensitive transducer materials for LAPS. Besides, there is an ohmic contact (e.g.,  $300 \text{ nm}$ ) in the rear-side.

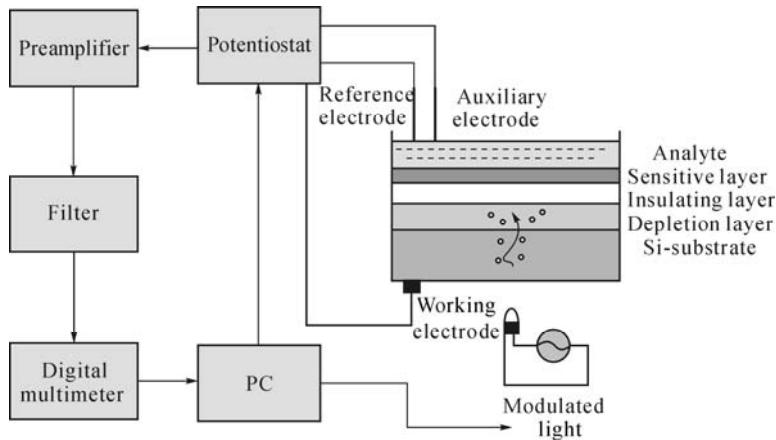
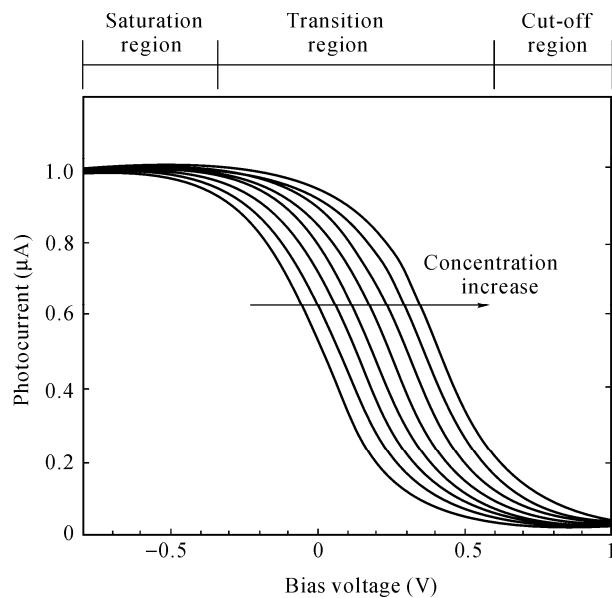
The characteristics of LAPS are investigated by means of current-voltage ( $I-V$ ) measurements. When a DC bias potential is applied to the silicon plate, the phase and the magnitude of the potential are adjusted so that the major charge carriers near the insulator/semiconductor interface are depleted by the electrical field effect. The width, and therefore, the capacitance of the depletion layer will vary with the potential at the solid/electrolyte interface, which is a function of the local value of the surface potential. The local value of the depletion capacitance can be read out with AC photocurrent that is generated when an intensity-modulated light source is shown at the bulk silicon surface (Ismail et al., 2001). The resultant AC photocurrent is then amplified with a preamplifier and converted into a DC voltage signal, which is acquired by the computer via an A/D converter. By measuring the photocurrent, which is dependent on the capacitance of the depletion layer, the variation in the phase boundary potential can be determined. Since the value of the potential difference at the phase boundary of solid/electrolyte depends on the concentration of the corresponding ions in the solution, the voltage shift in the current-voltage characteristics of the LAPS can be applied to measure the ion concentration in the solution (Mourzina et al., 2003).

#### 4.2.3.2 Measurement Circuit and Characteristics

The general system for the LAPS measurement is shown in Fig. 4.13, which uses the three-electrode method that consists of a working electrode, reference electrode and auxiliary electrode. This approach is less affected by a power supply noise. A reference electrode is used to provide a fixed bias voltage, and a current pass is formed between the auxiliary electrodes and the ohmic contacts on the semiconductor substrate. An alternating photocurrent is amplified and extracted through the lock-in amplifier and tracking band-pass filter. The photocurrent is generally converted into a voltage signal that can be measured.

The characteristic curve of the n-type silicon substrate LAPS is illustrated in Fig. 4.14. It can be divided into a cut-off region, transition region (linear region) and a saturation region, which depends on the characteristics of the silicon wafer.

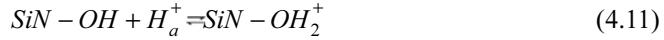
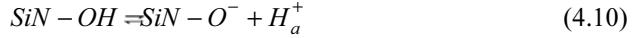
The shifting along the bias voltage axis in the characteristic curves corresponds to the response values of the sensitive layer, which is the basic principle for the measurement of LAPS. It should be noted that, the characteristic curves for the p-type silicon substrate LAPS are reverse to the n-type silicon substrate LAPS. The bias voltages are the highest for the saturated region and are lowest for the cut-off region.

**Fig. 4.13.** The measuring circuit of LAPS**Fig. 4.14.** The characteristic curves of LAPS

#### 4.2.3.3 pH-sensitivity

When LAPS is applied to the pH measurements, dissociation groups of  $\text{SiN-OH}$  are on the surface of the sensitive membrane  $\text{Si}_3\text{N}_4$ , which reacts with the  $\text{H}^+$  ions to maintain the electrochemical dissociation equilibrium (Zhang et al., 1999). Therefore, a net charge existed in the sensitive membrane surface apart from a point zero charge (PZC). The net charge will attract free ions with opposite charge

in the solution to form electric double layer. The ionization equilibrium is as follows:



where  $H_a^+$  means the hydrogen ions located in the interface between the sensitive membrane and the solution, the relationship between the concentration of  $H_a^+$  and the bulk  $H^+$  obeys the Boltzmann rule:

$$[H_a^+] = [H^+] e^{\frac{qE}{kT}} \quad (4.12)$$

where  $E$  is the interfacial potential between the sensitive membrane and the solution,  $q$  is the quantity of electric charge,  $k$  is the Boltzmann constant, and  $T$  is the absolute temperature.

Finally we can obtain the relationship between the pH value of the solution and the interfacial potential:

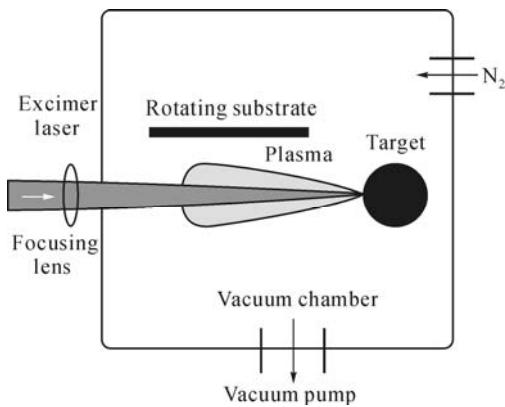
$$E = 2.303 \frac{kT}{q} (\text{pH}_{\text{pzc}} - \text{pH}) \quad (4.13)$$

where  $\text{pH}_{\text{pzc}}$  is the pH value of the zero charge point,  $\text{pH}$  is the pH value of the solution.

#### 4.2.3.4 Applications

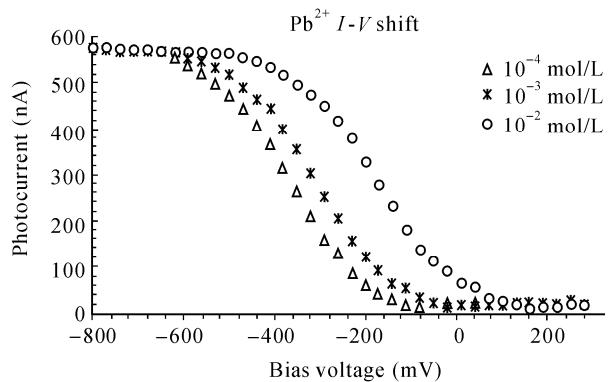
Since the introduction of LAPS in 1988, Hafeman et al. proposed a measurement device for biological applications, the first LAPS was mainly developed for biological investigations, e.g., a phospholipidbilayer membrane-based LAPS, a sandwich immunoassay for human chorionic gonadotropin (HCG) and an enzyme-based (urease) microchamber-LAPS device. Recently, concerns about the contamination of water by heavy metals such as  $Pb^{2+}$ ,  $Cu^{2+}$ ,  $Cd^{2+}$  and  $Hg^{2+}$  has been proposed because of the toxicity of such metals on a broad spectrum of organisms, including humans.

LAPS can be applied to various ions detection when deposited by different transducer materials on the sensor surface. Mourzina et al. (2001) described a novel chalcogenide glass ion-sensitive membrane LAPS device for the detection of  $Pb^{2+}$ . In this study, the Pb-Ag-As-I-S chalcogenide glass is deposited on the LAPS structure by a pulsed laser deposition (PLD) technique as a Pb-ion-selective transducer material for the first time. Fig. 4.15 shows the scheme of pulsed laser deposition technique.



**Fig. 4.15.** Pulsed laser deposition technique

The main potential-determining process, which takes place at the interface between the chalcogenide glass membrane and the solution, is the exchange of primary ions between the solution and the exchange sites at the modified surface layer of the glass. Fig. 4.16 shows the dependence of the AC photocurrent  $I$ , measured in the external circuit on the applied bias potential  $V$ , for different concentrations of  $\text{Pb}^{2+}$ -ions in the solution. The current-voltage curve moves reproducibly along the voltage axis depending on the  $\text{Pb}^{2+}$ -ion concentrations.



**Fig. 4.16.** Typical current-voltage characteristics of the Pb-LAPS

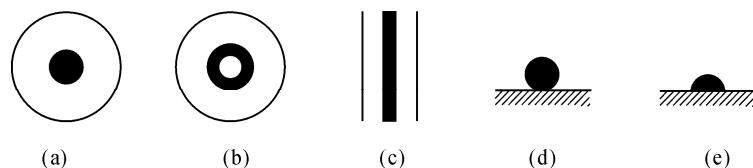
#### 4.2.4 Microelectrode Array

In the past few decades, the electrochemical sensors are developing towards miniaturization. Traditional electrochemical electrodes are large, so they cannot be mass produced, and the consistency is poor. Microelectrodes can not only meet the

needs of small occasional testing with little samples, but also possess lots of attractive features when compared with the traditional electrodes, such as enhanced mass transport, negligible ohmic drop, reduced charging current, small RC constant and enhanced signal-to-noise ratio. Thanks to advantageous properties of microelectrodes, new research fields of electrochemistry, biotechnology, medicine and environmental sciences are developing.

#### 4.2.4.1 Microelectrode

The definition of microelectrodes is ambiguous and it is very difficult to give a definition in terms of precise limits of its characteristic dimensions. Nevertheless, electrodes with one-dimension less than the diffusion layer are often called microelectrodes (Xie, 2005). This critical size can be the radius of a disk electrode or the thickness of band electrode, usually in the  $\mu\text{m}$ -level. 10 nm is the minimum size of micro-electrode, the electrodes below which are nano-electrodes. Similar to traditional electrodes, they are with different electrode types, such as disc, cylinder, band, ring, sphere, hemisphere, etc. (Fig. 4.17).



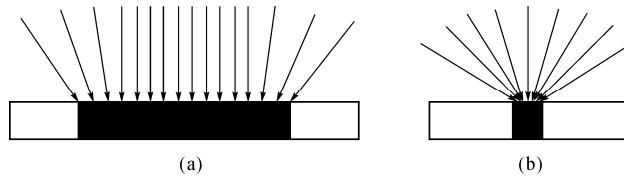
**Fig. 4.17.** The types of microelectrodes: (a) Disc; (b) Ring; (c) Band; (d) Sphere; (e) Hemisphere

The electrolytic process of microelectrode and the conventional electrode is the same in nature. When redox reaction occurs in the electrode system, concentration gradients are formed on the electrode surface, leading to the diffusion effects of the electro-active substance transfer from the bulk solution towards to the electrode surface. To disc electrode, for example, the diffusion equation is

$$\frac{1}{D} \frac{\partial c}{\partial t} = \frac{\partial^2 c}{\partial r^2} + \frac{1}{r} \frac{\partial c}{\partial r} + \frac{\partial^2 c}{\partial z^2} \quad (4.14)$$

where  $D$  is the diffusion coefficient,  $c$  is the bulk concentration of the solution,  $r$  is the electrode radius and  $z$  is the direction perpendicular to the surface of the electrode.

As shown on the right side of Eq. (4.14), the first two items show the radial diffusion, known as nonlinear diffusion, and the third item stands for the diffusion perpendicular to the direction of the electrode surface, called linear diffusion. For traditional electrodes, linear diffusion plays a leading role, but for the microelectrodes, nonlinear diffusion is the main component as shown in Fig. 4.18.



**Fig. 4.18.** The diffusion cross-sections of (a) the traditional electrode and (b) the microelectrode

For the steady-state, the mass transfer rate  $M$  for microelectrode is

$$M = \frac{4D}{\pi r} \quad (4.15)$$

It can be seen that the mass transfer rate become bigger when the radius of the microelectrode gets smaller.

In electrolytic cell, if the electrode potential step occurs, the relationship between the charging current  $i_c$  caused by the electric double layer and time  $t$  is as follows:

$$i_c \propto \frac{\Delta E}{R} \exp\left(-\frac{t}{RC_s}\right) \quad (4.16)$$

Where  $\Delta E$  is the amplitude of the step potential,  $R$  is internal impedance of the electrolytic cell,  $C_s$  is the capacitance of the double electric layer and  $t$  is the sustainable time of the potential.

The charging current  $i_c$  is exponential decay with the index  $t$ , and it is also an exponential relationship between  $i_c$  and the electrode surface area, for  $C_s$  is in direct proportion to the electrode surface area. The smaller the electrode radius, the faster the charging current  $i_c$  decreases. Therefore, a microelectrode is able to achieve steady state in a short time and can respond faster, so it can be used in the transient electrochemical methods including voltammetry.

The current on the electrode consists of the Faraday component and the charge current. The Faraday current density of a micro-electrode is large and the charge current decays quickly, leading to an increasing signal to noise ratio, improved sensitivity and lower detection limit. So microelectrodes are applicable to the determination of the trace substances.

Because of its small radius, the current density of microelectrode is significant, but the current intensity is very small for the small electrode surface area, only  $10^{-12} - 10^{-9}$  A, so the ohmic drop  $iR$  caused by the electrolytic cell system is negligible. It can be applied to the detection of high-impedance solution without supporting electrolyte.

#### 4.2.4.2 Microelectrode Array

The current of a single microelectrode is very small, which is at the pA – nA level.

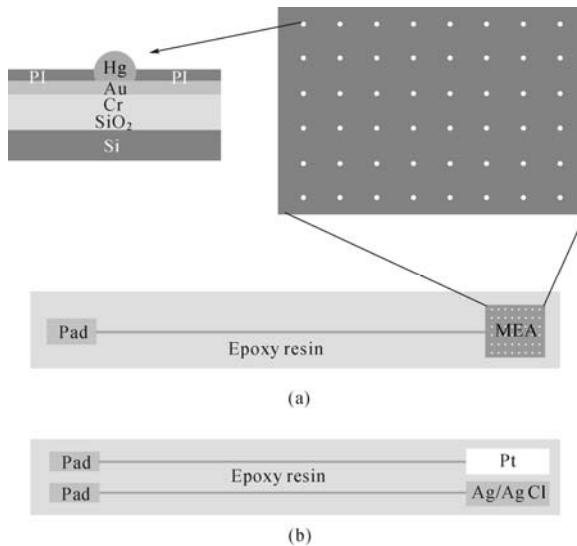
Microelectrode array consisting of a large number of microelectrodes can enhance the current signal without losing the characteristics of microelectrodes. The distance between the microelectrodes must be large enough to ensure that diffusion layers of microelectrodes do not overlap to get increased mass transfer capability. But the current density decreases when the spacing between electrodes increases. Empirically, microelectrode is ideal when the electrode spacing is 10 times the diameter of the electrode. The limited diffusion current for the disk microelectrode array is

$$I = 4mnFDrc \quad (4.17)$$

where  $m$  is the number of the electrode,  $n$  is the number of electrons transferred,  $r$  is the radius of the single electrode and  $c$  is the concentration of the electro-active substance.

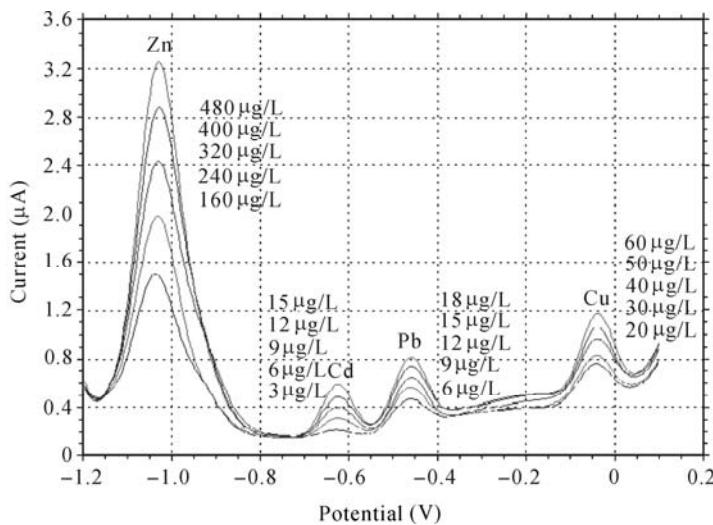
#### 4.2.4.3 Heavy Metals-sensitive MEA

Ping Wang et al., at Zhejiang University designed an Au-MEA for trace heavy metals detection. As shown in Fig. 4.19a, the Au-MEA consisted of  $30 \times 30$  Au microdisks of  $10 \mu\text{m}$  diameter separated by  $150 \mu\text{m}$  from each other. In Fig. 4.19b, a Pt foil as the counter electrode (CE) and an Ag/AgCl foil as the reference electrode (RE) were attached on the other side of printed circuit board and also encapsulated using epoxy resin. After mercury deposition was carried out on the Au, the MEA was ready to detect heavy metals such as Zn(II), Cd(II), Pb(II) and Cu(II).



**Fig. 4.19.** Structure of the MEA sensor: (a) On one side is silicon-based Hg-coated Au microelectrodes array; (b) On the other side is Pt and Ag/AgCl electrodes

Then the analytical performance of mercury-coated gold MEA was studied using differential pulse anodic stripping voltammetry (DPASV) for determination of Zn(II), Cd(II), Pb(II) and Cu(II) in the acetate buffer with pH 4.5 (Fig. 4.20). The detect sample consisted of Zn(II), Cd(II), Pb(II) and Cu(II) whose concentrations were 80 µg/L, 3 µg/L, 3 µg/L and 10 µg/L, respectively. After four additions, voltammograms for Zn(II), Cd(II), Pb(II) and Cu(II) were obtained and shown good linearity with their linear ranges separately in 10 – 600 µg/L, 1 – 100 µg/L, 1 – 200 µg/L and 2 – 300 µg/L.



**Fig. 4.20.** The DPASV for standard additions of Zn(II), Cd(II), Pb(II) and Cu(II)

### 4.3 Gas Sensors

Gas sensors are an important category in the family of chemical sensors. There are a variety of classification criteria. According to the gas sensitive materials and the mechanism of the interaction between gases and the sensitive materials, gas sensors can be divided into semiconductor gas sensors, solid electrolyte gas sensors, electrochemical gas sensors, optical gas sensors, surface acoustic wave gas sensors, infrared gas sensors and so on. This section focuses on the widely used electrochemical gas sensors, semiconductor gas sensors, solid electrolyte gas sensors and surface acoustic wave gas sensors.

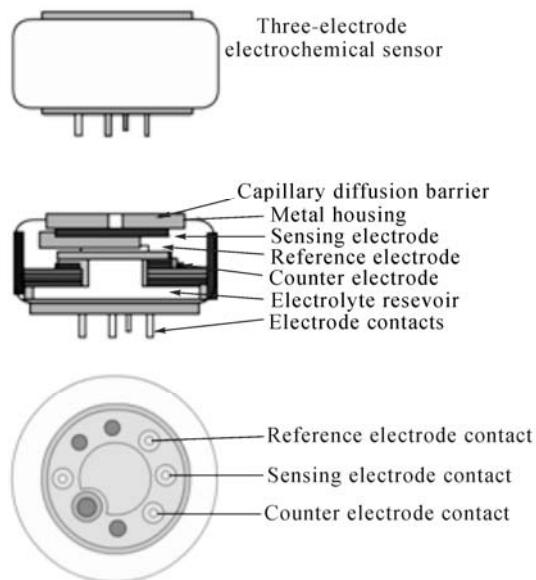
#### 4.3.1 Electrochemical Gas Sensors

Electrochemical gas sensors are used to detect and monitor low levels of toxic

gases and oxygen levels in both domestic and industrial situations where it is essential to ensure that the air is safe to breathe.

#### 4.3.1.1 Structure and Principle

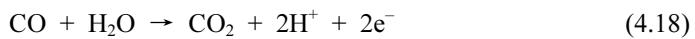
The most common type of electrochemical sensor is the 3-electrode fuel cell as shown in Fig. 4.21.



**Fig. 4.21.** Typical electrochemical sensor layout

Electrochemical gas sensors contain two or three electrodes, occasionally four, in contact with an electrolyte. The electrodes are typically fabricated by fixing a high surface area precious metal on to the porous hydrophobic membrane. The working electrode contacts both the electrolyte and the ambient air to be monitored usually via a porous membrane. The electrolyte most commonly used is a mineral acid, but organic electrolytes are also used for some sensors. The electrodes and housing are usually in a plastic housing which contains a gas entry hole for the gas and electrical contacts.

The air being measured diffuses into the cell through the diffusion barrier (capillary) and filters. When it comes into contact with the sensing electrode, the toxic gas present in the sample undergoes an electrochemical reaction. In the case of carbon monoxide, for example, the reaction is:



The carbon dioxide generated diffuses away into the air, while the positively charged hydrogen ions ( $H^+$ ) migrate into the electrolyte. The electrons generated charge the electrode but are removed as a small electric current by the external measuring circuit.

This oxidation reaction is balanced by a corresponding reduction reaction at the counter electrode:



So at one electrode, water is consumed while electrons are generated, and at the other, water is recreated and electrons are consumed. Neither reaction can occur if no carbon monoxide is present. By connecting the two electrodes, the small electric current generated between them is measured as directly proportional to the concentration of carbon monoxide in the air.

The reference electrode controls the whole process. It remains totally immersed in electrolyte, sees no gas and is not allowed to pass any current. The reference electrode always remains at the same electrochemical potential (known as “rest-air potential”, dependent on the material the electrode is made from, and the electrolyte used). The sensing electrode is electrically tied to the reference electrode ensuring its potential will not change even when it is exposed to its determinand and generating current. Usually the potential of the sensing electrode is maintained at exactly the same value as the reference electrode, but for some gases and some applications, performance benefits are gained by maintaining the potential of the sensing electrode at a fixed level above or below the potential of the reference. This is known as “biased” operation.

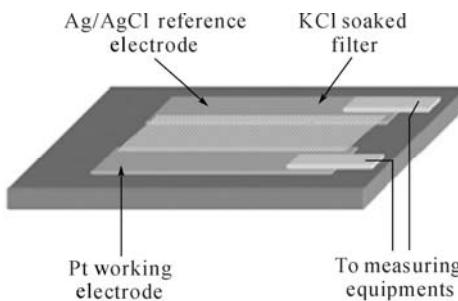
#### 4.3.1.2 Applications

Reliable and accurate blood pressure and oxygenation measurements within the cardiovascular system are important clinical applications. A method of electrochemical combined with PDMS was adopted by Goutam Koley et al. for oxygen content measurements within the heart and blood vessels (Koley et al., 2009).

The blood oxygen sensing was performed based on the change in current flowing between a Pt and an Ag/AgCl electrode kept in contact with KCl solution soaked filter paper. The current flowing between the electrodes, which were maintained at a potential difference equal to the reduction potential of dissolved oxygen, can respond to any change in the dissolved oxygen content in the KCl solution with high sensitivity. For estimating the oxygen content of a given test liquid, the sensor (and KCl soaked filter paper) can be separated from the liquid using a PDMS thin film as the intervening membrane. Due to high oxygen permeability of the PDMS membrane, the dissolved oxygen in the KCl solution will track the dissolved oxygen content in the test liquid quite accurately.

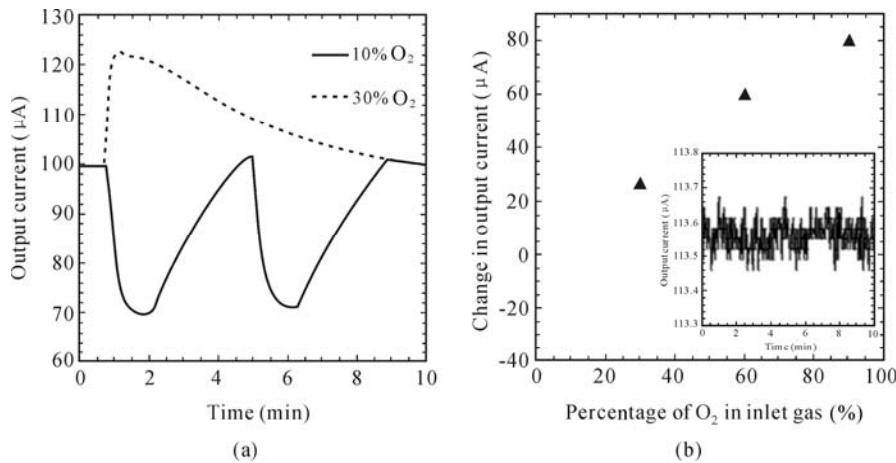
The fabricated sensor consisted of three layers that are a gas-permeable membrane (PDMS, film thickness: 30  $\mu\text{m}$ ), a membrane filter with the dimension

of 20 mm×12 mm (Isopore VMTP4700, Millipore Corp., USA) containing electrolytic solution (KCl 0.1 mol/L). The schematic diagram of the sensor is shown in Fig. 4.22, Pt working electrode and Ag/AgCl reference electrode are fabricated on the top layer with electron beam deposition (electrode thickness: 100 nm). The electrode is a simple stripe design that the width of the Pt electrode is 10 mm and that of the Ag/AgCl electrode is 5 mm. The sensor is fabricated by stacking the electrodes, gas-permeable membrane and solution-permeable filter together. The chemical reactions are the following:



**Fig. 4.22.** Schematic diagram of the oxygen sensing set up (reprinted from (Koley, 2009), Copyright 2009, with permission from Elsevier Science B.V.)

The sensor was subject to pure Ar, 10% and 30% O<sub>2</sub> and the responses are shown in Fig. 4.23, the output current was significantly reduced by 30 μA, when 10% O<sub>2</sub> gas (10% O<sub>2</sub> and 90% Ar) was flown into the air-filled chamber. The response time to reach a steady current level was approximately 40 s. The current began to recover right after the gas flow was stopped, as air started to flow in the chamber. The recovery time to return to the original level of current was almost four times higher than the decay response time, which, however, can be reduced by flowing fresh air into the chamber at a high flow rate. As shown in Fig. 4.23a, the sensor output shows repeatable current response in the presence of 10% O<sub>2</sub>, and the current was reduced by about 30 μA for each cycle. In contrast to the 10% O<sub>2</sub>, exposure to 30% O<sub>2</sub> made the output current changes in the reverse direction, and increase sharply by about 25 μA. This is because the oxygen current content in the sensor ambient was increased by 20% compared to the baseline value. The response time for the current to reach a steady value in this case was also about 40 s, similar to decay response time observed for 10% O<sub>2</sub>. However, the recovery time observed was even longer than the first case.



**Fig. 4.23.** Output current of the sensor to oxygen: (a) Time dependent electrode current as the ambient air is replaced by oxygen-argon mixture; (b) Variation of maximum electrode current with oxygen concentration. Inset shows the rms noise plotted as a function of time (reprinted from (Koley, 2009), Copyright 2009, with permission from Elsevier Science B.V.)

To determine the sensor performance over a large range of oxygen concentration, it was further exposed to 60% and 90%  $\text{O}_2$ . We observed that the change in output current is +60  $\mu\text{A}$  for 60%  $\text{O}_2$  gas, and +75  $\mu\text{A}$  for 90%  $\text{O}_2$ . Fig. 4.23b shows the change in output current as the sensor is exposed to different oxygen composition from the baseline air environment. We observe that the output current changes much faster with change in oxygen composition for lower oxygen concentration, but gradually tends to saturate for higher oxygen concentration. This is possible because the oxygen generated current starts to get affected by the diffusion-limitation of dissolved oxygen at the Pt electrode.

### 4.3.2 Semiconductor Gas Sensors

It is complicated to describe the sensitive mechanism of semiconductor gas sensors, while the fact of conductivity variation is distinct when the surface of the device absorbs the special gas molecular.

#### 4.3.2.1 Structure and Principle

Generally, the following models are used to explain the mechanism qualitatively.

##### *Surface space-charge layer model*

The surface space-charge layer will change, when the semiconductor adsorbs the

gas molecular, then it causes the conductivity to be changed. For the N-type semiconductors, the space-charge layers are widened and the barriers are heightened which reduces the conductivity, while they contact with the oxidative gas, vice versa.

#### ***Grain interface barrier model***

The grain interface barrier model takes into consideration that a barrier exists between grain interfaces. For N-type semiconductors, their conductivity reduces as a result of the interface barrier being heightened, when they contact with oxidative gas, vice versa.

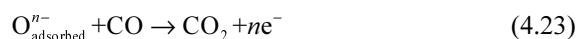
#### ***Adsorption effect model***

The adsorption effect model is based on the sinter grain model. In this model, electrons distribute uniformly in the center of the grain, whereas the jugular part and surface of the grain have lower electron density which makes the resistivity larger than the other part. When the semiconductor devices contact with the gas molecular, the internal resistance of grain is basically changeless. Then the resistance of semiconductor gas sensors is changed along with the type and concentration of gas. And the conductivity of the device is changed mainly by the alterations of the space-charge layer in the jugular part and surface.

The principle of semiconductor gas sensors has been mentioned above. Then the sensors will be classified into three types according to their sensitive mechanisms and structures.

#### ***Surface resistance controlling type***

It has been stated that the surface resistance increases for N-type semi-conductor gas sensors when the oxygen molecule is adsorbed on the surface of the device. Since an oxygen molecule captures electrons from the sensor's surface, it transforms into  $O_2^-$ ,  $O^-$ , and  $O^{2-}$ . Then the following reaction formulas take place when reductive gas, like  $H_2$  or  $CO$ , comes into contact with sensors.

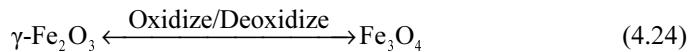


From the above equations, it can be seen that the electrons return to the semiconductor which reduces the surface resistance. This type of sensor uses the resistance on the surface to represent sensitivity. Presently, sensors of this type are fabricated into a porous sintered body, thin film or thick film.

For the purpose of adsorption and desorption, most of these devices are heated up to the temperature of 150 °C. Therefore, metal-oxide semiconductors with larger energy gaps and better thermal stabilities are used in preparation for these type of sensors. In order to improve the sensitivity of sensors, it is necessary to blend Pd and Pt with the original semiconductor.

### **Body resistance controlling type**

As the name implies, this type of sensors perform their sensitivity by variations of their body resistance. Due especially to the elimination of the stoichiometric ratio, the easily reduced metal-oxide semiconductor can change their resistance in gas with lower temperature. This characteristic is essential for gas detection. For example, the gas-sensing material  $\gamma\text{-Fe}_2\text{O}_3$  produces  $\text{Fe}^{2+}$  with the gas concentration increasing. Their oxidation-reduction reaction is expressed as follows:



This transformation is reversible. It returns to the original state when gas molecules depart. This is the work principle of  $\gamma\text{-Fe}_2\text{O}_3$  as a gas sensing device.

### **External resistance type**

The working principle is also to use the variation of the surface space-charge layer of the semiconductor, or metal-semiconductor barrier. The different point for this type of sensor is that it does not measure the resistance any more, but other parameters, for example, volt-ampere characteristics of a diode or FET. This type of device, such as a metal-semiconductor diode, metal-oxide-semiconductor (MOS) diode and MOSFET, can use the planar process to improve the stability, repeatability and integration level of the sensor device.

#### **4.3.2.2 Applications**

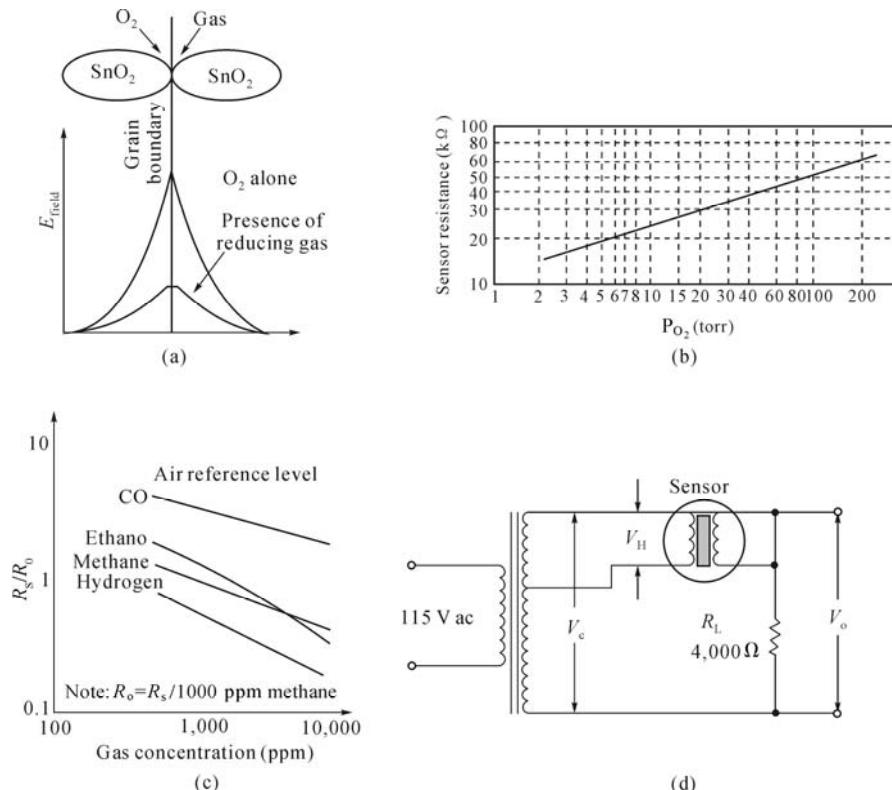
This part takes the  $\text{SnO}_2$  semiconductor gas sensor as an example to introduce a typical sensor for gas detection and its application information. This type of sensor device is developing fast, from sintered to thick and thin film, and has become the most widely used sensor in certain applications.  $\text{SnO}_2$  is a kind of white powder, and its parameters are relative density 6.16 – 7.02 g/m<sup>3</sup>, melting point 1,127 °C and boiling point over 1,900 °C. It does dissolve in a heated strong acid or alkali solution, but not the same as in water.

There are three main factors contributing to the gas sensitive effect. The first one is its structure, generally, the more oxygen vacancy, the more evidence for sensitive effect. The next is that additives can also affect the sensitive process. Table 4.1 shows that, to some degree, different additives can make some new specialties. The third one is about temperature during the sintering and heating process.

**Table 4.1** The additives to  $\text{SnO}_2$  sensors

Additives	Detection gas	Working temperature (°C)
PdO, Pd	CO, C <sub>3</sub> H <sub>8</sub> , Alcohol	200 – 300
PdCl <sub>2</sub> , SbCl <sub>3</sub>	CH <sub>4</sub> , CO, C <sub>3</sub> H <sub>8</sub>	200 – 300
Sb <sub>2</sub> O <sub>3</sub> , TiO <sub>2</sub> , TiO <sub>2</sub>	LPG, CO, Alcohol	200 – 300
V <sub>2</sub> O <sub>5</sub> , Cu	Alcohol, Acetone	250 – 400
Sb <sub>2</sub> O <sub>3</sub> , P <sub>2</sub> O <sub>5</sub>	Reducing gas	500 – 800

A number of sensor-based instruments on the market can measure the concentrations of reducing gases or vapors in the air. Examples include breath-alcohols analyzers used by police departments, carbon monoxide (CO) analyzers used in performing emission control measurements on vehicles, and methane detectors used to protect against explosions and other dangers from natural gas. All these applications have three things in common: They are at relatively low cost, they are operated by ordinary people rather than scientists and engineers, and they are manufactured using similar technology.



**Fig. 4.24.** Principle and application of the Figaro gas sensors: (a) Figaro gas/vapor sensor uses sintered; (b) Sensor resistance vs. partial pressure of oxygen; (c) Ratios for various gases and vapors; (d) Typical circuit diagram

The Figaro TGS gas sensors are based on a technology that uses powdered tin dioxide ( $\text{SnO}_2$ ) sintered onto a semiconductor substrate in Fig. 4.24a. In normal operation the sensor element is heated to approximately  $400^{\circ}\text{C}$ . Oxygen is adsorbed onto the surface of the  $\text{SnO}_2$ , where the oxygen molecules accept electrons. These electrons create a relatively high electrical potential barrier that is difficult for free electrons to cross. As a result, the electrical resistance is high and is a

function of the partial pressure of oxygen ( $P_{O_2}$ ) which is shown in Fig. 4.24b. When a reducing gas or vapor (e.g., CO, methane, methanol) is present, it is adsorbed onto the surface and reacts with the oxygen, thereby reducing the resistance of the device.

Fig. 4.24c shows the ratio of the actual sensor resistance  $R_S$  of TGS2442 to a standard resistance  $R_0$  for several different elements. The standard resistance  $R_0$  is the value of  $R_S$  in an atmosphere of 1,000 parts per million (ppm) methane gases.

A typical circuit for the TGS sensors is shown in Fig. 4.24d. The heater voltage  $V_H$  heats the sensor element to the required temperature, while the operating voltage  $V_C$  provides excitation to the sensor element. A load resistance  $R_L$  is used to convert current flowing in the sensor to an output voltage  $V_O$ . The values of  $V_C$  and  $V_H$  vary from one sensor to another, but are typically in the range of 0.5 to 12 V. Some Figaro gas sensors for the detection of toxic gas, including TGS2442, are shown in Fig. 4.25.



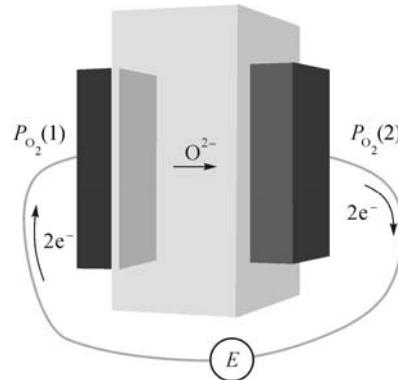
**Fig. 4.25.** Figaro gas sensors for the detection of toxic gas

### 4.3.3 Solid Electrolyte Gas Sensors

A solid electrolyte is one of the types of solid state materials with the same ionic conduction characteristic as the electrolyte solution, and the solid electrolyte gas sensor is one kind of chemical cell taking the ionic conductor as the electrolyte. It does not need to make the gas pass through the breather membrane and dissolve in the electrolyte, this can avoid such problems as solution evaporation and electrode waste. Because of the high conductivity, sensitivity and selectivity, these types of sensors are widely used in the fields of petrochemical, environmental protection, mining industry, and food industry and so on.

### 4.3.3.1 Structure and Principle

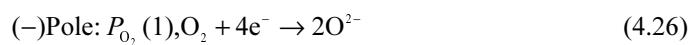
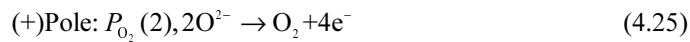
The solid electrolyte will have the obvious electrical conductivity only under a high temperature. Zirconia ( $\text{ZrO}_2$ ) is a typical material for solid electrolyte gas sensors. The pure zirconia is the clinohedral structure under normal temperature. When the temperature rises to about 1,000 °C, the allomorphism transformation will happen. Then the clinohedral structure turns into the polytropism structure, and follows the volume contraction and endothermic reaction, therefore it is an unstable structure.



**Fig. 4.26.** The structural principle of concentration cell

Mixing  $\text{ZrO}_2$  with the stabilizer such as alkali soils calcium oxide  $\text{CaO}$  or rare earth yttrium oxide  $\text{Y}_2\text{O}_3$ , the  $\text{ZrO}_2$  will become the stable fluorine cubic crystal. The stable degree is related to the density of stabilizer. The  $\text{ZrO}_2$  is sintered under 1,800 °C after being mixed with stabilizer, a part of zirconium ion will be substituted by the calcium ion, producing  $(\text{ZrO}\cdot\text{CaO})$ . Because  $\text{Ca}^{2+}$  is divalent ion,  $\text{Zr}^{4+}$  is quadrivalence ion, to maintain the electric neutrality, the oxygen ion  $\text{O}^{2-}$  hole will be generated in the crystal. This is why  $(\text{ZrO}\cdot\text{CaO})$  transfers oxygen ions at high temperature, and  $(\text{ZrO}\cdot\text{CaO})$  becomes oxygen ion conductor at 300 – 800 °C. But in order to pass oxygen ions actually, there must also be different partial pressure of oxygen (oxygen potentiometer) on the two sides of the solid electrolyte to form the so-called concentration cell. The structural principle is shown in Fig. 4.26, the precious metal electrodes are on both sides, forming sandwich structure with the intermediate dense  $(\text{ZrO}\cdot\text{CaO})$ .

Set the partial pressure of oxygen on both sides of the electrodes are  $P_{\text{O}_2}(1)$  and  $P_{\text{O}_2}(2)$  respectively, in the two electrode reactions occur as follows:



The electromotive force (EMF) of the reaction expressed by the Nernst equation:

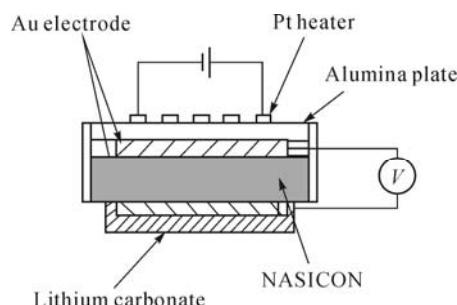
$$E = \frac{RT}{nF} \ln \frac{P_{O_2}(1)}{P_{O_2}(2)} \quad \text{or} \quad E = 0.0496T \ln \frac{P_{O_2}(1)}{P_{O_2}(2)} \quad (4.27)$$

As the above equation, fixing  $P_{O_2}(1)$  at a certain temperature, the oxygen concentration of the sensor's positive pole can be equated by the above formula.

In addition to measuring oxygen, the application of  $\beta\text{-Al}_2\text{O}_3$ , carbonate, NASICON solid electrolyte such as sensors, can also be used to measure CO, SO<sub>2</sub>, NH<sub>4</sub>, CO<sub>2</sub> and other gases. New gas sensors have emerged in recent years, using antimony acids, La<sub>3</sub>F, etc., can be used in low temperature and can be used to detect positive ions.

#### 4.3.3.2 Applications

Recently, accurate measurement of CO<sub>2</sub> concentration in offices and houses has become widespread, as CO<sub>2</sub> is a good indicator of air quality. A practical CO<sub>2</sub> gas sensor for air quality control is developed using a combination of a Na<sub>3</sub>Zr<sub>2</sub>Si<sub>2</sub>PO<sub>12</sub> (NASICON) as a solid electrolyte and Li<sub>2</sub>CO<sub>3</sub> as a carbonate phase by Kaneyasu et al. (2000).

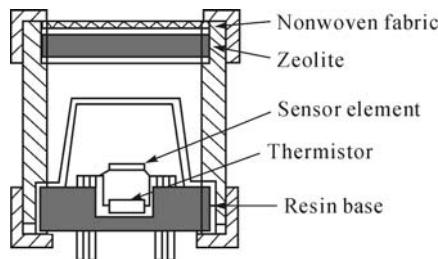


**Fig. 4.27.** Construction of the sensor element (reprinted from (Nakagaichi, 2000), Copyright 2000, with permission from Elsevier Science B.V.)

The construction of the CO<sub>2</sub> sensor element is shown in Fig. 4.27. The solid electrolyte sinter of NASICON—Na conductor, about 4 mm in diameter and about 0.7 mm in thickness—was used. A pair of gold electrodes was attached to both surfaces of the solid electrolyte by screen printing. A working electrode was pasted with lithium carbonate on one side of the electrode by screen printing and baked at 600 °C. A built-in Pt heater screen printed on an alumina plate was laminated on a reference electrode and sealed with glass. The sensor element was heated at 450 °C and EMF was measured by a high-impedance voltage meter.

The construction of the CO<sub>2</sub> sensor is shown in Fig. 4.28. The sensor element was mounted on a resin base and the gas entrance was covered with a filter

consisting of zeolite powder (Na/Y type, about 1 g) sandwiched between two non-woven fabrics. The size of the sensor was 24 mm in diameter and 31 mm in height.

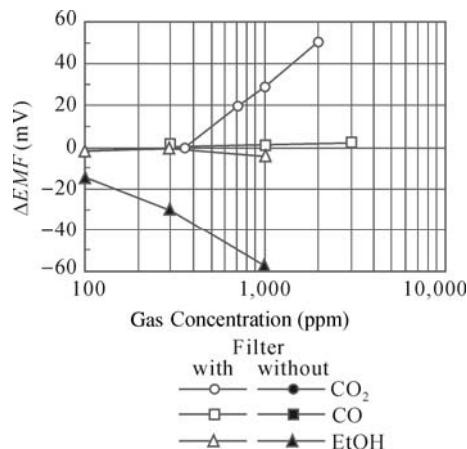


**Fig. 4.28.** Construction of the CO<sub>2</sub> sensor (reprinted from (Nakagaichi, 2000), Copyright 2000, with permission from Elsevier Science B.V.)

The sensitivity of various gases is shown in Fig. 4.29. In this figure, change in EMF ( $\Delta EMF$ ) is calculated according to the expression as follows:

$$\Delta EMF = EMF (\text{CO}_2=350 \text{ ppm}) - EMF (\text{measuring atmosphere}) \quad (4.28)$$

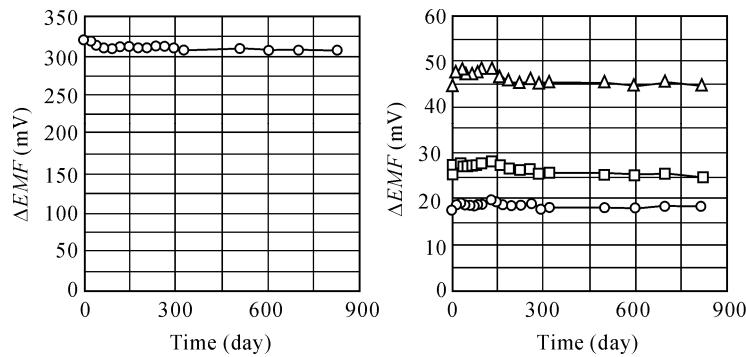
$\Delta EMF$  of the sensor showed a linear relationship with the logarithm of CO<sub>2</sub> concentration and was slightly affected by interfering gases, such as carbon monoxide and ethyl alcohol, because of the zeolite filter. The EMF of the sensor increased as the surrounding temperature rose, necessitating a correction in the temperature dependence using a thermistor.



**Fig. 4.29.** Sensitivity of various gases (reprinted from (Nakagaichi, 2000), Copyright 2000, with permission from Elsevier Science B.V.)

The heating condition stability of the EMF and  $\Delta EMF$  in indoor atmospheres is shown in Fig. 4.30. Both the EMF and  $\Delta EMF$  indicated excellent stability over

2 years. On the other hand, when the sensor was exposed to a high humidity atmosphere, the  $EMF$  decreased but  $\Delta EMF$  stayed fairly stable. It is therefore possible to measure  $CO_2$  concentration by calculating  $\Delta EMF$ .



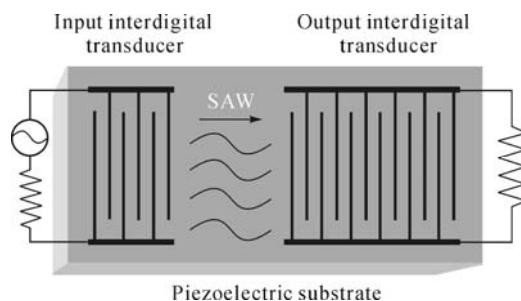
**Fig. 4.30.** Heating condition stability of the  $EMF$  and  $\Delta EMF$  in indoor atmospheres (reprinted from (Nakagaichi, 2000), Copyright 2000, with permission from Elsevier Science B.V.)

#### 4.3.4 Surface Acoustic Wave Sensors

A surface acoustic wave (SAW) is an acoustic wave travelling along the surface of a material exhibiting elasticity, with amplitude that typically decays exponentially with depth into the substrate.

##### 4.3.4.1 Structure and Principle

SAWs were first explained in 1885 by Lord Rayleigh. Named after their discoverer, Rayleigh waves have a longitudinal and a vertical shear component that can couple with any media in contact with the surface. This coupling strongly affects the amplitude and velocity of the wave, allowing SAWs to directly sense mass and mechanical properties.



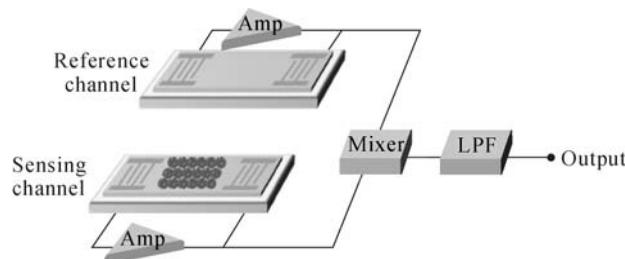
**Fig. 4.31.** Schematic picture of a typical SAW device design

SAWs normally use one or more interdigital transducers (IDTs) to convert acoustic waves to electrical signals and vice versa by exploiting the piezoelectric effect of certain materials (quartz, lithium niobate, lithium tantalate, lanthanum gallium silicate, etc.) as shown in Fig. 4.31. These devices are fabricated by photolithography, the process used in the manufacture of silicon integrated circuits. Staple et al., used the SAW sensor in z-Nose, realizing a high sensitivity mass sensor with a base frequency of 500 MHz, the sensitivity to the sarin gas can reach 10.34 Hz/pg.

#### 4.3.4.2 Applications

In recent years, many types of renewable energy are receiving increasing attention. In particular, hydrogen energy may become a new clean energy for daily use. But any leak of hydrogen over a wide range of concentration (4% – 75%) will result in an explosion, and if humans are exposed to it in a closed space, it can cause asphyxiation. Therefore, a method for precisely detecting the content of hydrogen at room temperature is very much needed in the development of a hydrogen energy economy. A SAW sensor with Pt coated ZnO nanorods as the selective layer has been investigated for hydrogen detection by Fu et al. (2009).

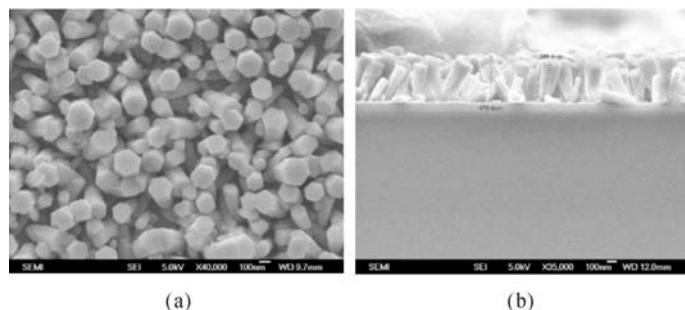
The SAW sensor was fabricated based on a 128° YX-LiNbO<sub>3</sub> substrate with an operating frequency of 145 MHz, the SAW resonator was then connected to an amplifier to configure an oscillator. A dual delay line system as shown in Fig. 4.32, which consisted of two counterparts in the oscillator (one is coated with the selective material and the other is bare to execute common mode rejection), was realized to eliminate external environmental fluctuations. To function as an active element, the coated one contributes to a frequency shift by the interaction between the sensing material and the target gas. By comparison, the reference one, which has a bare surface, gives the signal of the environmental effects.



**Fig. 4.32.** Schematic diagram of a dual delay line configuration

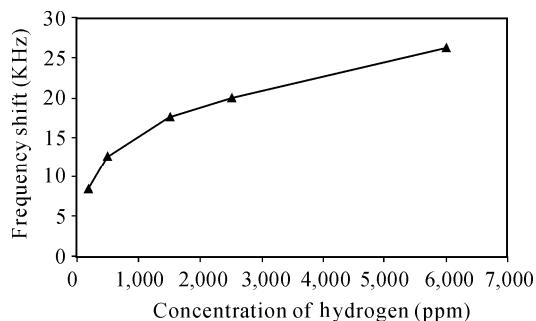
Pt coated ZnO nanorods were chosen as the selective layer due to the advantages of simple fabrication, high sensitivity to hydrogen at room temperature, and no reaction to moisture. First of all, a thin ZnO film was deposited on the SAW delay line, the as-prepared substrate was immersed into an aqueous solution

of zinc nitrate hydrate and methenamine at 95 °C for 5 h. Then, the substrate was rinsed with deionized water. Fig. 4.33 is a scanning electron microscope (SEM) image of the ZnO nanorods. Finally, a Pt film was coated over the ZnO nanorods as a catalyst by electron beam evaporation.



**Fig. 4.33.** SEM images of ZnO nanorods with growing time of 5 h: (a) Top view; (b) Side view (reprinted from (Fu et al., 2009), Copyright 2009, with permission from IOP Publishing)

The real-time responses of the dual-channel sensor to different H<sub>2</sub> concentrations are shown in Fig. 4.34. At the initial stage, the steady state of the base frequency was reached, and then nitrogen or hydrogen was led into the PDMS chamber. Testing cycles were implemented with constant exposure time and purge time to reach a new steady state or return to the baseline. The sensor was then exposed to different concentrations of hydrogen: 200, 500, 1,500, 2,500, and 6,000 ppm at room temperature. The responses were 8.36, 12.66, 17.47, 20, and 26.2 kHz respectively. It took less than 15 min to reach about 90% of the steady state, and the recovery time was about 2 – 3 min. The frequency shifts for different H<sub>2</sub> concentrations are shown in Fig. 4.34.



**Fig. 4.34.** Changes in frequency with H<sub>2</sub> concentration (reprinted from (Fu et al., 2009), Copyright 2009, with permission from IOP Publishing)

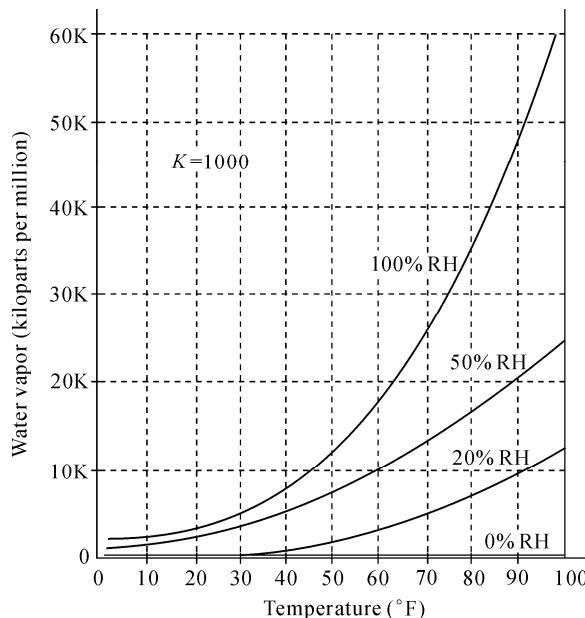
The results show that the Pt coated nanorod based SAW hydrogen sensor provides high sensitivity, fast response, and good repeatability while operating at room temperature. It is worth noting that the sensor can avoid the influence of humidity.

## 4.4 Humidity Sensors

Dry air is a gas consisting of approximately 78% nitrogen ( $N_2$ ) and 21% oxygen ( $O_2$ ); the remaining 1 percent encompasses “all others”. When water vaporizes, it becomes gaslike and enters into the air. Humidity is a measure of the water vapor content of air. Dry air has zero humidity, while air that holds all the water that it possibly can is said to be saturated.

Absolute humidity (AH) is measured in terms of water mass per unit volume of air (e.g.,  $\text{kg}/\text{m}^3$ ) and gives the amount of water in the air. The humidity most often quoted in weather forecasts is the relative humidity (RH), which is specified in terms of water parts per million parts of air, or as a percentage. By definition, relative humidity is defined as the ratio of the absolute humidity of the air to the saturated absolute humidity at the same temperature, or

$$RH = \frac{\text{mass H}_2\text{O} / m^3}{\text{mass H}_2\text{O} / m^3 \text{ saturated}} \quad (4.29)$$



**Fig. 4.35.** Graph of water vapor vs. temperature for three different relative humidities

Fig. 4.35 shows that humidity is a nonlinear function of air temperature. For any given temperature and relative humidity a maximum water vapor content is possible. If any more water tries to evaporate, the dew point is reached, and condensation (rain or fog) takes place.

The most important specifications to keep in mind when selecting a humidity

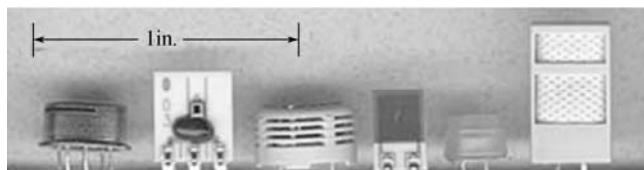
sensor are:

- Accuracy
- Repeatability
- Interchangeability
- Long-term stability
- Ability to recover from condensation
- Resistance to chemical and physical contaminants
- Size
- Packaging
- Cost effectiveness

Additional significant long-term factors are the costs associated with sensor replacement, field and in-house calibrations, and the complexity and reliability of the signal conditioning and data acquisition (DA) circuitry. For all these considerations to make sense, the prospective user needs an understanding of the most widely used types of humidity sensors and the general trend of their expected performance (Roveti, 2001).

#### 4.4.1 Capacitive Humidity Sensors

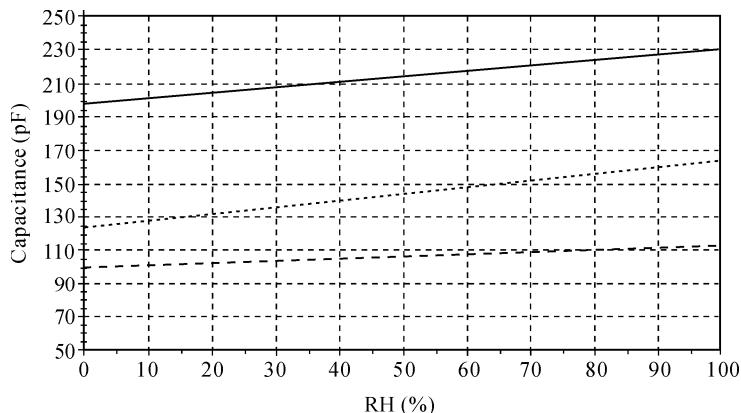
Capacitive relative humidity sensors (Fig. 4.36) are widely used in industrial, commercial, and weather telemetry applications.



**Fig. 4.36.** Capacitive RH sensors are produced in a wide range of specifications, sizes, and shapes including integrated monolithic electronics. The sensors shown here are from various manufacturers (reprinted from (Roveti, 2001), Copyright 2001, with permission from Questex Media Group, Inc.)

They consist of a substrate on which a thin film of polymer or metal oxide is deposited between two conductive electrodes. The sensing surface is coated with a porous metal electrode to protect it from contamination and exposure to condensation. The substrate is typically glass, ceramic, or silicon. The incremental change in the dielectric constant of a capacitive humidity sensor is nearly directly proportional to the relative humidity of the surrounding environment. The change in capacitance is typically 0.2 – 0.5 pF for a 1% RH change, while the bulk capacitance is between 100 and 500 pF at 50% RH at 25 °C. Capacitive sensors are characterized by low temperature coefficient, ability to function at high temperatures (up to 200 °C), full recovery from condensation, and reasonable resistance to chemical vapors. The response time ranges from 30 to 60 s for a 63% RH step change.

State-of-the-art techniques for producing capacitive sensors take advantage of many of the principles used in semiconductor manufacturing to yield sensors with minimal long-term drift and hysteresis. Thin film capacitive sensors may include monolithic signal conditioning circuitry integrated onto the substrate. The most widely used signal conditioner incorporates a CMOS timer to pulse the sensor and to produce a near-linear voltage output (Fig. 4.37).



**Fig. 4.37.** Near-linear response from different sensors of capacitance changes vs. applied humidity at 25 °C

The typical uncertainty of capacitive sensors is  $\pm 2\%$  RH from 5% to 95% RH with two-point calibration. Capacitive sensors are limited by the distance the sensing element can be located from the signal conditioning circuitry, due to the capacitive effect of the connecting cable with respect to the relatively small capacitance changes of the sensor. A practical limit is  $< 10$  ft.

Direct field interchangeability can be a problem unless the sensor is laser trimmed to reduce variance to  $\pm 2\%$  or a computer-based recalibration method is provided. These calibration programs can compensate sensor capacitance from 100 to 500 pF.

Thin film capacitance-based sensors provide discrete signal changes at low RH, remain stable in long-term use, and have minimal drift, but they are not linear below a few percent RH. These characteristics led to the development of a dew point measuring system incorporating a capacitive sensor and microprocessor-based circuitry that stores calibration data in nonvolatile memory. This approach has significantly reduced the cost of the dew point hygrometers and transmitters used in industrial HVAC and weather telemetry applications.

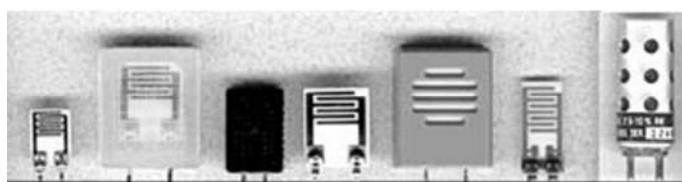
The sensor is bonded to a monolithic circuit that provides a voltage output as a function of RH. A computer-based system records the voltage output at 20 dew point values over a range of  $-40$  °C to  $27$  °C. The reference dew points are confirmed with a NIST-traceable chilled mirror hygrometer. The voltage vs. dew/frost point values acquired for the sensor are then stored in the EPROM of the instrument. The microprocessor uses these values in a linear regression algorithm

along with simultaneous dry-bulb temperature measurement to compute the water vapor pressure.

Once the water vapor pressure is determined, the dew point temperature is calculated from thermodynamic equations stored in EPROM. Correlation to the chilled mirrors is better than  $\pm 2$  °C dew point from -40 °C to -7 °C and  $\pm 1$  °C from -7 °C to 27 °C. The sensor provides long-term stability of better than 1.5 °C dew point drift/yr. Dew point meters using this methodology have been field tested extensively and are used for a wide range of applications at a fraction of the cost of chilled mirror dew point meters.

#### 4.4.2 Resistive Humidity Sensors

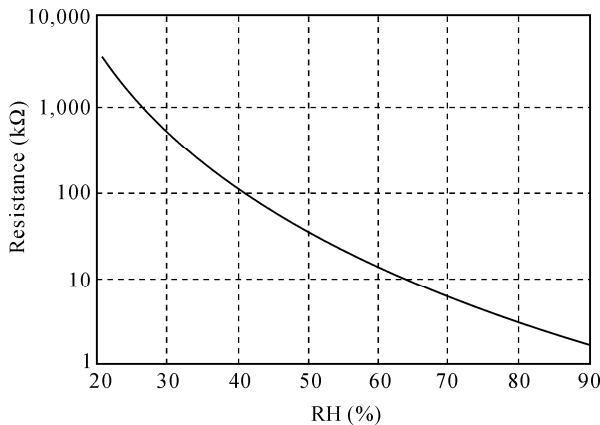
Resistive humidity sensors (Fig. 4.38) are applied to measure the change in electrical impedance of a hygroscopic medium such as a conductive polymer, salt, or treated substrate.



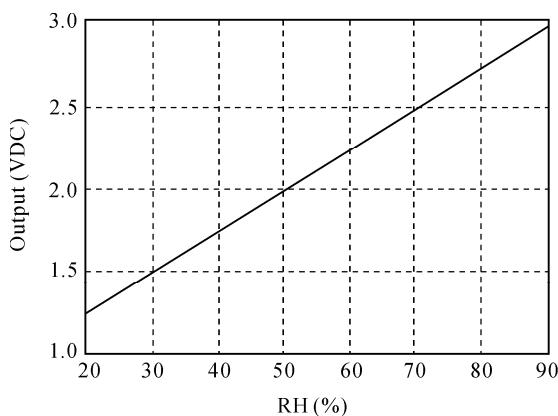
**Fig. 4.38.** Resistive sensors are based on an interdigitated or bifilar winding. After deposition of a hygroscopic polymer coating, their resistance changes inversely with humidity. The Dunmore sensor (far right) is shown 1/3 size (reprinted from (Roveti, 2001), Copyright 2001, with permission from Questex Media Group, Inc.)

The impedance change is typically an inverse exponential relationship to humidity (Fig. 4.39). Resistive sensors usually consist of noble metal electrodes either deposited on a substrate by photoresist techniques or wire-wound electrodes on a plastic or glass cylinder. The substrate is coated with a salt or conductive polymer. When it is dissolved or suspended in a liquid binder, it functions as a vehicle to evenly coat the sensor. Alternatively, the substrate may be treated with activating chemicals such as acid. The sensor absorbs the water vapor and ionic functional groups are dissociated, resulting in an increase in electrical conductivity. The response time for most resistive sensors ranges from 10 to 30 s for a 63% step change. The impedance range of typical resistive elements varies from 1 kΩ to 100 MΩ.

Most resistive sensors use symmetrical AC excitation voltage with no DC bias to prevent polarization of the sensor. The resulting current flow is converted and rectified to a DC voltage signal for additional scaling, amplification, linearization, or A/D conversion (Fig. 4.40).



**Fig. 4.39.** The exponential response of the resistive sensor, plotted here at 25 °C, is linearized by a signal conditioner for direct meter reading or process control



**Fig. 4.40.** Resistive sensors exhibit a nonlinear response to changes in humidity. This response may be linearized by analog or digital methods. Typical variable resistance extends from a few KΩ to 100 MΩ

In residential and commercial environments, the life expectancy of these sensors is >5 years, but exposure to chemical vapors and other contaminants such as oil mist may lead to premature failure. Another drawback of some resistive sensors is their tendency to shift values when exposed to condensation if a water-soluble coating is used. Resistive humidity sensors have significant temperature dependencies when installed in an environment with large (>10 °F) temperature fluctuations. Simultaneous temperature compensation is incorporated for accuracy. The small size, low cost, interchangeability, and long-term stability make these resistive sensors suitable for use in control and display products for industrial, commercial, and residential applications.

#### 4.4.3 Thermal Conductivity Humidity Sensors

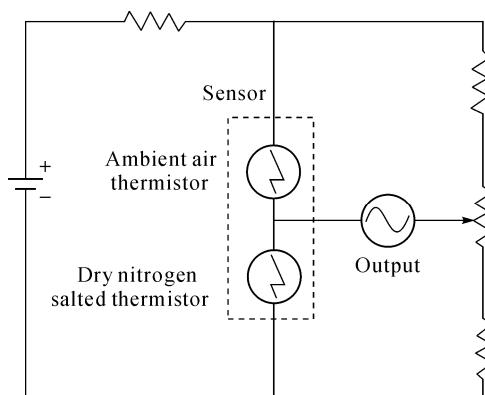
Thermal conductivity humidity sensors (Fig. 4.41) measure the absolute humidity by quantifying the difference between the thermal conductivity of dry air and that of air-containing water vapor.



**Fig. 4.41.** For measuring absolute humidity at high temperatures, thermal conductivity sensors are often used. They differ in operating principle from resistive and capacitive sensors. Absolute humidity sensors are left and center; thermistor chambers are on the right (reprinted from (Roveti, 2001), Copyright 2001, with permission from Questex Media Group, Inc.)

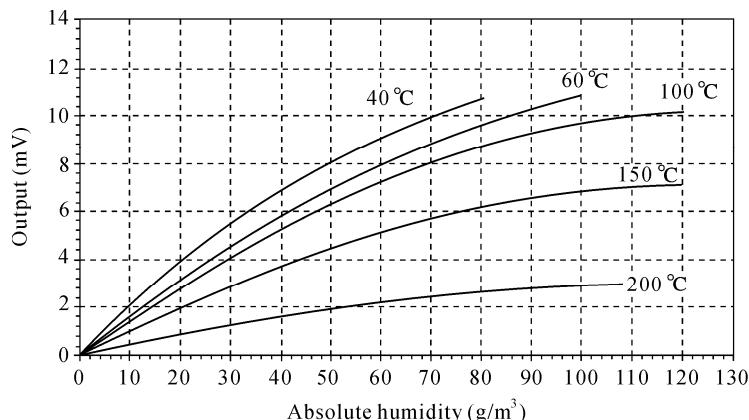
When air or gas is dry, it has a greater capacity to “sink” heat, as in the example of a desert climate. A desert can be extremely hot in the day but at night the temperature rapidly drops due to the dry atmospheric conditions. By comparison, humid climates do not cool down so rapidly at night because heat is retained by water vapor in the atmosphere.

Thermal conductivity humidity sensors (or absolute humidity sensors) consist of two matched negative temperature coefficient (NTC) thermistor elements in a bridge circuit; one is hermetically encapsulated in dry nitrogen and the other is exposed to the environment (Fig. 4.42).



**Fig. 4.42.** In thermal conductivity sensors, two matched thermistors are used in a DC bridge circuit. One sensor is sealed in dry nitrogen and the other is exposed to ambient. The bridge output voltage is directly proportional to absolute humidity

When current is passed through the thermistors, resistive heating increases their temperature to  $>200\text{ }^{\circ}\text{C}$ . The heat dissipated from the sealed thermistor is greater than the exposed thermistor due to the difference in the thermal conductively of the water vapor as compared to dry nitrogen. Since the heat dissipated yields different operating temperatures, the difference in resistance of the thermistors is proportional to the absolute humidity (Fig. 4.43).



**Fig. 4.43.** The output signal of the thermal conductivity sensor is affected by the operating temperature. Maximum output is at  $600\text{ }^{\circ}\text{C}$ ; output at  $200\text{ }^{\circ}\text{C}$  drops by 70%

A simple resistor network provides a voltage output equal to the range of  $0 - 130\text{ g}/\text{m}^3$  at  $60\text{ }^{\circ}\text{C}$ . Calibration is performed by placing the sensor in moisture-free air or nitrogen and adjusting the output to zero. Absolute humidity sensors are very durable, operate at temperatures up to  $575\text{ }^{\circ}\text{F}$  ( $300\text{ }^{\circ}\text{C}$ ) and are resistant to chemical vapors by virtue of the inert materials used for their construction, i.e., glass, semiconductor material for the thermistors, high-temperature plastics, or aluminum.

An interesting feature of thermal conductivity sensors is that they respond to any gas that has thermal properties different from those of dry nitrogen; this will affect the measurements. Absolute humidity sensors are commonly used in appliances such as clothes dryers and both microwave and steam-injected ovens. Industrial applications include kilns for drying wood; machinery for drying textiles, paper, and chemical solids; pharmaceutical production; cooking; and food dehydration. Since one of the by-products of combustion and fuel cell operation is water vapor, particular interest has been shown in using absolute humidity sensors to monitor the efficiency of those reactions.

In general, absolute humidity sensors provide greater resolution at temperatures  $>200\text{ }^{\circ}\text{F}$  than do capacitive and resistive sensors, and may be used in applications where these sensors will not survive. The typical accuracy of an absolute humidity sensor is  $\pm 3\text{ g}/\text{m}^3$ ; this converts to about  $\pm 5\%$  RH at  $40\text{ }^{\circ}\text{C}$  and  $\pm 0.5\%$  RH at  $100\text{ }^{\circ}\text{C}$ .

## 4.5 Intelligent Chemical Sensor Arrays

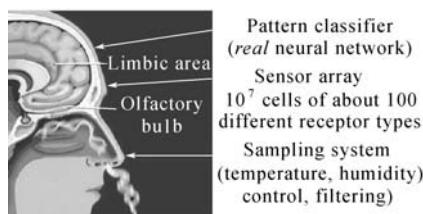
The intelligent chemical sensors generally include the sensor arrays and pattern recognition function. At present, the electronic or artificial nose (e-Nose) and electronic or artificial tongue (e-Tongue) have achieved great development.

### 4.5.1 e-Nose

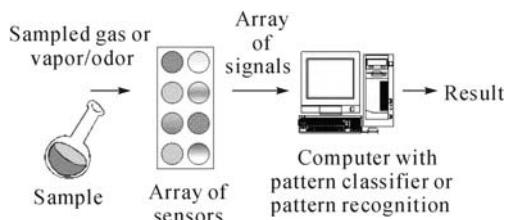
e-Nose is an instrument, which comprises a sampling system, an array of chemical gas sensors with differing selectivity, and a computer with an appropriate pattern-classification algorithm, capable of qualitative and/or quantitative analysis of simple or complex gases, vapors, or odors.

#### 4.5.1.1 Structure and Principle

One cannot discuss the e-Nose without first comparing it with the biological nose. Fig. 4.44 illustrates a biological nose and points out the important features of this “instrument”. Fig. 4.45 illustrates the artificial e-Nose. Comparing the two is instructive. The human nose uses the lungs to bring the odor to the epithelium layer; the e-Nose has a pump. The human nose has mucous, hairs, and membranes to act as filters and concentrators, while the e-Nose has an inlet sampling system that provides sample filtration and conditioning to protect the sensors and enhance selectivity. The human epithelium contains the olfactory epithelium, which contains millions of sensing cells, selected from 100 – 200 different genotypes that interact with the odorous molecules in unique ways. The e-Nose has a variety of sensors that interact differently with the sample. The human receptors convert the chemical responses to electronic nerve impulses. The unique pattern of nerve impulses is propagated by neurons through a complex network before reaching the higher brain for interpretation. Similarly, the chemical sensors in the e-Nose react with the sample and produce electrical signals. A computer reads the unique pattern of signals, and interprets them with some forms of intelligent pattern classification algorithm. From these similarities we can easily understand the nomenclature (Stetter and Penrose, 2001).



**Fig. 4.44.** The “Biological Nose”

**Fig. 4.45.** The basic design of the “e-Nose”

Although e-Noses are systems that, just like the human nose, try to characterize different gas mixtures, there are still fundamental differences in both the instrumentation and software. The Bio-nose can perform tasks still out of reach for the e-Nose, but the reverse is also true.

**Table 4.2** Comparing e-Nose with the human nose

Human nose	Electronic nose (e-Nose)
10 million receptors, self generated	5 – 100 chemical sensors manually replaced
10 – 100 selectivity classes	5 – 100 selectivity patterns
Initial reduction of number of signals (– 1,000 to 1)	“Smart” sensor arrays can mimic this?
Adaptive	Perhaps possible
Saturates	Persistent
Signal treatment in real time	Pattern recognition hardware may do this
Identifies a large number of odors	Has to be trained for each application
Cannot detect some simple molecules	Can detect also simple molecules ( $H_2$ , $H_2O$ , $CO_2$ , ...)
Detects some specific molecules	Not possible in general at very low concentrations
Associative with sound, vision, experience, etc.	Multisensor systems possible
Can get “infected”	Can get poisoned

Accordingly, an e-Nose is composed of two main components: the sensing system and the pattern recognition system, capable of recognizing simple or complex odors. And an individual sensor used for the detection of a particular substance, e.g., CO-sensor, is thus not e-Nose.

#### 4.5.1.2 Sensing System

The sensing system, which consists of a sensor array, is the “reactive” part of the instrument. When in contact with volatile compounds, the sensors react, which means they experience a change of electrical properties. Each sensor is sensitive to all volatile molecules but each in their specific way. Most e-Noses use sensor arrays that react to volatile compounds on contact: the adsorption of volatile compounds on the sensor surface causes a physical change of the sensor. A specific response is recorded by the electronic interface transforming the signal

into a digital value. Recorded data are then computed based on statistical models.

The more commonly used sensors include metal oxide semiconductors (MOS), conducting polymers (CP), quartz crystal microbalance, surface acoustic wave (SAW), and field effect transistors (MOSFET).

### **Gas sensor array**

Zhejiang University designed an electronic nose instrument CN e-Nose II used in lung cancer early stage diagnosis based on metal oxide gas sensor array. According to the research of Phillips et al., the exhaled gas of lung cancer patients contains some volatile organic compounds (VOCs) that can be taken as the biomarker of lung cancer, the corresponding diagnosis results can be obtained by detecting these VOCs.

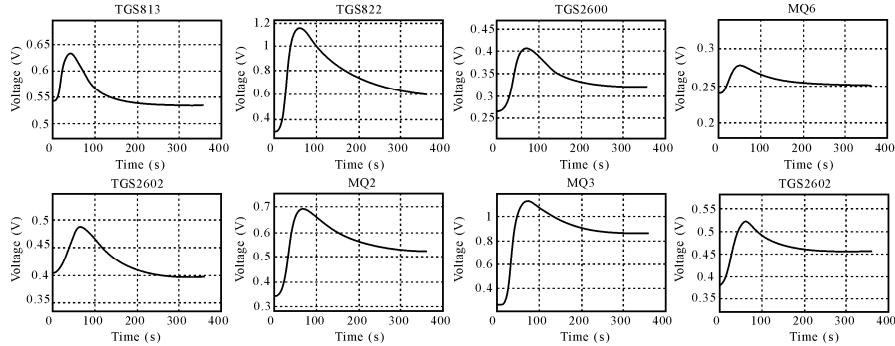
In the CN e-Nose II, five TGS MOS gas sensors and three MQ MOS gas sensors were used in the gas sensor array. The cross sensitivity between these sensors can be seen from Table 4.3, and they have different sensitivities to the homogeneous substances. The original intention of the CN e-Nose II lies in examining the concentration of biomarkers in a human's breath to represent the health condition. Taking this into consideration, 30% of the breath gas comes from the alimentary tract, which makes a contribution to health representation, and taking the digestive tract disease of an ulcer as an example, it will have ammonia in micro-scale from breathing. Therefore the sensor array includes not only eight metal-oxide semiconductor gas sensors shown in Table 4.3, but also one high sensitivity NE-NH<sub>3</sub> electrochemical sensor.

**Table 4.3** Characteristic parameter list of 8 MOS gas sensors according to the datasheets

Sensor model	Detectable gas	Detection range
TGS813	H <sub>2</sub> , Isobutene, Ethanol, CH <sub>4</sub> , CO	500 – 10,000 ppm
TGS822	Acetone, Ethanol, Benzene, n-Hexane, Isobutane, CO, CH <sub>4</sub>	50 – 5,000 ppm
TGS2600	H <sub>2</sub> , Ethanol, Isobutene, CO, CH <sub>4</sub>	1 – 100 ppm
TGS2602	Toluene	1 – 30 ppm
	H <sub>2</sub> S	0.1 – 3 ppm
	NH <sub>3</sub>	1 – 30 ppm
	Ethanol	1 – 30 ppm
	H <sub>2</sub>	1 – 30 ppm
TGS2620	Methane, CO, Isobutene, H <sub>2</sub> , Ethanol	50 – 5,000 ppm
MQ-2	Ethanol	100 – 200 ppm
	H <sub>2</sub>	300 – 5,000 ppm
	CH <sub>4</sub>	5,000 – 20,000 ppm
	Butane	300 – 5,000 ppm
MQ-3	Ethanol, Benzene, n-Hexane, LPG, CH <sub>4</sub>	0.1 – 10 mg/L
MQ-6	LPG, LNG, Butane, Propane	100 – 10,000 ppm

In the experiments of breath examination, low-concentration gas mixtures were prepared employing the possible biomarkers in the lung cancer patient's breath. Then the analysis was carried out after sample preconcentration.

Taking peak height, stable value and peak area as the characteristic values, the response curves from 8 MOS gas sensors are shown in Fig. 4.46.

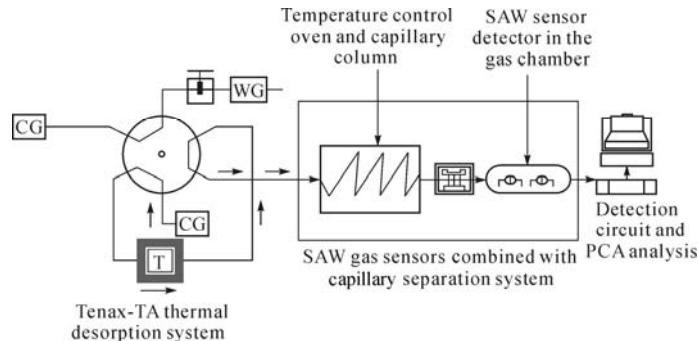


**Fig. 4.46.** Response curves of the mixed gas samples from 8 MOS gas sensors

#### *Virtual gas sensor array*

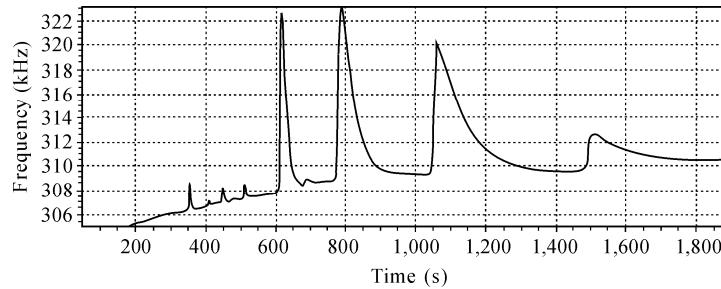
Zhejiang University designed an electronic nose instrument used in lung cancer early stage diagnosis based on a SAW gas sensor combined with a capillary separation technique.

The structure of the e-Nose is shown in Fig. 4.47. The respiratory gas is enriched by an adsorption tube, desorption happens in the inlet of the capillary at a high temperature, then the VOCs is carried into the capillary to be separated by the carry gas. When the VOCs come out from the capillary, there will be a frequency change because the VOCs can attach to the surface of the SAW sensor independently by reason of condensation, then the PCA and image analysis are used for pattern recognition after the signal is obtained and processed.



**Fig. 4.47.** Structure of the e-Nose based on SAW gas sensors combined with capillary separation system

One detect result of the mixed VOCs sample by the e-Nose system is shown in Fig. 4.48. As seen from the figure, the VOCs can be easily detected by the e-Nose system.

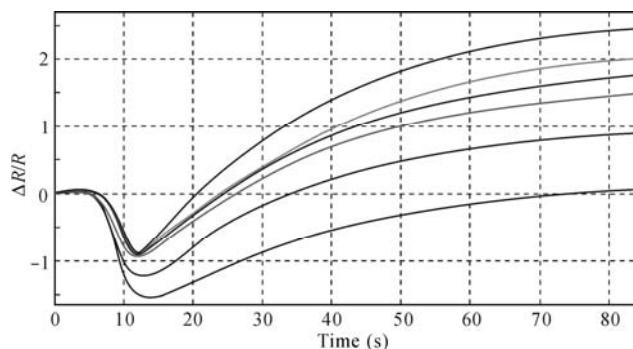


**Fig. 4.48.** Spectrogram of mixed VOCs sample

This e-Nose, which is based on the gas chromatography technology, causes its responses to have two components: the appearance time and the response intensity. We may know from the capillary separation technique that the peak time represents the time of each component used to pass through the capillary, as a result of the difference of physical and chemistry characteristics, different components use difference time to pass through the capillary, so the appearance time of the peak can be used for determining material. Because the capillary separating technique is applied, some disturbance factor in the environment is separated in the peak time; there will be no influence on the substance we need to detect, regardless of whether its density is high or low compared to the environmental disturbance. So the SAW gas sensor combined with the capillary separation technique can simulate a virtual sensor array containing hundreds of orthogonal (non-overlapping) sensors, which can detect and distinguish hundreds of different kinds of gases.

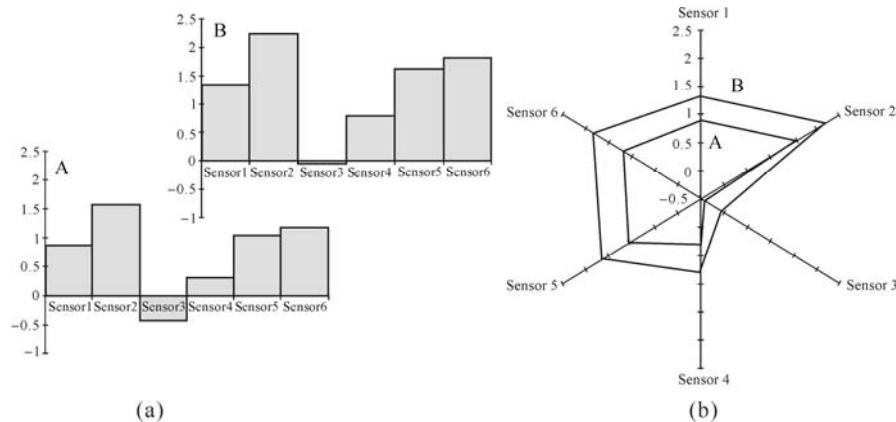
#### 4.5.1.3 Pattern Recognition System

The raw signal generated by an array of odor sensors is a typical collection of different electrical measurements vs. time curves (Fig. 4.49). These signals need to be processed in a more or less sophisticated manner in order to allow the recognition of a particular odor.



**Fig. 4.49.** Typical sensor response of a conducting polymer sensor array to a certain odorant

A basic method for observing a data set is simply to plot all variables, or a subset of variables, in a bar chart. Another form of output is a scaled polar plot (Fig. 4.50). Both forms can be obtained from the raw signal by integration of the curve over a distinct period of time. This way of visually displaying data is simple to interpret. Each vector on the polar plot represents the output from one sensor. As the relative response of each sensor changes when the sensor array is exposed to vapors from differing samples, the overall shape and appearance of the polar plot will vary.



**Fig. 4.50.** Bar graph (a) and Polar plot (b) generated from the raw data. A: Values taken as average signal from 15 to 75 s; B: Values taken as average signal from 55 to 75 s

In order to express the similarity or difference of two odors, it may be useful to calculate the distance of the two corresponding data sets.

As a chemical sensor system providing several variables, a multivariate distance measure is therefore more appropriate than a simple univariate distance measure. A multivariate distance is calculated in the original or a reduced variable space. There are two main methods to calculate multivariate distances. The euclidian distance (ED) is the length of the vector connecting two points in the variable space.

The ED can be calculated according to

$$ED = \sqrt{\sum_{i=1}^n (x_a - x_b)^2} \quad (4.30)$$

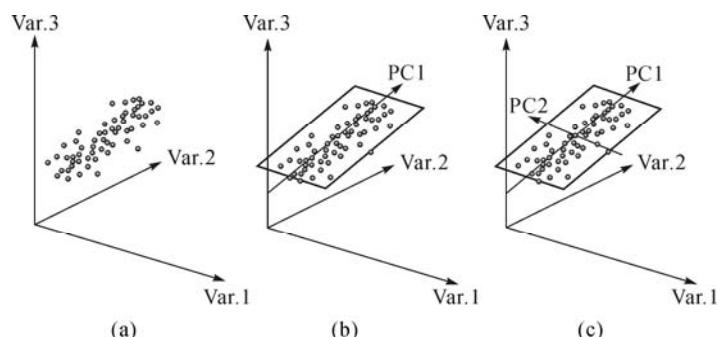
where  $x_a$  is the response of sensor number  $n$  produced by sample A and  $x_b$  is the response of the same sensor of  $x_a$  contacting with sample B.

However, the euclidian distance does not take the variation within classes into account. A more appropriate distance measure between classes is the statistical distance (also called Mahalanobis distance). The statistical distance is calculated as the ratio of the euclidian distance and the class variance in the direction of vector among class centres. Directions of high variance within the classes will thus give a

low statistical distance.

#### **Classification and dimension reduction**

Classification is the task of making a model capable of assigning observations into different classes. A classification is often combined with a dimension reduction in variable space. A multi-sensor system produces data of high dimensionality, i.e. a large number of variables characterizing each observation. It is difficult to visualize more than three dimensions simultaneously. Hence, methods to reduce the dimensionality of multivariate data sets are important. The variable space is an essential concept in order to grasp the ideas behind many data processing techniques. In the variable space, variables are seen as orthogonal basis vectors. An observation corresponds to a point in the sensor space, and a whole data set can be seen as a point swarm in this space. A way to reduce the dimensionality is to find new directions in the variable space and use only the most influential directions as new variables. A basis change is made and a dimensionality reduction is performed. In a principal component analysis, a transformation (projection) in the variable space is made (Fig. 4.51). Directions are found explaining as much of the variance in a data set as possible. These new directions, called principal components, are then used as the new variables. Keeping only principal components with high variation, leads to a dimension reduction. There are other methods to reduce the dimensionality in a variable space. All these methods are performed by finding new directions optimizing a specific criterion, and only the most influential directions are kept for the following visualization and classification.

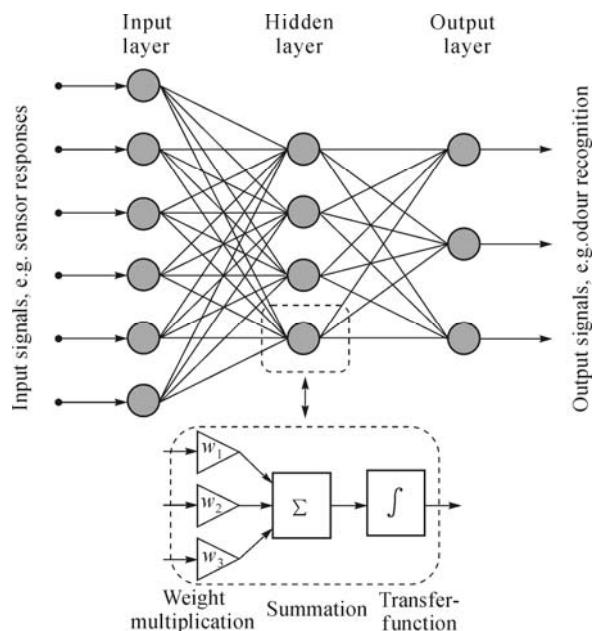


**Fig. 4.51.** Schematic picture of how a principal component score plot is made: (a) Raw data; (b) The first principal component is the direction with the most of the variance in the data set; (c) The low-dimensional projection of the data can be used as a simple but good approximation of the data set

#### **Artificial neural network**

An artificial neural network (ANN) is an information processing paradigm that was inspired by the way biological nervous systems, such as the brain, process

information. The key element of this paradigm is the novel structure of the information processing system. It is composed of a large number of highly interconnected processing elements (neurons) working in unison to solve specific problems (Fig. 4.52). ANNs, like people, learn by example. An ANN is configured for an application such as identifying chemical vapours through a learning process. Learning in biological systems involves adjustments to the synaptic connections that exist between the neurons. This is true of ANNs as well. For the electronic nose, the ANN learns to identify the various chemicals or odors by examples.



**Fig. 4.52.** Schematic of an artificial neural network. It consists of three interconnected layers of neurons. The computing neurons (hidden and output layers) have a non-linear transfer function. The parameters of the neurons are chosen with a minimization of the output error for a known training set

The basic unit of an artificial neural network is the neuron. Each neuron of the input layer receives a number of inputs, multiplies the inputs by individual weights, sums the weighted inputs, and passes the sum through a transfer function, which can be, e.g., linear or sigmoid (linear for values close to zero, flattening out for large positive or negative values). An ANN is an interconnected network of neurons. The input layer has one neuron for each of the sensor signals, while the output layer has one neuron for each of the different sample properties that should be predicted. Usually, one hidden layer with a variable number of neurons is placed between the input and output layer. During the ANN training phase, the weights and transfer function parameters in the ANN are adjusted such that the calculated output values for a set of input values are as close as possible to the

known true values of the sample properties. The model estimation is more complex than that for a linear regression model due to the non-linearity of the model. The model adaptation is made using the specific algorithm like back-propagation algorithm involving gradient search methods, where each weight is changed in proportion to the error which is caused.

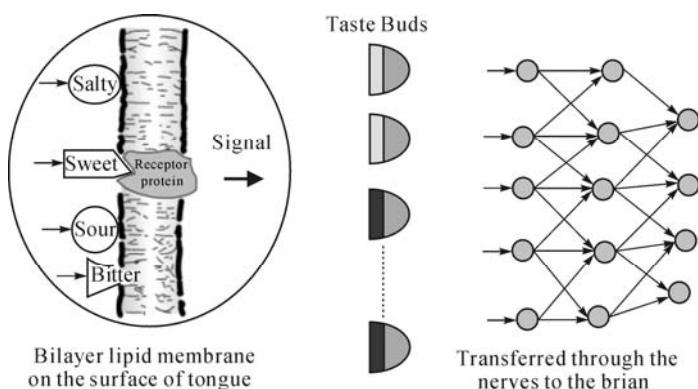
### 4.5.2 e-Tongue

e-Tongue is a sort of analytical equipment using multi-sensor array to detect the characteristic response signal of the liquid sample and process it by pattern recognition and expert system for learning identification to obtain qualitative or quantitative information. The most obvious difference between e-Nose and e-Tongue is that the former is for the gases while the latter is for the liquids.

The research on e-Tongue began only a few decades ago, so it is still not very mature. The most successful company in marketing e-Tongue systems is Alpha-MOS whose production accounts for more than 99% of the world's market. The e-Tongue systems are very useful in food, medical, environmental and chemical industry.

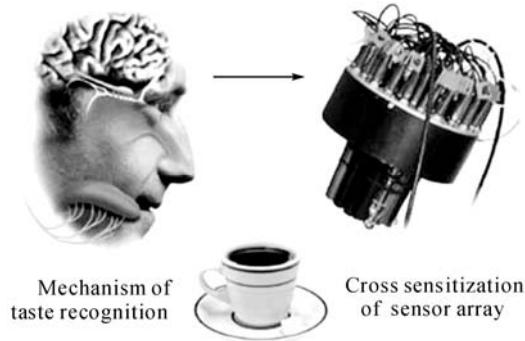
#### 4.5.2.1 Principle

The taste of organisms (Fig. 4.53) comes from taste buds on the surface of the tongue. The taste buds respond to different chemicals in the solution to generate signals which are transferred through the nerves to the brain. Then the brain does the analysis and processing to obtain the overall features of the signals and gives the distinction between different chemicals as well as the sensory information.



**Fig. 4.53.** Biological taste recognition

The initial design idea of e-Tongue originates from the biological mechanism of taste recognition (Fig. 4.54). Just like the tongue of organisms, the sensor array of e-Tongue responds to different chemical substances and collects a variety of signals to be transferred to the computer. Instead of the brain of the organism, the computer distinguishes the different signals, makes identification and finally gives sensory information of the various substances. Just as taste buds on the surface of the tongue, each individual sensor in the sensor array has cross sensitization. That is, a separate sensor not only responds to a chemical, but to a group of chemicals. In addition, while responding to specified chemicals, the sensor also responds to some other chemicals of a different nature.



**Fig. 4.54.** Design idea of e-Tongue

The realization of the e-Tongue technology is based on multi-sensor multicomponent analysis in the traditional analytical chemistry. Supposing that a specific sensor system with an array consisting of  $M$  sensors are applied to the analysis of a solution containing  $N$  components whose concentrations are  $C_1, C_2, \dots, C_N$  and all of the  $N$  components will be responded.  $P_i$  ( $1 < i < M$ ) represents the signal of sensor  $i$ . The  $M$ -sensor  $N$ -component analysis can be written as the following mathematical expressions:

$$\begin{aligned} P_1 &= A_{1,1}C_1 + A_{1,2}C_2 + \cdots + A_{1,N}C_N \\ P_2 &= A_{2,1}C_1 + A_{2,2}C_2 + \cdots + A_{2,N}C_N \\ &\dots \\ P_M &= A_{M,1}C_1 + A_{M,2}C_2 + \cdots + A_{M,N}C_N \end{aligned} \quad (4.31)$$

All of the  $M$  sensors are specific which means that a sensor only responds to one component. The constants  $A_{i,j}$  which are the ratio of the signal of sensor  $i$  to the concentration of component  $j$  are already known. As long as  $M \geq N$ , Eq. (4.31) can be solved by matrix operations to obtain the concentrations of all the  $N$  components.

The difference between the e-Tongue technology and the traditional multi-sensor

multicomponent analysis is that the e-Tongue employs cross-sensitive sensors instead of specific sensors in the array. In this way,  $A_{ij}$  in Eq. (4.31) become non-linear functions related to the concentration of component  $j$ . So Eq. (4.31) must be solved by a non-linear pattern recognition method such as artificial neural networks. The e-Tongue system should be trained by lots of sample solutions to establish self-learning expert system and then do the calculation.

#### 4.5.2.2 Characteristics

The structure of e-Tongue can be divided into three main parts which are the cross-sensitive sensor array, the self-learning expert system and the smart pattern recognition system which are respectively equivalent to the tongue, memory and brain calculation of organisms. The main characteristics of e-Tongue can be summarized as follows:

- The detection object is liquid samples.
- The signal obtained is the overall response to a solution, rather than the response to a specific component in the solution.
- The attributes of different samples are able to be distinguished through processing the original signal collected from the sensor array.
- The sample attributes derived by e-Tongue are different from the concept of taste of organisms.

#### 4.5.2.3 Functional Membranes

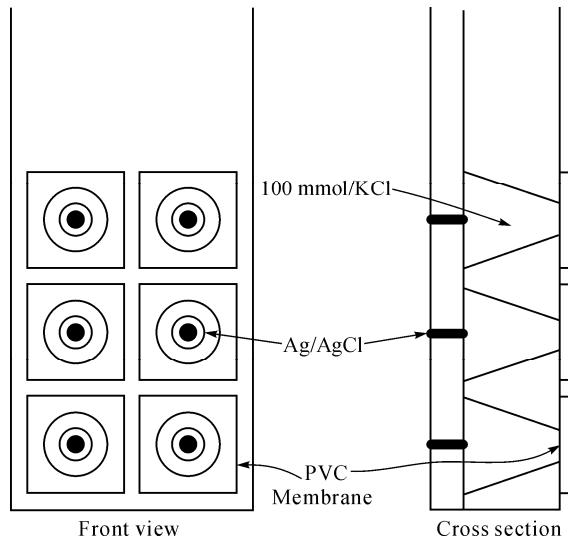
##### *PVC membrane*

The e-Tongue system based on PVC membrane sensor array invented by the research team of Toko K. in Kyushu University was the first e-Tongue system in the world. The PVC membrane sensor shown in Fig. 4.55 measured the open circuit potential with the Ag/AgCl reference electrode. The intensity of the affinity between taste substances and the PVC membrane modified with a variety of active materials was transformed into a potential signal. Such an e-Tongue system is generally composed of several electrodes respectively, providing a response to different taste substances, had the advantage that the data was relatively limited. So the test results represented by radar charts were directly corresponding to the characteristics of the taste substances.

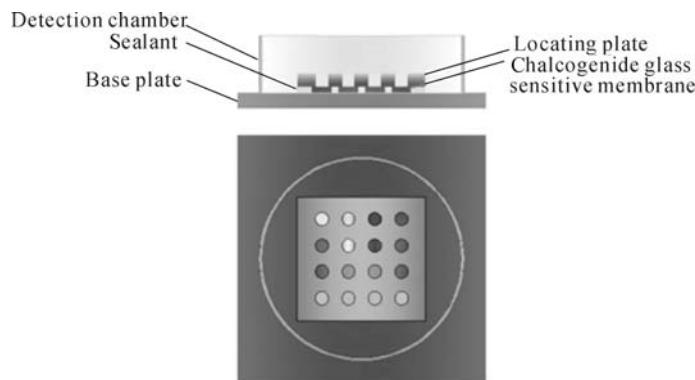
##### *Chalcogenide glass membrane*

Chalcogenide glass membrane sensor is a kind of solid-state ion selective electrode which has been applied in the detection of heavy metal ions for more than 30 years. E-Tongue with chalcogenide glass sensor array (Fig. 4.56) was originally invented by the research team of Legin A. and Vlasov Y. G. They developed many non-specific sensors based on chalcogenide glass materials such

as GeS-GeS<sub>2</sub>-Ag<sub>2</sub>S, Ag<sub>2</sub>S-As<sub>2</sub>S<sub>3</sub>, Ge-Sb-Se-Ag and AgI-Sb<sub>2</sub>S<sub>3</sub>. According to the principle of e-Tongue, a variety of chalcogenide glass sensors of high sensitivity and low selectivity were used to fabricate the sensor array to detect heavy metal ions and H<sup>+</sup> in the solution. This e-Tongue system has wide application in environmental assessment of water pollution, food quality assessment, etc.



**Fig. 4.55.** PVC membrane sensor array

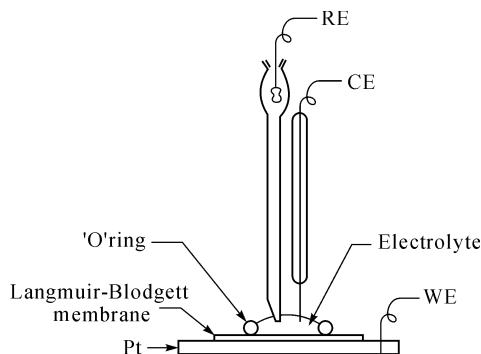


**Fig. 4.56.** Chalcogenide glass membrane sensor array

#### **Langmuir-Blodgett membrane**

The research team of Riul A. Jr. in Brazil invented an e-Tongue system based on Langmuir-Blodgett membrane sensor array (Fig. 4.57). They modified the platinum electrode surface with 10 nm-thick Langmuir-Blodgett membrane that consists of stearic acid, polyaniline, polypyrrole, etc. It was easy to detect the signal of the

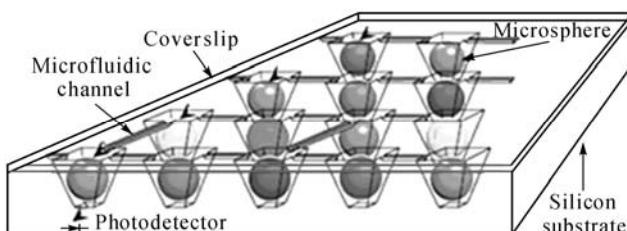
interaction between the sensors and the taste substances such as sour, sweet, bitter, salty and so on by electrochemical impedance spectroscopy. The results showed that the e-Tongue system was very sensitive to the taste substances and able to distinguish mineral water, beverages, wine, coffee, etc.



**Fig. 4.57.** Electrochemical detection device based on Langmuir-Blodgett membrane

#### 4.5.2.4 Bionic Taste Chip

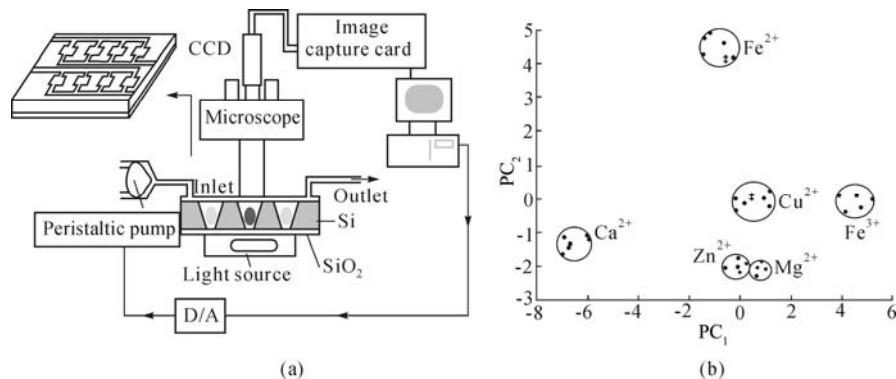
The research team in Austin University utilized ion-sensitive polymer microspheres as bionic taste buds to detect the constituents of a solution based on photochemical principles. The bionic taste chip was able to do the parallel, real-time and quantifiable measurement of a variety of constituents in the same solution. Synthetic microspheres whose diameters in the range of 50 – 100  $\mu\text{m}$  were fixed in micro grooves etched in the silicon wafer surface (Fig. 4.58). The chip and CCD were separately fixed on top of and under the platform. Modulated lights emitted by the blue light-emitting diodes went through the ball and the bottom of the platform and were absorbed by the CCD detector. The taste chip could preliminary determine the concentrations of  $\text{H}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Ce}^{3+}$  and sugar in the solution.



**Fig. 4.58.** The bionic taste chip

Based on the above work, the researchers developed algorithms of signal recognition to achieve the multi-ion identification automatically. Fig. 4.59a shows the detection system of the bionic taste chip. The analyte was pumped into the

reaction chamber and reacted with the sensitive materials adsorbed on the surfaces of microspheres whose colors changed in the reaction. Images of the microspheres were recorded by CCD through the microscope. RGB values were extracted from specified areas on the microspheres as the output of the taste chip. Principal component analysis (PCA) was applied to process the data to achieve the qualitative and quantitative measurement of the constituents of the analyte. Fig. 4.59b shows the PCA results which successfully distinguished the six kinds of metal ions in the same solution.



**Fig. 4.59.** Experiment of the bionic taste chip: (a) The detection system; (b) The PCA results of 6 kinds of metal ions

## 4.6 Micro Total Analysis System

Micro Total Analysis Systems ( $\mu$ TAS) have become the research hotspot in the world since its first appearance in 1990's, which is a distinct and novel field based on multidisciplinary fields such as analytical chemistry, micro-electro-mechanical systems (MEMS), computer science, electronics, materials sciences, and biology. It is also known as microfluidics or "lab-on-a-chip".

### 4.6.1 Design and Fabrication

As a unique and multidiscipline field, microfluidics is expanding into new areas of applications and the systems under development are becoming more complex. There is an increasing demand both for theoretical and experimental work on fundamental physical and chemical phenomena, and also for better modeling tools.

#### 4.6.1.1 Basic Principles of Microfluidic Chips

Fluid mechanics is one of the important disciplines to be further addressed, which may have great influences on the design of microfluidic devices and understanding the special effects related to fluid flow in micron-scale. Most of researchers working on microfluidics employ fluid mechanic modeling as a design tool, or as a way to correlate and explain experimental results. When dealing with flow in configurations of microns or less, some special effects and unexpected phenomena can be observed. Sir Eddington (1928) once said, “We used to think that if we know one, we know two, because one and one are two. We are finding that we must learn a great deal more about ‘*and*’”. Basically, the flows in macro and micro configurations are quite different. The unique features in micron-scale fluid flow are still far from being completely understood due to not much being known about the complex surface effects that play major roles in these events. This may excite researchers for years to search for the answers to these issues (Ho and Tai, 1998).

In many simple cases, the flow-pressure characteristics of a device are the fundamental quantities that can usually be dominated by one single restriction, which make it sufficient to use a simple analytical model well known from macroscopic fluid mechanics. This approach has been successfully applied to the modeling of some microfluidic components such as valves, and channels. In more complex structures or systems, numerical simulations are used. The most common method of numerical simulation is based on a subdivision of the complete structure into lumped elements, which can be described individually by simple analytical models, and for which simple relations between individual lumped elements can be formulated (Gravesen et al., 1993). These models and relations of interaction can then be fed into a dedicated or generally available computer program. In this way, micropumps, valves, flow sensors, and flow dispenser have been simulated using dedicated computer programs. In order to apply the approaches mentioned before to fluid mechanic modeling successfully by the direct utilization of analytical models or lumped element models, it is necessary to make correct assumptions as to types of flow. In micro flows, the Reynolds number is typically very small and shows the ratio between the viscous force and the inertial force. It is a common and practical method to determine whether a given flow pattern is laminar or tubular by evaluating the Reynolds number.

For the design of microfluidics, it is necessary to consider the following requirements carefully:

- The uniformity of the flow velocity in microfluidic chip, the reduction of dead volume;
- The evaporation and random flow of the fluid during reaction and storage, the uniformity of mixing and the avoiding of bubble formation;
- The calculation of reagent and production volumes, the compatibility, reliability, and reactivity of materials used in microfluidic chips;
- Interference of flow to the signal acquisition and the improvements on signal to noise ratio;
- Proper handling of the waste and used microfluidic chips.

Computer modeling and simulation is an important approach for the design of microfluidics, which can also be used to interpret the experimental data. It can provide beneficial prediction on the liquid-phase process of flow mechanics as well as modeling of device thermal fields and chemical concentrations. By this approach, the time and cost of the microfluidic chip design can be significantly reduced and various parameters can be optimized. Currently, there are some commercial softwares, such as Flume (Coventor, INC) for microfluidic chip design which are available.

#### 4.6.1.2 Microfluidic Chip Fabrication

It is very important to choose proper materials to fabricate microfluidic chips. Some of the main issues that should be addressed are as follows:

- Chemical and biological compatibility between microfluidic chip and working interface;
- Electric insulation and thermal properties of materials used;
- Optical properties related to the interference of signal detection;
- The modification properties of materials related to the generation of electroosmosis and solid immobilization biological molecules;
- The simplicity and low cost of microfluidic chip fabrication.

However, it is hard to have the kind of materials that can fully satisfy all the requirements mentioned above. The selection of materials for microfluidic chip fabrication is usually made according to the practical applications. At present, materials that are mainly used include silicon, glass, crystal, and organic polymer. Every type of material has its own advantages and disadvantages and some differences exist in the corresponding fabrication process. For example, silicon has excellent chemical inert and thermal stability. Also, the techniques for the production and micro fabrication are mature and have been widely used in semiconductor and integrated circuits. So, the initial microfluidic chips are usually fabricated on the basis of silicon.

The fabrication process of a microfluidic chip is very sensitive to the environment, and should be done in the clean room. The techniques used for microfluidic chip fabrication originated from the micro fabrication of semiconductors and integrate circuits, while they are different when compared to the silicon-based techniques for the two dimensional and depth fabrication of integrated circuit chips. The fabrication methods vary according to the different chip base materials. Some important methods are utilized such as lithography, etching techniques, soft lithography, molding, LIGA, ultraviolet laser, and deep reactive-ion etching (DRIE). For the details related to the fabrication process of microfluidic chips it is suggested that you refer to the related literatures.

Sealing is also a very important process for chip fabrication. Before sealing, chips with different structures and functional units must be very clean through strict washing and handling. For silicon and glass, some commonly used sealing techniques include heat sealing, anodic bonding, and cryogenic adhesive bonding.

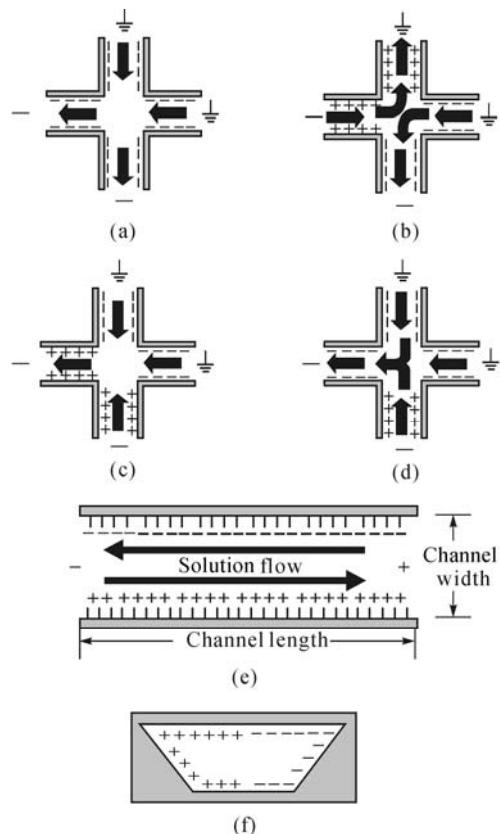
For polymer materials, various sealing methods, such as hot pressing method, heat or light catalysis adhesive technique, organic solvent adhesive method, automatic adhesive method, plasma oxidation sealing method, ultraviolet irradiation method, and cross-link agent regulation method, are available to be selected according to the different materials used.

After fabrication, the surface of micro channels usually needs to be modified according to their practical applications to improve some of the chemical or physical properties. The research on the techniques for chip surface modification is composed of a large percent of the research on the microfluidics. When the analysis system works, micro flow operation that depends on the inner surface properties of microchannels is usually worked in a passive mode. In the microfluidic chips that use electro osmotic flow driving, it is the most common used technique due to its simple operation, without requirements on extra devices, and without increase in the chip system volume. The basic principle of this technique is to determine the velocity and direction of the electro osmotic flow by changing the density and polarity of charges located in the inner surface of micro channels. As shown in Fig. 4.60, by the method of coating an electrolyte layer of polymer on the inner surface of micro channels, the direction of the micro flow can be changed and complex flow operations can be achieved (Barker et al., 2000). Furthermore, simultaneous flow in opposite directions can be achieved in a single micro channel. Another predominant application of this technique is to obtain the flow limitation. For example, hydrophobic glass micro channels can be achieved by the method of immobilizing a self-assemble monolayer of octadecyltrichlorosilane on the inner surface of micro channels. In the polymer micro channel, the hydrophobic and hydrophilic region can be achieved by depositing a layer of poly phenylene-2-methyl and silicon oxidize on the inner surface of micro channel.

#### 4.6.1.3 Driving and Control of Micro Flow

The basis of a microfluidic chip operation is the technique for micro flow driving and control. Since the invention of the microfluidic chip, it has always been an important topic in the basic research field of microfluidic chip and new techniques and methods are progressively appearing. Here, we will briefly introduce two techniques for micro flow driving, which are electro osmotic driving and micro pump driving. Also, two techniques for micro flow control will be introduced, which are electro osmotic control and micro valve control.

At present, electro osmotic driving is one of the most widely used methods in micro flow driving. Its basic principle is using the fixed charges on the inner surface of micro channels to drive the micro flow. Its advantages include lack of mechanical components, simple configuration, convenient operation, flat flow, and no pulsation. However, this method is sensitive to the influences of external electrical fields, channel surface, properties of micro flow, and the effect of heat transfer. So, it is not so stable and can only be applied to electrolyte solutions.

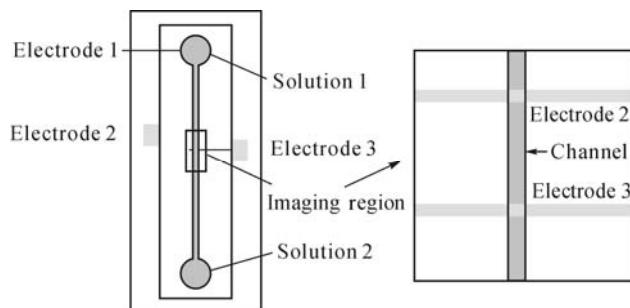


**Fig. 4.60.** Complex flow control can be realized by the surface modification of micro channel: (a), (b), (c), and (d) show various flow modes can be achieved by the control of charge polarity located on the inner surface of micro channel. (e) and (f) show the opposite direction flow can be realized in the single channel. (e) is the top view of the micro channel, while (f) is the cross section view

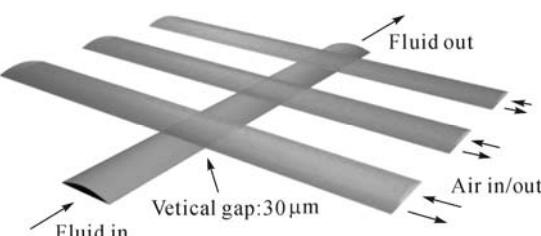
Electroosmosis can not only be used to directly drive the charged flow, but also can be used as the energy source of the micro pump, which is called the electro osmotic pump. The method to achieve this kind of electro osmotic pump is as follows: electrodes with certain intervals are fabricated on the surface of a chip basis by using lithography. Then it is sealed with PDMS micro channels to form a hermetic electro osmotic driving system. When it works, the voltage is applied to the electrodes to generate an electro osmotic flow. As the electro osmotic flow only exists between the two electrodes, the flow outside the two electrodes can be driven by the electro osmotic flow. Consequently, the function of the electro osmotic pump can be realized as shown by Fig. 4.61.

Pneumatic micro pump in the mechanical driving system is composed of multiple pneumatic micro valves. Its structure is shown in Fig. 4.62 (Unger et al., 2000). When the pressure is applied, PDMS thin film deformed under the effect of

gas pressure, leading to the block of channel and the closing of the valve. When there is no pressure applied, the restitution of the PDMS thin film can be achieved by its own elastic force, thus the channel becomes unobstructed and the valve sits open. By the sequential control of the opening and closing of three valves, the driving of micro flow can be obtained.



**Fig. 4.61.** Schematic diagram of electro osmotic pump structure

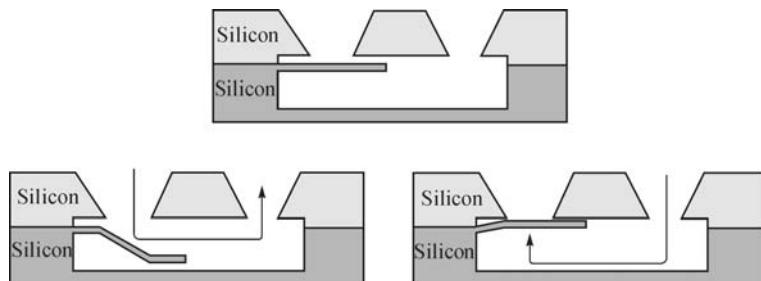


**Fig. 4.62.** Schematic diagram of pneumatic micro pump structure (reprinted from (Vnger et al., 2000), Copyright 2000, with permission from AAAs)

Micro flow control is the central principle of microfluidic chip operations. It is related to almost all the processes such as sampling, mixing, reaction, and separation, which are necessary to be finished in the controllable flow. Valve is the central component for flow control both in macro and micro scale. Due to its importance, micro valve has been deeply studied before the invention of the microfluidic chip. In the primary stage of its development, microfluidic chips are normally a kind of capillary electrophoresis on a chip, which is dependent on the electro osmotic driving. So, until now, electro osmotic driving is still the most widely used technique for micro flow control. In addition, the structure of channel, surface modification of the chip, laminar flow, and the effect of diffuse also play an important role in micro flow control.

There are various kinds of micro valves. Theoretically, all components that can control the opening and closing of the channel can be used as micro valves in microfluidic chips. An ideal micro valve can be characterized as follows: low leakage, low energy consuming, fast responses, linear operation, and wide range of adaptation (Gravesen et al., 1993). According to the necessity of the excitation

source during micro valve operations, micro valves can be classified into passive valves and active valves. Fig. 4.63 shows the structure of a type of passive one-way valve and how it works.

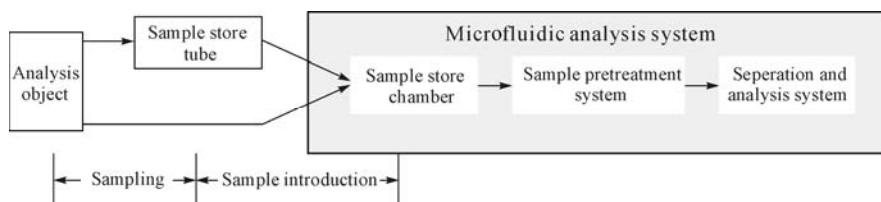


**Fig. 4.63.** Schematic diagram of passive one-way valve

Electroosmosis is a type of phenomenon that the solution in the micro channel can move in the desired direction along the inner surface of the channel under the effect of the electrical field. It has been widely used in micro flow control. Compared to other types of micro pumps, the most important feature of electro osmotic valves is their simple and flexible operation. The velocity and direction of flow can be controlled by adjusting the voltage applied to different nodes of the micro channel. Consequently, operations such as complex mixing, reaction, and separation can be realized. Besides the voltage, electro osmotic micro valves can be affected by such factors as the chemical composition of channel surface, ingredients of buffer solution, and temperature.

#### ***Sample introduction and pretreatment***

Sample introduction is the first step of microfluidic chip analysis. It includes the process of sampling from the analysis object and the introduction of sample into the micro channel for sample handling. Before detection, a series of pretreatment and reaction steps are necessary to be done to the sample, such as pre-separation, pre-concentration, and dilution. Fig. 4.64 is the schematic diagram of microfluidic system sample operation mode.

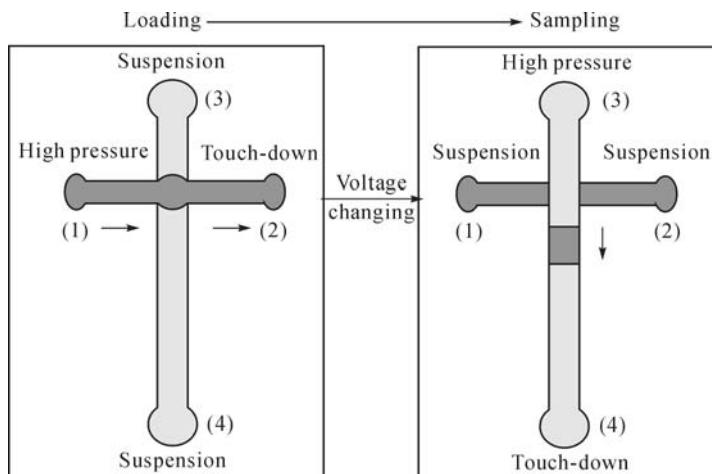


**Fig. 4.64.** Schematic diagram of microfluidic system sample operation mode

Currently, in most microfluidic analysis systems, sample, reagent, and buffer solution are stored in a well type storage chamber located on the chip. Sample

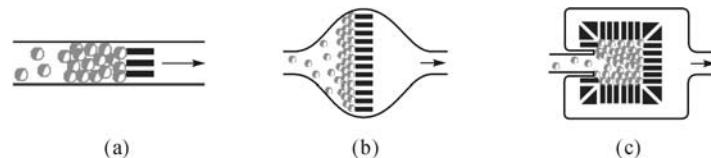
introduction methods usually add the sample to the well type storage chamber manually or automatically. Then the sample is imported into the channel for pretreatment or directly separated and analyzed. If various samples need to be measured, it is necessary to change the sample stored in the well type storage chamber manually or automatically in an intermittent way. Although the microfluidic analysis system has the ability of repeated measurement, the results of automatic continuous measurement are originated from the continuous measurement of the same sample. Very few of the results are originated from the different samples. There is no report on the applications of microfluidic analysis systems to the real-time process monitoring due to the lack of efficient sample changing methods.

There are two methods being used to solve the changing sample problem. One is the once-off sample introduction, such as the utilization of disposable chips, multi sample chambers on a single chip, multi analysis units on a single chip, and multi sample introduction before measurement. Another method is to use recyclable chips to realize continuous changing of the samples either manually or automatically. Generally, the sample source tends to provide continuous sample flow. In order to get output from the sample zone, some auxiliary methods are necessary. A commonly used method is to set an auxiliary channel on the chip, which is perpendicular to the sample processing channel. The sample zone can be generated in the cross of the two channels. This method is called the single channel aid sample introduction. It is the most studied and most representative sample zone introduction method. It includes two steps, loading and sampling. Loading refers to the process of sample load to the auxiliary channel through a storage chamber and is filled within the cross of channels. Sampling is the process of introducing the sample that is located in the cross to the sample processing channel by electrical forces or pressure. Fig. 4.65 shows the principle of the simple sample introduction method by electrical forces.



**Fig. 4.65.** Schematic diagram of simple sample introduction method by electrical forces: (1) Sample chamber; (2) Sample waste solution chamber; (3) Buffer solution chamber; (4) Buffer waste chamber

Sample for microfluidic analysis systems are usually related to the biological sample containing complex composition. So, the techniques for sample pretreatment are very important, which often include sample pre-separation and pre-concentration. Pre-separation includes liquid-liquid extraction, solid phase extraction, filtration, chromatography, and membrane separation. Pre-concentration includes iso-electric focusing, isotachophoresis, and field amplified stacking. Furthermore, multi phase laminar flow techniques and various micro filters (Fig. 4.66) can be used to sample pretreatment.



**Fig. 4.66.** Structure of various micro filters: (a) Micro channel-based filter; (b) Micro channel magnum-based filter; (c) Micro square cage-based filter

#### **Micro mixer, reactor and separator**

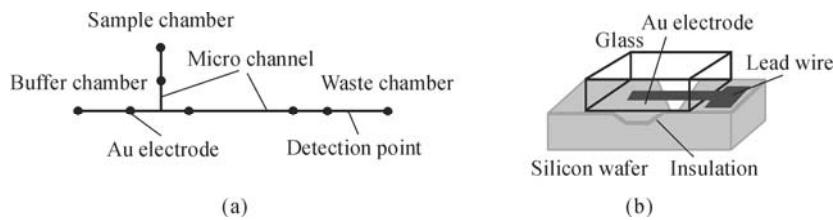
Reaction is the central process of chemical and biological experiments. Also mixing and separation are necessary for the process of reaction, especially in the micro scale. So, micro mixer, reactor, and separator are important components of microfluidic chips.

Micro mixer is very useful in the biological process that requires rapid responses such as the hybridization of DNA, cell activation, enzyme reaction, and protein folders. In microfluidic systems, the size of the channels is in the micro meter scale. The velocity of the solution is usually low and the solution mixing is mainly based on the mechanisms of laminar flow, which can be greatly influenced by the molecular diffusion. In order to improve the efficiency of laminar flow mixing, some principles should be followed: (1) extending the flow shear to increase the contact area of solution; (2) splitting and recombining the solution by the utilization of distributed mixing design, consequently reducing the solution thickness to realize more efficient mixing.

Micro reaction technique is the application of micro structure advantages to the process of chemical reaction. Micro reactor is a mini chemical reaction system with unit reaction interface in a micro meter scale. Its basic features include a small linear scale, high physical quality gradient, high surface to volume ratio, and low Reynolds number. Also, by its parallel units, a micro reactor can realize flexible and scale up production, and rapid and high throughput screening.

Recently, great progress has been achieved in the micro separation techniques. Now, various chromatography and electrophoresis separation modes can be realized on chips. Micro separator has become one of the fastest developing and maximum maturity technical units, which has greatly advanced the integration trends of microfluidic chips. Taking the integrated capillary electrophoresis chip as an example, microchannels and other functional units can be etched on the chip in a few centimeter square areas using micro processing technology. Consequently, a

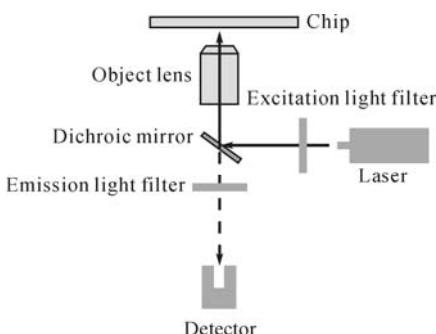
micro analysis device integrated with functions of sample introduction, separation, reaction, and detection can be realized, which is characterized with rapid, high efficiency, low sample consuming, low cost, and portability. Fig. 4.67 shows schematic diagram of the structure of an electrophoresis chip.



**Fig. 4.67.** Structure of (a) an electrophoresis chip and (b) its microelectrode and lead wire

### **Detection methods**

The detector of microfluidic chips is used to measure the desired composition of the sample as well as its quantity. The overall performance of detectors will have a great influence on the sensitivity, detection limit, and detection speed of the whole system. So, it is the key component of microfluidic chips. Compared to the conventional analysis systems, microfluidic chips have some special requirements on its detector. Detection techniques, which are characterized with the higher sensitivity and signal-to-noise ratio, higher response speed, miniaturization, and low cost, are greatly preferred for usage in microfluidic chips. Currently, a large number of detection techniques have been used in microfluidic chips. However, the optical and electrochemical detection methods are two of the most widely used methods. Because microfluidic chips take over some characteristics of capillary electrophoresis, optical detection methods such as laser induced fluorescence (LIF), chemiluminescence, and UV absorption, are still the mainstream detection methods. Fig. 4.68 shows the basic optical structure of a confocal LIF detector. Various electrochemical detection methods have also been used in microfluidic chips due to their advantages of simply structure, low cost, and easy integration. In addition, mass spectra detector plays an irreplaceable role in the research of proteomics due to its powerful capability of distinguishing and identification.

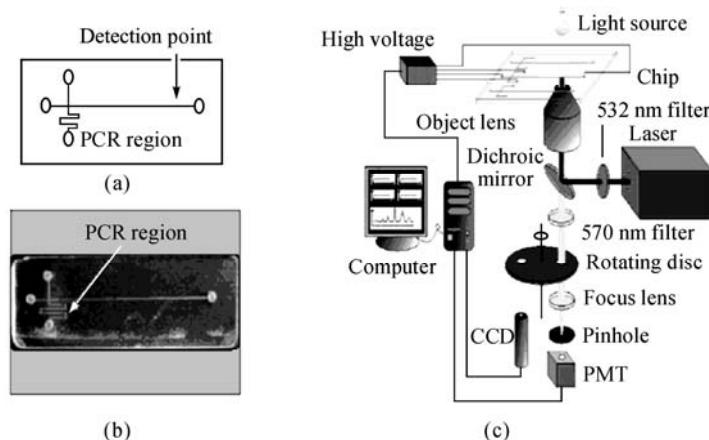


**Fig. 4.68.** Schematic diagram of basic optical structure of confocal LIF detector

#### 4.6.2 Applications

Microfluidic chips have great potential in microminiaturization, integration, and portability of analytical devices, which provide wider prospects for microfluidic chips to be applied in many different fields such as biomedicine, high throughput screen for drug synthesis, optimization of priority crops sterile, environmental monitoring and protection, health quarantine, judicial expertise, and biological warfare agent detection. Currently, nucleic acid research is still one of the most widely used application fields for microfluidic chips. It has broadened its application fields from analysis of simple nucleic acid sequences to complex genetic analysis and diagnosis. In the clinical laboratory, microfluidic chips can perform the detection of multiple diseases of multiple patients on a single chip, which can provide very helpful diagnostic information for doctors.

In 2003, with the large-scale outbreak of SARS, it was necessary to establish a rapid, non-invasive method for SARS virus detection, which was very important to the diagnosis and control of SARS (Lin and Qin, 2006). For this undertaking, a microfluidic chip integrated with functions of PCR and electrophoresis separation was developed. The structure of the microfluidic chip is shown in Figs. 4.69a and b. By the utilization of detection system (Fig. 4.69c), it can realize the amplification, separation, and detection of virus genes. It can dramatically reduce the time for SARS virus detection compared to the conventional methods.



**Fig. 4.69.** Microfluidic chip for SARS virus detection: (a) Structure of the microchip; (b) PCR microchip; (c) SARS virus detection by the laser-induced fluorescence system.

## 4.7 Sensor Networks

Benefiting from the progress of information and MEMS technology, sensor

networks, which are novel style of sensors, begin to show promising advantages.

Sensor networks are special networks composed of a group of sensors, wired or wireless, utilizing given methods or rules, whose targets are feeling, collecting and processing specialized information of certain objects through a united approach. The breakthrough within sensor networks benefits from the progress of sensor techniques, built-in process techniques, distributing information process techniques and communication techniques (Wang and Akilydiz, 2002).

As to the communication mode, either a wired mode or wireless mode is utilized. The optical fiber sensor network is a freshman in the sensor network family. It has an optical fiber but not an electronic wire/cable that works as a communication media. Since the placement of optical fiber is similar to traditional cable, the optical fiber sensor network is usually classified a wired sensor network.

#### **4.7.1 History of Sensor Networks**

The 1st-generation sensor network is based on a traditional analog output sensor with point-to-point translation. Such sensor networks were utilized widely in 1980s, but dropped behind due to high cost, complex placement and poor EMC performance.

An obvious feature of the 2nd-generation sensor network is the application of SMART sensors. A built-in processor, for example, MCU or DSP, acts as the center control unit of the sensor. It receives the analog signals from the sensitive unit, converts them to digital data, then stores or transmits accordingly. At the same time, more and more 2nd-generation sensor networks accept series digital bus as transport media, such as RS-232, RS-422 and RS-485 digital data bus.

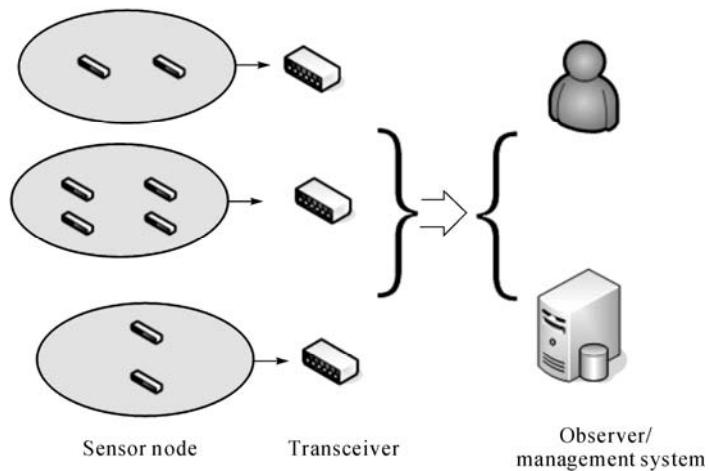
The 3rd-generation sensor network is a smart sensor network, which has an advanced field bus, which is a transport network that is all-digital, double-direction and open. The requirement for wire/cable placement, communication bandwidth is much less than earlier sensor networks. MPS (Michigan parallel standard) bus and Inter-IC ( $I^2C$ ) bus were two bus standards that were introduced successfully at an early stage. And now, controller area network bus (CAN bus) and Ethernet bus are two typical smart buses with universal applications.

The 4th-generation sensor network is in progress now, with features such as multi-function sensor units, self-organized network structures and wireless transport modes; it is called a wireless sensor network (WSN). This is a new type sensor network, which is constructed of basic nodes. A sensitive device, built-in micro processor, wireless interface, power supply unit, application software and security strategy are integrated in each node. Each node can be a basic sensor unit, transmit relay unit, even a local information collecting unit. Due to the characteristic of WSN, specialized communication agreement and route arithmetic are the key points for developing WSN techniques. And we predict that advances in MEMS technology will produce WSN that is even more capable and versatile.

#### 4.7.2 Essential Factors of Sensor Networks

##### **Sensor**

A sensor is composed of power, sensitive device, built-in processor, communication unit and respective software. Power provides energy to each part. Sensitive device detects and monitors information of targets, then changes the information to digital data, if necessary. Built-in processor controls the status of each part, especially collects the information from the sensitive device, and then sends it to the communication unit after processing.



**Fig. 4.70.** A typical sensor network

##### **Observer**

The observer is the user of sensor networks and the accepter of information acquired. Observer is a man, a computer or other devices. For example, either a scientist or a computer station can be an observer of certain sensor networks. One sensor network can support multi observers at the same time. An observer can check the information provided by sensor networks, then judge, conclude the information accordingly, or take corresponding actions to the objects.

##### **Object**

An object is the monitor target of a sensor network. Physical parameter, chemical process and biomedical status can be included. One sensor network can monitor multi objects in certain area. At the same, an object can be monitored by different sensor networks.

### 4.7.3 Buses of Sensor Networks

In a sensor network, sensor nodes are generally connected to a controller or a computer which provides linearization, error correction, and access to the network. The interface between sensor node and controller becomes more and more important. Though maybe not introduced for sensor networks originally, some digital interface standards provide extensive applications in the field of sensor networks. Some brief introductions to typical digital interfaces are included in the following sections (Sichitiu, 2004).

#### 4.7.3.1 RS-232 Bus

RS-232 is a standard for serial binary data signal connections between data terminal equipment and data circuit-terminating equipment. It is commonly used in computer serial ports. The standard defines electrical signal characteristics such as voltage levels, signaling rate, timing and slew-rate of signals, voltage withstand level, short-circuit behavior, and maximum load capacitance, interface mechanical characteristics, pluggable connectors and pin identification. Details of character format and transmission bit rate are controlled by the serial port hardware, often a single integrated circuit called UART that converts data from parallel to asynchronous start-stop serial form. Details of voltage levels, slew rate, and short-circuit behavior are typically controlled by a line-driver that converts from the UART's logic levels to RS-232 compatible signal levels, and a receiver that converts from RS-232 compatible signal levels to the UART's logic levels.

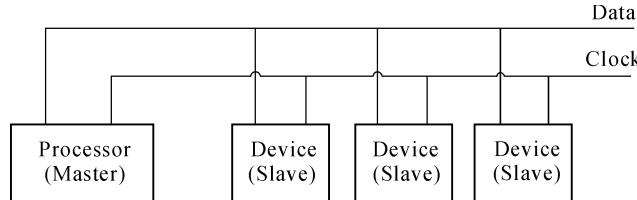
For data transmission lines (Tx<sub>D</sub>, Rx<sub>D</sub> and their secondary channel equivalents) logic one is defined as a negative voltage, and logic zero is positive and the signal condition is termed spacing. Control signals are logically inverted with respect to what one would see on the data transmission lines. When one of these signals is negative, the voltage on the line will be between 3 to 15 V. The active state for these signals would be the opposite voltage condition, between -3 and -15 V. In order to convert TTL level to RS-232 level, a MAX232 chip or another chip with similar function is often be used.

#### 4.7.3.2 I<sup>2</sup>C Bus

The I<sup>2</sup>C bus was introduced by Philips as a standard for connecting integrated circuits (IC) which may or may not include sensors. I<sup>2</sup>C is intended for application in systems which connect microcontrollers and other microcontroller-based devices or parts. It is a two-wire serial bus as shown in Fig. 4.71.

The serial data and serial clock carry information to every device connected to the bus, which has a unique address. The serial data wire is bi-directional but data may flow in only one direction at a certain time. Devices on the bus are defined as masters or slaves. A master, which is usually a microcontroller, initiates a data

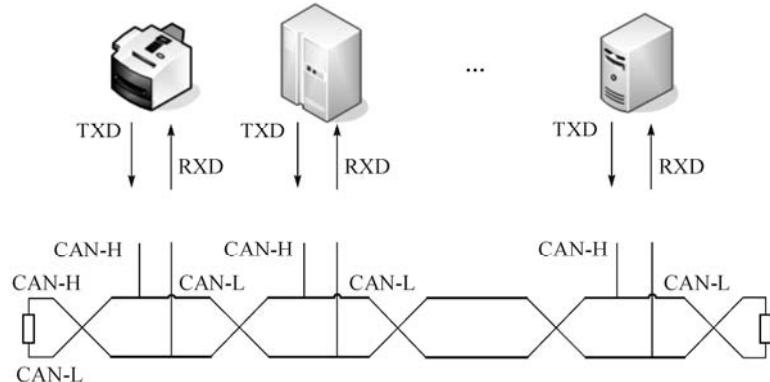
transfer on the bus and generates the clock, and generates the control signals which are placed on the data wire. The slave device is controlled by the master. A slave device can either receive or send data depending on the master. To save energy, some sensor nodes should be in sleep mode most of the time and woken up by a timer or sensing event.



**Fig. 4.71.** I<sup>2</sup>C bus structure

#### 4.7.3.3 CAN Bus

CAN bus is a vehicle bus standard designed to allow microcontrollers and devices to communicate with each other within a vehicle without a host computer, which is showed by Fig. 4.72. CAN bus standard was officially released in 1986 at the Society of Automotive Engineers (SAE) congress in Detroit, Michigan. The first CAN controller chip, which was produced by Intel and Philips, came on the market in the 1980s.



**Fig. 4.72.** CAN bus structure

A modern automobile may have as many as 70 electronic control units (ECU) for various subsystems, including the engine control unit, transmission, airbags, antilock braking, cruise control, audio systems, windows, doors, mirror adjustment, etc. Some of these form independent subsystems, but communications among others are essential. A subsystem may need to control actuators or receive feedback from the sensors. The CAN standard was devised to fill this requirement.

CAN is a multi-master broadcast serial bus standard for connecting electronic control units. The devices that are connected by a CAN network are typically sensors, actuators and control devices. A CAN message never reaches these devices directly, but instead a host processor and a CAN controller are needed between these devices and the bus. If two or more nodes begin sending messages at the same time, the message with the more dominant ID will overwrite other nodes' less dominant IDs, so that eventually only the dominant message remains and is received by all nodes. Each node requires the support from the host processor, CAN controller and transceiver.

#### 4.7.3.4 Serial Peripheral Interface

The serial peripheral interface bus (SPI bus) is a synchronous serial data link standard named by Motorola that operates in full duplex mode. Devices communicate in master/slave mode where the master device initiates the data frame. Multiple slave devices are allowed with individual slave select (chip select) lines. Sometimes SPI is called a “four-wire” serial bus, contrasting with three-, two-, and one-wire serial buses. The SPI bus specifies four logic signals, which are Serial Clock (SC), Master Output/Slave Input (MOSI/SIMO), Master Input/Slave Output (MISO/SOMI) and Slave Select (SS). The SPI bus can operate with a single master device and with one or more slave devices. Most slave devices have tri-state outputs so their MISO signal becomes high impedance when it is not selected. Devices without tri-state outputs cannot share SPI bus segments with other devices. At one time only one slave device could talk to the master with its chip select being activated.

To begin a communication, the master first configures the clock, using a frequency less than the maximum frequency of the slave device. The master then pulls the slave select low for the desired chip. Transmissions may involve any number of clock cycles. When there is no more data to be transmitted, the master stops toggling its clock. Normally, it then deselects the slave device. SPI bus is a full duplex communication standard, and has a higher speed than I<sup>2</sup>C bus mentioned above. SPI bus requires only extra 4 pins in hardware design, so it is much easier for layout design. Furthermore, some chips combine MOSI and MISO into a single data line (SI/SO). Usually it is called three-wire signaling.

#### 4.7.4 Wireless Sensor Network

At the beginning of this chapter, a typical WSN application example was given. With this example and moreover, the senses, technique challenges and wide applications of WSN will be introduced in detail.

#### 4.7.4.1 Typical Application

The early blue-green algae bloom in Taihu Lake (Fig. 4.73), which is the third largest fresh water lake of China, led to the tap water pollution and water supply crisis in May, 2007. It was a typical environmental hazard caused by chemical pollution and biological turbulence. To clarify the pollution status, a WSN monitor project has been carrying out since 2008.



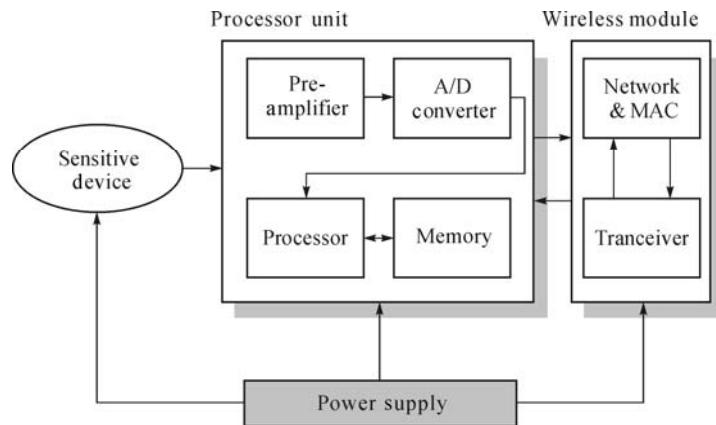
**Fig. 4.73.** The photo of Taihu Lake

#### 4.7.4.2 Typical Structure

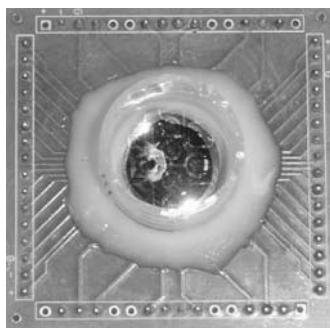
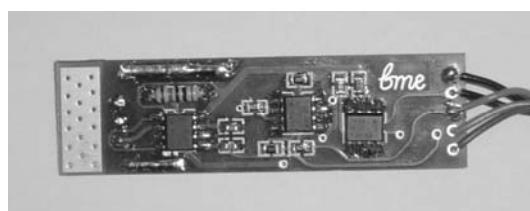
Sensor node and wireless network construction are two essential components of WSN.

##### *Sensor node*

Fig. 4.74 shows the structure of a WSN node, which will be deployed in the Taihu Lake project, focusing on chemical pollution monitoring. A WSN node is composed of sensitive device, processor unit, wireless communication module and power supply unit. The sensitive device detects the specialized information of a certain object. The processor unit receives the signal from the sensitive device, amplifies it if necessary, then converts it into digital data and sends it to the next process. The processor unit also controls the total sensor node as a commander. The wireless communication module answers the communications with the other nodes, sending/receiving data and exchanging information. The power supply unit provides energy to all the parts above. Usually a micro battery or solar energy solution is an advisable choice.

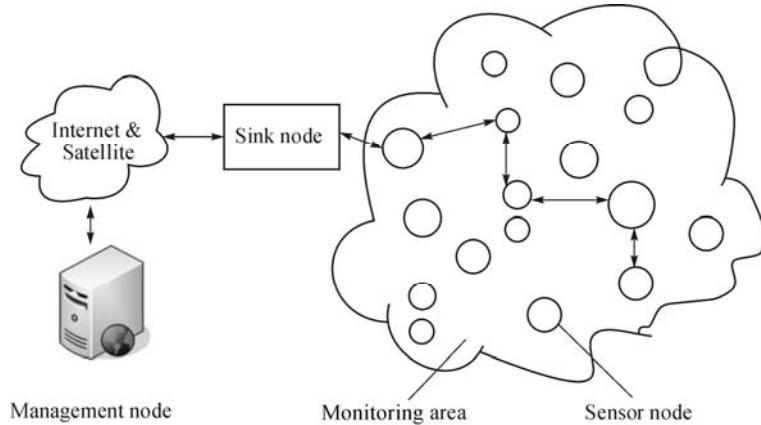
**Fig. 4.74.** Structure of sensor node

Furthermore, Fig. 4.75 shows the complex MEMS sensitive device of the WSN node in the Taihu Lake project. Up to 4 MEA (microelectrode array) and 4 LAPS (Light addressable potentiometer sensor), which all act as chemical sensors, are integrated on the complex chip. Based on different sensitive theories of MEA and LAPS, self-compensation and multi-parameter measurement are standout advantages of the complex device. Fig. 4.76 shows the pre-amplifier unit. Ultra low noise amplifier receives the weak signal from complex device and sends it to next stage after amplifying. The detect limit can be as low as 0.5 nA.

**Fig. 4.75.** MEMS sensitive device**Fig. 4.76.** Preamplifier unit

### Wireless network construction

A typical wireless network contains a sensor node, and sink node and management node. Sensor nodes, which are placed randomly, can locate themselves and send information point by point. A sink node will collect the information from sensor nodes and then re-send it to the management node by internet, satellite, etc. Finally, the research team can get the quantity of information from the management node.



**Fig. 4.77.** Wireless network construction

#### 4.7.4.3 Key Techniques

We now briefly describe three important techniques in WSN.

##### *Power efficiency control*

Many WSNs must aggressively conserve energy in order to operate for extensive periods without wired power sources. Since wireless communication often dominates the energy dissipation in a WSN, several promising approaches have been proposed to achieve power-efficient multi-hop communication in ad hoc networks. Topology control aims to reduce the transmission power by adjusting nodal radio transmission ranges while preserving necessary network properties. Power-aware routing protocols choose appropriate transmission ranges and routes to conserve energy used for multi-hop packet transmission. Both topology control and power-aware routing focus on reducing the power consumption when the radio interface is actively transmitting and receiving packets (Sichitiu, 2004).

##### *Network security*

Because sensor networks pose unique challenges, traditional security techniques used in traditional networks cannot be applied directly. Firstly, to make sensor networks economically viable, sensor devices are limited in their energy, computation,

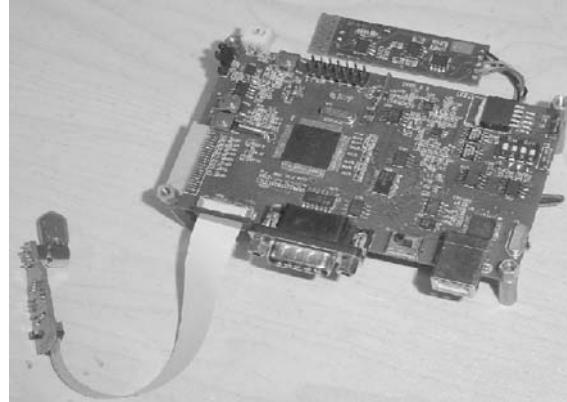
and communication capabilities. Secondly, unlike traditional networks, sensor nodes are often deployed in accessible areas, presenting the added risk of physical attack. And thirdly, sensor networks interact closely with their physical environments and with people, posing new security problems.

An adequate solution is in conjunction with secure group management, intrusion detection and secure data aggregation. First, interest in network data aggregation and analysis can be performed by groups of nodes, and the outcome of the group's computation is normally transmitted to a base station. Then, in order to look for anomalies, applications and typical threat models must be understood, and the use of secure groups may be a promising approach for decentralized intrusion detection. And last, depending on the architecture of the wireless sensor network, aggregation may take place in many places in the network. All aggregation locations must be secured (Perrig et al., 2004).

### ***Relative location estimation***

Self-configuration is a general class of estimation problems which we explore via the Cramer-Rao bound (CRB). Specifically, the sensor location estimation problem is explored for sensors that measure range via received signal strength (RSS) or time-of-arrival (TOA) between themselves and neighboring sensors. TOA ranging has been implemented using two-way or round-trip time-of-arrival measurements. Inquiry-response protocols and careful calibration procedures are presented to allow devices to measure the total delay between an original inquiry and the returned response. Ranging is also possible using RSS measurement, which can be measured from reception of any transmission in the network. In a frequency hopping radio, RSS measurements can be averaged over frequency to reduce frequency-selective fading error. RSS is attractive from the point of view of device complexity, but is traditionally seen as a coarse measure of ranges. Sensor location estimation with about 1 m RMS error has been tested using both TOA and RSS measurements (Meguerdichian et al., 2001).

Fig. 4.78 shows a multi-function sensor/sink WSN node, which is the basic unit of the Taihu Lake project, combined with key techniques mentioned above. With the special low power consumption MCU and related extern circuits. Average work current is lower than 20 mA, and idle mode current is as low as 100  $\mu$ A. Moreover, the relative location estimation program has been planted into the MCU as part of the firmware. At the same time, the network security issue is checked from both the hardware and software view. Using CPLD and FPGA programmed devices, the hardware of the circuit is difficult to be copied. And security arithmetic is integrated in the application software. Further research is even in progress, focusing on simplifying the hardware circuit. The final version node may include only 2 ICs (integrate chips): one is the MCU, including power management and interface module, the other is a complex analog chip, including all analog amplifiers, signal mixers, A/D and D/A converters.



**Fig. 4.78.** Multi-function sensor/sink WSN node

#### 4.7.4.4 Senses and Challenges

Recent advances in micro-electro-mechanical systems (MEMS) technology, wireless communications, and digital electronics have enabled the development of WSNs, which contain low-cost, low-power, multifunctional sensor nodes that are small in size and communicate by wireless media for short distances. These tiny sensor nodes, which consist of sensing, data processing, and communicating components, leverage the idea of sensor networks based on collaborative effort of a large number of nodes. WSN has the potential to revolutionize sensing (and/or actuating) technology in the future. Large numbers of cheap nodes can be placed in the area to be monitored. In contrast to traditional networks, WSN at least has the following advantages (Akyildiz et al., 2002).

The large number ensures that at least some of the sensors will be close to the phenomenon of interest and thus be able to have high quality measurements. In-network processing allows for the tracking of targets and the evolution of the studied phenomena. It also allows for substantial power savings and reduced bandwidth necessary to observe certain phenomena. The large number of sensors also increases the reliability of the system, as failure of a percentage of the sensor nodes will not result in system failure.

*Sensors can be positioned far from the observers.* In this sense, observers do not need to be near the actual position, which may be polluted, dangerous or hard to reach. So there are a wide range of applications envisioned for such sensor networks, including microclimate studies, groundwater contaminant monitoring, precision agriculture, condition-based maintenance of machinery in complex environments, urban disaster prevention and response, and military interests.

*Several sensors that perform only sensing can be deployed.* The positions of the sensors and communications topology are carefully engineered. They transmit time series of the sensed phenomenon to the central nodes where computations are performed and data are processed efficiently.

As a coinstantaneous result, due to its unique characters, WSN is facing some technical challenges, such as energy limitations, communication capacity/power confines, process performances and storage shortages (Chong and Kumar, 2003).

*Micro sensor node is usually powered by small size batteries, whose capacity is limited.* Since there are so many cheap sensor nodes in the target area, which sometimes human cannot reach, so it is very difficult, if not impossible, to change batteries and refresh the sensor notes by human operations. So the service time of sensor nodes is decided by capacity of the batteries and the power consumption of sensor nodes.

A sensor node includes a sensitive unit, processor and wireless communication devices. The consumption of the processor and the sensitive device is reduced with the advance of integrated chipset progress. Most power consumption happens in wireless communication circuits. Such modules have four statuses, sending, receiving, idling and sleeping. It is the essential research and development to find a way to let wireless modules be more efficient and reduce unnecessary power consumption.

*With the increase of communication distance, the power consumption arises accordingly.* Considering that the communication ability of each node is limited and the object area is often large, it is necessary to adopting a multi-point route. The distance of each node should be no more than 100 – 150 m.

The communication bandwidth and RF output power of each sensor node is also limited. The signal of each node may be degraded, or completely destroyed by complex surface circumstances or dreadful weather.

In order to reduce the cost and power consumption of each sensor node, a low-end but power-saving built-in processor is used, with a small size memory device. The sensor node is expected to monitor the object, convert the analog signal to digital data, save/process the data, communicate with other nodes, etc. It is a challenge to complete the missions with limited processor ability and memory size.

Fortunately with the improvement of low consumption chips and IC system design, many ultra low consumption processors are now available. Besides reducing absolute work current, module power supply and dynamic voltage scaling is supported by the new generation processors. When the duty of the processor is light, some unnecessary units of the processor will be closed, and the power voltage and operation frequency may be controlled to a relative lower level. So the processor will not be jammed with heavy duties, and the operation current will be saved in idle time.

#### 4.7.4.5 Forecasts

WSN has a lot of distributed sensor nodes. The concepts of micro-sensing and wireless connection of these nodes promises many new application areas, such as environmental monitors, military applications, etc.

Some environmental applications of sensor networks include tracking the

movements of birds, small animals, and insects; monitoring environmental conditions that affect crops and livestock; irrigation; macro instruments for large-scale Earth monitoring and planetary exploration; chemical and biological detection; precision agriculture; biological and environmental monitoring in marine, soil, and atmospheric contexts; forest fire detection; meteorological or geophysical research; flood detection; bio-complexity mapping of the environment; and pollution study. A pollution monitor project based on WSN is a typical example for such an application.

Furthermore, the rapid deployment, self-organization and fault tolerance characteristics of sensor networks make them a very promising sensing technique for military applications. In chemical and biological warfare, being close to ground zero is important for timely and accurate detection of the agents. Sensor networks deployed in the friendly region and used as a chemical or biological warning system can provide the friendly forces with critical reaction time, which drops casualties drastically. For instance, we can make a nuclear reconnaissance without exposing a team to nuclear radiation.

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## Chapter 5

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### Biosensors and Measurement

In this chapter, the enzyme biosensors and microorganism biosensors are introduced as catalytic biosensors. The antibody and antigen biosensors (immune sensors), nucleic acid biosensors (DNA sensors), as well as receptor and ion-channel sensors are introduced as affinity biosensors. Then, sensors for measurement of cellular metabolism, impedance, and electrophysiology are discussed in cell and tissue biosensors. Finally, some novel techniques such as biochips for microarrays and nanomaterials are also described, including typical sensing applications for biosensors.

#### 5.1 Introduction

The basic concept, most important properties, and the primary bioreceptor components of biosensors are described as the introduction for biosensing and measurement.

##### 5.1.1 *History and Concept of Biosensors*

###### *History*

In 1956, Professor Leland C. Clark, as the father of the biosensor concept, published his definitive paper on the oxygen electrode. Based on this experience and addressing his desire to expand the range of analytes that could be measured in the body, the concept was illustrated by glucose oxidase entrapped in a Clark oxygen electrode using a dialysis membrane (Historically, glucose sensing has dominated the biosensor literature and has delivered huge commercial successes to the field). So, the earliest biosensors were catalytic biosensor systems that integrated enzymes, cellular organelles, tissues or whole microorganisms with

transducers that convert a biological response into a digital electronic signal. The principal transducers used were electrochemical, optical, and thermometric.

The next generation of biosensors, affinity biosensors, capitalized on a similar range of measurement principles but with the addition of piezoelectric transducers (that interconvert mechanical deformation and voltage to measure mass or viscoelastic effects) and magnetic transducers. Affinity biosensors delivered real-time information about the binding of antibodies to antigens, cell receptors to their ligands, and DNA and RNA to nucleic acid with a complementary sequence.

### ***Concept***

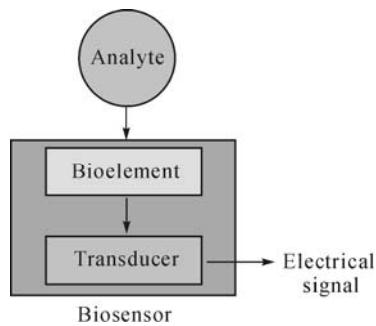
Generally, a biosensor is an analytical device which converts a biological response into an electrical signal. The term “biosensor” is often used to cover sensor devices to determine the concentration of substances and other parameters of biological interest even where they do not utilize a biological system directly. This very broad definition is even used by some scientific journals (e.g., *Biosensors & Bioelectronics*, Elsevier) but will not be applied to the coverage in this chapter.

Professor Anthony P F Turner (editor-in-chief of *Biosensors & Bioelectronics*, Cranfield University) once defined a biosensor as a compact analytical device incorporating a biological or biologically-derived sensing element either integrated within or intimately associated with a physicochemical transducer (Turner, 1996). The usual aim of a biosensor is to produce either discrete or continuous digital electronic signals which are proportional to a single analyte or a related group of analytes. Professor Turner’s name is synonymous with the field of biosensors. Generally, his definition has been used as the concept of biosensors.

Biosensors represent a rapidly expanding field, at the present time, with an estimated growth rate of more than 60% annually. The major impetus comes from the health-care industry (e.g., 6% of the western world population are diabetic and would benefit from the availability of a rapid, accurate and simple biosensor for glucose) but with some pressure from other areas, such as food quality appraisal and environmental monitoring. Research and development in this field are wide and multidisciplinary, spanning from biochemistry, bioreactor science, physical chemistry, electrochemistry, and electronics to software engineering.

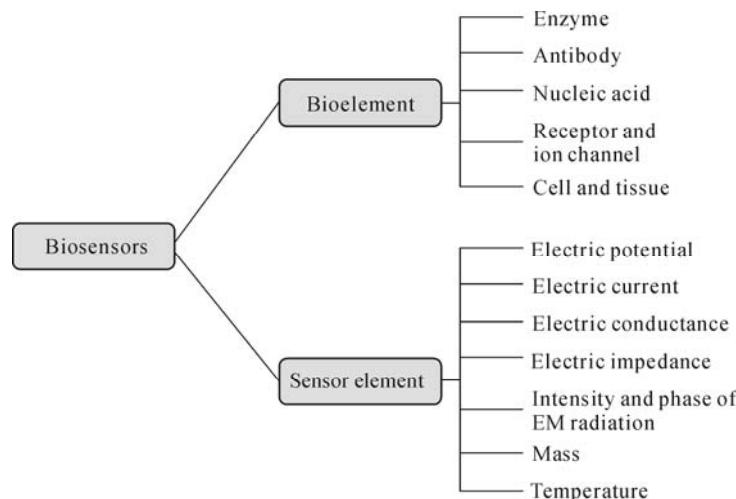
### ***5.1.2 Components of a Biosensor***

A biosensor generally consists of a biological sensing element, such as an enzyme, antibody or cell, in close contact with a physico-chemical transducer, such as an electrode or optical fiber. Measurement of the target analyte(s) is achieved by selective transduction of a parameter of the biomolecule-analyte reaction into a quantifiable electrical or optical signal (Fig. 5.1). The key part of a biosensor is the transducer which makes use of a physical change accompanying the reaction (Mohanty and Kougianos, 2006).



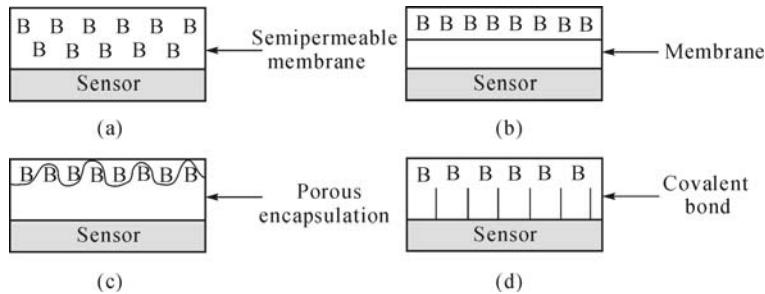
**Fig. 5.1.** A schematic representation of biosensors

A detailed list of differently applied bioelements and sensor-elements is shown in Fig. 5.2. Different combinations of bioelements and sensor-elements constitute several types of biosensors to suit a vast pool of applications (Mohanty and Kougianos, 2006).



**Fig. 5.2.** Elements of biosensors

The high specificity of biomolecules and biological systems with respect to intermolecular interactions of interest can be successfully exploited in biosensor devices only if there is highly efficient coupling between the biological and transducer components. The bio and the sensor elements can be coupled together in one of the four possible ways as illustrated in Fig. 5.3: membrane entrapment, physical adsorption, matrix entrapment, and covalent bonding (Mohanty and Kougianos, 2006).



**Fig. 5.3.** In biomaterial-sensor coupling, the bio and sensor elements can be coupled together in one of four ways: (a) Membrane entrapment; (b) Physical adsorption; (c) Matrix entrapment, and (d) Covalent bonding (reprinted from (Mohanty and Kougianos, 2006), Copyright 2006, with permission from IEEE)

In the membrane entrapment scheme, a semi-permeable membrane separates the analyte and the bioelement; the sensor is attached to the bioelement. The physical adsorption scheme is dependent on a combination of van der Waals forces, hydrophobic forces, hydrogen bonds, and ionic forces to attach the biomaterial to the surface of the sensor. The porous entrapment scheme is based on forming a porous encapsulation matrix around the biological material that helps in binding it to the sensor. In the case of the covalent bonding, the sensor surface is treated as a reactive group to which the biological materials can bind.

### 5.1.3 Properties of Biosensors

The two most important properties of any proposed biosensor are: (a) its specificity and (b) its sensitivity towards the target analyte(s) (Byfield and Abuknesha, 1994). The specificity of a biosensor is entirely governed by the properties of the biological component because this is where the analyte interacts with the sensor. The sensitivity of the integrated device, however, is dependent on both the biological component and the transducer because there must be a significant biomolecule-analyte interaction and a high efficiency of subsequent detection of this reaction by the transducer.

In comparison with chemical sensors, an inherent advantage that can be exploited in biosensor technology is the significantly higher specificity that can generally be achieved as a direct result of biologically-optimized molecular recognition. This is best typified by an antibody-antigen interaction (the antigen is the substance against which the antibody has been generated *in vivo*) where an antibody can recognize and bind its antigen with extremely high specificity. Minor chemical modification of the molecular structure of the antigen can dramatically lower its affinity for the original antibody (which has low cross reactivity). Similarly, enzymes such as glucose oxidase will recognize their natural substrate (glucose in this case) with a far higher affinity than other components in the

operating environment. Chemically similar molecules, in this case other small sugars, can elicit a small amount of cross-reactivity if present in sufficiently high concentrations. The level of specificity and, in many cases, sensitivity of detection and measurement that has been achieved to date in biosensors far exceeds that obtained for almost all chemical sensors.

In addition, arguably the most obvious disadvantage in exploiting the exquisite specificity and sensitivity of complex biological molecules is their inherent instability. Many strategies may be employed to restrain or modify the structure of biological receptors to enhance their longevity. So, stability is also a very important issue for biosensors.

#### **5.1.4 Common Bioreceptor Components**

The primary bioreceptor components can be classified into five groups.

##### ***Enzymes***

Enzymes are proteins that catalyze specific chemical reactions. These can be used in a purified form or be present in a microorganism or in a slice of intact tissue.

##### ***Antibodies and antigens***

An antigen is a molecule that triggers the immune response of an organism to produce an antibody, a glycoprotein produced by lymphocyte B cells, which will specifically recognize the antigen that stimulated its production.

##### ***Nucleic acids***

The recognition process is based on the complementary nature of the base pairs (adenine and thymine or cytosine and guanine) of adjacent strands in the double helix of DNA. These sensors are usually known as genosensors. Alternatively, interaction of small pollutants with DNA can generate the recognition signal.

##### ***Cellular structures or whole cells***

The whole microorganism or a specific cellular component (receptors), for example, a non-catalytic receptor protein, ion channels and lipid membrane, is used as the biorecognition element.

##### ***Other biomimetic receptors***

Recognition is achieved by use of receptors, for instance, genetically engineered molecules, artificial membranes, or molecularly imprinted polymers (MIP), that mimic a bioreceptor or ion channels. Those bioreceptors will not be discussed in this chapter.

Biosensors are typically classified by the above type of recognition element or transduction element employed (Turner, 2000). A sensor might be described as a catalytic biosensor if its recognition element comprised an enzyme or series of enzymes, a living tissue slice (vegetal or animal), or whole cells derived from microorganisms such as bacteria, fungi, or yeast. However, the sensor might be described as a bioaffinity sensor if the basis of its operation is a biospecific complex formation. Accordingly, the reaction of an antibody with an antigen or hapten, or the ligand with a receptor, could be employed.

## 5.2 Catalytic Biosensors

The most commonly used biological components in catalytic biosensors are enzymes. There is currently a wide selection range of enzymes available; especially, microorganisms which also take advantage of the complex enzyme systems, coenzyme, and all the physiological functions supplied by microorganism themselves for catalytic biosensors. Therefore, the enzyme biosensors and microorganism biosensors are described with some commercial applications.

### 5.2.1 Enzyme Biosensors

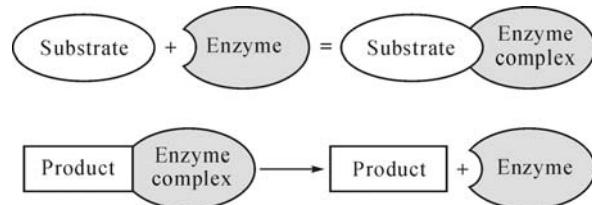
Enzyme biosensors utilize one or more enzyme types as the macromolecular binding agents and take advantage of the complementary shape of the selected enzyme and the targeted analyte. Enzymes are proteins that perform most of the catalytic work in biological systems and are known for highly specific catalysis. The shape and reactivity of a given enzyme limit its catalytic activity to a very small number of possible substrates. Enzymes are also known for rapidness, working at rates as high as 10,000 conversions per second per enzyme molecule. Enzyme biosensors rely on the specific chemical changes related to the enzyme-analyte interaction as the means for determining the presence of the targeted analyte. For example, upon interaction with an analyte, an enzyme may generate electrons, a colored chromophore or a change in pH (due to release of protons) as the result of the relevant catalytic enzymatic reaction. Alternatively, upon interaction with an analyte, an enzyme may cause a change in a fluorescent or chemiluminescent signal that can be recorded by an appropriate detection system.

#### 5.2.1.1 Enzyme

Enzymes are proteins (polypeptide structures) whose catalyse specific chemical

reactions are *in vivo*. They accelerate the rate of reaction of a particular chemical (the substrate) without being consumed in the process.

Fig. 5.4 shows the working principle of enzymes. An enzyme, upon reaction with a substrate, forms a complex molecule that, under appropriate conditions, forms the desirable product molecule releasing the enzyme at the end (Mohanty and Kougianos, 2006).



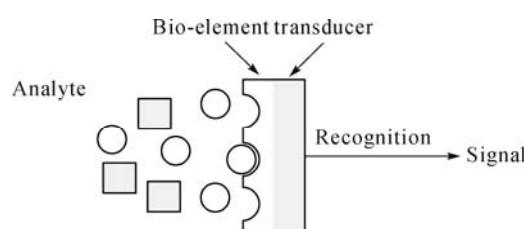
**Fig. 5.4.** Working principle of enzymes

Compared with chemical catalysts, enzymes demonstrate a significantly greater level of substrate specificity, primarily because of the constraints placed on the substrate molecule by the active site in the environment involving factors such as molecular size, stereochemistry, polarity, functional groups and relative bond energies.

The sensitivity of enzyme-based biosensors is directly dependent on the maximum limiting affinity underlying enzyme-substrate complication and the rate of subsequent transformation. And, after being isolated and incorporated into biosensor systems, the decrease in activity of enzymes can be minimized by suitable pH, temperature and other environment conditions. Enzyme immobilization techniques, such as adsorption, cross-linking, covalent bonding, physical entrapment, electrochemical polymerization and self-assembled monolayer, have well preserved the activity of lots of enzymes for many potential applications.

### 5.2.1.2 Enzyme Sensors

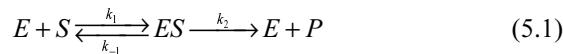
The enzymes are extremely specific in their action: enzyme *X* will change a specific substance *A*, not *C*, to another specific substance *B*, (not *D*), as illustrated in Fig. 5.5. This extremely specific action of the enzymes is the basis of biosensors.



**Fig. 5.5.** Specificity of enzymes is the basis of enzyme sensors

The mechanisms of operation of enzyme-based biosensors can involve: (a) conversion of the analyte into a sensor-detectable product, (b) detection of an analyte that acts as enzyme inhibitor or activator, or (c) evaluation of the modification of enzyme properties upon interaction with the analyte.

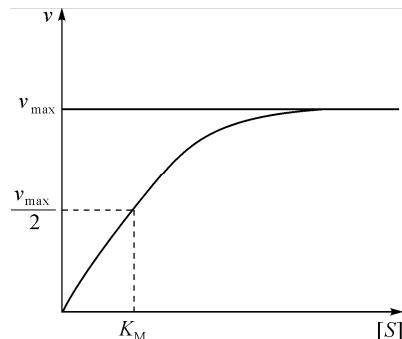
The relationships between the parameters of relevance in enzyme catalysis are given by Michaelis-Menten analysis as shown in the following enzyme-catalyzed reaction (Byfield and Abuknesha, 1994):



where  $E$  is the enzyme,  $S$  is the substrate,  $P$  is the product(s),  $k_1$  and  $k_{-1}$  are the forward and reverse rate constants for formation of the enzyme-substrate intermediate, and  $k_2$  is the rate constant for formation of product(s). The second part of the reaction can be considered as irreversible at the beginning of the reaction, when the concentration of product is significantly lower than the substrate concentration. In many reactions the *in vivo* and *in vitro* concentration of the enzyme is much lower than the concentration of the substrate. In this case, all enzyme molecules are involved in the catalysis, or they will become saturated. The Michaelis-Menten equation relates the initial reaction rate  $v_0$  to the substrate concentration  $[S]$ .

$$v_0 = \frac{v_{\max}[S]}{K_M + [S]} \quad (5.2)$$

Fig. 5.6 is the corresponding graph of a hyperbolic function. The maximum velocity is described as  $v_{\max}$ .  $K_M$  is Michaelis constant (Note that often the experimental parameter  $k_{\text{cat}}$  is used, but in simple cases this parameter equal to the kinetic parameter  $k_2$  for the elementary reaction from  $[ES]$  to  $E + P$ ).



**Fig. 5.6.** Michaelis-Menten plot relating the reaction rate  $v_0$  to the substrate concentration  $[S]$

In the medical diagnostic field, several manufacturers have marketed biosensors for the measurement of common blood chemistry components including glucose, urea, lactate, and creatinine. In general, enzyme-based biosensors employ semi-

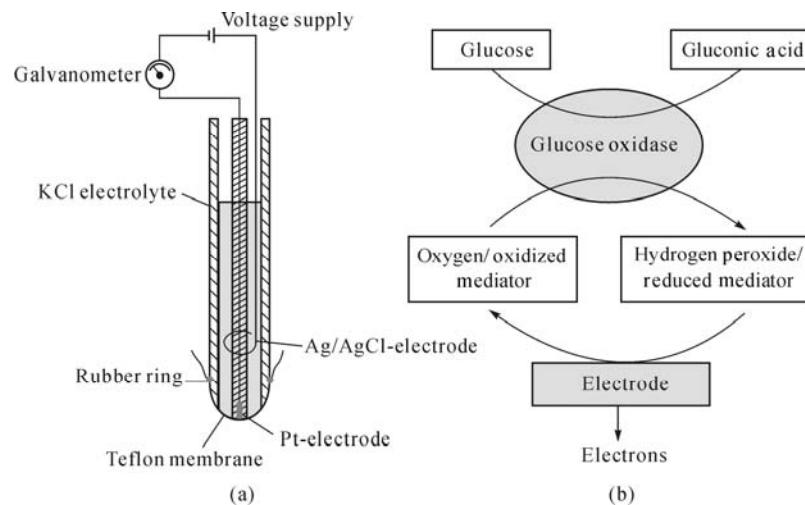
permeable membranes through which target analytes diffuse toward a solid-phase immobilized enzyme compartment. The major drawback of this type of sensor is that many enzymes are inherently unstable, necessitating a packaging approach to limit degradation of the biosensor performance.

### 5.2.1.3 Clark Oxygen Electrode Sensor

The most commercially successful biosensors are amperometric glucose biosensors. These biosensors have been made available in the market in various shapes and forms, such as glucose pens and glucose displays. The first historic experiment that served as the origin of glucose biosensors was carried out by Leland C. Clark. He used platinum (Pt) electrodes to detect oxygen (Fig. 5.7).

The Clark-type electrode is the most widely used oxygen sensor for measuring oxygen dissolved in a liquid (Mohanty and Kougianos, 2006). The basic principle is that there is a cathode and an anode submersed in an electrolyte. Oxygen enters the sensor through a permeable membrane by diffusion, and is reduced at the cathode, creating a measurable electrical current.

Electron flow to oxygen as a result of oxidative phosphorylation can be demonstrated using an oxygen electrode. The electrode compartment is isolated from the reaction chamber by a thin Teflon membrane. The membrane is permeable to molecular oxygen and allows this gas to reach the cathode, where it is electrolytically reduced. The reduction allows a current to flow. This creates a potential difference which is recorded on a flatbed chart recorder. The trace is thus a measurement of the oxygen activity of the reaction mixture. The current flowing is proportional to the activity of oxygen provided the solution is stirred constantly (stir bar) to minimize the formation of an unstirred layer next to the membrane.



**Fig. 5.7.** Clark electrode for glucose detecting: (a) Clark-type electrode; (b) Glucose detecting

The enzyme glucose oxidase (GOD) was placed very close to the surface of platinum by physically trapping it against the electrodes with a piece of dialysis membrane. The enzyme activity changes depend on the surrounding oxygen concentration. Fig. 5.7 also shows the reaction catalyzed by GOD. Glucose reacts with GOD to form gluconic acid while producing two electrons, and two protons, thus reducing GOD. The reduced GOD, surrounding oxygen, electrons, and protons (produced above) react to form hydrogen peroxide and oxidized GOD, which is in the original form. This GOD can again react with more glucose. The more the glucose content, the more oxygen is consumed. On the other hand, less glucose content results in less hydrogen peroxide. Hence, either the consumption of oxygen or the production of hydrogen peroxide can be detected with the help of platinum electrodes, and this can serve as a measurement for glucose concentration.

With oxygen electrodes or hydrogen peroxide electrodes and whether transfer mediators are used or not, glucose enzyme electrodes can be classified into oxygen electrode glucose sensors, hydrogen peroxide electrode glucose sensors, oxidized mediator glucose sensors, and direct electrochemical glucose sensors. Of course, Clark oxygen electrodes also have been well used for microorganism based biosensors and immune biosensors, which will be presented subsequently in this chapter.

### **5.2.2 Microorganism Biosensors**

Many enzymes are extracted from the microorganism cells. In 1977, GA Rechnitz et al., proposed the direct use of microorganism cells as recognition elements for biosensors to avoid separation and purification of the enzymes. There is not only the problem of high cost, but also the reduction and even the loss of enzyme activities in the separating and purifying process. Compared to immobilized enzyme sensors, those novel microorganism sensors also take advantage of complex enzyme systems, coenzyme and all the physiological functions supplied by microorganism themselves.

#### **5.2.2.1 Microorganism**

A microorganism is an organism that is microscopic (usually too small to be seen by the naked eye). Microorganisms are very diverse. They include bacteria, fungi, archaea, and protists; microscopic plants (called green algae); and animals such as plankton and the planarian. Microorganisms live in all parts of the biosphere where there is liquid water. Microorganisms are critical to nutrient recycling in ecosystems as they act as decomposers. Microorganisms are also exploited by people in biotechnology, both in traditional food and beverage preparation, and in modern technologies based on genetic engineering. However, pathogenic microbes are harmful, since they invade and grow within other organisms, causing diseases

that kill millions of people, other animals, and plants.

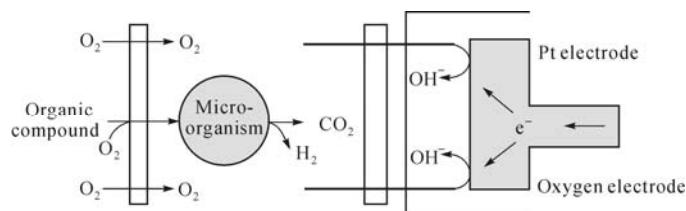
The structure of a microorganism sensor is immobilizing microbial cell membranes onto sensors, which is just like the enzyme membrane immobilized onto the enzyme sensors. The methods of immobilizing microorganisms are basically the same as those of immobilizing enzymes. Therefore, the methods of adsorption, embedding, cross-linking or covalent combining can also be used. Currently, the commonly used method is making microorganisms adhere to the membranes, such as a cellulose acetate membrane, is filtering paper or nylon membrane by centrifugation, filtration or combined culturing. Such a method of embedding microorganisms has high sensitivity.

### 5.2.2.2 Microorganism Sensors

Microorganism pathways are activated by some analytes, such as pollutants. These pathways are involved in metabolism or nonspecific cell stress that results in the expression of one or more genes. For example, immobilized yeast is one of the most commonly used sensors. It has been used in the detection of formaldehyde and toxicity measurements of cholic acids. The changes in metabolism indicative of the analyte were detected via O<sub>2</sub> electrode measurements or extracellular acidification rates.

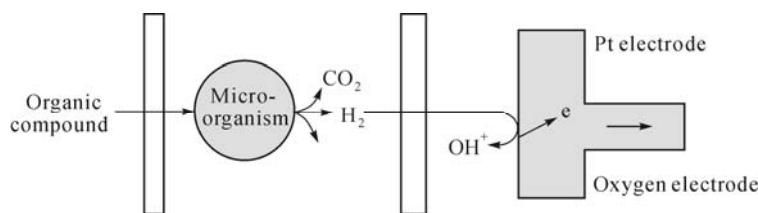
In the electrochemical detecting system, the cultured (or immobilized) microorganism was covered appropriately on the surface of the corresponding electrochemical sensor. It uses the selective catalytic reactions of enzyme in cells, such as hydrolysis, ammonolysis, or oxidation, as well as the selective detecting reactants of electrochemical sensor elements to measure the information of substrate.

Respiratory activity microorganism sensors consist of the aerobic microorganism immobilized membrane and oxygen electrodes, based on the activity of microbial respiration (Fig. 5.8). When the sensor is inserted in the test solution with dissolved oxygen maintained, the organic compounds in the test solution are assimilated by microorganisms. The microbial respiration enhancement leads to the reduction of diffusion oxygen on the electrode and the sharp drop of current value. Finally, the oxygen diffusion rate in the solution and the oxygen consumption rate of microorganism achieve a balance. When the oxygen spreading to the electrode tends to be constant, a constant current value can be obtained, which has correlation with the concentration of organic compounds in the tested solution.



**Fig. 5.8.** The respiratory activity microorganism sensors

The measurement of metabolic microorganism microbial sensors is based on microbial metabolic activities (Fig. 5.9). When microorganisms take organic compounds and generate a variety of metabolites containing electrode active materials, the ammeter can measure the hydrogen, formic acid, a variety of NADPH and other metabolites, while the electricity meter can measure  $\text{CO}_2$ , organic acids ( $\text{H}^+$ ) and other metabolites. From this we can get the concentration information of organic compounds.



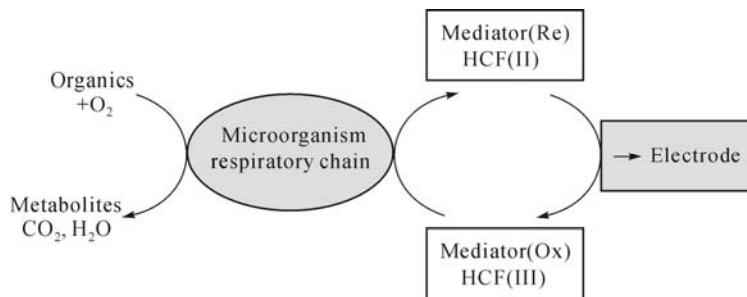
**Fig. 5.9.** The measurement of metabolic activity microorganism sensors

### 5.2.2.3 Microorganism Sensors for BOD

One of the areas where microbial biosensors are widely used is in environmental treatment processes. This is done by detecting the biochemical oxygen on demand (BOD). Most of the BOD sensors consist of a synthetic membrane with immobilized microorganisms as the biological recognition element. The bio-oxidation process is registered in most cases by means of a dissolved  $\text{O}_2$  electrode. A wide variety of microorganisms have been screened during the construction of BOD sensors.

Recently, it has been shown that certain redox-active substances could be reduced by certain microorganisms (Yoshida et al., 2000). It can serve as electron shuttling between microorganisms and electrodes. These mediators have been applied to the fabrication of microbial fuel cells and to microbial detection. It has been suggested that reduction of the redox mediator, rather than oxygen, is due to metabolic reactions of microorganisms. Thereafter, instead of oxygen, potassium hexacyanoferrate(III) [HCF(III)] has been used as an electroactive compound for the development of amperometric biosensors using microorganisms. Fig. 5.10 shows the principle of the amperometric microbial sensor using HCF(III). Usually, organic substances are oxidized by microorganisms during aerobic respiration. However, when HCF(III) is present in the reaction medium, it acts as an electron acceptor and is preferentially reduced to HCF(II) during the metabolic oxidation of organic substances. The reduced HCF(III) is then reoxidized at a working electrode (anode) which is held at a sufficiently high electric potential. As a result, a current is generated and detected using the electrode system.

The microbial strains selected are chosen for their ability to assimilate a suitable spectrum of substrates. BOD sensors based on a pure culture have the advantages of relatively good stability and longer sensor lifetime, but are restricted by their limited detection capacity for a wide spectrum of substrates.



**Fig. 5.10.** Principle of the amperometric-mediated BOD biosensor

### 5.3 Affinity Biosensors

Affinity biosensors depend on an essentially irreversible binding of the target molecules (e.g., affinity sensors based on antibodies, nucleic acids, or receptors). The primary sensing event does not result from catalysis (e.g., enzymes or microorganisms).

#### 5.3.1 Antibody and Antigen Biosensors

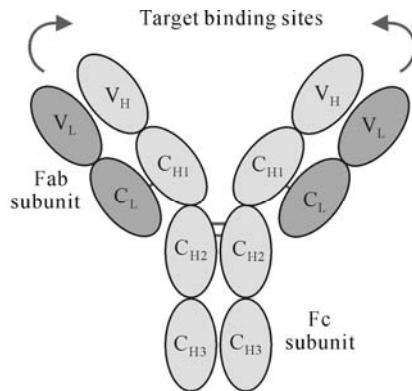
Immunosensors transduce antigen-antibody interactions directly into physical signals. The design and preparation of an optimum interface between the biocomponents and the detector material are the key parts of immunoSENSOR development. Recently, lots of novel investigated sensing techniques have greatly improved the detection schemes.

##### 5.3.1.1 Antibody and Antigen

Antibodies are serum proteins which are produced by B lymphocytes and plasma cells, in response to a foreign (non-self) substance (Byfield and Abuknesha, 1994). The foreign substance is termed an immunogen, so-defined because it evokes an immune response. With very high affinity constant and low cross-reactivity, the antigen-antibody reaction may therefore be regarded as highly specific.

Antibodies consist of four polypeptide sub-units comprising two heavy chains (H chains) and two light chains (L chains) (Fig. 5.11). The carbohydrate residues in antibodies are covalently bonded to the C-terminal half (Fc). The key portions of the antibody molecules that contain the antigen binding sites are termed the Fab fragments; each Fab fragment comprises an entire light chain and a segment of the heavy chain. An antigen (Ag) will interact with an antibody (Ab) raised to one of

its antigenic determinants (portion of structure to which antibodies are produced) with a high binding affinity. The strength of the interaction is dependent on the complementarities of the fit of the antigenic determinant to the binding site of the antibody. The binding forces present in the Ag-Ab complex are non-covalent forces, such as electrostatic attraction (major contribution) hydrogen bonding, hydrophobic bonding and Van der Waals forces.



**Fig. 5.11.** Structure of an antibody showing two identical heavy chains (V<sub>H</sub> - C<sub>H1</sub> - C<sub>H2</sub> - C<sub>H3</sub>) and two identical light chains (V<sub>L</sub>-CL). The locations of the antigen binding sites are shown

A very important step in the preparation of immunoassay biosensor is to immobilize antibodies or antigens on the sensor surface, so as to detect the corresponding antigen or antibody. Besides common methods of immobilizing bio-recognition elements, there are biotin-avidin system (BAS), self-assembled monolayer (SAM), glutaraldehyde cross-linking method, protein A and other indirect fixation methods can be used in the preparation of immune sensors with well immobilizing efficiency, fixed-layer adaptability and reacting sensitivity.

### 5.3.1.2 Immunosensor

The high specificity and high sensitivity which typifies an antigen-antibody reaction (Ag-Ab) has been used in a vast range of laboratory-based tests that incorporate an antibody component (immunoassay). The analytes detected and measured have included many medical diagnostic molecules such as hormones (for example, pregnancy related, steroids), clinical disease markers, drugs (therapeutic and abused), bacteria, and environmental pollutants such as pesticides. There is a crucial distinction to be made, between an immunoassay and an immunosensor, which is the technology relevant to this analysis.

The affinity of an antigen for a corresponding antibody, which is a measurement of the strength of the binding forces in the resultant Ag-Ab complex, is expressed as a molar association constant ( $K_a$ ) detailed in Eq. (5.4) for a reaction scheme shown in Eq. (5.3).



$$K_a = \frac{[\text{Ag-Ab}]}{[\text{Ag}][\text{Ab}]} = \frac{k_f}{k_r} \quad (5.4)$$

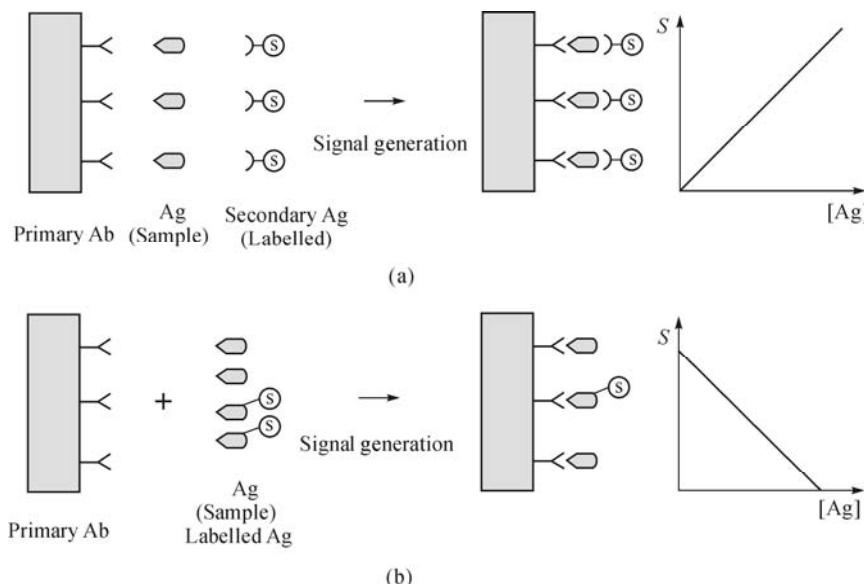
where  $k_f$  is the forward rate constant (for complex formation) and  $k_r$  is the reverse rate constant for dissociation of the antigen away from the antibody. The vast majority of antigen-antibody interactions under conventional pH, temperature and buffer conditions,  $k_f >> k_r$ , gives a very high value of  $K_a$ . Values of  $K_a$  up to  $10^{15}$  mol/L have been measured. For comparison, the majority of enzymatic  $K_m$  values, or protein affinities, are in the range  $10^3 - 10^6$  mol/L, while binding constants for typical chemical binding reactions are usually lower still. The much greater magnitude of  $k_f$  relative to  $k_r$  for Ag-Ab interactions is a consequence of the highly optimized, multi-site attractive forces between Ag and the binding pocket of the Ab.

An immunoassay is a laboratory-based multi step diagnostic test which is based on the recognition and binding of the analyte by an antibody. The two major types of immunoassay are shown in Fig. 5.12 and are known as (a) sandwich assay (non-competitive assay), and (b) competitive assay (Byfield and Abuknesha, 1994). Both are based on a solid-phase immobilization matrix. It can be seen in both cases that the use of a specific and high affinity Ab-Ag binding reaction to detect and measure the concentration of an analyte (the Ag) requires the presence of a signal-generating tracer in immunoassay formats. A tracer is therefore used to generate a signal (for example, optical, electrochemical, radioisotopic decay) which enables quantification of the amount of bound Ag relative to unbound Ag. The most common types of tracers are fluorescent molecules (fluoro-immunoassay (FIA)), enzymes (enzyme-linked immunosorbent assay (ELISA)) or radio-isotopes (radio-immunoassay (RIA)).

In contrast to immunoassays, the aim of immunosensor research is essentially to develop more simple antibody-based diagnostic tests, which combine the great specificity and sensitivity of laboratory-based immunoassays, with the much greater versatility with respect to operating environment offered by enzyme biosensors or chemical sensors. Direct (-acting) immunosensors therefore are those in which analyte quantification is carried out by direct detection and measurement of the Ag-Ab binding reaction, where generally either Ag or Ab is immobilized on a surface prior to sample addition. The binding of Ag to immobilized Ab, or vice versa, is accompanied by a small amount of conformational perturbation of the Ab polypeptide structure and alteration in localized electrostatic interactions.

In the study of electrochemical immune sensors, compared to non-labeled immunoassay sensors, the labeled immune sensors have more practical applications at present, and a number of enzyme immune sensors have been applied in clinical research with the biochemical amplification based on marked enzyme catalyzing to its substrate. These types of sensors require fewer samples,

generally several microlitre to a few tens of microlitre. They have high sensitivity, good selectivity and can be used as a routine method. The disadvantages are the requirement of adding markers and the complicated operation process.



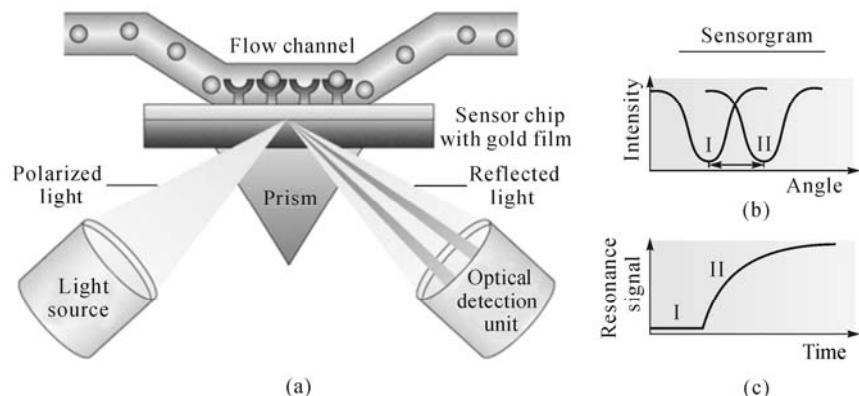
**Fig. 5.12.** The two main types of immunoassay: (a) Sandwich assay (non-competitive); (b) Competitive assay. The signal is proportional to analyte concentration in the former configuration, and inversely proportional in the latter

### 5.3.1.3 SPR-based Immunosensor

Conventional chemical analytical techniques such as optical spectroscopy, nuclear magnetic resonance, or electrochemical measurements are largely incapable of resolving the small change in signals above background noise or non-specific binding of other components in the sample. Interest has tended to focus on alternative methods of transducing changes in physicochemical properties of the solution-surface interface, such as refractive index and dielectric constant. The surface plasmon resonance (SPR) based biosensor systems (i.e., BIAcore<sup>TM</sup>, AutoLAB<sup>TM</sup>), which have developed and are commercially available, represents a significant breakthrough in molecular sensor, particularly used in immunosensor techniques (Fig. 5.13).

SPR analysis has shown promise in providing direct measurement of Ab-Ag interactions occurring at the surface-solution interface (Cooper, 2002). SPR is a phenomenon which occurs when a beam of light is directed onto a glass-metal interface usually a glass prism on a gold or silver metal layer. At a specific angle (the resonance angle), a component of the electromagnetic lightwave propagates in the metal along the plane of the interface in the form of surface plasmons. The

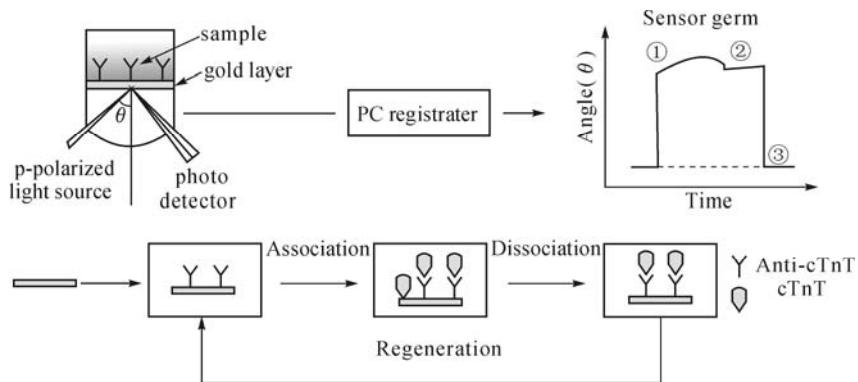
resonance angle is sensitive to changes in refractive index and dielectric constant at the interface up to a distance of  $-1000$  nm from the actual metal surface, with an exponential fall in sensitivity with distance from the surface. Immobilization of an Ab on the surface causes a measurable shift in the resonance angle; on binding of Ag to the immobilized Ab, a further change will occur. This binding-induced shift in resonance angle (expressed as resonance units, RU) is approximately linearly proportional to concentration of bound Ag (or Ab, if Ag is preimmobilized) for typical biological systems.



**Fig. 5.13.** SPR detects changes in the refractive index in the immediate vicinity of the surface layer of a sensor chip. SPR is observed as a sharp shadow in the reflected light from the surface at an angle that is dependent on the mass of material at the surface: (a). The SPR angle shifts (from I to II in the lower left-hand diagram) when biomolecules bind to the surface and change the mass of the surface layer (b). This change in resonant angle can be monitored non-invasively in real time as a plot of resonance signal (proportional to mass change) versus time (c) (reprinted from Cooper, 2002), Copyright 2002, with permission from Nature Publishing Group)

The AutoLAB<sup>TM</sup> system shows considerable promise in enabling determination of low concentrations of important clinical substances in whole serum samples (Dutra and Kubota, 2007). Determination of cTnT (a cardiospecific highly sensitive marker for myocardial damage and is immediately released to bloodstream during the acute myocardial infarction (AMI)) with a detection limit of 0.01 ng/mL in 5 min was achieved (Fig. 5.14). It was possible to measure the cTnT without dilution of the human serum with good specificity and reproducibility.

With the development of SPR, it has been increasingly used in many types of biosensors. It can be used to detect the dynamic relationship of the biomolecular combination, the structure and function of biomolecular. The BIA of BIAcore<sup>TM</sup> from the General Electric Company is the abbreviation of “Biomolecular Interaction Analysis”, which just means that SPR is able to provide real-time observation of biomolecular interaction. Therefore, in the study of DNA sensors, enzyme sensors, and even the cell biosensors, SPR sensors have all played an important role.



**Fig. 5.14.** Schematic representation of the principle of SPR immunosensor for cTnT determinations (reprinted from (Dutra and Kubota, 2007), Copyright 2007, with permission from Elsevier Science B.V.)

### 5.3.1.4 QCM-based Immunosensor

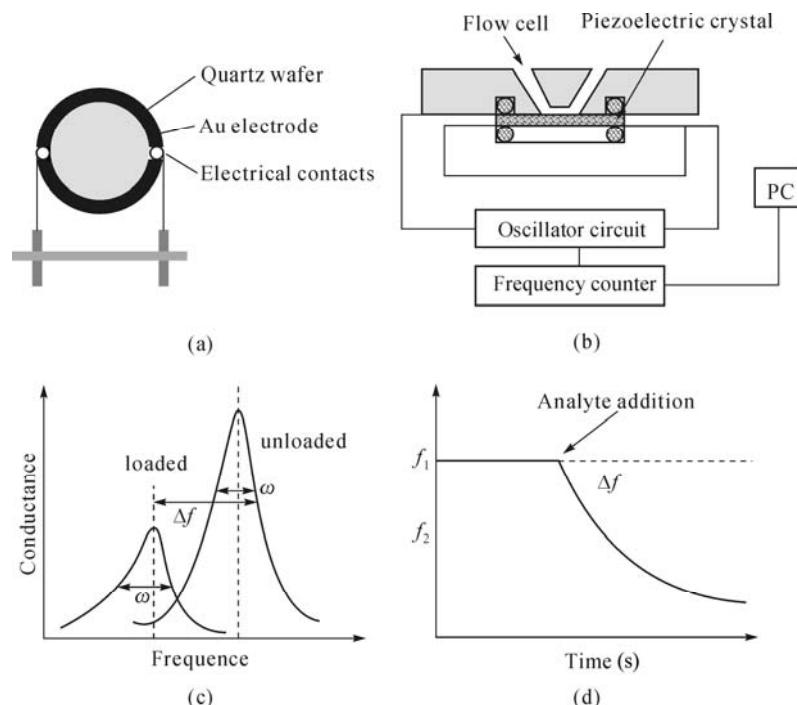
Piezoelectric sensors are made from the characteristics of piezoelectric effect. The piezoelectric quartz crystal sensor has gained good business development (such as the commercialization of the Sweden Q-sense™ series) based on the wide application of biosensors. As the sensor is based on the principal theories of close relationship between the resonant frequency change of piezoelectric quartz crystal and the change of crystal surface mass loading, such piezoelectric sensors are often called quartz crystal microbalance (QCM) (Fig. 5.15).

As a sensitive surface mass sensor, QCM has been extensively applied as transducer in biosensing (Carmon et al., 2005). A QCM (i.e., commercial Q-sense AB™) sensor utilizes an AT-cut piezoelectric quartz crystal film with gold electrodes deposited on both surfaces. Application of a radio frequency voltage at the resonant frequency of the crystal excites the crystal into oscillation. The principle of QCM detection is based on the frequency changes of the crystal that is proportional to the mass changes on the crystal surface. The details about QCM can be found in Chapter 3 sensors based on piezoelectric resonators. The quantitative relationship between the frequency shifts and the mass changes of the crystal is given by the well-known Sauerbrey equation:

$$\Delta f = -\frac{2f^2}{\sqrt{\rho_q \mu_q}} \Delta m = -C_f \Delta m \quad (5.5)$$

where  $\Delta f$  is the frequency shift resulting from the additional mass per area ( $\Delta m$ ),  $f$  is the intrinsic crystal frequency,  $\rho_q$  is the density of the quartz, and  $\mu_q$  is the shear modulus of the quartz film. For a 5 MHz crystal,  $C_f = 56.5 \text{ Hz} \cdot \text{cm}^2/\mu\text{g}$ . When the added mass is not a rigid solid, as with many biological samples (i.e., antibody

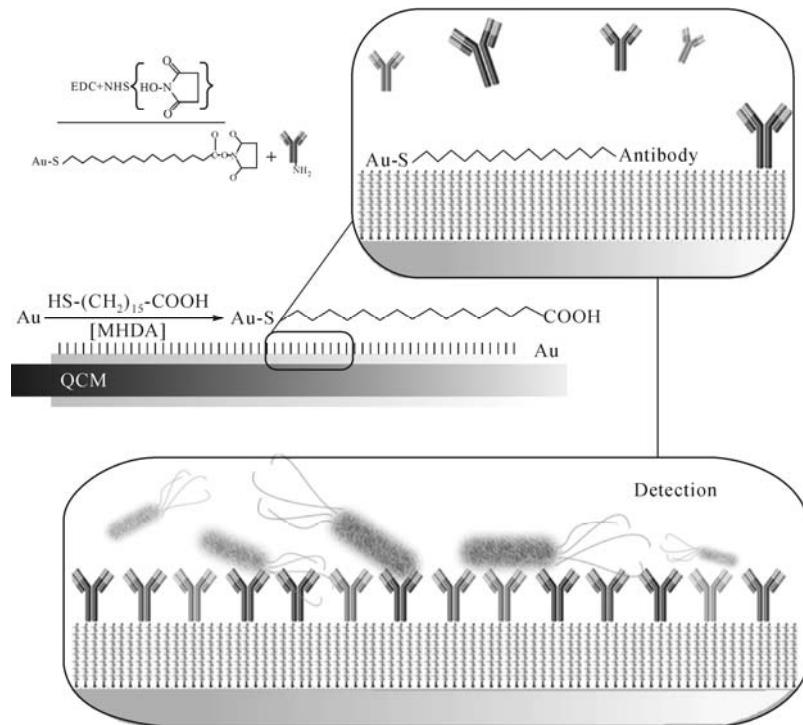
or nucleic acid), the frequency response is damped, resulting in a frequency shift that is less than predicted using Sauerbrey equation. Recent papers have addressed this deviation from the Sauerbrey equation with efforts to account for the viscoelastic properties of the film on the surface. This expands the versatility of the QCM methodology and enables application of the technology to biological systems. Using QCM sensing, with an appropriate antibody immobilized on the sensor surface, it is possible to detect a specific antigen in solution.



**Fig. 5.15.** QCM based sensor: (a) Typical QCM device; (b) Scheme of a flow cell for piezoelectric crystals in liquid media, including the oscillating system and frequency counter; (c) Impedance analysis is based on electrical conductance curve; (d) The central parameters of measurement are the resonance frequency and the bandwidth. The variation in quartz crystal frequency as a function of time. ( $f_1$ ) initial frequency; ( $f_2$ ) frequency after the addition of analyte. Schematic mass loading induced frequency change ( $\Delta f$ )

For example, the immune biosensor for *E. coli* O157: H7 detection can be constructed by the self-assembly monolayer (SAM) (Wang et al., 2008). In order to immobilize antibodies on the gold surface of QCM, a layer of dense protein-coupled interface is formed on gold with 16-mercaptopo-acid (MHDA) molecules using SAM firstly. MHDA is a long chain thiol acid with a carboxyl-terminal. One thiol end can form self-assembled film on gold surface through the strong Au-S bond, and the formed free end of SAM is carboxyl. Amino-terminal of antibody protein and self-assembled carboxyl-terminal should be cross-linked with an

activator. Mostly, carboxyl group can be changed into a more active intermediate using NHS/EDC, making it more convenient for condensation reaction between amino group and carboxyl group. After the treatment of SAM and activation, its monoclonal antibodies are immobilized on the sensor surface. The preparation and testing process of QCM immunosensor are shown in Fig. 5.16.



**Fig. 5.16.** QCM-based immune biosensor for *E. coli* O157: H7

*E. coli* O157:H7 is an enterohemorrhagic strain of the bacterium *Escherichia coli* and a cause of food-borne illnesses. Infection often leads to hemorrhagic diarrhea, and occasionally to kidney failure, especially in young children and the elderly. Its virulence is very strong, each intake of 10 viable cells can cause sickness (while 10<sup>6</sup> or more to normal *E. coli* pathogenic). QCM-based immune biosensors can be used to detect pathogenic O157: H7. Using such classic MHDA molecular self-assembly methods to fix antibodies and capture target bacteria have obtained good results with the detection scope between 10<sup>2</sup> CFU / mL and 10<sup>8</sup> CFU / mL.

Although the same as SPR sensors, the advantage of QCM biological sensors are real-time detection without labeling. However, the sensitivity of QCM sensors in the liquid environment is less than that in the gas environment. There are excessive and inconsistent interference factors in volatile liquid, such as pH, viscosity, action time and ion concentration, which may reduce the accuracy and precision for detecting the target. How to effectively eliminate or reduce the

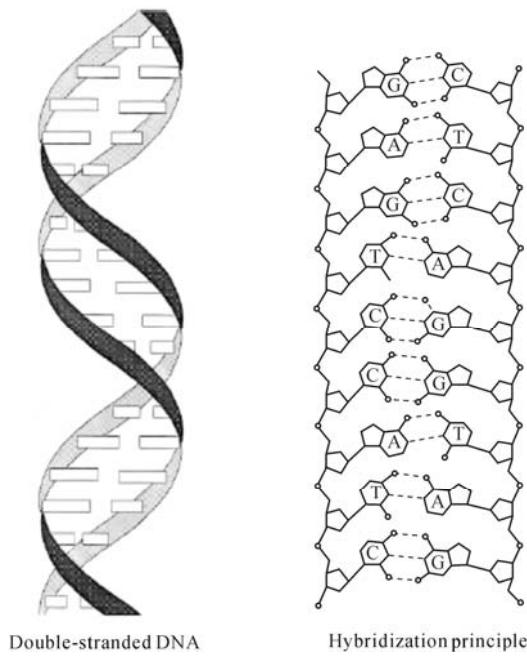
interference factors and optimize the test conditions, still needs continuous studies in the future.

### 5.3.2 Nucleic Acid Biosensors

Another biorecognition mechanism involves hybridization of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), which are the building blocks of genetics. In the last decade, nucleic acids have received increasing interest as bioreceptors for biosensor and biochip technologies.

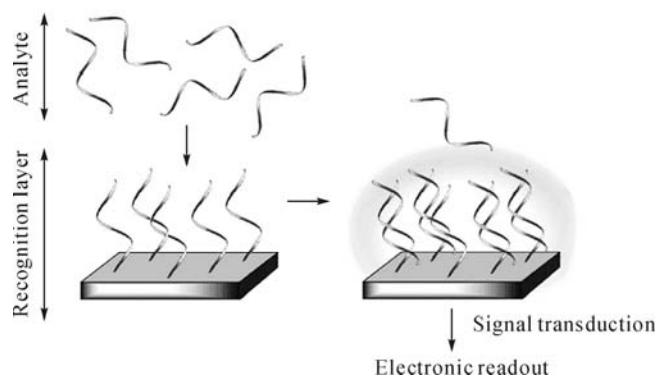
#### 5.3.2.1 Nucleic Acid

The complementarities of adenine : thymine (A : T) and cytosine : guanosine (C : G) pairing in DNA (Fig. 5.17) forms the basis for the specificity of biorecognition in DNA biosensors, often referred to as genosensors. If the sequence of bases composing a certain part of the DNA molecule is known, then the complementary sequence, often called a probe, can be synthesized and labeled with an optically detectable compound (e.g., a fluorescent label). By unwinding the double-stranded DNA into single strands, adding the probe, and then annealing the strands, the labeled probe will hybridize to its complementary sequence on the target molecule.



**Fig. 5.17.** DNA and the hybridization principle

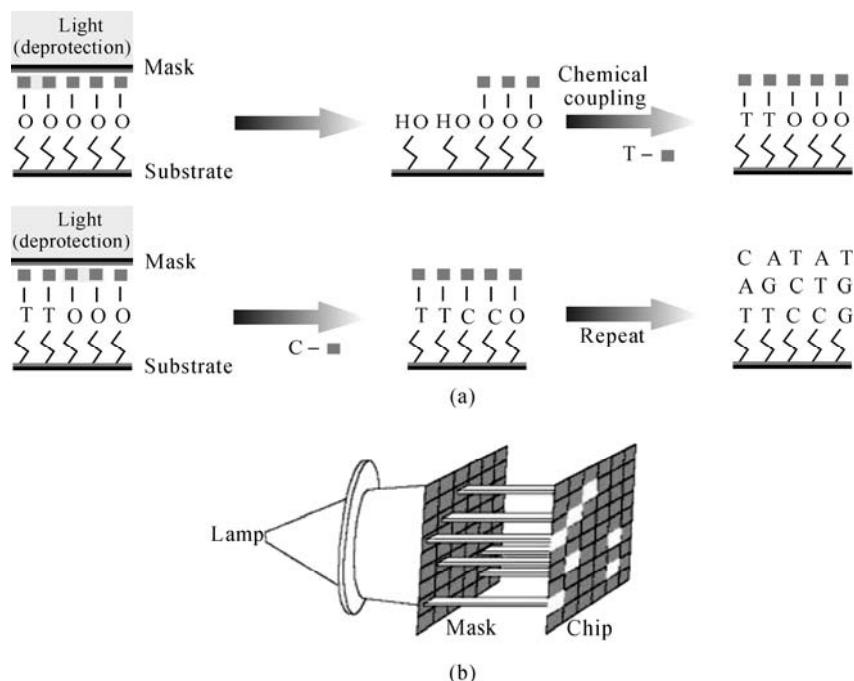
Nucleic acid technology is based on the hybridization of known molecular DNA probes or sequences with complementary strands in a sample under test. In the case of nucleic acid biosensors, target DNA is captured at the recognition layer, and the resulting hybridization signal is transduced into a usable electronic signal for display and analysis (Fig. 5.18). In the case of electronic and electrochemical biosensors, signal transduction is greatly simplified, because the incoming signal is already electronic in origin (Vo-Dinh and Cullum, 2000).



**Fig. 5.18.** General DNA biosensor design

Nucleic acid analysis in general requires extensive sample preparation, amplification, hybridization, and detection. In theory, nucleic acid analysis provides a higher degree of certainty than traditional antibody technologies, because antibodies occasionally exhibit cross reactivity with antigens other than the analyte of interest. The real time detection of hybridization events has been demonstrated in numerous optical or electrochemical systems.

In order to firmly connect DNA probes on the electrode surface, it often needs effective physical and chemical methods. In addition to the previously described methods for fixing biological elements, there are covalent bonding and electrical polymerization methods which can also be used to probe fixation. The fabrication of hundreds of thousands of polynucleotides at high spatial resolution in precise locations on a surface is a very important landmark of DNA chips developed by Affymetrix (Lipschutz et al., 1999). The combinatorial photolithographic process was originally destined for peptide syntheses. Highly structured lateral oligonucleotide libraries on glass supports are accessible by initially modifying the surface with photolabile protection groups (Fig. 5.19). Illumination through a microstructured photomask leads to the deprotection of selected areas, to which the first phosphoramidite building block is covalently attached. Since the coupled nucleotides also contain photolabile protection groups, the iterative repetition of the process generates new patterns, which lead to two dimensionally structured oligonucleotide arrays.



**Fig. 5.19.** Oligonucleotide array fabrication processes using polymeric photoresists: (a) Light directed oligonucleotide synthesis. A solid support is derivatized with a covalent linker molecule terminated with a photolabile protecting group. Light is directed through a mask to deprotect and activate selected sites, and protected nucleotides couple to the activated sites. The process is repeated, activating different sets of sites and coupling different bases allowing arbitrary DNA probes to be constructed at each site; (b) Schematic representation of the lamp, mask and array (reprinted from (Lipschutz et al., 1999), Copyright 1999, with permission from Nature America Inc.)

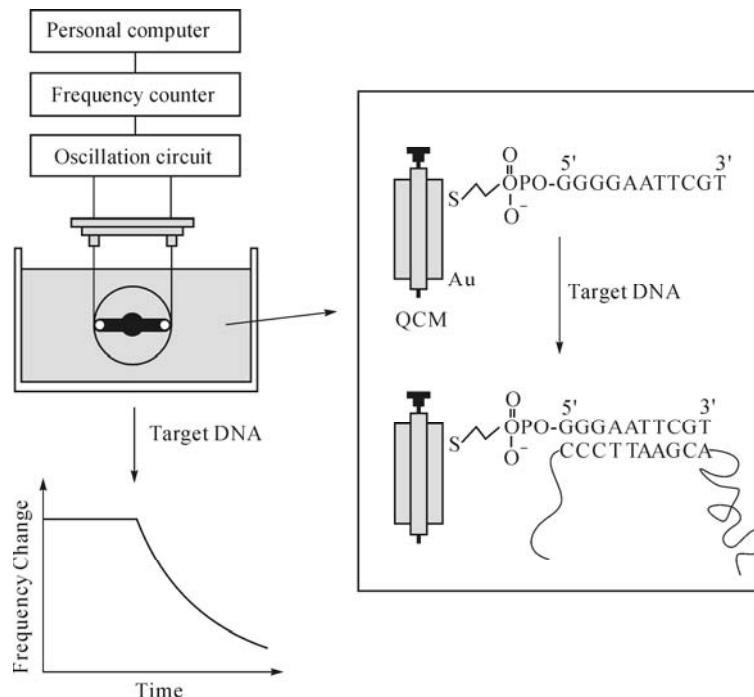
### 5.3.2.2 Nucleic Acid Sensors

Nucleic acid sensors allow for an easy, fast and reliable DNA testing, giving the possibility to analyze simultaneously many samples without labeling. These sensor technologies exploit transducers to convert the hybridization event into electrical or optical signals.

Electrochemical DNA sensors consist of the electrodes that support DNA probes and the detecting systems for electrochemical activities for target probes. Probe molecules are modified to the electrode surface to constitute a DNA electrode, and hybridized selectively with the target sequence to form double-stranded DNA, leading to electrochemical changes on the electrode surface. The differences in changes before and after hybrid can also be identified by hybrid indicators with electrical activities for target sequences or a specific gene. The electrochemical DNA sensors with enzyme-linked amplification have similarities with early enzyme

marked immune sensors. Enzyme is marked in the DNA molecule as a recognition element. When it acts on the substrate, it leads to an electrochemical response. The target DNA can be detected indirectly by the electrochemical signals.

Recently, DNA sensors based on quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) have been applied with success, for instance, for specific detection of DNA sequences. Fig. 5.20 was an example of a nucleic acid sensor based on QCM as a commercial example



**Fig. 5.20.** DNA biosensor design based on QCM

Traditional QCM sensors have high sensitivity in mass changes, often respond to any loading and unloading on the electrode, however, without the effect of selectivity loading materials. DNA hybridization is based on the principle of base pairs. Only sequences with whole or partly complementarities can hybrid with high selectivity. In respect to the above advantages, piezoelectric DNA sensors can be constructed with high sensitivity and specificity. For example, ssDNA of staphylococcal enterotoxin B (SEB) and *E. coli* O157: H7 can be modified on the QCM gold electrode by silane methods to construct a DNA sensor for rapidly detecting the genes of these pathogens. The amount of the electrode modified DNA probe can be determined by the amount of nucleic acid before and after solidification by the protein nucleic acid analysis system. The amount of ssDNA solidified on the crystal surface can be determined by the DNA dot blot color revealed by biotin-labeled DNA phosphoryl enzymes. Studies have found that,

DNA fragments with relatively short length have better hybridization and well reusing capabilities.

And, the study on SPR-based DNA sensors also has become more and more developed. The relevant mechanism can be seen in the section of immune sensors with SPR.

### 5.3.2.3 Nucleic Acid Sensor and DNA Chip

Compared to conventional nucleic acid detection, DNA biosensors have the following advantages (Kricka, 2001; Wang, 2000):

- *Hybridization in liquid-phase.* Conventional nucleic acid detecting is mainly hybridization in solid-phase. DNA sensors can detect DNA of target materials quantitatively by the changes of sound, light, electricity signals in the liquid phase reaction.
- *It can carry on real-time detection of DNA.* Combined with microfluidic chips, the dynamic reaction process of DNA can be monitored in real-time. And, DNA can be measured quantitatively and timely, achieving online and real-time detection of DNA.
- *It can carry on dynamic detection of nucleic acid in vivo.* Currently, there is no effective method studying nucleic acid *in vivo* directly. DNA sensors provide the possibility for studying of dynamic processes of nucleic acid metabolism transfer *in vivo*.
- *It can carry on a large number of intelligent DNA detections.* The combination of DNA sensors and artificial neural networks can filter out sensitive components with better selectivity and activity. The developing multi-functional or intelligent DNA sensors will detect a variety of DNA samples at the same time.
- *It has high sensitivity.* DNA sensors can directly detect the target materials. If combined with polymerase chain reaction (PCR) and other techniques, the sensitivity of sensors will be greatly improved for low copy nucleic acid.
- *It has high specificity.* A DNA sensor is based on the principle of complementary combination. Thus, it has high specificity.
- *It is clean.* It does not need isotopic labeling, and avoids harmful substances.

In the varieties of reported DNA sensors, most of the hybridization time is about 10 min to 1 h, which is a great progress when compared to typically traditional overnight hybridization (20 h), but still too long for sensors. Not only does it greatly reduce the advantages of dynamic monitoring, but also the deadly disadvantage of measuring large quantities of samples. The hybridization time and hybridization volume (sensitivity) is in itself a pair of contradictory elements. How to shorten hybridization time in the premise of ensuring adequate sensitivity is the main problem that needs to be solved. The development of a biochip (mainly DNA chips) based on micro-array hybridization has played a significant role for DNA sensors.

DNA sensors and DNA chips both developed with the combination of molecular biology, modern physics, modern chemistry, and micro-electronics technologies. These two technologies penetrate mutually and develop parallelly, which will bring major breakthroughs both in the diagnosis and treatment of diseases.

Recognizing molecules of DNA sensors and DNA chips are both based on the principle of base pairing complementarily. DNA sensors have dynamic monitoring functions with ultra-miniature, micro-system, multi-parameter, and finally for bedside monitoring, *in vivo* monitoring, non-invasive monitoring, and cell monitoring. However, DNA chips have the characteristics of high-density, which can detect tens and even thousands of genes simultaneously. The research goal of the DNA chip is to achieve overall and rapid diagnosis of a patient's entire genome change by a chip and to make it become conventional technology. Now, DNA sensor researches still focus on improving detection sensitivity and shortening response time as well as enhancing the stability and usability; while DNA chip researches have paid emphasis on specific DNA microarrays for disease-related gene fragments. The new generation of DNA chips should be based on microelectronics principles, with the dynamic monitoring characteristics of DNA sensors and multi-gene synchronous detection function of the DNA chip. A good hybrid of these two chips is also one of the main directions of the DNA biosensor development.

### 5.3.3 Receptor and Ion Channel Biosensors

Molecular receptors are cellular proteins (often membrane-bound) which bind specific chemicals in a manner which results in a conformational change in the protein structure. The conformational change triggers a cellular response, for example, opening an ion channel or secreting an enzyme.

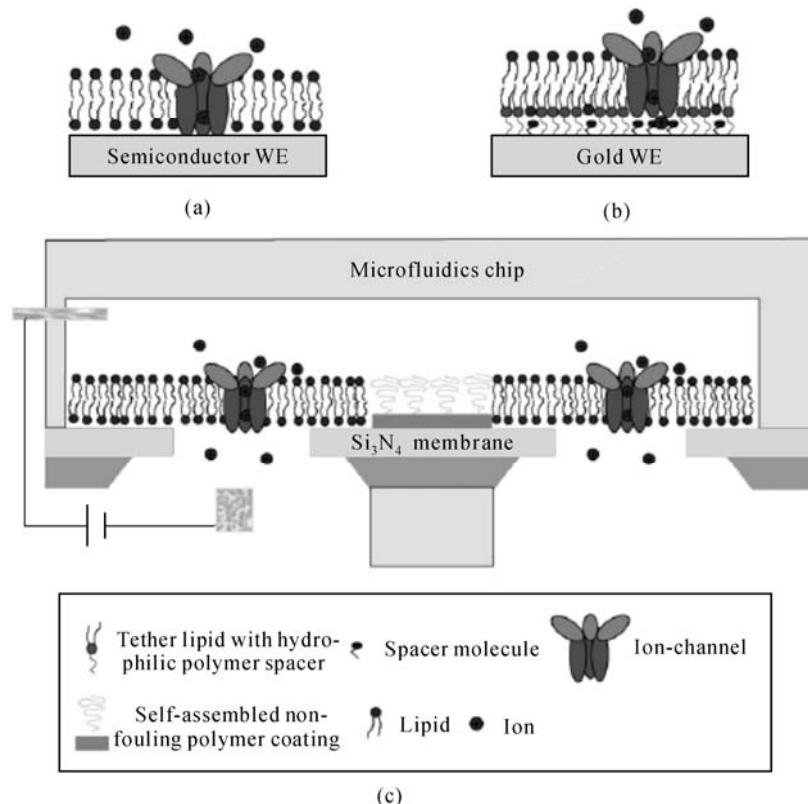
Receptors and ion channels are interesting and provide important opportunities for the development of biosensors for three principal reasons (Subrahmanyam et al., 2002). First, receptors and ion channels possess high affinity and specificity refined by the evolutionary process. Second, receptors and ion channels are natural targets for toxins and mediators of physiological processes, and due to this, they can be used for monitoring these compounds in clinical and environmental analyses and in the development and screening of drugs. Third, the research of receptors and ion channels is an important area and novel sensors can be useful for real-time elucidation of receptor-ligand interactions.

#### 5.3.3.1 Receptor and Ion Channel

The fact that many ion channels and receptors can be purified and reconstituted in black lipid membranes (BLMs) for studies of function and pharmacology has

spurred initial interest in the development of ion channel/receptor-based biosensors. However, ion channels especially those pertaining to mammalian physiology, cannot be considered robust in BLMs or isolated membrane patches due to the well-known property of ion channel “rundown” or “washout”. In the absence of integral intracellular machinery provided by cells needed to maintain function, ion channels typical of mammalian physiology presently do not constitute practical biosensors.

To preserve the integrity of the receptor with the immobilizing conditions to closely resemble the natural environment, biosensors are often achieved by reintegrating receptors into lipid membranes.



**Fig. 5.21.** A selection of membrane sensor platforms for ion channels or receptors (reprinted from (Reimhult and Kumar, 2008), Copyright 2007, with permission from Elsevier Science B.V.)

Fig. 5.21 shows a selection of membrane sensor platforms to support receptors and ion channels (Reimhult and Kumar, 2008). (a) Supported lipid bilayer on hydrophilic support. Typically a hydrophilic semiconductor or oxide substrate has to be used for direct assembly of a supported lipid bilayer (SLB). The support will act as a working electrode (WE) for electrochemical measurements; (b) Tethered

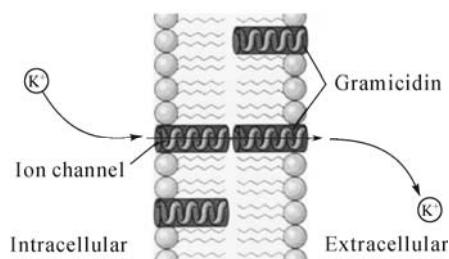
supported lipid bilayer. Covalently attached hydrophobic molecules with a hydrophilic linker, often derived from lipids, are used to tether and support the lipid membrane to a gold WE; (c) Free-spanning membrane or black lipid membrane as it could look in a self-assembled membrane sensor array. Lipid membranes containing ion-channels are separated into functional spots, for example by non-fouling polymer barriers, and span apertures in a solid support. It has free liquid access on both sides to accommodate large proteins and to easily conduct electrochemical measurements. The schematics show how such a platform combined with microfluidics could be used for parallel voltage clamp measurements on different single transmembrane proteins.

Based on the above lipid supported biosensor system, “Gigaohm seal” enabling measurements of single ion-channels and transporters can be obtained and single ion channel sensitivity can be also achieved. Membrane stable  $>1$  d for screening applications also has been achieved in several formats.

### 5.3.3.2 Receptor and Ion Channel Sensor

The *in vitro* receptor molecule sensors have advantages in sensitivity and selectivity. However, there are many difficulties in the separation and purification of membrane receptors, fixation of *in vitro* receptor molecules and conversion and amplification of receptor sensors, all of which affect the development of *in vitro* receptor biosensors. The glutamate receptor and dopamine receptor sensors using lipid membrane supporting system have created a certain foundation on receptor sensors.

The ion channel sensors are mainly designed with gramicidin supported by lipid bilayer, as shown in Fig. 5.22 (Liu et al., 2008).



**Fig. 5.22.** The model of ion channels based on Gramicidin

Gramicidin A is a kind of polypeptide extracted from the *Brucella* consisting of 15 hydrophobic amino acid alternating array. One of its main biological functions is to form selective transmembrane ion channels, especially the unit price ion channels, enabling passive transportation of easily diffused ions such as  $\text{Na}^+$ ,  $\text{NH}_4^+$  and  $\text{K}^+$ . As the formation of lipid bilayer, gramicidin polymerization forms ion channels in the lipid bilayer in the form of dimer, which can

dramatically increase membrane ion permeability. The dielectric properties of lipid bilayer, such as the membrane capacitance, change with the gramicidin embedded in. Thus, channel currents can be measured to obtain information about its permeability properties over a very wide range of permeate ion concentrations or applied potentials.

### 5.3.3.3 Electrifying Cell Receptor Sensors

Modifying receptors on the surfaces of cells to enable them to interact with proteins that are not their natural partners is one way of controlling signaling processes in cell biology. And, when isolated from their natural environment, these re-engineered proteins could be used for various sensing and drug screening applications.

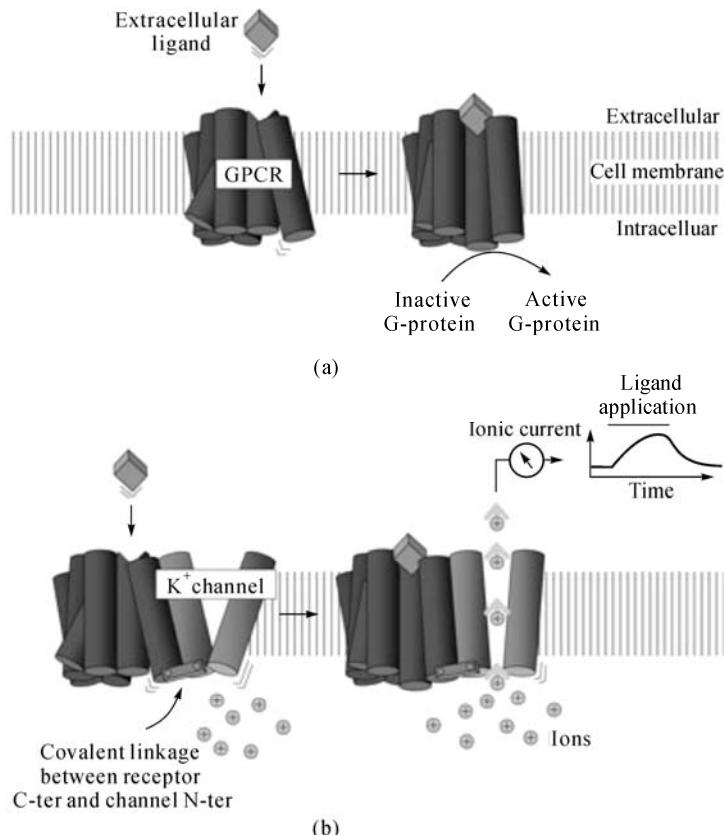
The challenge in developing such biosensors lies in functionally connecting a molecule detector to an electrical switch. In order to facilitate the study of ion channel-coupled receptors, recently the Western Reserve University and the University of North Carolina researchers have started G protein-coupled receptor clone and expression, based on the use of six amino acid (hexaglycine) such as C-terminal cross-linking the receptors with the inward rectifier potassium channel (Kir 6.2) of the N-terminal for coupling (Moreau et al., 2008). This method of using bio-engineering will be the coupling of receptor proteins and ion channel technology, which is easy to set up as a new type of bio-molecular sensing detection technology, allowing receptor-binding information to be detected by electrophysiological parameters, which are called electrical activity of cell receptors (electrifying cell receptor) (Fig. 5.23) (Abbas and Roth, 2008).

The studies were carried out on platforms of lipid bilayer, which facilitates by keeping the biological activity of receptors through the support of lipid bilayers. Also it can simulate the transduction of receptor signals based on ion channel coupling and transform stimulated signals into action potentials, which are bioelectrical signals available for biosensor detection.

## 5.4 Cell and Tissue Biosensors

Cell and tissue biosensors on the other hand offer a broad spectrum detection capability. Moreover, using cells as the sensing elements provides the advantage of *in-situ* physiological monitoring along with analyte sensing and detection (Pancrazio et al., 1999; Wang and Liu, 2009). A cell by itself encapsulates an array of molecular sensors. Receptors, channels, and enzymes that may be sensitive to an analyte are maintained in a physiologically stable manner by native cellular machinery. In contrast with antibody approaches, cell and tissue sensors are expected to respond optimally to functional, biologically active analytes. Cell and tissue biosensors have been implemented using microorganisms, particularly for

environmental monitoring of pollutants. Sensors incorporating mammalian cells have a distinct advantage of responding in a manner that can offer insight into the physiological effect of an analyte. Several approaches for transduction of cell sensor signals are available and these approaches include measurements of cell metabolism, impedance, and extracellular potentials.



**Fig. 5.23.** Electrifying cell receptor of ion channel-coupled receptors: (a) Upon binding of its ligand at an extracellular site, a transmembrane G-protein-coupled receptor (GPCR) adopts a new conformation that triggers activation of intracellular G-proteins; (b) The GPCR is attached to an ion channel in such a way that both proteins are mechanically coupled. When the GPCR binds a ligand and changes conformation, this change is directly transmitted to the channel and results in a change in gating and in the ionic current through the channel (reprinted from (Moreau et al., 2008), Copyright 2008, with permission from Nature Publishing Group)

In many cell and tissue biosensor applications, primary cells or tumor-derived cell lines are selected as the source of cells. Primary cells are extracted directly from animals, while a cell line refers to a genetically identical cellular population that actively divides *in vitro*. Primary cells have the advantages of cell type availability, and the likelihood to have *in vitro* functionality similar to that found

*in vivo*. However, the process of harvesting is inefficient and presents ethical issues for large-scale operations. Tumor-derived cells, when the desired cell type is available, offer the advantage of convenience of preparation. Nevertheless, they are typically derived from abnormal cells and may suffer the disadvantage of not retaining the desired functionality of *in vivo* cells.

Since the last decade, there has been significant interest in the characterization of totipotent embryonic stem cells which are capable of maturing into any cell types. Stem cells provide an alternative between tumor-derived cell lines and terminally differentiated primary cells, because they can be dissociated from tissue, grown indefinitely in a culture, and induced to differentiate into mature cells. The technique of “embryonic stem cell test” has also been developed and used *in vitro* to screen new medicines and other chemicals. The test has shown merits such as no requirement of the sacrifice of animals, no side effects on humans, higher sensitivity, and even higher accuracy. Therefore, stem cells have provided a renewable novel source of cells for cell and tissue biosensor applications (Liu et al., 2007a; 2007b).

### 5.4.1 Cellular Metabolism Biosensors

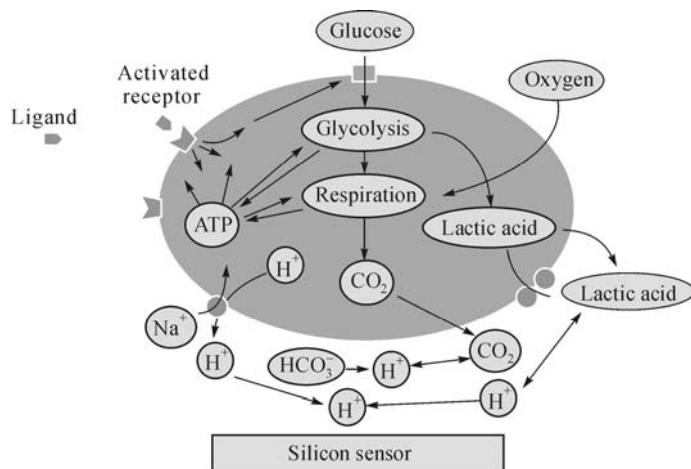
A category of cellular biosensors relies on the measurement of energy metabolism, a common feature of all living cells. This is especially useful in testing drugs as well as in cancer research. The combined application of microfabrication technology to microfluidics has aided in the development of portable sensors. The changes in the cell metabolism due to the effect of a chemical reagent are transduced into electrical signals that are read out and analyzed.

#### 5.4.1.1 Cellular Metabolism by Cytosensor

In the 1990s, Molecular Device Corporation presented a biological application of light addressable potentiometric sensors (LAPS) and invented the Cytosensor®, which is often called a microphysiometer. It is a system for performing bioassay, which integrates living cells with LAPS. Acidic products of energy metabolism acidify cellular environments and the microphysiometer measures the rate of proton excretion from cells, as in Fig. 5.24 (Hafner, 2000).

A general feature of living heterotrophic cells is the uptake of metabolites (carbon sources), the production of energy (ATP) and the excretion of acid waste products (e.g., lactic and carbonic acid). Carbon sources are sugars, amino and fatty acids. In regular culture conditions, glucose and glutamine are present in high concentrations and are taken up by cells and broken down into energy and waste products. Under aerobic conditions, glucose is converted via pyruvate and acetyl CoA into CO<sub>2</sub> yielding energy (respiration). The corresponding pathways are glycolysis, citric acid cycle and oxidative phosphorylation. The extracellular

acidification of cells sitting in a flowing chamber can be measured with the Cytosensor and thus, a functional response of cells upon receptor stimulation can be monitored under non-invasive conditions and in real time. Upon stimulation of a membrane-bound receptor, which can be either G protein-coupled, tyrosine kinase-coupled or an ion channel, a signal transduction cascade is initiated. Many steps in this cascade are either directly or indirectly energy-dependent. Under steady state conditions, one cell produces about  $10^8$  protons per second. After receptor stimulation, this will be raised to between 10% and 100%, depending on the cell-type, the receptor and the coupling pathway. The result is an acidification of the extracellular medium, which can directly be detected with the cytosensor system.



**Fig. 5.24.** Microphysiometer studies based on light addressable potentiometric sensor. Schematic representations of metabolic pathways in which extracellular pH changes occur (reprinted from (Hafner, 2000), Copyright 2000, with permission from Elsevier Science B.V.)

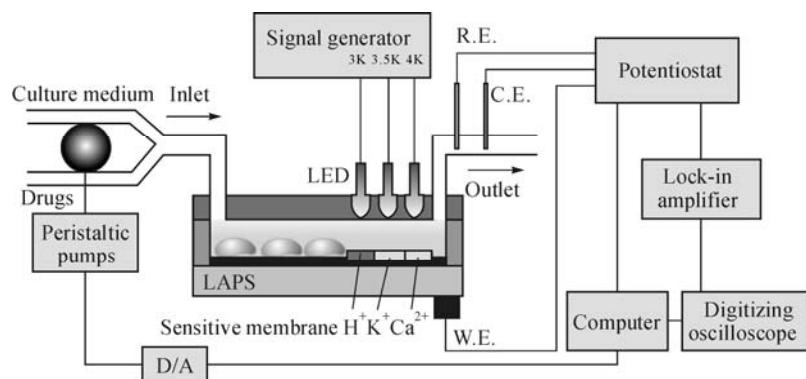
Large numbers of experiments on ligand-receptors have proved that the combination of receptor and agonist increases the rate of extracellular acidification (ECAR). A microphysiometer is able to estimate the effect of chemotherapy by ECAR. So it can be used to evaluate and screen drugs and even suborganelle, such as, G protein-coupled receptors, receptors with intrinsic catalytic activity, ligand gated ion channel, and receptors linked with cytoplasmatic tyrosine kinase.

#### 5.4.1.2 Microphysiometer Based on LAPS

Getting more information about the multi-functional cellular processing of input- and output-signals in different cellular plants is essential for basic research as well as for various fields of biomedical applications. The concentrations of the extracellular ions, such as Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, may change along with the alteration of cell physiology. In order to analyze simultaneously the relations of the extracellular

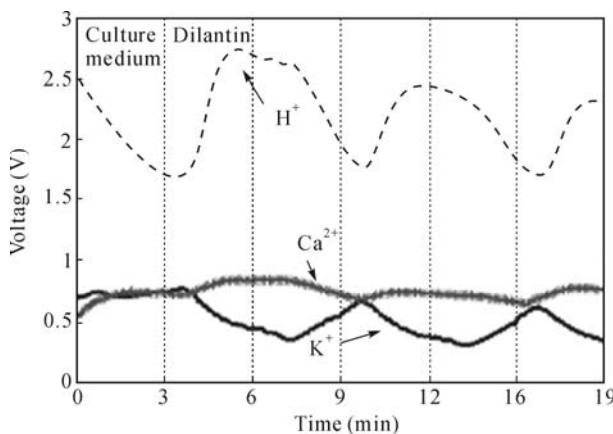
environmental  $H^+$ ,  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$  under the effects of drugs, our laboratory has developed a novel microphysiometer based on multi-LAPS (Wu et al., 2001; Zhang et al., 2001). The surface of the LAPS is deposited with different sensitive membranes by the silicon microfabrication technique and the PVC membrane technique. When we fabricated the  $K^+$  sensitive membrane, we used 2 mg valinomycin as the electro active substance, 1.3 mL tetrahydrofuran (THF) and 1.3 mL cyclohexanone as solvent, 0.066 g PVC powder as bulk material, and 1–2 gutta di-butyl phthalate as plasticizing agent. When we fabricated the  $Ca^{2+}$  sensitive membrane, we used 0.24 mL formamide as an active substance, 4.72 mL tetrahydrofuran as solvent, 0.15 g PVC powder as bulk material, and paucity tetraphenylboron sodium as the plasticizing agent.

The different sensitive membranes are illuminated in parallel with light sources at different frequencies, and measured online by parallel processing algorithm Fig. 5.25.



**Fig. 5.25.** The schematic drawing of the system of the multi-LAPS to different extracellular ions ( $H^+$ ,  $K^+$ , and  $Ca^{2+}$ ) (reprinted from (Wu et al., 2001), Copyright 2001, with permission from Elsevier Science B.V.)

The amplitude of each frequency component can be measured on-line by the fast Fourier transform (FFT) analysis. On the sensor, a different sensitive ( $H^+$ ,  $K^+$ ,  $Ca^{2+}$ ) membrane is illuminated simultaneously with three light sources at different frequencies (3 kHz for  $K^+$ , 3.5 kHz for  $Ca^{2+}$ , 4 kHz for  $H^+$ ), the photocurrent comprises the three frequency components. The amplitude of each frequency component might be measured on-line by FFT (as shown in Fig. 5.26). Dilantin, i.e., phenytoin sodium, is a kind of anti-epilepsy drug, which has significant effects on transqualizing and hypnotic and anti-seizure. Moreover, dilantin is also one of the anti-arrhythmia drugs. It has been proved that dilantin has membrane stabilizing actions on neural cells because it can reduce pericellular membrane ions ( $Na^+$ ,  $Ca^{2+}$ ) permeability, inhibit  $Na^+$  and  $Ca^{2+}$  influx, stave  $K^+$  efflux, thus, prolonging the refractory period, stabilizing the pericellular membrane, and decreasing excitability. This mechanism can be proven by our experiments (Wu et al., 2001; Liu et al., 2007c).



**Fig. 5.26.** Microphysiometer based on LAPS. Illuminate simultaneously at the three sensitive membranes with three light sources at the three modulation frequencies.  $H^+$ ,  $K^+$ ,  $Ca^{2+}$  analyze simultaneously by multi-LAPS (reprinted from (Wu et al., 2001), Copyright 2001, with permission from Elsevier Science B.V.)

The extrusions or intrusions of protons and ions are very general parameters involved in the activation of nearly all kinds of membrane-bound receptors. These parameters can be measured with the novel microphysiometer. In addition, the microphysiometer works under regular cell culture conditions, so cells can be repeatedly stimulated with drugs within a few hours. Thus, a functional response of cells upon receptor stimulation can be monitored under non-invasive conditions and in real-time. The responses depend on the cell-type, the receptor and the coupling pathway. The microphysiometer makes it easy to complete a dose-response curve and  $EC_{50}$  (the concentration of agonist that provokes a response halfway between the baseline and maximum response) determination. Furthermore, by comparing different curves and  $EC_{50}$  values under different conditions (such as different cell types), drug effects may be evaluated. With the microphysiometer, no knowledge about the kind of receptor coupling is necessarily prior to the experiment. This makes it easier to work with orphan receptors and ligands from unknown receptors. However, by blocking certain signaling pathways inside the cell by means of signal transduction probes, specificity can be brought into the system and the exact coupling of a membrane bound receptor can be easily elucidated, and drugs like agonists can be screened.

#### 5.4.2 Cellular Impedance Biosensors

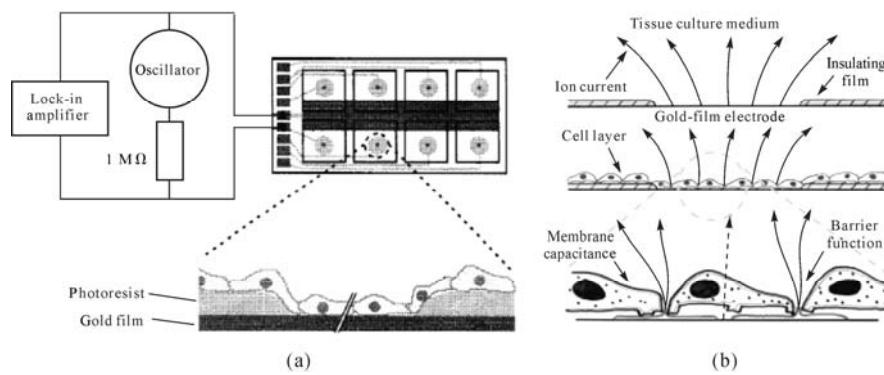
Vertebrate cell behavior in tissue culture is normally studied by periodic microscopic examinations of cell density and morphology. If a continuous record of behavior is required, it is generally obtained by using cinematographic arrangements. In recent years, lots of researchers have described novel methods in

which cells can be monitored continuously using electric fields.

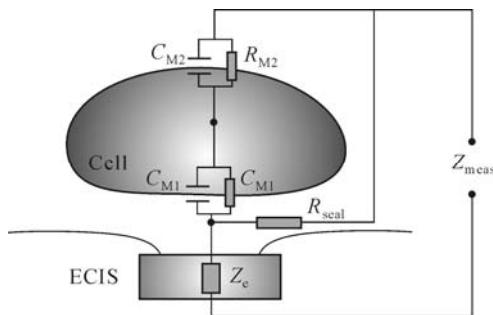
#### 5.4.2.1 Electric Cell-substrate Impedance Sensing

By culturing cells over one or more electrode contacts, changes in the effective electrode impedance permit a non-invasive assay of cultured cell adhesion, spreading, and motility (Giaever and Keese, 1993). Fig. 5.27 shows the schematic of an impedance sensor called an electric cell-substrate impedance sensing (ECIS<sup>TM</sup>), which is used to monitor attachments and the spreading of mammalian cells quantitatively and in real time. This technology was invented by Drs. Ivar Giaever and Charles R. Keese while working at General Electric Corporate Research and Development, and then they formed Applied BioPhysics, Inc. as a private company to develop, commercialize and market this and other biophysical technologies.

A schematic view of a cell positioned over ECIS electrodes is shown in Fig. 5.28. The total measured impedance consists of the electrode impedance  $Z_e$ , the resistance between the electrode and the bulk electrolyte due to the thin layer of medium between the cell and the passivation layer  $R_{\text{seal}}$ , the membrane capacitance and ion channel resistance over the electrode  $C_{m1}$  and  $R_{m1}$ , the membrane capacitance and ion channel resistance of the top and sides of the cell  $C_{m2}$  and  $R_{m2}$ , the solution resistance and the counterelectrode impedance (omit in the Figure). In reality,  $R_{\text{seal}}$  is distributed with the capacitance and conductance of the membrane in the region over the passivated layer and the result of AC impedance is mainly affected by  $R_{\text{seal}}$ . For this measurement, the counter electrode impedance, both the solution resistance and electrode impedance should be negligible (impedance of platinized electrodes is relatively small), so the impedance measurement is dominated by the seal resistance and the cell membrane properties. It is clear that  $R_{\text{seal}}$  must be on the same order (or larger than) the membrane impedance if changes in membrane properties are to be observed. When living cells are cultured on the surface of chips, some important factors including the speed of propagation, moving and adherence, etc. can be obtained from the impedance using ECIS.



**Fig. 5.27.** The schematic (a) and principle (b) of an impedance cell sensor



**Fig. 5.28.** Schematic of a cell positioned over an electrode (not to scale). The measured impedance consists of the electrode impedance, the resistance between the electrode and the bulk electrolyte due to the thin layer of medium between the cell and the passivation layer, the membrane capacitance and ion channel resistance over the electrode

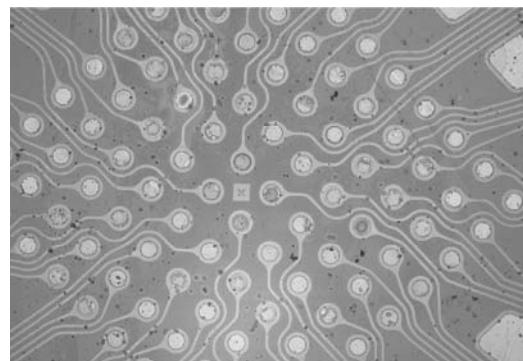
This method is based on measuring changes in AC impedance of small gold-film electrodes deposited on a culture dish and used as growth substrates. The gold electrodes are immersed in the tissue culture medium. When cells attach and spread on the electrode, the measured electrical impedance changes because the cells constrain the current flow. This changing impedance is interpreted to reveal relevant information about cell behaviors, such as spreading, locomotion and motility. They involve the coordination of many biochemical events. They are extremely sensitive to most external parameters such as temperature, pH, and a myriad of chemical compounds. The broad response to changes in the environment allows for this method to be used as a biosensor. Impedance techniques are theoretically capable of dynamic measurements of cellular movement at the nanometer level, with resolution above that of conventional microscopy. Impedance measurements have been used to assess the effect of nitric oxide on endothelin-induced migration of endothelial cells. From the standpoint of biosensors, changes in cell migration or morphology tend to be somewhat slow; and marked changes in impedance in the presence of cadmium emerged only after 2 – 3 h of exposure. Thus for real time tracking and monitoring the effects of analytes, a sensing technique based on impedance measurements would be slow and cumbersome.

#### 5.4.2.2 Applications of Cell Impedance Biosensors

Impedimetric analysis on adherently growing cells by micro-electrodes provides information related to cell number, cell adhesion and cellular morphology. In recent years, cancer is rapidly becoming the number one killer in many countries. And, chemotherapy (anti-cancer drugs) is still one of the most important treatment methods in clinics. In pre-clinical testing studies, there is a great demand to develop more rapid and simpler techniques for studying cancerous cells, especially for understanding their interactions with drugs and toxins. There is a

need to develop minimally invasive, reliable, inexpensive, and easy to use instrumentation for studying real-time biological events *in vitro*.

In our study, cell-based biosensors with micro-electrode array (MEA) were used to monitor the culture behavior of mammalian cancer cells and evaluate the chemosensitivity of anti-cancer drugs using electrochemical impedance spectroscopy (Liu et al., 2009). The platinum electrode arrays were fabricated by semiconductor technology to a  $10 \times 10$  pattern, with a diameter of  $80 \mu\text{m}$  for each electrode (Fig. 5.29).

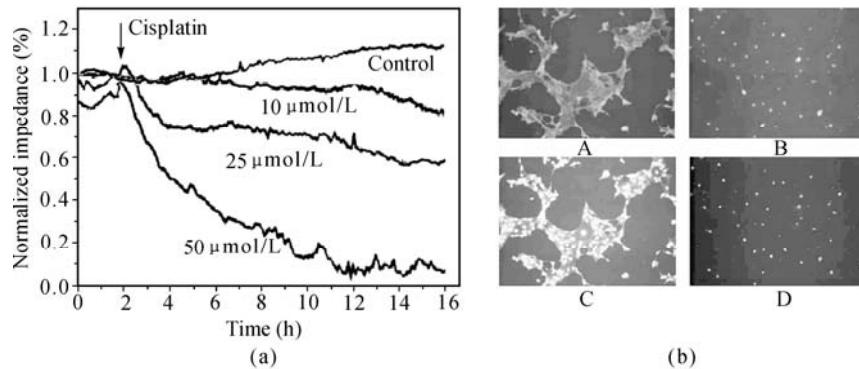


**Fig. 5.29.** Photo of the micro-electrode arrays (reprinted from (Liu et al., 2009), Copyright 2009, with permission from Elsevier Science B.V.)

The human oesophageal cancer cell lines (KYSE 30) were cultured on the surface of the electrode with the help of fibronectin, the connecting protein for tumor cells metastasis and adhesion in an extracellular matrix. Morphology changes of cell adhesion, spreading, and proliferation can be detected by impedimetric analysis in a real time and in a non-invasive way. The anti-cancer drug cisplatin was added to cells for potential drug screening applications (Fig. 5.30). The experimental results show that this well-known drug has characterized chemosensitivity effects detected by MEA. The cancer cell chip provides a useful analytical method for cancer research.

Cell adhesion in an extracellular matrix is the precondition for tumor metastasis, and then a tumor clone is formed with unbounded cell proliferation. The current adhesion assay is an *in vitro* method which is used to determine the rate or strength of adhesion for different cell types to extracellular matrix proteins by fluorescent labeling. At the same time, cellular morphology is recognized as one of the most important parameters in cancer biology. Especially, most of anti-cancer agents currently employed that target the cytoskeleton, do so through interactions with the microfilaments. The cytoskeleton consists of a complex network of filamentous proteins which are involved in regulation of cell morphology and adhesion. Moreover, the concept that when tumor cells are exposed to anti-cancer drugs, they usually die from apoptosis has become a widely held tenet of modern cancer treatment. Therefore, the cell chip with micro-electrode was used to monitor the culture behavior of mammalian cancer cells and evaluate

the chemosensitivity of anti-cancer drugs using an electrochemical impedance spectroscopy.



**Fig.5.30.** Analysis chemosensitive of cisplatin to KYSE 30 cells: (a) Cells were grown onto electrodes until confluent and treated with cisplatin ( $10 \mu\text{mol/L}$ ,  $25 \mu\text{mol/L}$ , and  $50 \mu\text{mol/L}$ ), and impedance was determined for up to 16 h; (b) Fluorescence imaging of KYSE 30 cells cultured on the surface of the micro-electrode. A: Phalloidin imaging specific for microfilament after cells cultured 48 h; B: Phalloidin imaging after cells treated with cisplatin 12 h; C: Propidium iodide specific for nucleic acid imaging after cells cultured 48 h; D: Propidium iodide specific for nucleic acid imaging after cells treated with cisplatin 12 h (reprinted from (Liu et al., 2009), Copyright 2009, with permission from Elsevier Science B.V.)

### 5.4.3 Extracellular Potential Biosensors

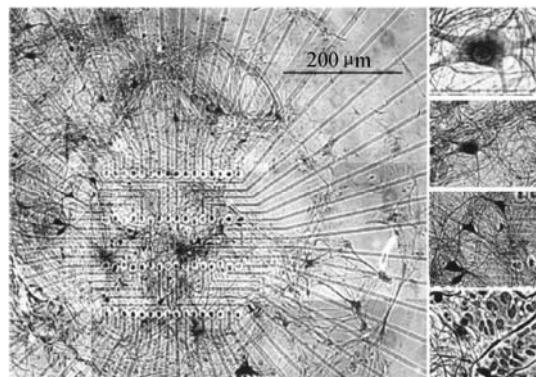
Living cells grown *in vitro* are particularly attractive as potential detector elements. The electrophysiological signals of excitable cells depend on the cellular functional information. Excitable cells, such as neural cells, and myocytes, are evoked into action potential when subjected to stimulus. Patch clamps can be used to detect the cellular membrane potential directly, but the measurement is limited to a fixed recording site, so it cannot be used to measure the coupling of cells. Furthermore, the invasive nature of intracellular recording, as well as the voltage-sensitive and optical-sensitive dyes, limits the utility of standard electrophysiological measurements and optical approaches. As a result, planar microelectrode arrays (MEA) were developed and have become powerful tools, which offer a non-invasive and long-term approach to the measurement of excitable cells action potential, or communication between cardiac or neural tissues.

#### 5.4.3.1 Extracellular Potential Detection by MEA

MEA typically consisting of 16 – 64 recording sites presents a tremendous conduit for data acquisition from networks of electrically active cells. As a result, planar

MEA have emerged as a powerful tool for long-term recording of network dynamics. Extracellular recordings have been achieved from dissociated cells as well; which is more useful in specific chemical agent sensing applications.

Gross and colleagues at the University of North Texas over the past 20 years have demonstrated the feasibility of neuronal networks for biosensor applications (Gross et al., 1995). Neurons cultured over microelectrode have shown regular electrophysiological behavior and stable pharmacological sensitivity for over 9 months. Fig. 5.31 shows neuronal cultures on a 64 microelectrode array of Multichannel Systems (Roboocyte®, Germany) (Kovacs et al., 2003). In fact, their precise methodological approach generates a co-culture of glial support cells and randomly seeded neurons, resulting in spontaneous bioelectrical activity ranging from stochastic neuronal spiking to organized bursting and long-term oscillatory activity.



**Fig. 5.31.** Typical dissociated neuron (mouse spinal) culture atop a microelectrode array. Insets at right illustrate the variety of morphologies seen in the culture (reprinted from (Gross et al., 1995), Copyright 1995, with permission from Elsevier Science B.V.).

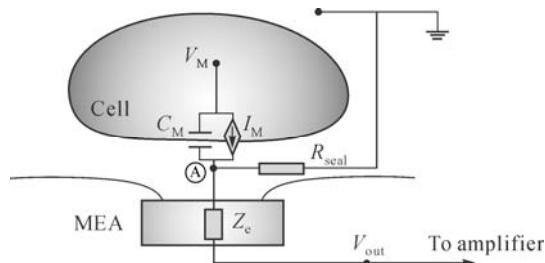
As shown in Fig. 5.32, the coupling model of cell with MEA for extracellular potential detection.  $R_{\text{seal}}$  is the seal resistance between cell and electrodes, and it is necessary to make the seal resistance  $R_{\text{seal}}$  as large as possible. The current  $I_{\text{total}}$  through the electrode is:

$$I_{\text{total}} = C_M \frac{d(V_M - V_J)}{dt} + \sum_i I_m^i = C_J \frac{dV_J}{dt} + \frac{V_J}{R_{\text{seal}}} \quad (5.6)$$

In the equation,  $I_{\text{total}}$  is the total current,  $C_M$  is the membrane capacitance,  $V_M$  is the membrane voltage,  $V_J$  is the coupling voltage,  $I_M$  is the total current of extracellular ions,  $C_J$  is the capacitance of the coupling layer. So, when  $R_{\text{seal}}$  is larger, it means that the creepage current is less and the recorded current and it is mainly coming from the cell's electrophysiological signals.

When the electrode is coupled with cells,  $R_{\text{seal}}$  is the seal impedance, which is used to illustrate the leaking current between the interfaces of the cell and

electrode and the width of the gap. When  $R_{\text{seal}}$  is large, the gap is small and more available for a larger voltage signal  $V_J$  with a high S/N ratio. However, when the  $R_{\text{seal}}$  value is small, it means there is a large part of the effective current leakage to the ground through point A as shown in Fig. 5.32, and the junction voltage  $V_J$  will be small. So in experiments, the whole impedance should be smaller or in the same magnitude with  $R_{\text{seal}}$  for a better detection of the electrical extracellular signals.



**Fig. 5.32.** Conceptional drawing of a cultured cell coupled to a microelectrode and the simplified circuit schematic of the cell-electrode junction

Microelectrode arrays coupled with “turnkey” systems for signal processing and data acquisition are now commercially available. In spite of the obvious advantages of the microelectrode array technology for determining the effect of chemical analytes at the single cell level, it becomes essential to pattern the dissociated cells accurately over microelectrodes. Single cell sensing forms the basis for determining cellular sensitivity with a wide range of chemical analytes and determining the cellular physiological changes. Also analyses of the extracellular electrical activity, which results in unique identification tags associated with cellular response to each specific chemical agent are also known as “signature patterns”. Beside the merits of long-term and non-invasive applications, paralleled recording makes it possible to conveniently study the signal transmission at the synapse of neuronal network or at the junction gap between cardiac cells. With these abilities, MEA has been the fundamental tool in neuroscience for network study and widely used in the pharmacological field.

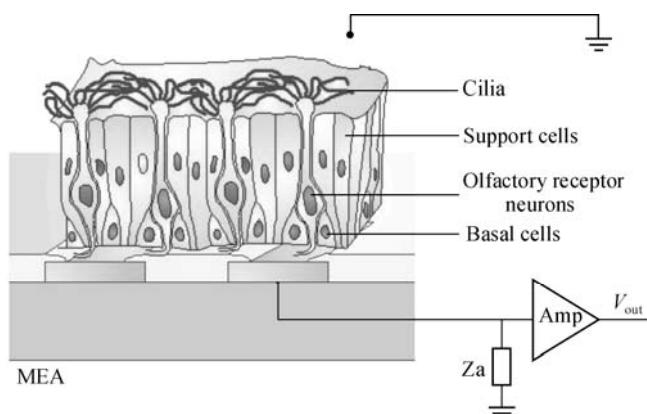
#### 5.4.3.2 Applications of Extracellular Potential Detection

Since olfactory and gustatory systems play important roles in detecting environmental conditions, a lot of olfactory and gustatory research has been carried out due to their potential commercial applications. e-Nose and e-Tongue belong to these technologies, which mimic animals’ olfactory and gustatory systems to detect smell and taste by exploiting sensitive materials. The detection ability of these devices mainly depends on the absorbability or catalysis of sensitive materials to special chemicals. Although great achievements have been made, this method still has limitations in sensitivity and specificity, compared with

the biology binding of specific odorants or tastants to the olfactory receptor neurons or taste receptor cells.

It was considered to be beneficial to establish both artificial olfaction systems for odor detection and useful platforms for olfactory research. In recent years, some groups have used olfactory cells and receptors coupled with sensors, such as surface plasmon resonance (SPR), quartz crystal microbalance (QCM) and field effect transistor (FET), as a bioelectronic nose to detect odorant molecules. These methods have relatively high specificity and sensitivity with the interaction between the olfactory receptor and the odorant successfully recorded. However, subsequent intracellular changes, which result from changes in ionic currents and the membrane potential in cells, especially in olfactory receptor neurons, cannot be detected.

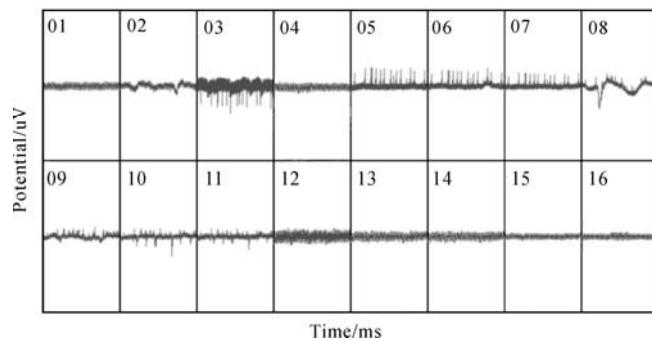
Based on microelectronic sensor chips, cell and tissue biosensors can collect the electrophysiological responses directly relating to cellular functions (Liu et al., 2006). In our studies, we have extracted olfactory cells and tissues from rats and cultured them on MEA, and recorded extracellular potentials of olfactory receptor neurons following odorant-receptor binding (Liu et al., 2010a; 2010b). We find that MEA might be suitable for recording the electrical signals produced by olfactory cells and thus could be used as olfactory cell based biosensors (Fig. 5.33). Especially, we have managed to combine the intact olfactory epithelium with MEA for a bioelectronic nose of olfactory receptor neurons. Compared to the cultured olfactory cells, the intact olfactory epithelium can be obtained conveniently with the primary cell structure well-preserved, which can mimic the *in vivo* process of gas sensing, and is a good candidate for the biological elements of a bioelectronic nose.



**Fig. 5.33.** Recording extracellular potentials of olfactory receptor neurons in intact epithelium by microelectrodes (reprinted from (Liu et al., 2010), Copyright 2010, with permission from Elsevier Science B.V.)

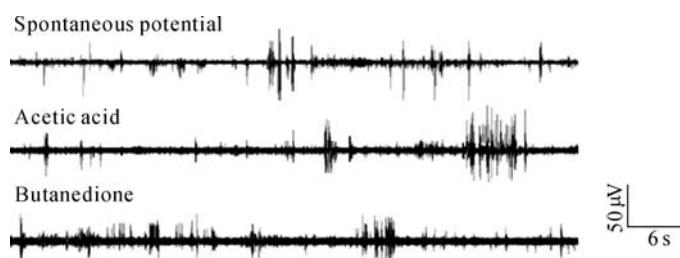
MEA can record the multisite potentials simultaneously and has great potential in electrophysiological detection in a long-term and non-invasive way. The *in vitro* tissue was kept bioactive, where olfactory receptor neurons fired spontaneously,

with amplitude and duration of peaks were about 50 – 100  $\mu$ V and 10 – 20 ms. For example, Fig. 5.34 displays the 16-channel signals as an example. It can be seen that Channel 03 recorded the negative peaks, while Channels 05, 06 and 07 recorded spontaneous potentials in cluster with similar positive peaks, whose average amplitude was larger than that in the other channels. Channel 08 recorded small positive peaks with baseline drifting. Meanwhile, Channels 10 and 11 recorded both positive and negative peaks with smaller amplitudes. Other channels had no obvious peaks but were only recorded with steady baseline levels during this time. There were peaks appearing in these channels at other times. The correlation pattern analysis of neighboring channels provides more information about the neural activity during various experimental conditions.



**Fig. 5.34.** Multi-channel recording electrophysiological signals of olfactory epithelium (reprinted from (Liu et al., 2010b), Copyright 2010, with permission from Elsevier Science B.V.)

On the basis of effectively recording spontaneous potentials, we recorded electrophysiological signals after odor stimulations. Fig. 5.35 shows the stimulated potentials compared with spontaneous potentials in one of the channels on the MEA. Spontaneous potentials were fired every second. The release of potentials after acetic acid stimulation was mainly about sustained signals with significant amplitudes in a short time, while the release of potentials after butanedione stimulation was mainly about long-term signals with low amplitudes. The frequency and amplitude of the olfactory epithelium potential changed with stimulation of different odors, showing some characteristics of the discharge modes.



**Fig. 5.35.** Changes of electrophysiological signals after the stimulation of acetic acid and butanedione (reprinted from (Liu et al., 2010), Copyright 2010, with permission from Elsevier

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And, the recorded signals may be analyzed for individual spikes, the extracellular correlates of an action potential generated by a neuron, or the spikes of small populations of cells detected by one electrode. The overlapping potentials of larger populations of cells creates low-frequency components in the recording (local field potentials, LFP) that may reveal additional information not to be gained from spike data. So, the defined epithelial strata afford facile identification of extracellular electrophysiological recording sites with microelectrodes. In the study, we can distinguish the different discharge modes of spontaneous signals from those after the stimulation of butanedione and acetic acid both in time and frequency domain. The differences of spatio-temporal analysis may provide powerful support with pattern recognition for a practical bioelectronic nose system in the future.

## 5.5 Biochips

The completion of the human genome has revolutionized the traditional medical and biological research. It opens the door to tremendous analytical opportunities ranging from diagnostic tests for mutations to the assessment of medical treatment. Wide-scale DNA testing, the analysis of complex DNA samples and acquisition of sequence and expression information require the high throughput, parallel process analyzing, and sensitive techniques. The urgent demand for global analytical tools drives the rapid development of the biochip technology. The most attractive features of these devices are the miniaturization, speed and accuracy. DNA chips are the first kind of biochip. A number of terms, like DNA microarrays, gene chips or biochips, are often being intermixed to describe these devices. Such use of DNA microarrays is currently revolutionizing many aspects of genetic analysis. Soon, other types of biochips emerged, such as protein microarrays, tissue chips, cell chips, and lab-on-a-chip (Kricka, 2001). The microarray technology integrates molecular biology, advanced microfabrication/micromachining technologies, surface chemistry, analytical chemistry, software, robotics and automation. All these chips share some common design characteristics, based on the specific hybridization, microarrays, optical readout, and software analyzes. Therefore, they all belong to the “biochip” category. In the following text, the basic fabrication processes, the detecting systems and the related applications will be presented.

### 5.5.1 *Chips of Microarray*

Biosensors are small devices which utilize biological reactions for detecting target analytes. Such devices commonly couple a biological recognition element with a physical transducer that translates the recognition event into a useful detectable

signal, such as an electrical and light signal. And the biochips are characterized by the high-throughput parallel analyzing arrays (Wang, 2000). However, at the individual spot of microarray, it can be seen as a biosensor, where the hybridization information or other characteristic information is converted to a recognizable signal, such as fluorescence intensity. Therefore, generally speaking, the biochip is a biosensor array. Compared to biosensors, biochips can detect multiple analytes at the same time in a small area chip. That improves the analyzing efficiency greatly and decreases the volume of the analytes and reagents.

However, there are many differences between biosensors and microarray chips:

*The range of the sensitive element:* For the biosensor, DNA and enzymes are the most common used sensitive elements; in addition, the antibody, microorganism and cells can also be used to construct the biosensors. In the field of microarray chips, DNA chips have been commercialized. Protein arrays, tissue arrays and cell arrays are being developed as well.

*Signal transforming methods:* Many detection methods are employed by the biosensors, such as electrochemical analyzing, ion-selective electrodes, ion-selective FET, piezoelectric devices, optical methods; but the biochip is primarily based on the fluorescence detection and the following image process. Therefore, the related information can be readout.

*Application fields:* Biosensors have been applied broadly, such as agriculture, food, and environment and have not been restricted in the biomedical fields; the biochip is mainly used in the basic research for analyzing the DNA, protein, cell, and tissue.

### 5.5.2 Gene and Protein Chips

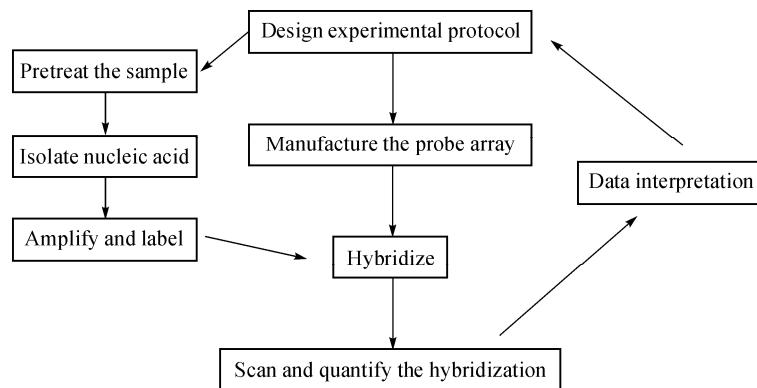
Microarray technologies of gene chips and protein chips have become crucial tools for large-scale and high-throughput biology. They allow fast, easy and parallel detection of thousands of addressable elements even in a single experiment.

#### 5.5.2.1 DNA Microarray

DNA is a very uniform and stable molecule which binds its complementary targets by means of a well-defined base-pairing principle. Based on this principle, DNA microarray is well developed. Currently a variety of DNA microarray and DNA chip devices and systems have been commercialized, which allow the DNA and/or RNA hybridization analysis to be carried out in microminiaturized highly parallel formats. Such array technology integrates molecular biology, advanced microfabrication/micromachining technologies, surface chemistry, analytical chemistry, software, robotics and automation (Heller, 2002).

The afterward microarray chips just adopt the basic process of DNA microarray. Fig. 5.36 shows a complete generic process diagram for microarray experiments. A complete microarray process includes a designing array probe, namely a capture molecule, selecting support material and manufacturing the chip; on the other hand, the analytes need pre-treating and labeling. In appropriate experimental conditions, capture molecules hybridize with target molecules. Then the chip is scanned and the data is post-processed and interpreted. Combining the bioinformatics resources, the experimental design can be further optimized.

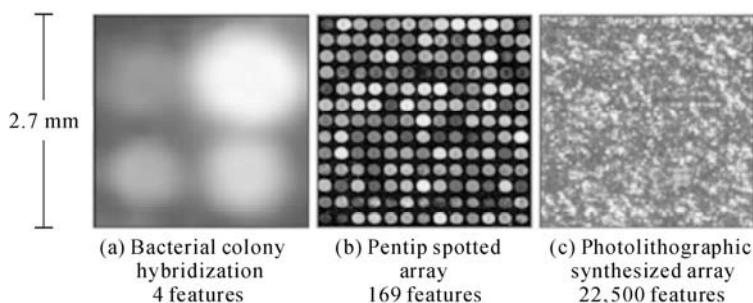
The microarray system mainly consists of four integrated parts: a disposable DNA probe array, the test sample preparation and pre-label, the interaction between the probe and the target molecules, and a scanner to read the data, along with corresponding software to control the instrument and process the data.



**Fig. 5.36.** Fabrication procedures and systems of DNA microarray

The fabrication of the DNA microarray is based on the supporting materials, such as glass, silicon, plastic, nylon membrane, and a nitrocellulose membrane. It is essential to activate the surface for a covalent attachment of the oligonucleotide probes. Then the probes are arranged in the solid support, and each site has its own physical ID. According to the density of the sites, the DNA microarray is classified into low-density and high-density DNA chips. The capture molecules can be immobilized by either a *situ* synthesis or spotting method. A *situ* synthesis method includes the use of photolithography for the *in situ* synthesis of high-density DNA microarrays, developed by Affymetrix, as well as the electronic-based addressing of microarrays developed by Nanogen. Many microarray spotting technologies and techniques now exist. Two of the more important spotting techniques used are the pin-based fluid transfer systems and the piezo-based inkjet dispenser systems. Once the probes are immobilized onto the solid support, a disposable DNA probe array is completed and can be used to detect the target molecules. The well-developed methods improve the density of the microarray and promote the manufacture of high-density DNA chips. Fig. 5.37 illustrates three kinds of microarray.

Sample preparation includes isolating and amplifying the desired form of nucleic acid molecules. At the same time, label molecules can be incorporated during synthesis of amplification products. Nucleic acid amplification can be accomplished through reverse transcription of RNA or via polymerase chain reactions (PCR) or a combination of these. The most common dyes are fluorescence molecules, such as Cy3, Cy5, FITC, F12.



**Fig. 5.37.** Feature density of representative microarray. Each image shows a 2.7 mm subregion: (a) Bacterial colony spots on nylon from the 1980s; (b) Ink-jet *in situ* synthesized oligos spots on glass; (c) Photolithographic synthesized array (reprinted from Stoughton, 2005), Copyright 2005, with permission from Annual Reviews)

Hybridization is the most important step in the detection. The fundamental parameters are time, stringency, concentration, and complexity of the sample, as well as density of available binding sites. Secondary parameters include the distribution of fragment lengths, steric effects of dye molecules, and surface chemistry. Under optimal conditions, hybridization can be well conducted. Then washing off the unbound sample after hybridization is also a crucial step.

The detection of the DNA hybridization relies on the signal generated by the binding events, therefore scanning or imaging the chip surface is essential for obtaining the complete hybridization pattern. Fluorescence imaging and mass spectroscopy are commonly used for such “reading” of the chips. Bioinformatic tools are used to translate the complexity of the data into useful information. Electrochemical detection of DNA hybridization is another important method; it meets the needs for point-of-care diagnosis and is ideal for shrinking the hardware. Scanning of a fluorescent hybridization signal can be done with CCD imaging, but now it is more commonly done with laser confocal scanners. The laser confocal approach has fundamental geometric advantages that tend to provide better signal-to-background ratios and less photo bleaching of the labels. Most devices have lasers and filter sets compatible with common fluorescent label pairs such as Cy3 and Cy5. New options for brighter individual labeling units, such as quantum dots and plasmon resonance particles, may finally allow single-molecule detection efficiency, further easing requirements on amplification and on the amount of input required for the biological sample amounts.

In general, DNA microarray hybridization applications are usually directed at

gene expression analysis or for point mutation/ SNP analysis. In addition to these important molecular biological and genomic research applications, microarray systems are also used for pharmacogenomic research, for infectious and genetic diseases and cancer diagnostics, and for forensic and genetic identification purposes. Table 5.1 shows some typical uses of the DNA microarray (Stoughton, 2005).

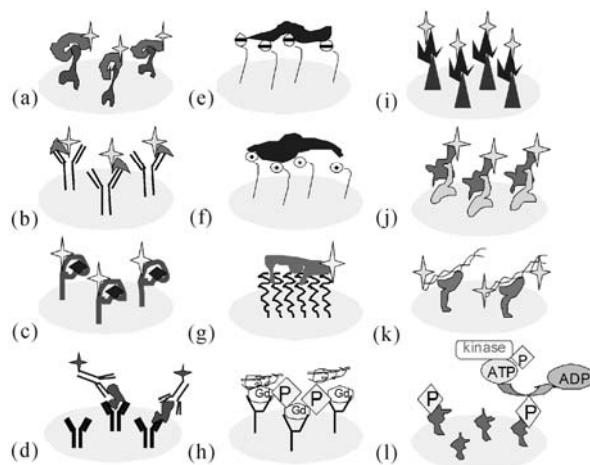
**Table 5.1** Modes of use for DNA microarray

Purpose	Target sample	Multiplexed reactions	Demultiplexing probes on array
Expressing profiling	mRNA or totRNA from relevant cell cultures or tissues	Amplification of all mRNAs via some combination of RT/PCR/IVT	Single- or double-stranded DNA complementary to target transcripts
Pathogen detection and characterization	Genomic DNA from microbes	Random-primed PCR, or PCR with selected primer pairs for certain target regions	Sequences complementary to preselected identification sites
Genotyping	Genomic DNA from humans or animals	Ligation/extension for particular SNP regions and amplification	Sequences complementary to expected products
Resequencing	Genomic DNA	Amplification of selected regions	Sequences complementary to each sliding N-mer window along a baseline sequence and also to the three possible mutations at the central position
Find protein-DNA interactions	Genomic DNA	Enrichment based on transcription factor binding	Sequences complementary to intergenic regions

### 5.5.2.2 Protein Microarray

The cellular functions of a living cell are mediated by the proteins and the accurate description of biological processes which requires in-depth knowledge of protein expression and the functional state of proteins. Proteomics development demands a powerful high-throughput tool to identify and quantify the target proteins. DNA microarray technology revolutionized gene analysis successfully, and therefore a protein microarray can be fabricated in the same fashion. Compared to the DNA microarray, the protein microarray offers more diversity. It can be used for the analysis of interactions between proteins with other proteins, peptides, low molecular weight compounds, oligosaccharides or DNA (Fig. 5.38). Technologies established for DNA chip applications have been adopted to cater for the needs of protein microarray-based research. The basic process of the protein microarray is

similar to the DNA chip, such as selecting the solid supports, modifying the support, immobilizing the capture molecule, detection methods and data interpret methods (Templin et al., 2003).



**Fig. 5.38.** Types of protein interaction and protein capture microarrays. Specific protein capture on microarrays by affibodies (a), antibodies (b), aptamers (c) or antibody sandwich formation (d). Unspecific capture is based on electrostatic (e, f), van der Waals-hydrophobic (g) or metal-chelate (h) interactions. Specific interaction microarrays have been described for receptor-ligand (i), protein-protein (j), protein-DNA (k) and enzyme-substrate interactions (l)

As shown in Fig. 5.38, there are many types of capture molecules in protein microarray, such as monoclonal antibodies, polyclonal sera, scFV/Fab, diabodies, affinity binding agents, scaffolds, affibodies, aptamers, and DNA/RNA/peptide. Monoclonal antibodies represent a virtually unlimited source of uniform, pure and highly specific binding molecules. However, antibodies, be they polyclonal or monoclonal, have some disadvantages in terms of generation, cost and overall application. The most promising alternative technologies in this field involve phage display techniques combined with highly diverse synthetic libraries. This enables the fast and efficient production of ultra diverse protein molecules and leads to the selection of binder molecules which can be directed against nearly any target within a few weeks. Aptamers are also a kind of good candidate for the capture molecules of the protein microarrays.

The lack of specific capture molecules is the main factor which still limits the broader use of protein microarray technology. There is no one-by-one interaction as observed in DNA base pairing. Proteins exhibit very diverse and individual tertiary molecular structures. Their binding interaction takes place by different means such as electrostatic forces, hydrogen bonds and/or weak hydrophobic Van der Waals interactions. In addition, individual proteins can even interact with different binding partners at the same time and in a synergistic way. At present, there is no way which would allow the prediction of high affinity protein capture molecules only on the basis of their primary amino acid sequence. Steady or

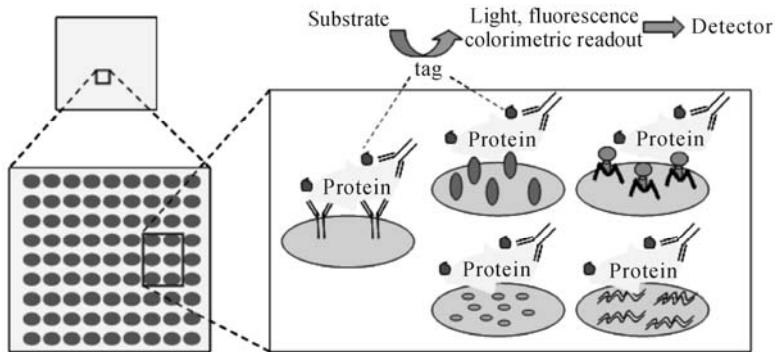
dynamic post-translational modifications like glycosylation, phosphorylation, and acetylation must also be taken into consideration. Table 5.2 illustrates some distinct properties in microarray technology between DNA and proteins (Templin et al., 2003).

**Table 5.2** Properties of DNA and proteins with respect to their applications in microarray technology

Properties	DNA	Protein
Structure	Uniform Hydrophilic acidic backbone Stable	Individual types Hydrophobic and/or hydrophilic domains Fragile
Functional state	Denatured, no loss of activity, can be stored dry	3 D structure important for activity, Avoid denaturizing
Interaction sites	1 by 1 interaction	Multiple active interaction sites
Interaction specificity	High	Dependent on individual protein
Activity prediction	Well defined, based on primary nucleotides sequence	Not possible yet. Efforts are undertaken to predict models that are based on sequence homologies, structure, etc.
Amplification	Established (PCR)	Not available yet

Capture molecules are immobilized in a microarray format in the same way as in DNA microarrays. The principles of solid support selection and probe immobilization are similar to that of a DNA microarray, and for the individual capture molecule, some special details should be considered. To detect the target proteins efficiently, the sample should be labeled by the markers, such as fluorescent molecules, enzyme, chemiluminescent substances, etc., and then be incubated on the array. Subsequently, bound proteins can be detected with the instruments that are used for conventional DNA microarray technology, such as laser confocal scanners. In addition, the protein microarray can be detected directly by the mass spectrum instrument.

Protein microarrays have broad applications. For proteomics, protein-protein interactions, enzyme-substrate assays, protein-DNA interactions, carbohydrate-protein interactions, and protein-small molecule interactions can all be conducted by using the protein microarray. Fig. 5.39 shows a proteomic array. The arrays are made by immobilizing antibodies or ligands in distinct spots. The array is incubated with a population of sample proteins, which are either directly or indirectly tagged with an enzyme or dye detected by fluorescence, light generation or colorimetric readout. The level of signal on each capture spot is proportional to the concentration of the target protein in the original sample (Liotta and Petricoin, 2000).



**Fig. 5.39.** Protein microarray

### 5.5.3 Tissue and Cell Chips

Tissue microarrays (also tissue chips) consist of paraffin blocks in which up to 1,000 separate tissue cores are assembled in array fashion to allow multiplex histological analysis. While, cell chips highlighted the multiparameters can be detected simultaneously in a range of cellular functions.

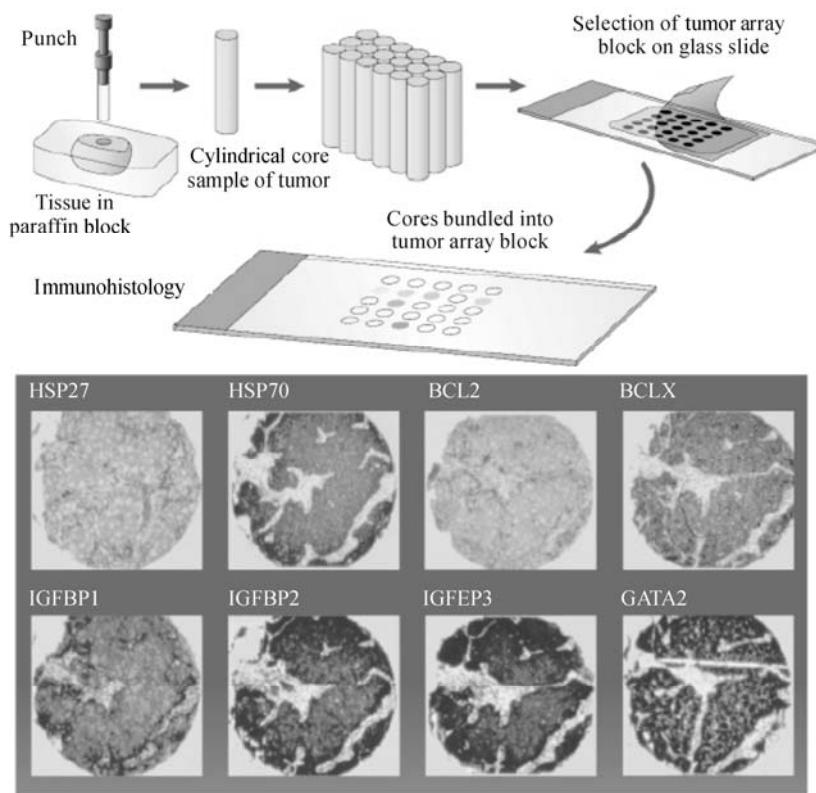
#### 5.5.3.1 Tissue Chips

Tissue chips follow the same modes of the DNA and protein microarray and a large number of specimens are arranged on one chip and treated simultaneously in an identical manner. In the past, immunohistochemistry or *in situ* hybridization, has been applied “one slide at a time” to a patient’s tissue sections. Consequently, to screen hundreds of specimens from patients, it was necessary to stain hundreds of microscopic glass slides, each containing a tissue slice. That is a rather slow and labor-intensive process. Parallel processing of a large number of histological samples will dramatically increase the throughput. Therefore, tissue microarrays are powerful research tools when it comes to screening a large number of samples for well-defined parameters, but should be considered with caution when using them as a diagnostic tool for individual cases.

Taking Fig. 5.40 as an example, we will explain the fabrication process of tissue chips briefly (Liotta and Petricoin, 2000). The array consists of 1,000 cylindrical tissue samples, each from a different patient, all distributed on a single glass slide. Each tumor is represented by a minute disc-shaped tissue section, 0.6 mm in diameter and 4 – 8  $\mu\text{m}$  in thickness. Fixed tumor tissue embedded in paraffin in blocks is sampled with a punch to generate cylindrical cores. The cores are packed together into a new block that contains cores from hundreds of patients. The composite array block is sliced into sections that are placed on a glass slide.

The slide now contains hundreds of tumor samples. Immunohistochemical staining or *in situ* hybridization can be used to query the array for specific molecules such as insulin-like growth factor binding protein (IGFBP1), apoptosis related proteins (BCL2), heat-shock proteins (HSPs) and a transcription factor (GATA2).

The potential of tissue array technology has been tested by assembling two replicas of breast cancer tumors, each with 645 samples, and screening the arrays using known breast cancer markers. The data confirmed many of the clinic pathological correlations of gene amplifications, or immunostaining reactions, reported with conventional techniques on the basis of whole-tumor analysis. Tissue arrays cannot be applied to study normal epithelium or pre-malignant lesions. This is because the punch biopsy diameters are so small that they will often miss the branching ducts or glands dispersed in the tissue. Conversely, tumor arrays are ideal for comparing large numbers of solid-tumor samples. Full automation of tissue array creation and screening is envisioned as a means to expeditiously correlate marker levels over large panels of tumors. Many continuing studies are now using tumor arrays to amplify leads from DNA arrays.

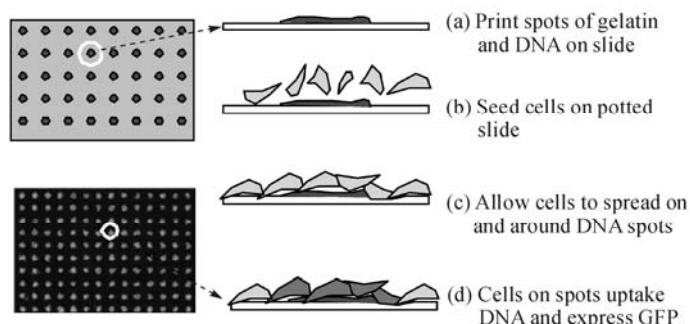


**Fig. 5.40.** Tissue microarray (reprinted from (Liotta and Petricoin, 2000), Copyright 2000, with permission from Macmillan Magazines, Ltd.)

### 5.5.3.2 Cell Chips

Generally speaking, there are two kinds of cell chips, namely cell microarray and microfluidic cell chips. The concept of “cell chips” is a general description. Sometimes microfluidic chips and lab-on-a-chips are included in the same category when they are handling the cells as the target elements. Investigators took inspiration from printing strategies used to create DNA and protein microarrays and applied them to living-cell assays, this is called cell microarray. This platform arranges the cells in the microarray in order to improve the miniaturization and parallelization and is especially useful for the high-throughput analysis of gene expressing profiling and drug screening. The cell chip based on the microfluidic technology, explores the MEMS technology and sensing techniques, to detect the parameters related to the living cells, such as cellular electrophysical signals, cellular metabolism and proliferation, intracellular ions and proteins. The greatest advantage of the microfluidic cell chip is that the multiparameters can be detected simultaneously in one chip (Wu et al., 2002).

One typical manufacturing process of the cell microarray is illustrated in Fig. 5.41. DNA is mixed with gelatin and spotted as a high-density array on a glass slide, and it is dried and stored until needed for an experiment; To perform an experiment, the array is rehydrated, and cells are seeded uniformly across the array; Cells attach and spread across the array, and those cells residing above DNA spots take up the underlying nucleotides and become transfected in a spatially localized fashion. Importantly, results from cell microarray experiments, in contrast to microtiter plate assays, must always be quantified using automated microscopy and image-based analysis because the experiments are all performed on the same substrate in the same culture solution (Yarmush and King, 2009).



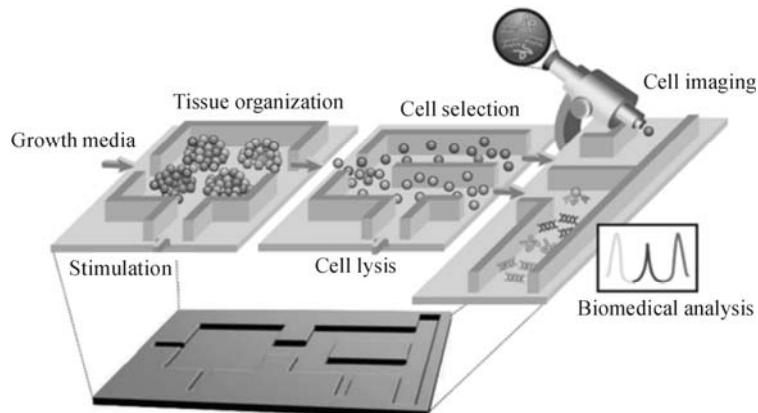
**Fig. 5.41.** Cell microarray

Cell microarrays have been applied to a broad range of applications in basic and applied biology, for example, overexpressing the defined cDNA to study the corresponding functions, to study intracellular localization of a library of genetic fusion proteins, or the Loss-of-Studies using plasmid-based siRNAs. Cell microarrays have advantages over traditional methods of expression cloning

because the identity of each cDNA is known from its array coordinates. This eliminates the need to isolate the cDNAs responsible for phenotypes of interest, a process that often requires substantial work involving fluorescence-activated cell sorting or repeated rounds of sib selection. In addition, cell microarrays can be used to identify gene products that regulate cell cycle and cell growth. The high-throughput and real-time monitoring are the most distinguished advantages.

#### 5.5.4 Lab-on-a-Chip

Lab-on-a-chip and microfluidic cell chips are all based on the microfluidic technology. Microfluidics is aimed at creating microscale closed-volume networks of channels that have emerged as a promising technology for miniaturizing and parallelizing fluid-addressable cultures and creating continuous-flow living-cell microarrays, and realizes the real-time spatial and temporal control of soluble cellular microenvironments (Yi et al., 2006). The detailed introduction of microfluidic chips has been presented in the section of Chapter 4.



**Fig. 5.42.** Tissue organization, culture and analysis in microfluidic cell chip (reprinted from (El-Ali et al., 2006), Copyright 2006, with permission from Nature Publishing Group)

The cell chip in Fig. 5.42 integrated many functional units into one small chip (El-Ali et al., 2006). Advanced tissue organization and culture can be performed in this chip by integrating homogeneous and heterogeneous cell ensembles, 3D scaffolds to guide cell growth, and microfluidic systems for transport of nutrients and other soluble factors. Soluble factors—for example, cytokines for cell stimulation—can be presented to the cells in precisely defined spatial and temporal patterns using integrated microfluidic systems. Microsystems technology can also fractionate heterogeneous cell populations into homogeneous populations, including single-cell selection, so different cell types can be analyzed separately.

Microsystems can incorporate numerous techniques for the analysis of the biochemical reactions in cells, including image-based analysis and techniques for gene and protein analysis of cell lysates. This makes microtechnology an excellent tool in cell-based applications and in the fundamental study of cell biology. As indicated by the yellow arrows, the different microfluidic components can be connected with each other to form an integrated system, realizing multiple functionalities on a single chip. However, this integration is challenging with respect to fluidic and sample matching between the different components, not least because of the difficulty in simultaneously packaging fluidic, optical, electronic and biological components into a single system. All these sequent events can be realized in one chip, and even much more complex functions can also be conducted. Therefore it is a kind of lab-on-chip or “micro-total-analysis-system” ( $\mu$ TAS) (Andersson and Berg, 2003).

Cell chips have great potentials in many aspects.

#### ***Microfabricated cell cultures***

Culturing cells *in vitro* is one of the cornerstones of modern biology. Nevertheless, even for intensively studied tissues, many of the factors that induce or stabilize differentiated phenotypes are poorly understood and difficult to mimic *in vitro*. One approach to increase control over cell-cell and soluble cues typical of *in vivo* cell environments is to combine microfabrication of 3D ECM structures and microfluidic networks that transport soluble factors such as nutrients and oxygen. Microfluidics has the additional advantage of being capable of creating mechanical strain, through shear, in the physiological range.

#### ***Cell stimulation and selection***

The control of cellular microenvironments via microfluidic systems potentially represents a valuable tool for fundamental studies of cell biology. Biological insight into the pathways that control cell phenotype and behavior can be gained by monitoring cellular responses to controlled perturbations in the extracellular environment. A wide range of Microsystems are therefore emerging with the express aim of facilitating the basic study of biochemical pathways, cell-fate decisions and tissue morphogenesis. In the next two sections, we provide examples of some techniques being applied to cell-based assays.

#### ***Biochemical analysis of cell lysates***

Currently, almost every analytical tool available in a conventional biology lab has an equivalent microfabricated counterpart. A significant research effort has been devoted to the development of integrated tools for microscale biochemical analysis. Quantitative analysis of complex biochemical mixtures, such as cell lysates, remains challenging, and with many devices success has only been achieved with low-complexity samples. The problems of low abundance and high complexity are generally handled in one of two ways: by linking sample preparation steps such as physicochemical separation and concentration before analysis, or by using high selectivity in the analytical system, typically through affinity methods based on antibodies. In this aspect, the microfluidic cell chip has profound application prospects.

## 5.6 Nano-Biosensors

In the past decade, nanotechnologies have greatly changed the state of science and technology. Nanotechnology involves the study, creation, manipulation and use of materials, devices and systems typically with dimensions ranging from 1 nm to 100 nm. The most commonly used nanomaterials include nanowire, nanotube, nanocapsule, nanopartical, nanochannel array, nanoporous membrane, etc. Now, nanotechnology is also playing an increasingly important role in the development of biosensors. Sensitivity and performance of biosensors can be improved by using nanomaterials, which display unique physical and chemical features due to effects such as the quantum size effect, mini size effect, surface effect and macro-quantum tunnel effect. Based on their submicron dimensions, nanobiosensors have allowed simple and rapid analyses *in vivo*. Even portable instruments which are capable of analyzing multiple components are becoming available.

A lot of works have reviewed the status of the various nanotechnology-based biosensors, especially at the molecular level (Chen et al., 2004; Helmke and Minerick, 2006; Kripasramanam et al., 2006). However, the application of nanotechnology to biosensor design and fabrication is also promising to revolutionize diagnostics and therapy at the cellular level. The convergence of nanotechnology, biology, and photonics makes it possible to detect and manipulate atoms and molecules by using a new class of nanoprobes and nanosensors for a wide variety of medical application at the cellular level. Nanosensors have the potential for monitoring *in vivo* biological processes within/without a single living cell, e.g., the capacity of sense individual chemical species in specific locations of a cell, which will improve our understanding of cellular functions greatly, thereby revolutionizing cell biology. In this section, we will give some examples, such as nanomaterials, nanoparticles, nanopores, nanotubulars and nanowires for nano-biosensor studies.

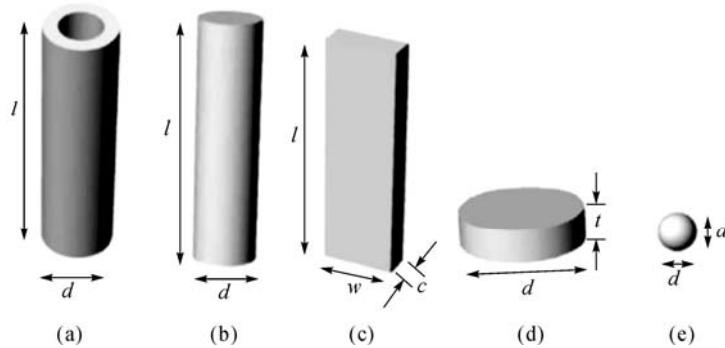
### 5.6.1 Nanomaterials for Biosensors

Nano-biosensors combined the chemistry and materials science of nanomaterials and biomolecules with their detection strategies, sensor physics and device engineering. The important types of nanomaterials for sensory applications will be introduced in this section.

#### 5.6.1.1 Nanomaterials

A long time ago, Nobel Prize winner Richard Feyman proposed that, when the dimension of the material is reduced to the similar values of its fundamental physical state, it would have a significant impact on the nature of the material. The emergence and development of nanoscience verified his prediction. In the early

stages of nanomaterials development, nanomaterial is a nanoparticles, nanofilms or solid state, composed by nanoparticles. As shown in Fig. 5.43, nowadays generalized nanomaterial is composed of basic units of material; at least one dimension within the three-dimensional space is at least in the nanoscale range (Chopra et al., 2007).



**Fig. 5.43.** Shows various forms of nanostructures with typical dimensions: (a) Nanotube,  $l$ : length (greater than 1,000 nm),  $d$ : diameter (less than 100 nm); (b) Nanowire,  $l$ : length (greater than 1,000 nm),  $d$ : diameter (less than 100 nm); (c) Nanobelt,  $l$ : length (greater than 1,000 nm),  $w$ : width (less than 500 nm),  $c$ : depth (less than 100 nm); (d) Nanodiskette,  $t$ : thickness (less than 100 nm),  $d$ : diameter (generally between 500 – 1,000 nm); (e) Nanoparticles,  $d$ : diameter (order of few nanometers) (reprinted from (Chopra et al., 2007), Copyright 2007, with permission from Taylor & Francis Group, LLC)

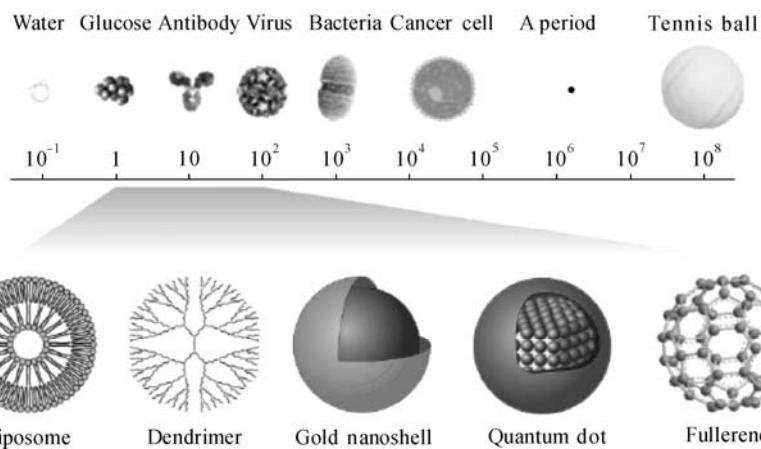
For a dot with the diameter of 1 mm, the surface atomic proportion of the total volume is only 1%, and when the diameter decreases to 10 nm, the proportion is 25%, while the diameter is about 1 nm, it is 100%, that is, all of the atoms are distributed on the surface in this case. Forces between atoms as well as changes in the proportion of non-equivalent atoms (under-coordinated atoms) determine that nanomaterials are different from their bulk materials. Therefore, new physical and chemical properties will generate in such a system.

### 5.6.1.2 Nanomaterials in the Biosensor Application

A combination of biology and materials science in the nanoscale will have a revolutionary impact on many fields of science and technology (Mcneil et al., 2005). The nanoscale in biology field is significantly relevant, for the reason that biomacromolecules such as proteins, DNA, as well as the scale of many important subcellular structures fall within the range of 1 – 1,000 nm (Fig. 5.44).

Recent research on the nanostructured materials, found that these nanomaterials have a huge potential to develop the unique capabilities of new devices and sensors. These nanomaterials have good biocompatibility, chemical stability, and changes of sensitive electrical characteristics when chemical composition changes.

They are close to biomolecules in size, which become the sensitive materials in chemical-biological sensors. In the next few years, the development of material physics and chemistry will significantly change the development of biological molecules and tissue optical, magnetic, and electrical sensing. Controlling the state of material in the nanoscale will allow for the construction of new biosensors. This new system will allow for single molecule tests in living cells, which can also be integrated to test multiple signals in parallel, and handle a number of different reactions simultaneously. Some of the sensors based on the nano-technology platform will allow the carrying out of the electrical tests of biological and chemical substances directly, without labels. This platform uses functionalized nanomaterials such as nanoparticles, nanotubes or nanowires, which bind sensitively and specifically with the tested objects including DNA, RNA, proteins, ions, small molecules, cells and pH, and many other components. Therefore, the nanostructures are widely applied in biosensors with different characteristics and special uses, and the different nanostructure biosensors will be described respectively in the following sections.



**Fig. 5.44.** The size distribution of different biological molecules and nanostructured materials (reprinted from (Mcneil et al., 2005), Copyright 1997, with permission from the Society for Leukocyte Biology)

### 5.6.2 Nanoparticles and Nanopores Biosensors

Nanoparticles and nanoporous materials possess large specific surface areas, and high sensitivity to slight changes in environments. They are the most widely used nanomaterials in the biomedical field.

### 5.6.2.1 Nanoparticle Biosensors

#### *Characteristics of nanoparticles*

Nanoparticles are usually larger than 1 nm, and are one of the most interesting nanomaterials. The control of nano-particle size, particle size distribution, shape, surface modification as well as their photoelectric chemistry application is the key to nanoparticle research. This new material level between the micro- and macro-, has many unique characteristics.

*Small size effect:* changes of macrophysical properties caused by smaller particle size are known as the small size effect. For the nanoparticle, the size decreases, while its specific surface area increases significantly, the electronic energy levels of surface atoms are discrete, the energy gap widens, the lattice changes, and the surface atom density decreases, resulting in a series of new properties. For example, for special optical properties, all the metals show black in their ultrafine particles state. The size is smaller, the color is darker, and the light reflection rate is less than 1%. Another example is the special thermal properties. The melting point of ultrafine particles is much lower, and becomes even more obvious with a size of less than 10 nm.

*Quantum size effect:* nanoparticles between atoms, molecules and bulk solid, split a continuous energy band of the bulk material into discrete energy levels, and the energy gap increases as the particle size decreases. If the thermal energy, electric field or magnetic energy is lower than the average energy gap, nanoparticles will present a series of abnormal characteristics compared with macroscopic material.

*Surface effect:* the ratio of surface atom numbers and total atom numbers increases sharply as the particle size decreases, resulting in a change of characteristics. The surface area of spherical particles is proportional to the square of the diameter, while the volume is proportional to the cube of the diameter, so the surface area/volume ratio (namely, specific surface area) is inversely proportional to the diameter. As the diameter of spherical particles decreases, their surface area will increase significantly, therefore, they have higher surface chemical activity. Surface effect is manifested mainly in lower melting points, specific heat increases and so on. Surface effect manifests itself in a lower melting point and higher specific heat.

*Macroscopic quantum tunnel effect:* the tunnel effect is one of the fundamental quantum phenomena, that is, when the total energy of microparticles is less than the barrier height, the particles can still pass through this barrier. In recent years, scientists discovered some macroscopic physical quantities including microparticle magnetization, the magnetic flux and charge in quantum coherent devices, also have the tunnel effect. They can cross the barrier of macrosystems to cause changes, so it is called the macroscopic quantum tunnel effect.

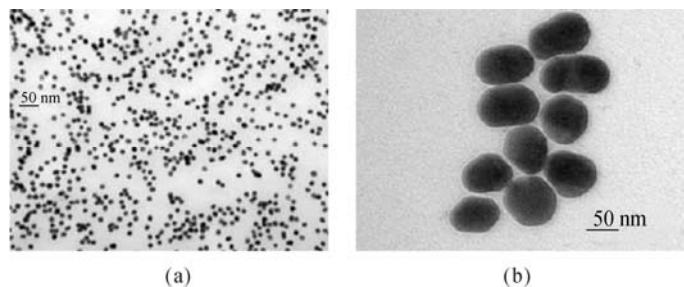
*Volume effect:* the nanoparticles contain few atoms due to their minimal size, therefore, many physical and chemical properties related to the state of interfaces, such as adsorption, catalysis, diffusion, sintering are significantly different from

that of traditional large particle materials. Consequently, the characteristics cannot be illustrated by the characteristics of the bulk material which has an unlimited number of atoms.

#### ***Preparation and modification of nanoparticles***

In addition to the characteristics of nanoparticles, their composition is also important for their applicability, for example, composition determines the compatibility and matching of nanoprobe and analyte, and also determines detection accuracy. The most common raw materials used for preparation of nanoparticles are gold, silicon and semiconductors (e.g., CdSe, ZnS, CdS) (Wang et al., 2008).

Gold nanoparticles are tiny gold particles, and usually form colloidal gold in aqueous solutions (Fig. 5.45). The sodium citrate reduction method is the most classic preparation method of colloidal gold. Different sizes of colloidal gold can be prepared by a reducing agent with different types and concentrations in the laboratory. Moreover, the method is simple and the raw materials are low cost. Colloidal gold has an absorption peak within the visible spectrum wavelength of 510 – 550 nm, and the absorption wavelength increases when the diameter of the gold particles increases. When the particle size changes from small to large, the apparent color shows a pale orange yellow, wine red, dark red, and blue-purple in turn. The properties of colloidal gold depend on the diameter and surface properties of gold particles. The diameters of the most important biomolecules (such as proteins, nucleic acids, etc.) are between 1 – 100 nm, so gold nanoparticle can be used as a probe into the biological tissue to detect biomolecule physiological functions, revealing life processes at the molecular level. Meanwhile the unique color change of gold nanoparticle particles is also an important basis for biochemistry applications.



**Fig. 5.45.** Electron micrograph of gold nanoparticles: (a) and (b) are nanoparticles with the diameter of 18 nm and 70 nm, respectively (reprinted from (Wang et al., 2008), Copyright 2008, with permission from Springer)

Silicon has been widely used in bioanalysis, such as biosensors, biochips, etc. It can be synthesized by a variety of processing techniques to prepare nanoparticles, transparent film and solid flat material. Preparation of silica nanoparticles has two classic methods. One is the reverse microemulsion method,

which is mainly used for synthesis of dye-doped silica particles and ultra-small magnetic silica particles; the other is the Stöber method for preparation of pure silicon particles and organic dye-doped silica particles. Synthetic silica particles are characterized by properties of dimension, optics or magnetics. The diameter of pure silicon particles can be confirmed by Transmission electron microscope (TEM), or scanning electron microscope (SEM), generally between 60 – 100 nm. Dye molecules in dye-doped silicon particles can be ruthenium bipyridine ( $\text{RuBpy}_2$ ), rhodamine, tetramethyl dextran, and fluorescein dextran, etc. The size and optical properties of this silicon particle are the most significant factors to determine the applications. Magnetic silica particles include  $\text{Fe}_3\text{O}_4/\text{SiO}_2$  and  $\text{Fe}_2\text{O}_3/\text{SiO}_2$  with diameter about 2 – 3 nm, which are close to super-paramagnetic material, consequently, the size and magnetic properties will determine the best synthesis conditions of magnetic silica particles.

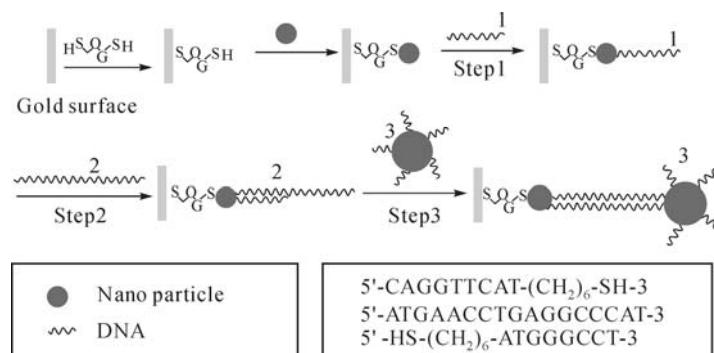
Quantum dots (QDs) are semiconductor crystal materials in nanoparticles, within 10 nm in diameter, and the volume of normal cells is thousands of times larger than that of QDs. It has a wide range of absorption wavelength and a narrow range of emission wavelength. Using different materials will result in different fluorescents. Cadmium selenide ( $\text{CdSe}$ ), zinc sulphide ( $\text{ZnS}$ ), indium arsenide ( $\text{InAs}$ ) are used for QDs, and the investigations have focused on  $\text{CdSe}$  in recent years. Synthesis of QDs offers a great deal of variety, such as the traditional fluorescent quantum dots and elongate nanobar for measuring anisotropy. The bottom-up one-step reaction is one of the classic methods, which allow for the inorganic chemical transformation and nanocrystallization processes in the same container.

### ***Biological sensing applications of nanoparticles***

Gold nanoparticles are widely applied in the sensor field, and gold nanoparticles have good catalytic activity, a strong surface-enhanced resonance, surface tension, and nonlinear optical properties, which can be used as structural and functional units to fabricate sensors. The formation of multilayer film extends the properties and application of nanoparticles, while their biological effects can enhance the sensitivity of biosensors. Gold nanoparticles can serve as the surface modification material of DNA sensors to enhance their sensitivity. For example, when gold nanoparticles are introduced into the sensitive membrane preparation, the performance of chemical and biological sensors will be greatly improved.

Another example is identification of the target gene by gold nanoparticle-DNA probes (Liu et al., 2004). These studies have shown that nanotechnology plays an important role in the DNA sensor sensitivity, stability and specificity. Scientists found that gold nanoparticles can be used to enhance the fluorescence of fluorophore indirectly in the immune optical biosensors. Fluorescence effects can be enhanced after gold nanoparticles were fixed at the right distance from the fluorophore. They also found that nanoparticles combined with the biocompatible solvent can expand by tens of times the fiber optic biosensor signal, as well as cardiac markers which can be accurately quantified to the level of 0.1 pmol.

Experiments of gold nanoparticles to enhance DNA sensor sensitivity have been carried out. Gold nanoparticles can be used as an amplifier. Besides, the sensitivity of DNA detection can exceed  $10^{-16}$  mol/L in a quartz crystal microbalance (QCM) system modified by gold nanoparticles (Fig. 5.46), and is much higher than a QCM sensor without gold nanoparticles modification. The enhancement of antibody fragments adsorption is applied in immunosensor platforms, which is based on gold nanoparticles, and QCM was directionally used as the transducer to adsorb antibody fragments. This enhancement of immobilization technology in nano-gold particles is expected as the immunosensor platform in solid-phase measurement, affinity chromatography and so on. Experiments showed that the method has good performance with high sensitivity, fast response rate, and operational stability.



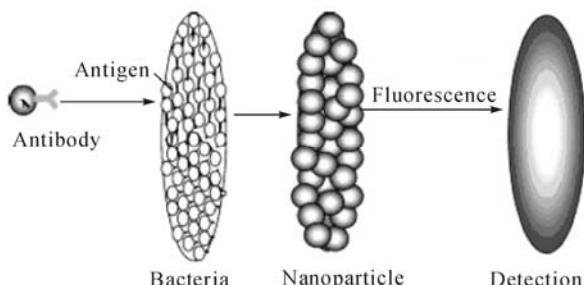
**Fig. 5.46.** DNA detection mechanism by QCM with gold nanoparticles (reprinted from (Liu et al., 2004), Copyright 2004, with permission from Elsevier Science B.V.)

At the same time, the study of enzyme immobilization by gold particles with diameter less than 10 nm also showed that gold nanoparticles can significantly improve the glucose oxidase (GOD) electrode sensitivity and lifetime. Hydrophilic and hydrophobic gold nanoparticles have good conductivity, which can be used as the electron transfer medium between the electrode surface and the GOD intermolecular to improve the electron transfer process in electrodes. Compared to using three kinds of nanoparticles, compound nanoparticles composed of  $\text{SiO}_2$  and gold or platinum can significantly improve the glucose sensor's current response.

In the biological sensors for bacterial detection, nano-particles improve and enhance the bacterial concentration and separation, signal amplification and other processes. Immunoassay based on a fluorescence signal of biomodification nanoparticles can even detect individual bacteria. Many bacterial surface antigens can recognize antibodies and combine with them, and nanoparticles can amplify the signals. The surface of every bacterial will combine thousands of nanoparticles, providing an enhanced fluorescence signal (Fig. 5.47). And a single *E. coli* can be detected within 15 min by this approach (Tan et al., 2004).

In the detection process of *E. coli* O157:H7 DNA by QCM, frequency changes of QCM caused by DNA which captures bacterial is too weak (<1 Hz). Therefore, after the electrode surface is fixed with a probe and hybrid with a target DNA, it is

necessary to introduce  $\text{Fe}_3\text{O}_4$  nanoparticles, binding it to the target DNA chain to amplify the signal by increasing the mass of nanoparticles. The QCM with amplification nanoparticles DNA sensor can detect *E. coli* of  $2.67 \times 10^2$  CFU/mL, and also found that the frequency change was linear in the concentration from  $2.67 \times 10^2$  CFU/mL to  $2.67 \times 10^6$  CFU/mL. From this result, scientists designed a resistance biosensor based on interdigitated microelectrode array for *E. coli* O157:H7 rapid detection. Biotin-labeled specific antibodies combined with streptavidin-coated magnetic nanoparticles, are used to separate enriched bacteria from the minced beef juice sample. The microelectrode array can detect bacteria concentration of  $8.0 \times 10^5$  CFU/mL, and the time from preparing sample to detecting result is about 35 min.



**Fig. 5.47.** The individual bacteria detection mechanism by nanoparticles fluorescence enhancement (reprinted from (Tan et al., 2004), Copyright 2004, with permission from Wiley Periodicals, Inc., A Wiley Company)

### 5.6.2.2 Nanopores Biosensors

#### *Characteristics of nanopores*

Nanopore material can be divided into nanopore and nanopore membranes, and carbon, silicon, silicates, metal oxides, polymer materials are common materials that can be used. Alumina nanopore membrane prepared by anodizing is used as a template material to assemble and design nanostructured material and function devices, which is a hot topic in the nanomaterials research field. Such nanopore membranes have good heat resistance and insulativity, even and orderly holes distribution, controllable size, etc. In addition to the native characteristics of the material, nanopores with special structures also have other characteristics.

(1) Large specific surface area, controllable pore size, morphology and distribution, expand the chemical properties of the material surface. Under specific temperature and pressure conditions, nanopore materials with good penetrability and selectivity, can be used for selective separation of gases. Large specific surface areas of nanopore material can contain or absorb more biochemical substances with a small size. Therefore, it can be used as the catalyst carrier and the response platform in the catalytic reactions.

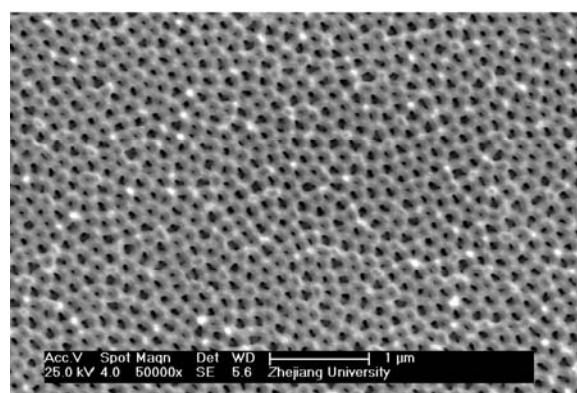
(2) The nanopore materials with large interior space may also be used for gas or liquid loading and storage, however, it is still in the preliminary study stage.

(3) Nanopore diameter of nanomaterials membrane match with the single biomolecule, so nano membrane material can also be used as the shell membrane for drug delivery and biological encapsulation to transport macromolecular drugs into the living body, and play a significant role in DNA sequencing, and single-molecule analysis as well.

### ***Preparation of nanopores***

Using the nanopore membrane as a template to synthesize other orderly nanostructures, the pore size and length of the template determine the size of the nanomaterials to a certain extent. Furthermore, it will determine the properties and functions of the nanomaterials. Consequently, preparation of different diameter and thickness of the nanoporous alumina templates is the key step in the preparation of nanomaterials.

Anodic oxidation of aluminum began in the 1920s, which was mainly for the manufacturing of electrolytic capacitors. For decades, many scientists have carried out deep and extensive research on properties, micro-structure and the growth mechanisms of anodized aluminum. Anodic oxidation of aluminum is now mainly applied in the sulfuric acid, phosphoric acid, oxalic acid, or other acidic electrolytes. Sulfuric acid has been widely used in particular due to its low cost, high transparency oxide film, good corrosion wear resistance, easy dye and electrolytic coloring.



**Fig. 5.48.** SEM micrograph of porous anodic alumina membrane

As shown in Fig. 5.48, porous alumina film has a unique and highly ordered nanopore array structure, forming a number of hexagonal micropores, which are perpendicular to aluminum substrate with a small diameter. The diameter of micropores is between 10 – 500 nm depending on different oxidation conditions. The density of micropores is very large, between  $10^{13}$  –  $10^{14}$  per square meter. And the diameter of porous alumina film and other parameters changes under different

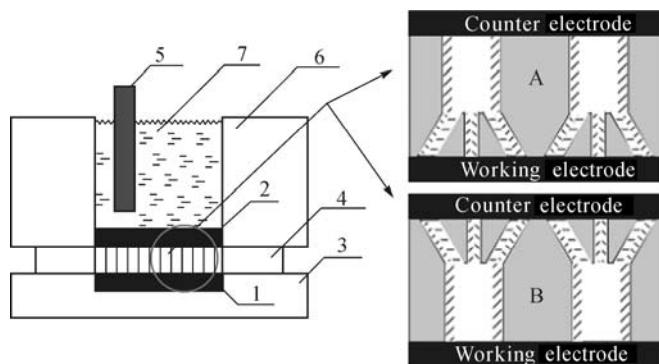
conditions. However, the porous alumina film maintained its fixed shape and structure features, so porous alumina film has the benefit of good heat resistance, stability and insulation, even pore distribution and higher pore density compared to the polymers.

### ***Biological sensing applications of nanopores***

In recent years, due to humidity sensors and ammonia sensors in food quality tests and the importance of meteorological research, development of these sensors have aroused people's attention. Among them, Pennsylvania State University, developed a humidity sensor and an ammonia body sensor, and did a print out of an Au electrode with different nano apertures on one side of an alumina film by an evaporation mask, and using an anodic porous alumina membrane as the sensitive medium. Studies have shown that the device's response to the behavior of ammonia and humidity strongly depends on membrane pore size and operating frequency. In the 5 kHz frequency, with an average pore diameter of 13.6 nm sensor ammonia and argon at work, the magnitude of its impedance increased two orders; while at the same frequency, the same sensor at 20% – 90% relative humidity environment, the magnitude of impedance can increase three orders, showing good sensitivity properties.

Nanopores with dimensions comparable to the sizes of biological polymers, such as short DNA and peptides, have been successfully. Researchers from Mexico State University have used single nanopores to sensor DNA hybridization via ionic conductance (Vlassiouk et al., 2005). Their study showed that nanoporous alumina modified with covalently linked DNA can be used to detect target DNA by monitoring the increase in impedance of the electrode upon DNA hybridization, which resulted from blocking the pores in the ionic flow (Fig. 5.49). Using cyclic voltammetry, direct current conductance, and impedance spectroscopy, they confirmed the importance of pore size: the effect is observed with 20-nm-diameter pores and is absent for 200 nm pores. So, nanoporous alumina with covalently linked ss-DNA on its surface can be used for electrical detection of complementary target DNA sequences without modification.

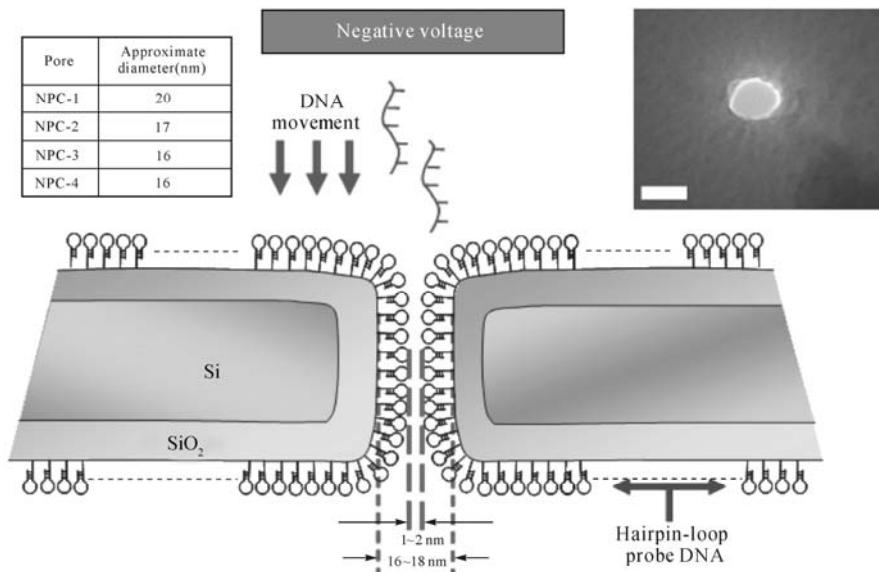
At the same time, solid-state nanopores have emerged as possible candidates for next-generation DNA sequencing devices. Researchers from Purdue University reported that the DNA sequence would be determined by measuring how the forces on the DNA molecules and also the ion currents through the nanopore, and ion current change as the molecules pass through the nanopore (Iqbal et al., 2007). Functionalized with a “probe” of hair-pin loop DNA, nanopores can selectively transport short lengths of “target” ssDNA that are complementary to the probe under an applied electrical field. As shown in Fig. 5.50, even a single base mismatch between the probe and the target can result in longer translocation pulses and a significantly reduced number of translocation events. The results can be explained in the conceptual framework of diffusive molecular transport with particle-channel interactions.



**Fig. 5.49.** DNA sensor based on nanoporous alumina. Two options for filter (4) orientation [A, working electrode (1) at the 20 nm side of the membrane; B, at the 200 nm side] in the homemade electrochemical cell with a stainless steel screen counter electrode (2) and reference minielectrode (5), immersed in solution (7). The working electrode is made of Pt. (reprinted from (Vlassiouk et al., 2005), Copyright 2005, with permission from American Chemical Society)

Putting the binding properties of receptors together with a transducer to form biosensor systems for a variety of applications has been achieved by reintegrating receptors or ion channels into lipid membranes, because of their immobilization conditions closely resemble the natural cellular environment. Recently, advances in the development of nanoporous substrates for electrochemical characterization of membrane protein-containing lipid bilayers have greatly improved techniques for lipid membrane self-assembly and membrane protein incorporation on these substrates. Anodic aluminum oxide is one of the particular interesting nanoporous membranes due to its excellent biocompatibility as well as its established fabrication process. With precise pore diameter and length achieved, anodic aluminum oxide can be used as solid supported membranes to lipid membranes, and appear to be well suited for the development of membrane biosensors with fully functional transmembrane ion channels. Instead of single nanopores, porous arrays consisting of densely packed pores will avoid current leakages and be capable of working with lower protein concentrations or alternatively with proteins that have a low charge translocation rate, such as ion-transporters. Several channel peptides and proteins have been studied with single channel recordings on the platforms. Ion-channel and receptor protein functionality was measured for up to 10 h and the blocking capacity of the membrane was observed for up to 6 d.

And, because of its excellent biocompatibility as well as the established fabrication process, nanoporous anodic aluminum oxide is of particular interest in cell culture and monitoring cell response. Alumina surfaces incorporating porous features on the nanoscale show significant biointegration and cell ingrowth; and, in addition, the cell response can be improved with nanoscale architecture. It has already been extensively used as a substrate for tissue construction.



**Fig. 5.50.** Cross-section of the solid-state nanopore channels (NPC) functionalized with HPL-DNA molecules (not drawn to scale). The inset table shows dimensions of the various NPCs used in this study. The inset TEM image shows the NPC-2 before functionalization (scale bar, 20 nm) (reprinted from (Iqbal et al., 2007), Copyright 2007, with permission from Nature Publishing Group)

### 5.6.3 Nanotubes and Nanowires Biosensors

Since carbon nanotube was discovered in 1991, one-dimensional nanomaterials have attracted the interest of researchers. One-dimensional nanomaterials have a new structure, which can be defined as material, whose mean free path for its nanomaterials charge carriers is greater than a two-dimensional scale. Based on morphology characteristics shown by the electron micrograph, one-dimensional nanomaterials are divided into nanowires, nanorods, nanobelts, nanotubes, etc. A lot of work on these materials has been carried out in synthesis, characterization, and applications.

#### 5.6.3.1 Characteristics of nanotubes and nanowires

One-dimensional materials have a high apparent ratio (scale limitation in both directions), so the specific surface area is large, and the Debye length (the role distance of either charge electric field in the plasma) is close to the material size. As a result, the surface chemical processes have high sensitivity due to these properties. Because of the scale limitation effect, these materials have an adjustable band gap, high optical gain and rapid response, along with other characteristics.

One-dimensional nanomaterials have attracted extensive attention among scientists, for the reason that they provide many methods for mesoscopic physics research, and also provide material for the preparation of nanodevices. One-dimensional materials can be used to study dependence on the dimension and scale reduction caused by electronic heat transfers and mechanical properties, which is expected for connection parts and functional units to play an important role in the preparation of electronics, optoelectronics, electrochemical and electro-mechanical devices, etc. in nanoscales.

Some metal nanowires will act as the semiconductor, after the diameter reaches a certain scale. Some consider that the conduction band and valence band move backwards extending the band-gap due to the quantum constraints. Some metal generates the ballistic effects of electronic conduction in the nanowires pattern. Semiconductor materials in nanowire patterns maintain the original electrical properties (e.g., 17.6 nm GaN nanowires), others were insulators (e.g., 15 nm Si nanowires). In addition, nanowires have many important photoelectric properties, such as field emission, surface plasmon resonance, photoconductivity and optical switching characteristics, and so on. One-dimensional nanomaterials have many different characteristics from those of bulk materials, and will not be repeated here.

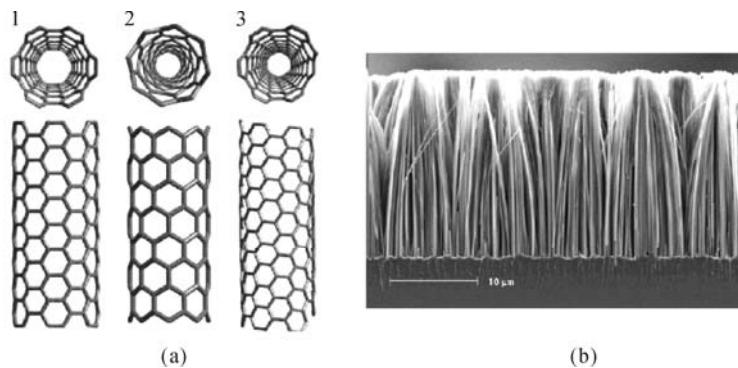
One-dimensional materials have a lot of unique characteristics, so that they are extensively studied as the sensitive material of sensors. The properties include the following aspects:

- One-dimensional nanomaterials have large specific surface areas, which mean that a large proportion of atoms or molecules are on the material surface, and are involved in surface reactions.
- The Debye length of most semiconductor oxide nanowires is equivalent to the radius in a wide temperature range and the doping level, which means that their electrical properties will be strongly influenced by the surface processes. It can be seen that the conductance of nanowires can change with the surface processes in the state of complete insulation and high-conductivity, which can improve test sensitivity significantly.
- For oxide semiconductor nanowires, they have a more certain chemical ratio, more complete crystal form, and are more stable than the multi-particle oxide sensors.
- Nanowires can build field-effect transistor (FET) devices easily, which make it possible to combine with existing preparation device technology. The construction of three-terminal FET devices can control the energy band position in the Fermi level. Furthermore, it can influence and control surface processes by electrical means.

### 5.6.3.2 Preparation of nanotubes and nanowires

The growth mechanism and crystallization process of one-dimensional mainly includes nucleation and growth processes. When the concentration of solid construction components (atoms, ions or molecules) is high enough, they are

gathered into small clusters (or cores) by homogeneous nucleation. Larger structures are produced by a continuous component supply. The growth process should be reversible, and the rate is controllable. According to the synthetic environment, preparation methods of one-dimensional materials can be generally divided into vapor deposition and liquid deposition. And there are also the corresponding physical and chemical reactions that need to be considered, during these processes. One-dimensional structures for many materials have been synthesized by different methods, including simple substance and compound materials, such as carbon nanotubes, silicon nanowires, tin nanowires, GaN nanowires, and various one-dimensional nanostructures of metal oxides. At present, carbon nanotubes, and silicon nanowires are two hot topics in science and technology studies, shown in Fig. 5.51.



**Fig. 5.51.** Carbon nanotubes and silicon nanowires: (a) 1, 2, 3 are carbon nanotubes of the armchair, zigzag and chiral structures, respectively; (b) Electron micrograph of silicon nanowires

Arc discharge is the main method to produce carbon nanotubes. As early as 1991, the Japanese expert in electron microscopy, Sumio Iijima, discovered by accident, carbon molecules (namely carbon nanotubes) which consisted of tubular coaxial nanotubes, when he observed spherical carbon molecules produced by graphite arc equipment under high-resolution TEM. A carbon nanotube is several layers to tens of layers of coaxial pipe, also consisting of hexagonal carbon atoms, with a fixed distance about 0.34 nm between layers and a diameter of 2 – 20 nm. Because of their unique structures, carbon nanotubes research has important theoretical significance and potential application value. For example, its unique structure is an ideal one-dimensional model material; with a large aspect ratio, that is expected to be used as a tough carbon fiber, and its strength is 100 times stronger than steel with only 1/6 the weight of steel; besides, it can be used as molecular wires, nanosemiconductor materials, catalyst carrier, molecular absorbent, near field emission materials and so on.

Semiconductor properties or metallic properties of carbon nanotubes are determined by the curl direction, which is difficult to control effectively during the preparation of carbon nanotubes, making it difficult to perfectly prepare

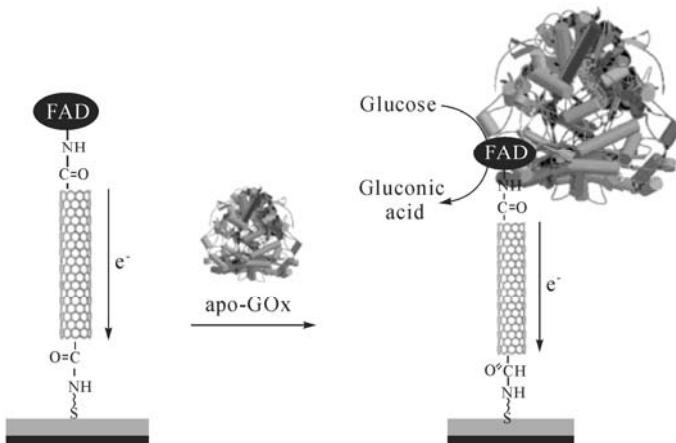
semiconductor properties or metal properties of carbon nanotubes. Therefore, it limits the applications of carbon nanotubes in electronic devices. While one-dimensional silicon nanomaterials have stable semiconductor properties and are compatible with modern semiconductor technology, consequently, silicon nanomaterials have better practical value in microelectronic fields. Silicon nanowires were prepared by lithography technology as well as the method of using a scanning tunneling microscope, but the output was very low, which restricted the practical application. Until 1998, a large number of silicon nanowires were prepared successfully by a light burning technique, and this allowed for the faster development of silicon nanowires. Since then, synthesized silicon nanowires had also been prepared successfully by chemical vapor deposition (CVD), hot gas-phase deposition, organic solvent growth and other methods, respectively.

### 5.6.3.3 Applications

#### *Carbon nanotube biosensors*

Carbon nanotubes are formed from carbon atom layers of graphene sheet rolled into a seamless, hollow tank; its radial diameter is between 1.4 nm to 60 nm, and with an axial length range from a few microns to more than one centimeter. According to the wall configuration, the number of layers of carbon atoms can be divided into single-walled carbon nanotubes (single-walled nanotube, SWNT) and multiwalled carbon nanotubes (multiwalled nanotube, MWNT). As a one-dimensional nanomaterial, carbon nanotubes have good mechanical properties, have better toughness and strong compression capabilities, and will help build biochemical sensors; wall carbon nanotubes, however, have a large number of topological defects, have greater reactivity, and offer both metal and semiconductor conductivity. These unique properties can make carbon nanotubes develop into different types of nano-scale bio-electrodes and sensors.

In enzyme-based biosensors, the electrical contacting of redox enzymes with electrodes has been a subject of extensive research in the last decade, with important implications for developing biosensing enzyme electrodes. Tethering of redox-relay units to enzymes associated with electrodes, the immobilization of enzymes in redox-polymers, and the reconstitution of enzymes on relay-cofactor units associated with electrodes were reported as means to establish electrical communications between redox proteins and electrodes. Single-walled carbon nanotubes (SWCNTs) were reported to have been used as a nanoconnector that electrically contacted the active site of the enzyme and the electrode (Patolsky et al., 2004). The electrons are transported along distances greater than 150 nm and the rate of electron transport is controlled by the length of the SWCNTs. The compatibility of SWCNTs with the preparation of novel biomaterial hybrid systems may have fascinating new properties for enzyme biosensors (Fig. 5.52).



**Fig. 5.52.** The reconstitution of glucose oxidase on a flavin-adenine-dinucleotide (FAD)-functionalized single-walled carbon nanotube (SWCNT) associated with an Au electrode yields an electrically contacted biocatalyst. The efficiency of the electrical contact is controlled by the length of the SWCNT (reprinted from (Patolsky et al., 2004), Copyright 2004, with permission from Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim)

In addition, the immune sensors, combined with the electrochemical analysis method, based on carbon nanotubes immune sensors provide the advantages of being low-cost, and offering fast and convenient methods for testing. The anti-Ig (anti-immunoglobulinG) and IgG fixed with carbon nanotube arrays, using electrochemical impedance spectroscopy detection of unlabeled antigen-antibody binding conditions, can be tested by changing the parameters to improve the detection sensitivity. In addition, the carbon nanotubes-DNA and carbon nanotubes-RNA complex, are also increasingly applied to gene diagnosis *in vivo* experiments.

After enzymes, antibodies, DNA, and other biological molecules have been fixed on carbon nanotubes, the promising nano-sensors can be designed. Produced through a variety of models with specific biological features, devices can be used for drug delivery and cell pathology research. The use of the spiral structure of carbon nanotubes is also an easy method for splitting chiral molecules. The disadvantage is that the current preparation processes, due to the complexity of the carbon nanotubesands, make it more difficult to obtain pure carbon nanotubes, for which scientific research has brought certain difficulties, and is not conducive to the carbon nanotubes in industrial applications. It is also believed that with improvements in the preparation and purification processes, carbon nanotubes will open up a whole new broader area for biosensor development.

#### **Silicon nanowire biosensors**

Silicon nanowire is a new one-dimensional nanomaterial, and their line diameter is about 10 nm, which is a single crystal silicon nuclei, and the outer coating is

covered with SiO<sub>2</sub>. Doped silicon nanowires, over carbon nanotubes and other nanomaterials, have better field emission properties, in the flat panel display technology, and also have better value than the carbon nanotubes, with excellent transmission performance and a better stability of the electronic properties of semiconductors (Palotsky and Lieber, 2005). Transistors are the basis for preparation of nano-electronic device components; doped silicon nanowires can be used to fabricate with excellent performance FET.

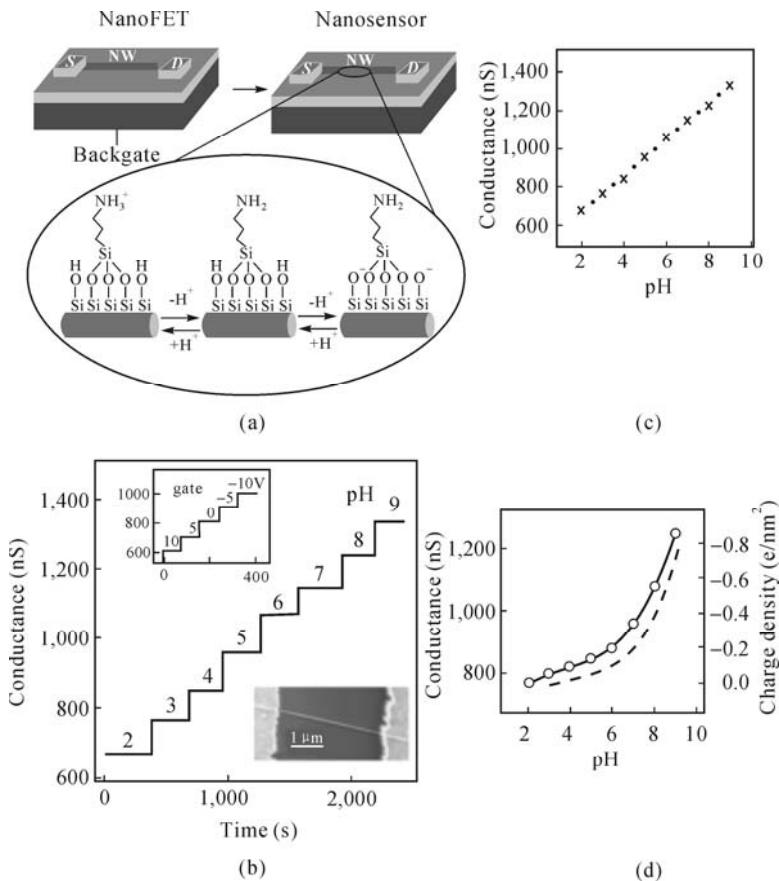
The ability of nanowire field-effect devices to detect species in liquid solutions was demonstrated for the case of hydrogen ion concentration or pH sensing (Cui et al., 2001). A basic *p*-type Si nanowire device had the silicon oxide surface modified with 3-aminopropyltriethoxysilane, which yields amino groups at the surface along with the naturally occurring silanol (Si-OH) groups of the oxide (Fig. 5.53). The amino and silanol moieties function as receptors for hydrogen ions, which undergo protonation/deprotonation reactions, thereby changing the net nanowire surface charge. Significantly, the nanowire devices modified in this way increase in conductance as the pH is increased gradually from 2 to 9. The nearly linear increase in conductance with pH is attractive from the standpoint of a sensor, and results from the presence of two distinct receptor groups that undergo protonation/deprotonation over different pH ranges.

The key role that the surface receptor plays in defining the response of the nanowire sensors was further tested by probing the pH response without modifying the silicon oxide surface. When only the silanol group function serves as a receptor for hydrogen ions, measurements of the conductance as a function of pH exhibit two different response regimes, unlike nanowire surfaces containing both amino and silanol receptors, where the conductance change is small at low pH (2 to 6) but larger compared to the high pH (6 to 9). This comparison in these early experiments clearly demonstrated that the sensing mechanism was indeed the result of a field effect analogous to applying voltage by a physical gate electrode.

### **Nanorods biosensors**

With the development of the nanotechnology, devices of nanoscale dimensions became capable of probing the innerspace of single living cells, leading to new information on the inner workings of the entire cell. As a novel approach for system biology research, it can greatly improve our understanding of cellular functions. These nano-sensors could be fabricated to have extremely small sizes, which make them suitable for sensing intracellular/intercellular physiological and biological parameters in microenvironments.

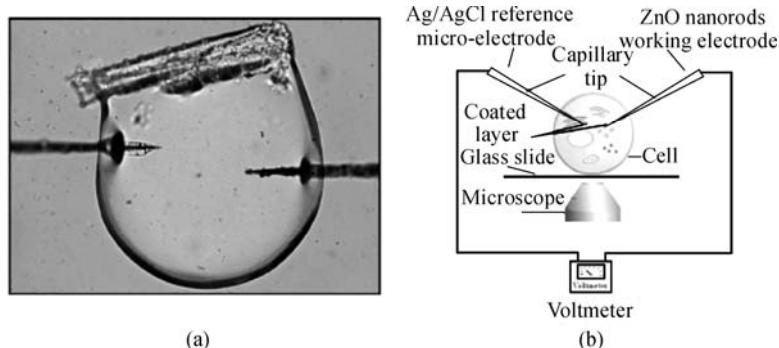
Zinc oxide (ZnO) has received considerable attention because of its unique optical, semiconducting, piezoelectric, and magnetic properties. ZnO nanostructures exhibit interesting properties including high catalytic efficiency and strong adsorption ability. Recently, researchers have been focusing toward the application of ZnO in biosensors because of its high isoelectric point, biocompatibility, and fast electron transfer kinetics (Kumar and Chen, 2008). Such properties indicate that ZnO is one of the more promising materials for biosensor applications.



**Fig. 5.53.** Nanowire pH sensors: (a) Schematic of an amino-functionalized nanowire device and the protonation/deprotonation equilibria that change the surface charge state with pH; (b) Changes in nanowire conductance as the pH delivered to the sensor varied from 2 to 9; inset is a plot of conductance data versus pH; (c) Schematic of an unmodified nanowire sensor containing silanol groups and the protonation/deprotonation equilibria that change the surface charge state with pH; (d) Conductance of an unmodified Si nanowire device (red) versus pH. The dashed green curve is a plot of the surface charge density for silanol groups on silica as a function of pH (reprinted from (Cui et al., 2001), Copyright 2001, with permission from the American Association for the Advancement of Science)

The focus of the current biosensor study is the fabrication of nanostructure ZnO nanorods suitable for intracellular pH sensing. Some authors have reported ZnO nanorods as an intracellular sensor for pH measurements (Al-Hilli et al., 2007). Their main effort has been directed toward the construction of tips capable of penetrating the cell membrane as well as optimization of the electrochemical properties. In this study, ZnO nanorods with a diameter of 80 nm and length of 700 nm, grown on the glass capillary (0.7 μm in diameter), were used to create a highly sensitive pH sensors for monitoring H<sup>+</sup> within single cells. The ZnO

nanorods, functionalized by proton  $\text{H}_3\text{O}^+$  and hydroxyl  $\text{OH}^-$  groups, exhibit a pH-dependent electrochemical potential difference versus an Ag/AgCl microelectrode (Fig. 5.54). The potential difference was linear over a large range, which could be understood in terms of the change in surface charge during protonation and deprotonation. Therefore the nanoelectrode devices have the ability to enable analytical measurements in single living cells and the capability to sense individual chemical species in specific locations within a cell.



**Fig. 5.54.** Optical image and schematic diagram illustrating intracellular pH measurements: (a) Performed in a single human fat cell using ZnO nanorods as a working electrode with Ag/AgCl reference microelectrode; (b) Schematic diagram (reprinted from (Al-Hilli et al., 2007), Copyright 2007, with permission from American Institute of Physics)

Besides intracellular detecting, some groups have also reported nanosensors for extracellular studies. For example, field-effect transistor arrays of silicon nanowire have been used to record neuronal signals. In their studies, hybrid structures of nanowire arrays were integrated with the individual axons and dendrites of live mammalian neurons. And, they think those nanoscale junctions can be well used as biosensors for highly sensitive detection, stimulation, and/or inhibition of neuronal signal propagation, with simultaneous measurement of the rate, amplitude, and shape of signals propagating along axons and dendrites. It was also a very important and successful application of nanotechnology for biosensor research.

As a summary and conclusion, biosensors have the potential to provide low cost detection and measurement technology for accurate and highly-specific quantification of low levels of important analytes in multi-component samples. The major commercial application areas for biosensor technology are medical diagnosis, food and hygiene analysis, environmental monitoring, security, and industrial process monitoring. The relatively slow rate of progress of biosensor technology from inception to fully functional commercial devices for these applications is due to both technology-related and market factors. Biosensor research has advanced to its present state in which a range of biological molecules and systems have been successfully coupled, in a relatively stable form, to transducers (generally electrochemical or optical) to provide specific analytical

devices, often with high sensitivity of detection. Future work is likely to focus on the development of strategies for enhancing biomolecule stability (that is, extending sensor lifetime) and for permitting reversibility of response and/or regenerable sensors. Most importantly, the next generation of biosensors must be able to maintain acceptable performance in complex and often potentially interference-inducing sample matrices such as whole blood.

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