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Neurochip based on light-addressable potentiometric sensor with wavelet transform de-noising*

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Abstract: Neurochip based on light-addressable potentiometric sensor (LAPS), whose sensing elements are excitable cells, can monitor electrophysiological properties of cultured neuron networks with cellular signals well analyzed. Here we report a kind of neurochip with rat pheochromocytoma (PC12) cells hybrid with LAPS and a method of de-noising signals based on wavelet transform. Cells were cultured on LAPS for several days to form networks, and we then used LAPS system to detect the extracellular potentials with signals de-noised according to decomposition in the time-frequency space. The signal was decomposed into various scales, and coefficients were processed based on the properties of each layer. At last, signal was reconstructed based on the new coefficients. The results show that after de-noising, baseline drift is removed and signal-to-noise ratio is increased. It suggests that the neurochip of PC12 cells coupled to LAPS is stable and suitable for long-term and non-invasive measurement of cell electrophysiological properties with wavelet transform, taking advantage of its time-frequency localization analysis to reduce noise.

Key words: Neurochip, Light-addressable potentiometric sensor (LAPS), Wavelet transform, Threshold, De-noising
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1 Introduction

Neuron electrophysiology is one of the main research fields in the neuroscience. By the record of extracellular potentials, electrical activities of living cells can be analyzed. In order to record the extracellular potentials of neurons, researchers have developed neurochips of microelectrode array (MEA) or field effect transistor (FET) array based on cell-based biosensors, using microelectromechanical systems (Fromherz *et al*., 1991; Fromherz, 2003; Maher *et al*., 1999; Neher, 2001; Kovacs, 2003). Being an in vitro recording system, neurochip is a technology of culturing neurons on the surface of MEA or FET array, where cells can couple to the electrodes or gates of FET in a thin layer of electrolyte. The novel biochip allows the signal transmission from cells to chips, as well as monitoring the electrical activities of neurons in a long-term and non-invasive way. With these merits, neurochips have been primarily applied to biomedical studies such as drug screening and environmental monitoring (Bousse, 1996; Pancrazio *et al*., 1999; Stenger *et al*., 2001; Wang *et al*., 2005; Wang and Liu, 2009).

Light-addressable potentiometric sensor (LAPS) is one type of the newly developing sensors with the characteristics of high repeatability, good accuracy, and linearity (Hafeman *et al*., 1988). In recent years, one availability is to make use of the surface potential sensor coupled with living cells for a novel design of

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neurochips (Parak *et al*., 2000; Ismail *et al*., 2003; Stein *et al*., 2004). Extracellular potentials of electrogenic cells coupled to LAPS can be monitored by the measurement of the relevant surface potential alteration, which allows to measure extracellular potentials at any position, for there is not any restriction for fixing recording locus (Ismail *et al*., 2003; Stein *et al*., 2004). However, during the application of cell-based LAPS biosensor, the signal-to-noise ratio of the signals measured by LAPS is low and the range of baseline drift is wide, especially in the detection of signals fired by neurons (i.e., cortical cells and olfactory cells) that are adhered to the chip surface directly (Xu *et al*., 2005; Liu *et al*., 2006). These factors limit the further studies of neurochips based on LAPS.

The pheochromocytoma (PC12) cell line is obtained from the rat adrenal medulla (Chalfie and Perlman, 1976; Greene and Tischler, 1976). The cells stop dividing and terminally differentiate to neurons after they are treated with nerve growth factors. The cellular electrophysiology has been well studied with existing methods like patch-clamp (Hegg and Miletic, 1996; Hassenklöver *et al*., 2006). It makes PC12 cell line widely employed as sensing elements of convenient neuronal models for a variety of cell-based biosensor studies (Huys *et al*., 2008; Slaughter and Hobson, 2009).

In this study, based on the theories of LAPS detecting extracellular potential system, PC12 cells are cultured on LAPS to construct a new kind of neurochip, which is used to detect the characteristics of cell discharging instead of primary cells in the previous studies. And, because wavelet transform is the localized space-time frequency analysis, it has good resolution both in the time domain and frequency domain in biosensor studies (Manganiello *et al*., 2002; Artursson and Holmberg, 2002; Zhu *et al*., 2009). According to the characteristics of the detected signals, wavelet transform is chosen to analyze and de-noise the signals. At last, both the neurochip of PC12 cell line-based biosensor and the de-noising technique based on wavelet transform are discussed for a wide using of this platform.

2 Theories

2.1 Neurochip based on PC12 cell line hybrid with LAPS

LAPS is an insulated semiconductor biosensor device, with electrolyte insulator semiconductor (EIS) as the basic structure as shown in Fig. 1a (Hafeman *et al*., 1988). When the chemical or biological properties of electrolyte change, surface potentials that reflect electrical charges at an electrolyte-solid interface can be recorded. While light is illuminating on LAPS, the semiconductor is absorbing energy, leading to the transition of energy band and the production of electron-hole pairs. When the depletion layer of LAPS is biased, the width is a function of local potential value of the surface.

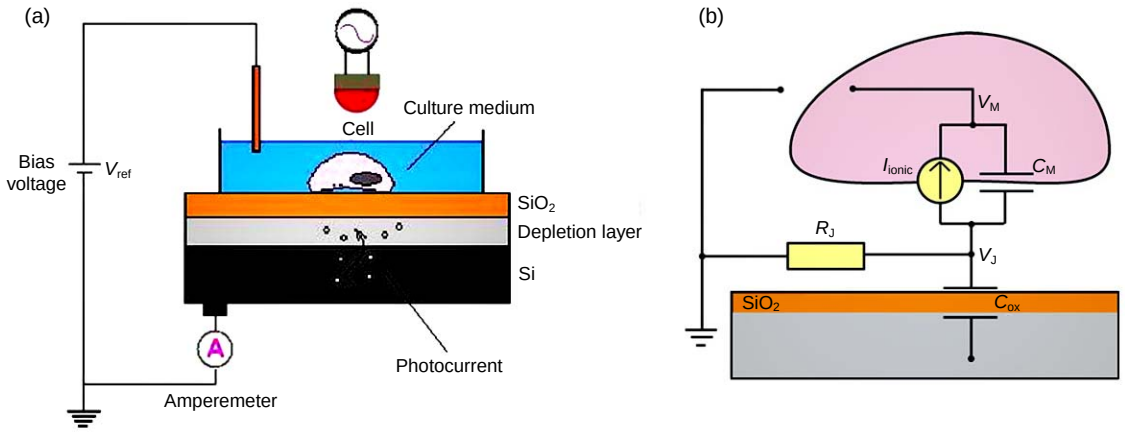

Fig. 1 Schematic diagram of the neuron-LAPS hybrid system

(a) Schematic of the PC12 cell and LAPS hybrid system; (b) The basic detection principle is that excited cells (PC12 cells) are well adhered to the LAPS surface, and the ionic currents produced by cells can bring photocurrent fluctuating of the silicon surface. V_{ref} is the potential of reference; V_M is the potential of transmembrane; C_M is the capacitance of the cell membrane; V_J is the transductive extracellular potential; R_J is the seal resistance; C_{ox} is the insulator capacitance per unit area; I_{ionic} is ionic current

If cells are appropriately positioned on the insulated semiconductor surface, the electric fields within the semiconductor can be modulated by transmembrane potentials. Based on this basic theory, LAPS has been tried to construct different types of cell-based biosensors, including cardiac cells, brain cortex cells, olfactory cells, and taste cells (Xu *et al*., 2005; Liu *et al*., 2006; 2007).

When neurons coupled to the oxidized silicon surface of the LAPS, the cell-semiconductor interface can be simplified as the equivalent schematic circuit, which has been illuminated in Fig. 1b (Fromherz *et al*., 1991; Liu *et al*., 2006). Combined with the Hodgkin and Huxley (1952) model, the relationship between transmembrane potential and ionic currents (I_{ionic}) that flow through membrane channels of neuron cells coupled to the LAPS can be calculated by Eq. (1):

$$\frac{V_{\text{J}}}{R_{\text{J}}}+C_{\text{ox}}\frac{\text{d}V_{\text{J}}}{\text{d}t}=C_{\text{M}}\frac{\text{d}(V_{\text{M}}-V_{\text{J}})}{\text{d}t}+I_{\text{ionic}}, \qquad (1)$$

where V_{J} is the transductive extracellular potential, C_{ox} is the insulator capacitance per unit area, C_{M} is the capacitance of the cell membrane, V_{M} is the potential of transmembrane, and R_{J} is the seal resistance. When V_{M} changes, which is caused by the cell activity, capacitive and ionic currents of the membrane flow into and out of the cell. Currents coming out from the cell are supposed to go through R_{J} and the capacitive coupling to the underlying semiconductor C_{ox}, especially when pulses contain high-frequency components. There are accompanied currents along the cleft, producing V_{J} between the cell and the surface of the oxidized silicon, equivalent to the bias voltage change of LAPS.

Previous studies on LAPS mainly used primary cultured cells. However, cell lines are convenient to culture and obtain. In the sensor detection, cell line culture can overcome the differences caused by primary culture cells such as different kinds of animals, different ages and weights, and all varieties of factors in the process of operation that may cause the weak repeatability of the biosensor. Thus, PC12 cell line was of great significance for the study of neurochip based on LAPS.

2.2 De-noising technique of wavelet transform

Usually, signals collected from neurochip based on LAPS contain some noise affecting later analysis. Consequently, they need to be de-noised to facilitate further study of neurochip for wider application. In the process of de-noising based on wavelet transform, we decompose extracellular potentials intermixed with some noise by discrete wavelet transform (DWT), which facilitates the computer analyzing and processing (Manganiello *et al*., 2002; Artursson and Holmberg, 2002; Zhu *et al*., 2009). A threshold is selected to apply on each detail coefficient level. The absolute coefficient values larger than the threshold are supposed to be parts of the extracellular potentials while those smaller than the threshold are parts of the noise, which can be put to zero. Thus, the signal can be reconstructed based on the series of new coefficients with less noise. DWT is defined as follows:

$$W_f(j,k)=2^{j/2}\int f(t)\psi^*(2^j t-k)\text{d}t, \qquad (2)$$

where $f(t)$ is the function of the original signal in time (t) domain, and j is the sequence number of a layer. When j is defined as different integers ranging from 1 to 5, the results of a 5-level decomposition orthogonal wavelet basis are illustrated in Fig. 2a. After the first decomposition into an approximation part and a detail part, it just processes the approximation part of the signal and further decomposes it into another two separated parts, with detail coefficients at all of the five levels (D1, D2, D3, D4, and D5) and approximation coefficients at deepest decomposition level (S5, Fig. 2a). The decomposition scale is higher, while the frequency of the signal is lower. The time resolution becomes better (small Δt) for high frequencies and the frequency resolution becomes better (small Δf) for low frequencies shown in Fig. 2b. Thus, according to the characteristics of wavelets, we can decompose the signal obtained by LAPS into multi-scales and reveal it onto each scale, so they can be processed on different frequency bands separately (Nakagawa and Yamamoto, 1997; Sardy *et al*., 2001).

Low pass filter, i.e., finite impulse response (FIR) filter, with its design based on the well-known concept of the Fourier transform, is a usual choice to de-noise signals (Liu *et al*., 2006), but it limits only to the frequency analysis. Wavelet transform illustrates signals both in time domain and frequency domain, which enables wavelet analysis to be used in more and more fields, including the neuroscience.

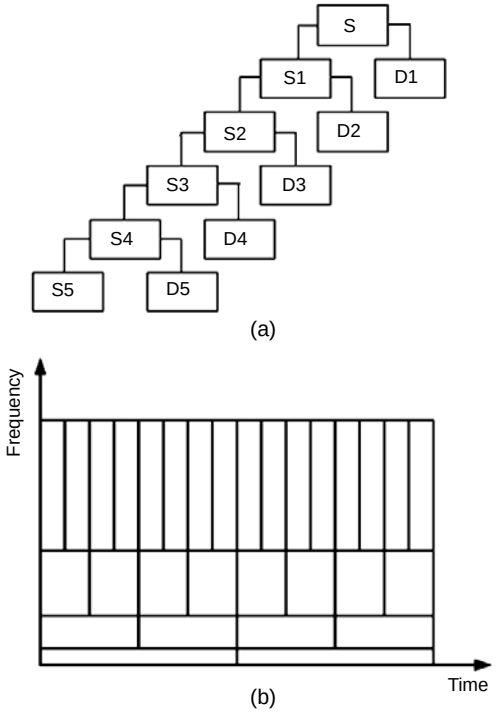

Fig. 2 Schematic diagram of wavelet decomposition. (a) Five-level multi-resolution tree of wavelet transform. S represents the original signal. S1, S2, S3, S4, and S5 represent the first, second, third, fourth, and fifth layers of the low-frequency part (or approximation part), respectively. And D1, D2, D3, D4, and D5 represent different layers of high-frequency part (or detail part); (b) Time frequency plane for the wavelet transform. The length of each block on time axis represents time resolution and the length of each square on frequency axis represents frequency resolution

3 Materials and methods

3.1 LAPS device

We chose *n*-type silicon (100) as the substrate to fabricate the LAPS chip (Xu *et al*., 2005; Liu *et al*., 2006). The silicon was covered with a 30-nm-thick SiO_2 layer on the positive side, and thermally oxidized at 1180 °C for 20 min. The sputtering of aluminum membrane with the thickness of 1 μm on the back of the wafer was to form an ohmic contact layer using the method of vacuum coating (vacuum $<2\times10^{-5}$ Pa). Finally, a Petri dish with the height of 30 mm and the diameter of 50 mm was formed around the chip for culturing cells.

3.2 LAPS system

During experiments, LAPS chip was fixed in the detecting system under the microscope to position the target cell. The modulated light (543.5 nm in wavelength and 5 mW in power), produced by a He-Ne laser (Coherent Co., USA), was focalized to the diameter of no more than 10 μm onto the target cell. If extracellular potential changed, LAPS photocurrent showed the fluctuating and was transferred by working electrode of potentiostat (EG & G, Princeton Applied Research, M273A, USA) into peripheral equipments. Finally, a 16-bit data-acquired card and laboratory virtual instrument engineering workbench (LabVIEW)-controlled potentiostat's collector collected signals.

3.3 Cell culture

PC12 nerve cells were seeded on the LAPS surface and measured after being cultured for 48 h with ordinary culture flasks. Prior to seeding cells, the LAPS chip surface was coated with poly-L-ornithine (100 μg/ml) and laminin mixture (8 μg/ml) to improve the attachment of cells to LAPS. Then, we seeded PC12 cells and plated them in culture dish with the density of 1500 cells/cm^2. They were maintained in LAPS device in humidified air with 5% CO_2 under standard conditions with the solution containing 10% (w/v) fetal calf serum and the temperature at 37 °C for 2–3 d. The solution was changed every one day with fresh Dulbecco's modified Eagle's media (DMEM).

3.4 De-noising by wavelet transform

In the process of de-noising by wavelet transform, we chose Daubechies wavelet as the base function, which has the capability of representing local features of the signal and is good for detecting abrupt changes. The scale of Daubechies wavelet is integral power of 2 and is limited support in time domain and focused in frequency domain. According to the selected wavelet, the signal was de-noised based on Matlab (Mathworks Inc., Natick, Massachusetts, USA) as following steps: First, we used wavedec function to calculate the coefficients of every layer based on the wavelet basis of db2, one kind of Daubechies wavelet. Second, the threshold value of each layer for de-noising was returned by ddencmp function. Then, the coefficients of layers that describe the noise were set to zero and the others were kept the same to construct a new series of coefficients. At last, signal was reconstructed according to the new coefficients based on the threshold values.

4 Results

4.1 Recording of PC12 cell spontaneous activity

The parental cells were round and some had rather short protrusions with the length no more than 5 μm after being cultured for 1 d. Two days after plating, the protrusions of PC12 reached a length of 100 μm. And, the cells presented neuritic processing networks in 3 d as shown in Fig. 3. Lots of cells extended their dendrites to others forming a cluster. Therefore, without the limitations of geometrical restrictions (such as MEA and FET), neurons preserved their morphological topology while building up neuronal networks on LAPS.

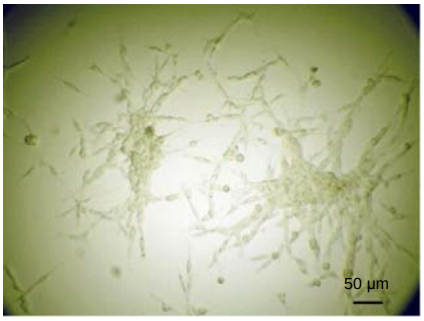

Fig. 3 PC12 cells grown on the LAPS surface observed by a microscope. Lots of cells extended dendrites to others while building up neuronal networks on LAPS

During recording, the LAPS cultured with PC12 cells was fixed under the microscope lens. The modulation frequency of the LAPS is determined by the frequency of laser (4 kHz). The sampling frequency was 40 kHz and the sampling time lasted for 60 s for each auto save. The extracellular potential was calculated from the photocurrent signal by the characteristic current-voltage curve (I-V curve) of LAPS (Xu *et al*., 2005). The potential acquired by the system is shown in Fig. 4a. The obtained overall signal lasted for 60 s, containing baseline drift and extracellular potential signals, which have the relatively large amplitude. Fig. 4b shows the single extracellular potential signal. We analyzed the detailed characteristics of each extracellular potential signal, and found it contained a sharply-raising segment and dropping segment. The duration of all parts of the signal is about 20 ms.

Those recordings accorded with our previous studies (Liu *et al*., 2006; 2007), and the segments may be related to the depolarization and repolarization phases of the active potential recorded by patch clamp.

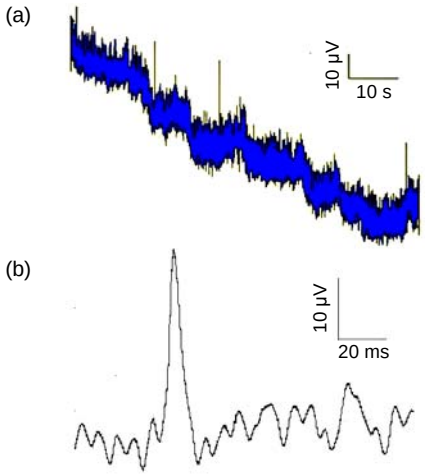

Fig. 4 Extracellular potentials (actual signals) collected by LAPS
(a) The overall signal with 60 s in duration; (b) The single extracellular signal

4.2 De-noising results of wavelet transform

For the purpose to reduce the baseline drift and biological noise in the collected signal, the signal was processed based on the wavelet transform and was first decomposed into different scales shown in Figs. 5a–5e, which are the reconstruction of the detail coefficients in different layers. Fig. 5a corresponds to the reconstructed signal of the detail coefficients in the fifth layer; Fig. 5b corresponds to the reconstructed signal of the detail coefficients in the fourth layer; Fig. 5c corresponds to that of the third layer; Figs. 5d and 5e correspond to the second and first layers, respectively. After decomposition, coefficients of the extracellular potential reveal relatively large values almost on the same positions in the third, fourth, and fifth layers. But the first and second layers do not have the obviously large amplitudes for they are the coefficients of high-frequency noise.

After the analysis in time domain, in order to see the frequency distribution of each reconstructed layer, we analyzed the frequency spectrum by fast Fourier transform (FFT) as shown in Fig. 6. In which, Fig. 6a shows the spectrum of the fifth layer, whose frequency range concentrates within 0 to 2 kHz with the highest value of amplitude at the point of 1 kHz. Figs. 6b–6e show the spectra from the fourth to the first

layers, respectively. The largest frequency of the previous layer doubles the later one, and so does the frequency point with the largest amplitude. This is because each decomposition is equivalent to being filtered by band-pass filter, whose lower cut-off frequency is (almost exactly) half of the higher cut-off frequency. The high-frequency part of every layer is shown in the Fig. 6 and the low-frequency part is for further decomposition.

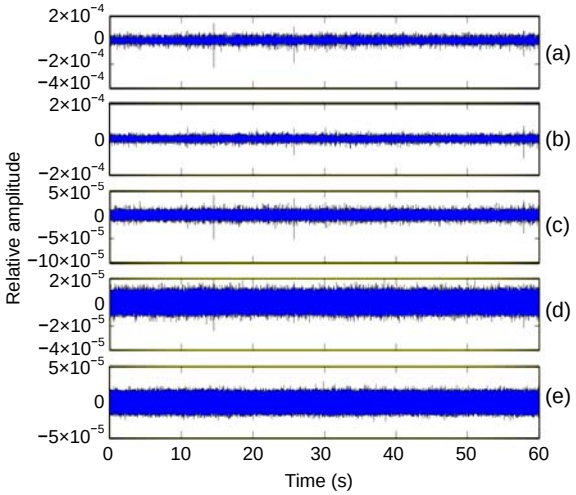

Fig. 5 Reconstruction of each layer of wavelet transform
The reconstructed signal of the detail coefficients in the fifth (a), fourth (b), third (c), second (d) and first (e) layers, respectively

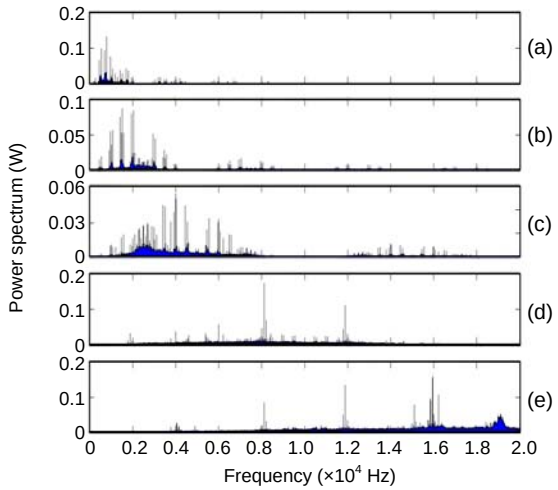

Fig. 6 Frequency spectrum of reconstructed signal on each layer of wavelet transform
(a), (b), (c), (d), and (e) correspond to the fifth, fourth, third, second, and first layers, respectively

Based on the analysis of frequency domain on each layer, coefficients of the first and second layers contributed to the noise, so they were all set to zero in our study. The coefficients of the other three layers were kept the same. Thus, a new set of coefficients was constructed for signal reconstruction. And the reconstructed results are shown in Fig. 7. In contrast to the original signal shown in Fig. 4a, the baseline drift was removed efficiently and the peak-to-peak value was increased obviously. Thus, we calculated the signal-to-noise ratio of the highest peak-to-peak extracellular potential signal, which increased from 6 to 16 dB. The corresponding reconstruction of a single extracellular potential is shown in Fig. 7b. The abrupt change parts of the signal were retained and the peak-to-peak values increased but the whole potential signal was distorted, for we set the approximation coefficients to zero when we constructed new coefficients. As for the peak-height of the signal, both signals before and after filtering were correct, for we calculated the peak-to-peak value in biological signals. Therefore, although the signal was distorted, the de-noising method in our study obviously increased the signal-to-noise ratio.

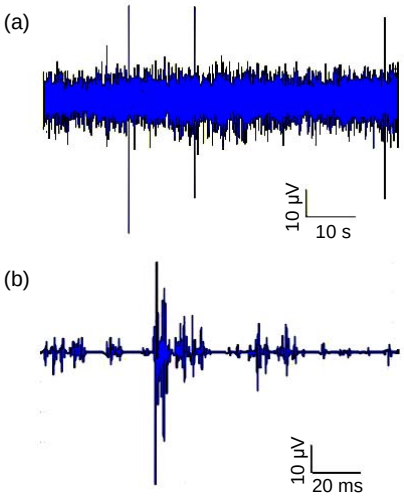

Fig. 7 Reconstructed signal after wavelet transform
(a) The overall signal with 60 s in duration; (b) The single extracellular signal

In order to ensure that the efficient frequency range was retained after de-noising, we did FFT analysis of the original signal and the de-noised signal shown in Fig. 8. The frequency spectrum of the de-noised signal centralized in the frequency less than 4 kHz consistent with the frequency ranges of the fifth and fourth layers shown in Fig. 6. However, within the frequency range of 2 to 4 kHz, the amplitude was

relatively small, because the new coefficients were processed based on the threshold returned by the function of ddencmp before reconstruction. Also, the frequency spectrum of de-noised signal was more concentrated and the high-frequency part was obviously removed.

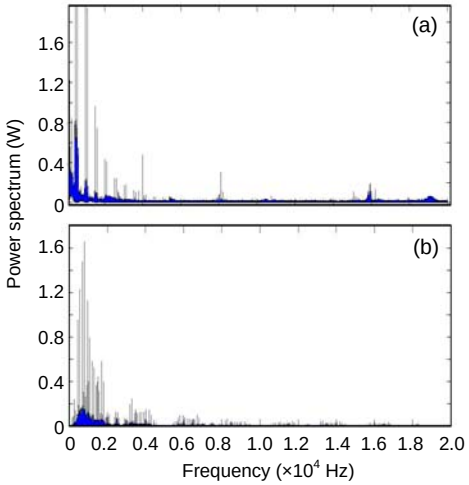

Fig. 8 Frequency spectra of the signals before (a) and after (b) de-noising

Clearly, PC12 cells could couple to the surface of LAPS as a form of networks and the electric activity could be detected at any point. And, from the de-noised results and signal spectrum analysis, we can conclude that the method of wavelet transform is efficient to analyze signals obtained from the neurochip in the time-frequency domain for further study.

5 Discussion

5.1 Neurochip of PC12 cell line-based biosensor

LAPS is characterized by simple preparation, low package requirement, good potential stability, high sensitivity and fast response (Fanigliulo *et al*., 1996). Unlike the cells that must be cultured on electrodes or gates of MEA and FET arrays, neurochip based on LAPS has revealed advantages in detecting cell signal due to no limitations in shape and the characteristics of light addressability.

In many previous studies of cell-based biosensor applications, some tissues of animals or primary cells were selected as the sensing elements. Tissues and primary cells were stripped from animals directly, which have the similar functionality in vitro and in vivo. However, there were varieties of occasional factors affecting every operation, thus leading to weak repeatability of experimental results. Compared to primary culture cells, neuron tumor cell lines have the characteristics of pureness, fast growth and reproduction, short culturing period, and being easily used and controlled. In this study, when PC12 cell line was grown in vitro on the LAPS surface, spontaneous potentials were recorded successfully. Due to the advantages of cell lines, the LAPS hybrid system with PC12 cells will be a very useful neurochip for biomedical studies, such as drug screening and environment detecting. Moreover, the neurochip can be extended for other neurons (e.g., hippocampal cell cultures) to detect extracellular potentials in neuroscience and neuron engineering.

5.2 De-noising technique based on wavelet transform

In measurement of biological signals from cells on micro-electronic sensor chips, there are electronic noise, interference from external sources, and biological noise (Gilchrist *et al*., 2005). The methods of de-noising include traditional Fourier transform and wavelet transform (Manganiello *et al*., 2002; Artursson and Holmberg, 2002; Liu *et al*., 2006). Fourier transform can analyze only signals in frequency domain. Usually, it is supposed to remove noise and retain the high frequency component of the signal as well. When the signal and the noise distribute in near the same frequency band, it is difficult for traditional de-noising methods, such as low pass filter (LPF), to distinguish. Wavelet transform is a localized space-time frequency analysis. Based on the method of multi-scale analysis, we can observe the local signal features of different accuracies in different scales. In the study of neurochips based on LAPS, we have dealt with the signal in steps including decomposing the signal into five layers according to the selected wavelet, processing coefficients of each layer to construct a new series of coefficients, and reconstructing the signal, in which constructing new coefficients is the most important because it decides the quality of the reconstructed signal. Besides, for a certain signal, if it is analyzed using different wavelet basis, the results will differ greatly. When we choose the right wavelet basis to make the signal and noise overlap as little as possible in the

corresponding coordinate, signal and noise can be separated. The best wavelet basis for a given signal is the one that can obtain maximum coefficients in time scale plane.

In the study of neurochip, analysis of different frequency components may acquire important biomedical information. Our previous studies showed that oscillatory potential released by olfactory receptor neurons can be effectively recorded by LAPS, and the signal frequency components were studied by Fourier transform analysis (Liu *et al*., 2006). Wavelet transform has combined the advantage of Fourier transform. Each layer can be analyzed by FFT. Thus, the signal can be processed in different scales by gradually changing the threshold of coefficients based on the spectrum. The further application of wavelet transform contributes to further study of cell sensing. We will use classical electrophysiological methods like patch-clamp as comparison and find a statistical correlation between the two methods in later studies to prove the experimental results and the reconstructed signals in a more empirical way.

6 Conclusion

In conclusion, in this study, we recorded extracellular potentials of PC12 neural cell lines cultured on LAPS and studied the de-noising process by wavelet transform for a novel neurochip of PC12 cells based on LAPS hybrid system. When the signal collected was de-noised by the method of wavelet transform, the baseline drift in the signal was removed and the level of signal-to-noise ratio was improved. All of these suggest that the cell line-LAPS hybrid system can be employed as a simple and convenient neurochip for biomedical studies.

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