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Design of Biomimetic Electronic Nose And Electronic Tongue

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The design of biomimetic electronic nose and electronic tongue is presented. First, the smell and taste sensors mimicking mammalian olfaction and gustation are described; then, some mimetic signal processing methods for the recognition of odorants and tastants are also developed. Finally, olfactory and gustatory cell-based biosensors are presented, which are based on the mimetic bioelectronic nose and bioelectronic tongue research, trying to culture living olfactory and taste cells on the surface of chips, that can detect odorants and tastants using microelectronic chips such as those based on field effect transistors (FETs), microelectrode arrays (MEAs), and light-addressable potentiometric sensors (LAPSs) to record action potential and identify the extracellular chemical and biological substances.

1. Introduction

As two of the basic senses of human beings, smell and taste play a very important role in daily life. Olfactory and gustatory sensor systems are very useful in the food industry and for environmental protection. At present, people have a considerable amount of basic knowledge about vision and hearing. A robot possessing these senses has been realized already. However, the study of smell and taste is still in an early stage of development. Only a few types of olfactory and gustatory sensor systems are in commercial use.

Therefore, the artificial-olfaction electronic nose and artificial-gustation electronic tongue are very important for future developments. Some research work has already been carried out in these fields. In our previous work,[1] we used a smell and taste sensor array combined with pattern recognition to implement the electronic nose and electronic

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tongue. Some integrated sensor arrays were produced. In order to optimize the system, a method that could adjust the structures of both the sensor array and the artificial neural network (ANN) and chaotic analysis algorithms were introduced in the system. Since the pattern recognition of smell and taste is carried out by the brain using neural networks and the responses of olfaction and gustation have some chaotic characteristics, the pattern recognition method including ANN and chaotic recognition was applied to mimic human pattern recognition. Due to the unusual characteristic of the light-addressable potentiometric sensor (LAPS), it is a very promising method of implementing olfactory and gustatory cell-based biosensors. Some successful applications of LAPS in mimetic olfactory and gustatory cell and receptor-based biosensors and in the bioelectronic nose and bioelectronic tongue can also be found in our recent research work.

2. Smell Integrated Sensor Array

Micromachining has been the key technology for the rapid development of solid-state chemical sensors in recent years. It has emerged as an important and expanding extension of integrated circuit technology. Basically, micromachining is a combination of precise etching, deposition, insulator-to-silicon or silicon-to-silicon bonding, packaging, connection between layers, and standard integrated circuit methods used to fabricate precise three-dimensional silicon-based microstructures of great diversity, including thin films, microbridges, and miniature cantilever beams. These micromachined structures, combined with special-purpose thin films and high-performance electronics, have been successfully employed to realize a large variety of solid-state sensors for determining gas composition, molecular concentration sensors, and other chemical sensors.(2) We introduce an integrated smell sensor array using micromachine techniques.

A smell sensor array was produced using microelectronic fabrication techniques. The recent advancement in micromachine techniques, such as chemical anisotropic etching and sacrificial layers, adds a new dimension to the advancement of chemical sensor research. Controlled temperature and operating conditions can be accomplished using these techniques and it is possible to construct low-mass, low-driving-power devices. A sensor array can be constructed for a multichannel sensing system for identifying various gases and their concentrations. A thin oxide-based olfactory sensor and other chemical sensors will be used to illustrate the advantages of this novel sensor structure. This work includes the design of a novel integrated olfactory sensor array with a heat insulation structure as shown in Fig. 1, microelectronic fabrication techniques, the design of an independent gas sensing element and its control of operating temperature, and the methods of recognizing various gases, among others.(3)

The experimental results show that the above-mentioned design methods for an integrated smell sensor array have obvious advantages over a separated gas sensor array in terms of size and integration. Secondly, the temperature distribution of the integrated gas sensor, which should be taken into account in manufacturing design, is discussed in the previous work.(4) The thermal analysis model derived from the array's exact structure and physical features, the equation and its boundary condition are obtained from this model. According to the complexity of the bounds, in the analysis of an array

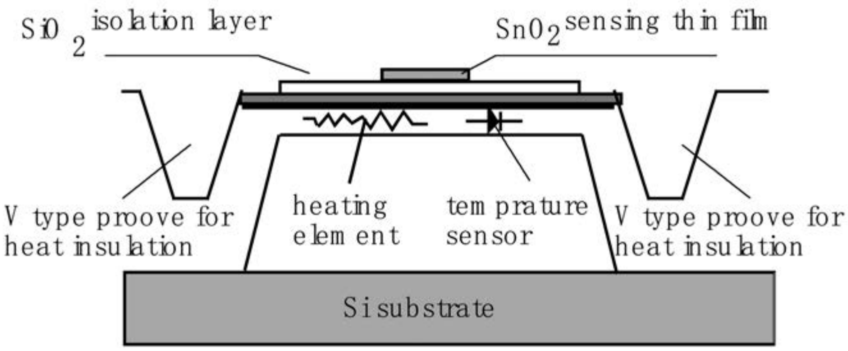


Fig. 1. Structure of one integrated smell sensor including temperature and heating elements.

model, finite element analysis (FEA) software is often used. In this analysis procedure, the result is obtained using the special FEA software. We also suggest a gas detection method that adjusts the element temperature in an array; the detection result can be processed by the clustering method.

3 Flexible Structure of Smell Sensor Array

At present, most smell sensors are made of metal-oxide-semiconductor sensing material or biological and polymer organic sensing material. Among them, metal-oxide-semiconductor sensing material is the most widely used because of its broad range of gases, high sensitivity, rapid response and ease in manufacturing.(5) With the development of microelectronic and microprocessing technology, sensors made of this material have potential for good accuracy, miniaturization and integration of the sensor array. A general defect of odor sensors is their poor selectivity, stability and sensitivity. After in-depth research on the functional membrane component surface status and membrane technology of semiconductor sensing material based on SnO_2, we applied the advanced nanometer technique used in material science to the manufacture of odor sensors. We achieved nanometer-order construction of odor-sensing material using the sol-gel technique. The sol-gel preparation technique makes it possible to make semiconductor sensing material on the molecular level. In the processing of sol-gel, components are mixed with each other in the liquid phase at the molecular level to prepare a homogeneous pure material or a homogeneous mixed material with the required components, which finally form nanometer-size crystalline grains. The technique clearly improves the accuracy and stability of sensors and offers a basis for the optimally designed odor sensor array and its stable function.

We propose a flexible design of artificial olfactory sensors as follows: generally, olfactory sensors work at constant levels of temperature t and humidity h. Here, we make gas-sensing elements work at different temperatures according to the design of optimum feature space in pattern recognition, so that gases to be recognized take up different pattern areas in the feature space. It is known from theoretical analysis and experiments that semiconductor gas sensors have different sensitivities and selectivities at different temperatures. The relationship can be expressed as $R = F(c,t,h)$ and this feature can be used to improve the similarity of the sensor array response mode. The basic method is described as follows:

1) Determine experimentally the relationship between working temperature and the response of each sensing element exposed to measurable gases.
2) Determine experimentally the relationship between working humidity and the response of each sensing element exposed to measurable gases.
3) Define the features of sensor response to measurable gases and complete the optimal feature extraction.
4) Compute the nonsimilarity between different gases of every sensing element under given working temperature and humidity levels.
5) Determine the optimal working temperature and humidity of every sensing element according to the criterion of maximum nonsimilarity.

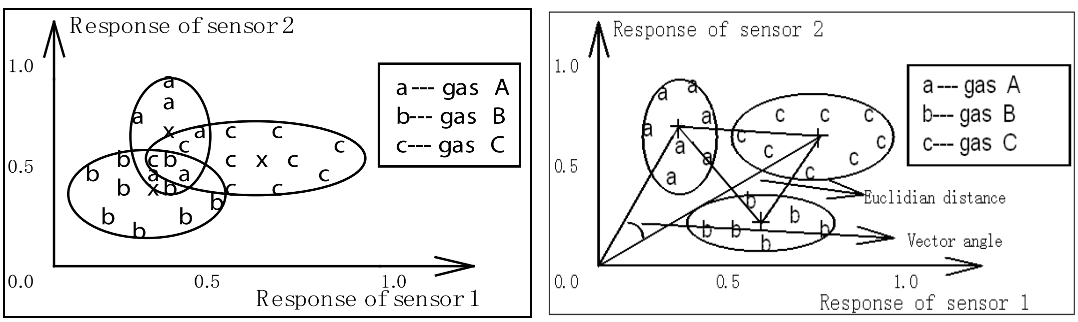
The design and comparison results of a flexible olfactory sensor array are shown in Fig. 2.[6]

4. Methods of Identifying Mixed Gases Based on Flexible Neural Network

4.1 *Existing methods*

Considerable interest has recently arisen in the use of arrays of gas sensors together with an associated artificial neural network technique to structure an artificial olfactory system or electronic nose. However, recent ANNs are characterized by network structures that have no flexibility or adaptability. That is, the network structure including hidden layers and neurons cannot be adaptively adjusted when measured gases are different.

We introduce a neural network with a flexible structure and its algorithm for an olfactory sensory system. This method involves two steps: first, we built a larger



(a) (b)

Fig. 2. Responses of two elements to three gases (ratio of voltage change). (a) Response of two sensors to three types of gas at given temperatures t_1 and t_2 before optimal design; (b) response of two sensors to three types of gas at optimal temperatures t_1^* and t_2^* after optimal design.

network structure with more hidden nodes, which is completed off-line according to tested gases; second, by on-line learning and calculating the correlations between hidden neurons and the dispersivity of the hidden neurons, useless or hardly active neurons were merged or deleted to obtain a feasible smaller network. Moreover, depending on the tested gas, the network can adaptively adjust the transfer characteristics of each neuron, i.e., activation functions, to obtain an optimum active characteristic. The experimental results show that the above-mentioned flexible design methods for a neural network can reduce the structure of the network considerably in the artificial olfactory system, and can enhance its flexible or adaptive characteristics. In addition, this design can reduce the training cycle of the neural network, while at the same time improve the accuracy in recognizing mixed gases.

At present, many ANN paradigms are used in artificial olfactory systems or electronic noses such as back propagation (BP) and self-organizing maps (SOMs).[7,8] However, the disadvantages of the above-mentioned ANNs include the fact that these network structures have no flexible capability; therefore, the training times are increased and the stability and sensitivity are also reduced. In fact, because measured gases and the effect of the environment are often changing, the neural networks in olfactory systems should have adaptive responses to mixed gases; i.e., they should be flexible. In general-purpose neural networks, the input and output layers are determined on the basis of practical problems, but the selection of hidden layers is very difficult. Theories show that a network with one hidden layer can realize arbitrary continuous monotonic nonlinear maps, and with an additional hidden layer, it has an appropriate output for representing odors. Therefore, networks with one or two hidden layers are often adopted. On the other hand, the choice of hidden units of ANN is very random; therefore, the ANN construction is not optimal.

4.2 *Flexible recognition algorithm*

We presented an adaptive structure for a neural network and its algorithm for an olfactory sensory system, namely, the electronic nose. The comparison of results before and after the addition of a flexible structure is shown in Fig. 3.[9,10]

The basic method is described as follows:

(i) Build ANN off-line.
(ii) Determine hidden layer and its neural units.
(iii) Train weights and thresholds.
(iv) Adjust ANN on-line.
(v) Input samples of gases.
(vi) Calculate correlation between units.
(vii) Calculate dispersion of each neuron.
(viii) Compress redundant hidden nodes.

4.3 *Fuzzy logic recognition algorithm*

Brain science tells us that the recognition procedure of the brain is fuzzy and that the brain uses fuzzy logic not fixed logic. For this reason, an improved SOM, which is an artificial neural network (ANN) with a fuzzy learning algorithm, was applied in the

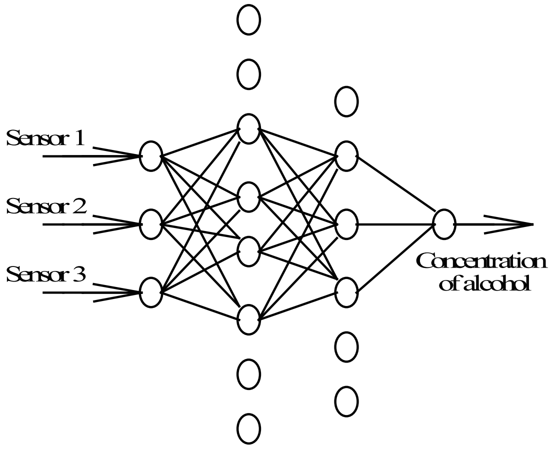


Fig. 3. Structure of flexible neural network.

pattern recognition of the five basic taste senses. We proposed an improved learning algorithm based on the fuzzy c-means algorithm (FCMA).[11] Instead of the winner-take-all rule that SOM uses, the FCMA uses the Euclidian distance to calculate the membership between the sample and the class. Also, when the FCMA updates its weight, all of the samples are incorporated, while the SOM uses only one sample to update its weight at one time. For this reason, SOM spends more time than FCMA in the learning procedure and may cause local oscillation. From this point of view, FCMA is fuzzy and parallel, and can cluster more accurately and faster. Another important property of the improved method is that the classification result of this method uses fuzzy logic, which can be found in the real biological system. Therefore, this result accurately simulates the taste sense of the actual biological system.

5. Taste Sensor Array

Existing taste sensors can only detect some physicochemical taste properties of the taste substance, and they cannot simulate the taste sense of the real biological system. These measurements are often disturbed by tasteless substances. In addition, the acquired properties cannot indicate the relationship between the different taste substances, for example, the inhibition and harmony effect. On the other hand, these taste sensors are designed to improve their selectivity to obtain a better measurement of the taste substance, but it is very difficult to manufacture a sensor that has a good selectivity for several taste substances. An effective method of solving this problem is to use a material that is very similar to the one existing in the real biological system as the sensitive membrane. Japanese scientists applied the biosensor mechanism and biological sensitive material to the realization of the taste sensor and some valuable results were obtained.[12,13] At present, the sensor ability is markedly improved and the discrimination ability is superior to that of humans by one order of magnitude in terms of the concentration of taste substances.[14,15]

In previous research,[16,17] we introduced an experiment that uses a multichannel electrode taste sensor made of biolipid material to detect the five basic types of taste (sourness, sweetness, saltiness, bitterness, and ‘umami’). We adopted the dip method to make the lipid membrane; this is a different approach from that adopted by the Japanese researchers. This method improves the characteristic of the membrane. Due to the fact that the thickness of the membrane is decreased by the dip method and the uniformity of the membrane is improved by it. When making the membrane, tetrahydrofuran (THF) was used as the solvent. Three lipids, namely, oleic acid, lecithin and cholesterol, were mixed with the plasticizer and dissolved in THF. The silver electrodes were immersed into the solution and then lifted up at a given speed. In the given environment in which temperature and time were precisely controlled, the solution formed a uniform thin membrane on the surface of the electrodes. Several experiments were carried out in order to find the optimal combination of these parameters. The Ag/AgCl electrode was chosen as the reference electrode. The finished electrodes were preserved in a 1 mM KCl solution. In another study, we improved this design by adopting the light-addressable potentiometric sensor (LAPS) technique, in order to integrate many sensor devices on a single semiconductor substrate.

In the experiment, five chemicals were used as basic taste substances: glucose for sweetness, HCl for sourness, NaCl for saltiness, quinine for bitterness, and monosodium glutamate for ‘umami.’ From the results of the experiment, we discover that this type of taste sensor, using a biological lipid membrane, only responds to substances that have taste and not to tasteless ones. The characteristics of the membrane, especially the responses to saltiness, were improved greatly in the experiments compared with those of other researchers. These sensors had a long-time drift as well as a short-time drift and both drifts were also reduced. The short-time drift is relative to the reaction equilibrium time of the reaction, but the long-time drift is relative to the aging of the lipid membrane. The reason for this phenomenon is that the artificial lipid membrane does not have the activation property of the biological membrane. The biological membrane has a metabolism and the ability to self-adjust; thus, it can have a continued reaction and maintain the stability of the membrane potential. However, the artificial membrane can only be used several times before it ceases to function. In spite of the limited number of taste substances, the taste substances in the experiment stand for the five basic tastes and the results are satisfactory as shown in Fig. 4.[17]

6. Chaotic Taste Sensor

There are different kinds of vibrations correlative to various information processing mechanisms in a physiological system, especially in the nervous system of animals, such as olfactory identification and visual identification. Recently, in the nervous system, the most important problem that interests us is the relationship between the dynamics of chaos and information processing. Chaos shows more emphasis on the common

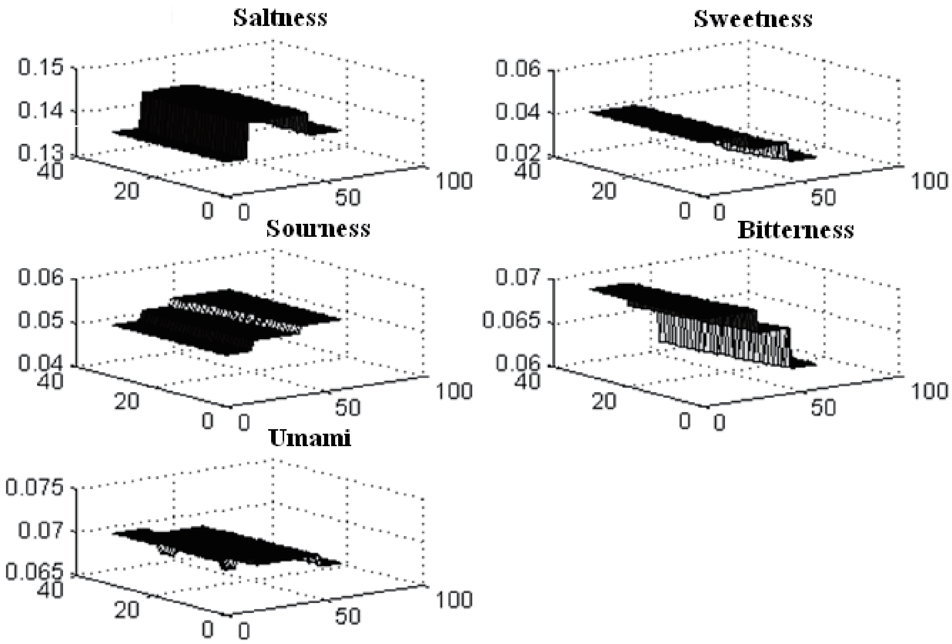


Fig. 4. Experimental results of taste images for five basic taste solutions.

characteristics. In an independent system, the study of chaos and other characteristics is also very important. It is possible to demonstrate the nature of the system via Chaos; for example, the original characteristics of the system may be demonstrated in the affinity structure in Chaos. Chaos itself is a random movement based on nonlinear performance, in which nonlinearly vibrated self-excited elements have been found in a variety of biological systems, for example, muscle cell aggregates and the olfactory system. We have studied many nonlinear phenomena involving the periodic stimulation in the giant axon of squid or in the giant nerve cell of Onchidium. By studying the response to the periodic stimulation, we can provide detailed information regarding the system. From here, we can see that chaos is an indicator for learning unknown stimulations and the initiator of unknown phenomena; in an engineering perspective, chaos may be regarded as a sensor with high sensitivity to the dynamic change of some parameters because of high sensitivity to the parameters of the relevant system.(18)

We use an experimental system that is similar to that used by Saida *et al.*(19) as shown in Fig. 5, in order to verify the response of the chaos model. In this study, we use a DOPH membrane as a lipoid membrane, the representative material of sour, bitter, sweet, spicy, and salty tastes, and a sine-signal current to simulate the sensor.

The results of the experimental and simulated data are shown in Fig. 6, of which the left corresponds to the preparatory periodic state, because the curve θ_n reflected to the position of attraction has no local minimum (or maximum). However, the right shows a

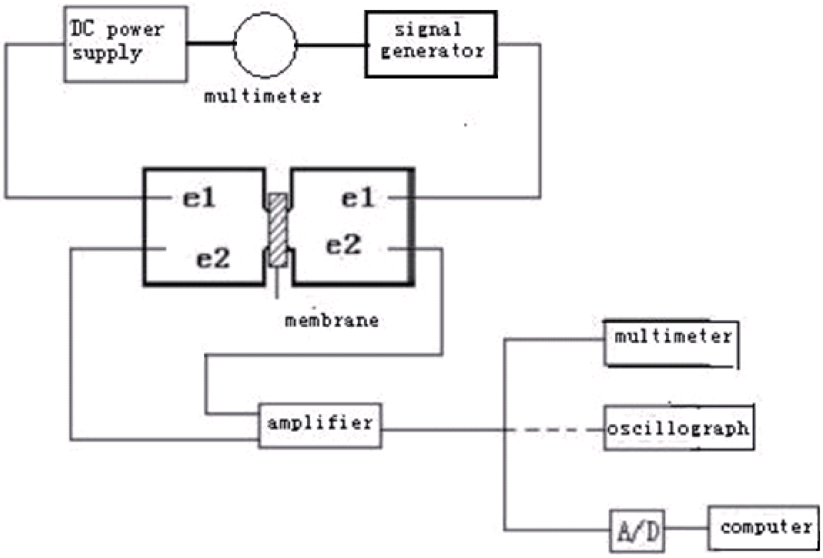


Fig. 5. Structure of chaotic taste sensor system.

chaos state because the curve has a local minimum (or maximum). It can be proved that chaos is produced by calculating the exponent λ to obtain a result in which $\lambda > 0$.[20,21]

7. Bioelectronic Nose And Tongue Using Cell-Based Biosensors

The light-addressable potentiometric sensor (LAPS), commonly used in the form of a semiconductor chip, usually consists of an electrolyte–insulator [SiO_2]–semiconductor [Si] (EIS) structure that is fit for detecting the biophysiological parameters of an extracellular microenvironment[22] as shown in Fig. 7(a). When a certain light pointer illuminates the LAPS chip, the semiconductor absorbs energy, which leads to an energy band transition, i.e., produces electron–hole pairs. Electrons and holes would recombine soon and no current would be detected by the peripheral circuit. If LAPS is biased in the depletion region, the width of the depletion layer is a function of the local value of the surface potential. When LAPS is reverse biased, the depletion layer is enlarged. The local value of the bias voltage can be read out with an ac photocurrent that is generated when a modulated light pointer is illuminated at the bulk silicon.

7.1 *Theory of LAPS detection*

To understand how an action potential is generated in electrically active cells, Hodgkin and Huxley empirically modeled the ionic currents that flow through the channels of excitable membranes. They combined this model with core conductor theory to predict the total transmembrane current (H-H theory) as shown in Fig. 7(b):

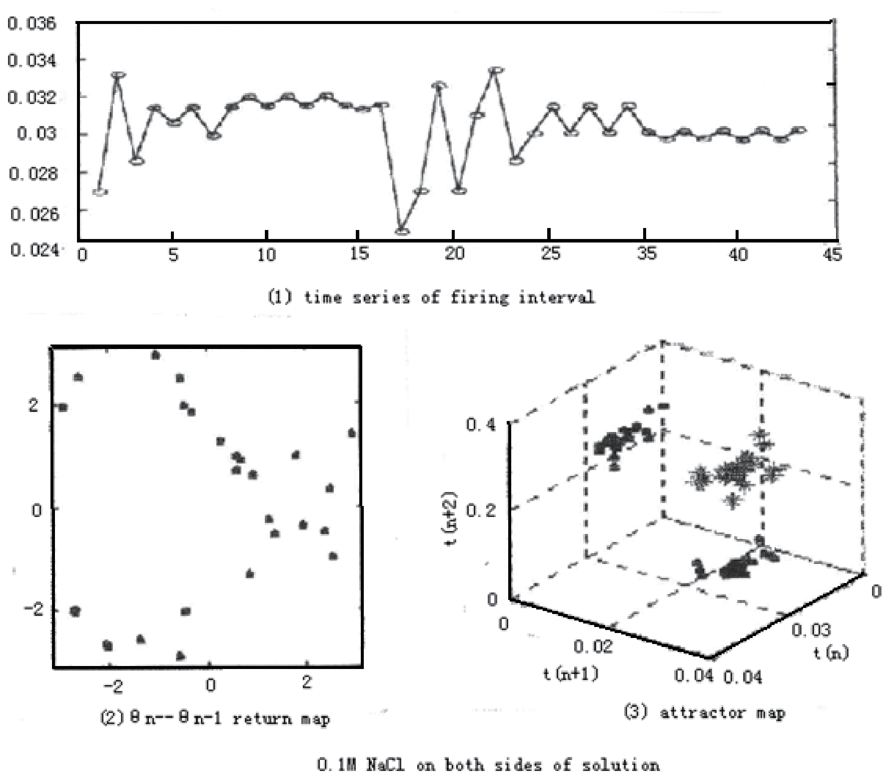


Fig. 6. Preparatory periodic property of the measurement of the taste sensor.

$$I_M = C_M \frac{dV_M}{dt} + I_{ionic} \tag{1}$$

where V_M is the transmembrane potential, C_M is the membrane capacitance per unit area, and I_{ionic} is the current due to the flow of ions through ionic channels in the cellular membrane.

According to H-H theory, when cells are cultivated on the surface of SiO_2, there is a cleft between the cells and the chip as discussed above. Considering the simplified schematic circuit of the cell/semiconductor interface shown in Fig. 7,(23,24) the relationship is obtained as

$$\frac{V_J}{R_J} = \frac{V_M - V_J}{R_{JM}} + c_M + \frac{d(V_M - V_J)}{dt} \tag{2}$$

where V_J represents the general extracellular potential detected by LAPS, R_J is the seal resistance, and R_{JM} is the membrane resistance.

To improve the signal V_J, one of the important schemes is to make the seal resistance

(a)

(b)

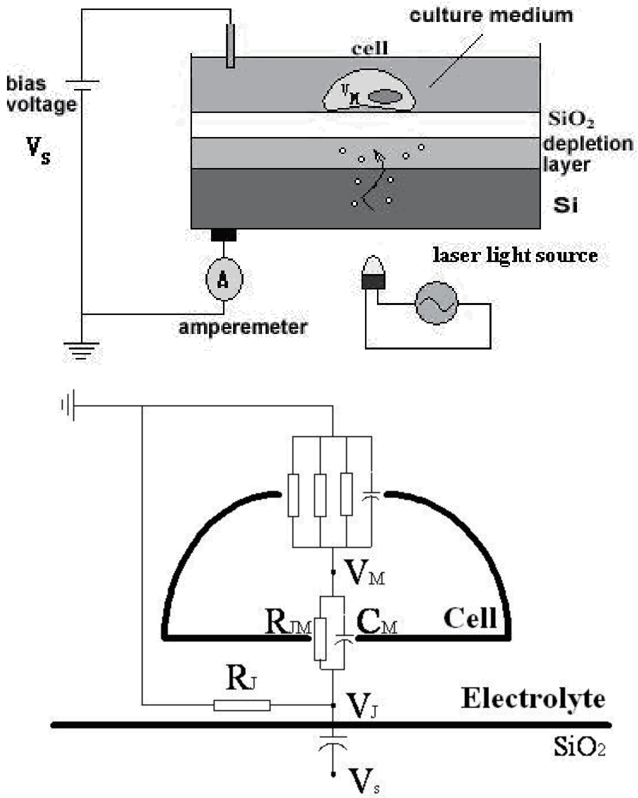


Fig. 7. Working principle scheme of cell based on LAPS. (a) Structure of LAPS device. (b) Simplified schematic circuit of the cell and LAPS interface.

R_J as large as possible. The cell-chip interaction through the core-coat conductor is enhanced by increasing thickness, cell-to-semiconductor distance, the resistance of the sealing region and water permittivity. The latter two factors are always invariable; thus, the thickness becomes the focus of concern.

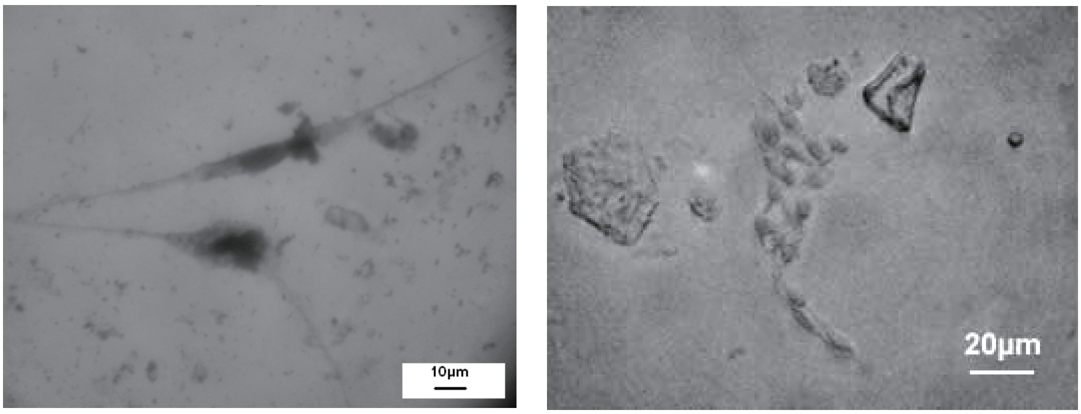
7.2 *Cellular metabolism detection*

The mechanism of signal detection and transduction in olfaction is an electrophysiological process, mainly taking place among olfactory epithelium receptor cells and their corresponding mitral cells in the olfactory bulb, after which the signals are transferred to the olfactory cortex. To improve the biocompatibility of the silicon device, the surface of the LAPS chip was coated with a poly-L-ornithine and laminine mixture prior to cell seeding, which means the deposition of a layer that can promote the attachment of cells to the surface of the chip. Figure 8 (a) shows olfactory neurons growing on the surface of LAPS for 7 days; some neuronal networks have even been formed among the cells. Figure 8 (b) shows taste bud cells that have remained on the surface of LAPS for 2–5 days.

The LAPS consists of an electrolyte-insulator [SiO_2]-semiconductor [Si] (EIS) structure. A 12×5×2 mm^3 microchamber was formed using polydimethylsiloxane on the chip to culture cells. During experiments, the LAPS chip with cultured cells was mounted under a microscope objective in the setup. The experimental system is shown in Fig. 9. The light source, which is driven by a special power supply (LDC202, THORLABS Inc.), has a wavelength of 635 nm and a power of about 2.5 mW. A chopper (Stanford Research Systems, SR540) modulates the laser beam with a diameter of 2 mm and generates modulated pulse light with a pulse frequency of 4 kHz. Then the modulated light is focused to a diameter of 10 μm by a lens onto the desired single cell. When the cell produces an action potential under drugs, there is a change in bias voltage and LAPS can detect the change and immediately generate a corresponding photocurrent, which is transmitted to peripheral equipment through the working electrode. The electrodes of a potentiostat (EG & G Princeton Applied Research, M273A) are used to detect the current.

The light from the light source of is modulated and focused vertically above the desired single cell cultured on the LAPS. When the drugs and culture medium are pumped into the chamber alternately, the cell responds to them and changes its status, which induces the cellular action potential. PC controls the pump, valve and the temperature of the microchamber. All measurements are performed at 37 ± 0.2°C.[25,26]

To primarily test the feasibility of using the LAPS for odor detection, a solution with different concentrations (1 μM, 25 μM, and 50 μM) of acetic acid (CH_3COOH,



(a) (b)

Fig. 8. Connected neuronal network among mitral cells (triangle) and granular cells (bipolar) cultivated on the LAPS.

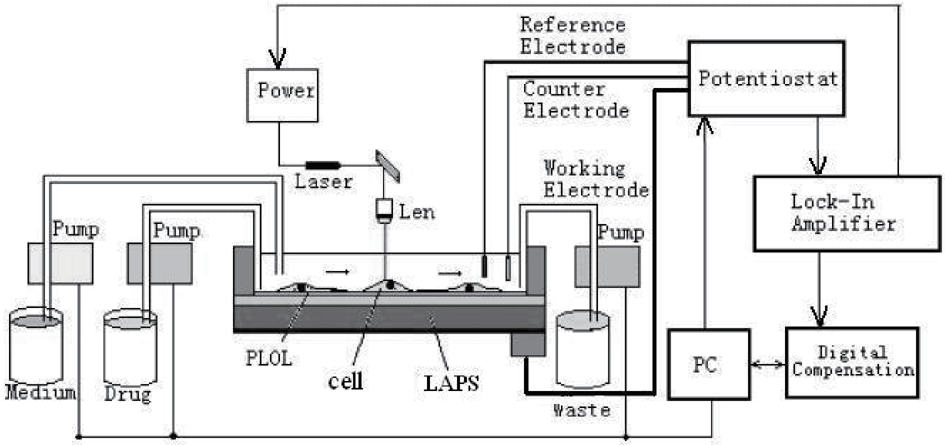
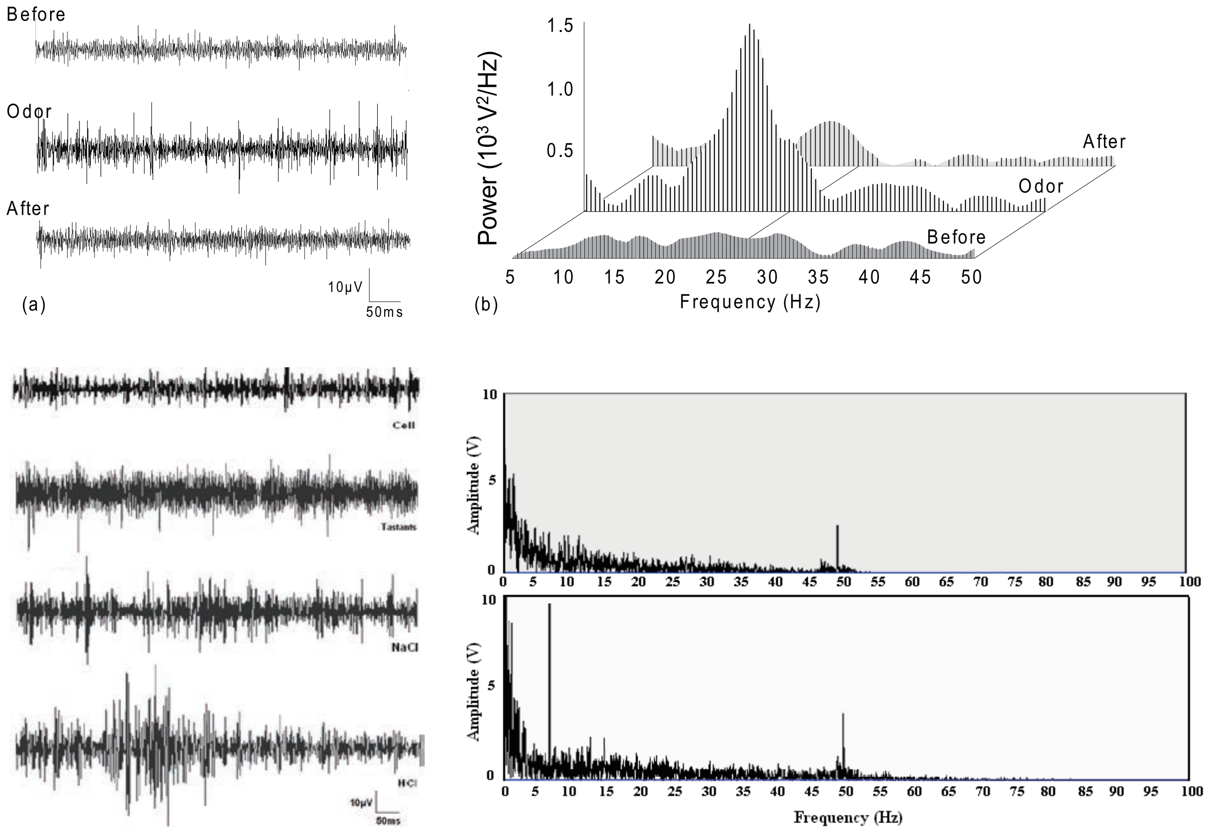


Fig. 9. Schematic drawing of the system.

an organic acid, with a distinctive pungent odor) is used as a stimulant for olfactory receptor neurons. The typical peaks were obtained, similar to those of mitral cells in Fig. 10(a). It was sustained throughout the course of the acetic acid's stimulation of the receptor cells. The result proved the excitability and desensitization of Glu to mitral cells more convincingly. Using fast fourier transform (FFT) analysis, we also found that olfactory receptor neurons showed a specific appearance frequency of 24 Hz, which occurred repeatedly with exposure to the stimulant. The amplitude of the ac signal increased in a concentration-dependent manner, and became negligible 10 min after the odor stimulation was stopped as shown in Fig. 10(b). Thereafter, we used acetic acid to stimulate mitral cells. Neither potential signals nor ac signals were found. This fact suggests that the ac signal represented the binding of the odor to the receptor neurons, and only the receptor neurons gained odor sensitivity.

Foliate and vallate taste buds were removed from the epithelium by gentle suction with a fire-polished pipette (tip inner diameter 50-100 μm) and plated on the surface of the chip. Taste bud cells were maintained for 2–5 days on the chip at 37°C under standard conditions of humidified air with 5% CO_2 and fed every 2 days with fresh DMEM. We monitored the response after four basic tastants (NaCl, HCl, Sucrose and $MgSO_4$) were applied on the taste cell cultured on the chip. Taste receptor cells treated with the culture medium produced sporadic bursts due to their electrical excitability, which were employed as control, as shown in Fig. 10(c). After applying a tastant mixture composed of 0.3 NaCl, 0.01 HCl, 0.03 $MgSO_4$, 0.5 surcose and 0.1 glumate (mol/L), we recorded the extracellular signal produced by the taste cell. The amplitude of the bursts was 10–30 µV, and the peaks centralized just at the beginning of the stimulation of the tastant mixture. These phenomena may be explained by the adaptation of a specific taste. The FFT was utilized to analyze the frequency components of each signal with MATLAB. In comparison with the control shown in Fig. 10(c), the burst shape and



(c) (d)

Fig. 10. Extracellular recording of olfactory cells. (a) Response of mitral cell under the effect of 1 μM Glu. (b) Odor-elicited extracellular potential of the olfactory receptor cells before, during and after odor exposure. (c)Recording of gustatory cells for measured tastants. After applying the tastant mixture, we recorded the extracellular signal produced by the taste cell. The burst shape and amplitude changed with a specific frequency component in the region of 7–10 Hz shown in (d).

amplitude changed with a specific frequency component in the region of 7–10 Hz, shown in Fig. 10(d). This result implies that 7–10 Hz frequency component may represent important information regarding the tastant molecule combined with those specific receptors distributed on the taste cell membrane, which reflects the peculiar character of taste perception in TRCs.

8. Conclusion

Alhough the history of the study of the biomimetic electronic nose and electronic tongue is not long, its progress has been rapid. Many methods have been applied in both the manufacture and signal processing of the sensor system, and some positive results have been obtained. On the basis of existing research, it can be anticipated that some kind of sensor system with practical commercial use will appear in the next few years.

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