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ORIGINAL ARTICLE

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Unconstrained and noninvasive measurement of bioelectric signals from small fish

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Abstract Recently, the technique of fish bioassay has attracted attention as a method for constant monitoring of aquatic contamination. The respiratory rhythms of fish are considered to be an efficient indicator for the monitoring of water quality, since they are sensitive to chemicals and can be measured indirectly from the bioelectric signals generated by their breathing. However, no method has yet been established to measure signals in small free-swimming fish. In this article, we propose a system to measure bioelectric signals in small fish and monitor the frequency component in real time. To cover the large measurement range required in a free-swimming environment, the signals are measured using multiple electrodes. Further, the system focuses on the frequency component of the signal to assess the condition of the fish using frequency analysis and a band-pass filter. Experiments were conducted with the purpose of enabling remote sensing and environment estimation. First, it was verified that the measured signals were synchronized with the breathing of the fish. Then, a remote sensing experiment was performed using medaka (*Oryzias latipes*) that were allowed to swim freely in a measurement aquarium. The results confirmed that bioelectric signals which were synchronized with breathing could be measured in unconstrained and noninvasive conditions.

Key words Bioassay · Bioelectric signals · Small fish

1 Introduction

Currently, incidents involving the contamination of water sources by industrial effluent are reported every year in Japan. Accordingly, the quality of tap water is monitored in water treatment plants to prevent contaminated water from being supplied to homes. During this monitoring, the levels of chemical concentrations in the water are analyzed and checked to ensure that safety standards for tap water are met. However, not all tests can be performed frequently because of limitations in terms of time and cost. As a result, only three items are inspected each day, and other checks are carried out just once a month.[1] Consequently, aquatic contamination may not be discovered until it causes a health hazard after a contamination incident occurs. This situation has led the Ministry of Health, Labour and Welfare to recommend introducing the bioassay system together with chemical analysis.[2]

Bioassay is a method of estimating environmental changes from biological responses. In general, fish are used in the examination of water. Since it has been reported that bioelectric signals from fish are sensitive to changes,[3] these signals are expected to make early detection possible, and several research projects on a bioassay system using bioelectric signals have therefore been conducted. For example, Shedd and van der Schalie[4,5] proposed a system that calculates the ventilatory frequency from bioelectric signals and evaluates aquatic contamination from changes in breathing. However, this system limits the range of movement of fish in order to improve the quality of the signal measurement, and this can cause stress and influence their breathing conditions. Taue and Hashimoto[6] proposed a system using small fish in free-swimming conditions. However, their system can only assess whether the fish are dead or alive, as it relies solely on the amplitude of the signal for the assessment.

The aim of this study was to develop a bioassay system using bioelectric signals from small fish in free-swimming conditions. As the first step, we propose a method to measure the bioelectric signals of medaka (*Oryzias latipes*) in unconstrained and noninvasive conditions to minimize their levels of stress. Instead of amplitude information, the system utilizes frequency information that has been proven to be quite stable in free-swimming conditions.

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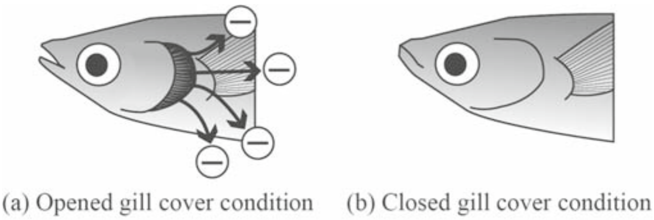

Fig. 1. Relationship between gill cover movement and ion movement

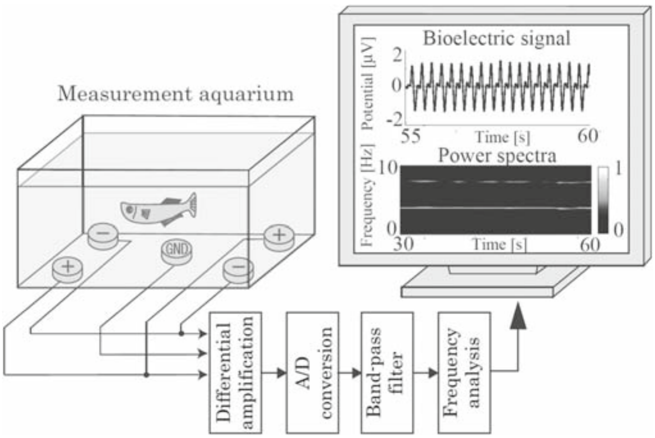

Fig. 2. Structure of the measurement and signal analysis system

2 Ventilatory signals

The medaka is suitable as a test fish for the bioassay system because it is relatively sensitive to chemicals, and is recommended in the OECD Guidelines for the Testing of Chemicals.[7] In our research, ventilatory signals were selected as the measurement target. It is already known that it is possible to observe the electrical field around a fish's body by which the periodic potential is generated.[8] The main source of the potential difference is considered to be the difference in ionic concentration between the inside and outside of the body caused by the osmotic mechanism.[9] As shown in Fig. 1a, when the gill cover is opened, ions move to the outside of the body, generating electric potential. On the other hand, when the gill cover is closed, ionic movement is shut off (Fig. 1b). The potential around the fish is thus synchronized with the opening and closing movement of the gill covers.[10,11]

3 The ventilatory signal measurement system

The system established to measure the ventilatory signals of the medaka is shown in Fig. 2, and consists of a signal measuring part and a signal processing part. This section describes the system configuration.

3.1 Signal measuring part

The signal measuring part plays the role of inputting measured signals into a PC, and is composed of a measurement aquarium, electrodes, amplifiers, and A/D converters.

Signals are measured using disposable medical electrodes (Ag–AgCl). n pairs of active electrodes (+, −) and a reference electrode (GND) are placed in the aquarium to allow differential amplification for signal denoising. Since the measured signals are faint (i.e., of the order of μV), they are amplified using a bioelectric amplifier (time constant, 3 ms; high cutoff frequency, 30 Hz; Nihon Kohden Corporation). AD processing (sampling frequency 1000 Hz) is then conducted to input the signals into a PC using an interface module (PCI-3521, Interface).

3.2 Signal processing part

In the signal processing part, the input signals are filtered and converted into the frequency domain, and both are monitored on the PC screen. First, input signals are filtered by band-pass filters (low cutoff frequency, 0.053 Hz; high cutoff frequency, 10 Hz). Then frequency analysis is conducted using an AR model, which is less influenced by unexpected noise. The AR model is given by the following equation:

$$x(n) = -\sum_{k=1}^{K} a(k)x(n-k) + \varepsilon(n), \tag{1}$$

where $x(n-k)$ is the measured signal, and $\varepsilon(n)$ is the prediction error (white noise). This model predicts future data $x(n)$ from measured signals by appropriately adjusting the AR parameter $a(k)$.

The power spectrum density (PSD) $P(f)$ is calculated for every second using an AR model of order K = 200 using Eq. 2.

$$P(f) = \frac{\sigma_\varepsilon^2}{\left|1 + \sum_{k=1}^{K} a(k)e^{j2\pi kf}\right|^2}, \tag{2}$$

where σ_ε^2 is the prediction error variance. $P(f)$ is normalized in the range 0–10 Hz using Eq. 3 to monitor the peak frequency per unit of time.

$$P_n(f) = \frac{P(f)}{\max_f(P(f))} \quad : (f = [0;10]) \tag{3}$$

Normalized PSD $P(f)$ is displayed in gray-scale on the PC screen, as shown in Fig. 2. This system can measure the ventilatory signals of medaka in real time and monitor the frequency component.

4 Measurement under constrained conditions

The ventilatory signal measurements of medaka were conducted under constrained conditions as a basis for later experiments in free-swimming conditions. First, synchroni-

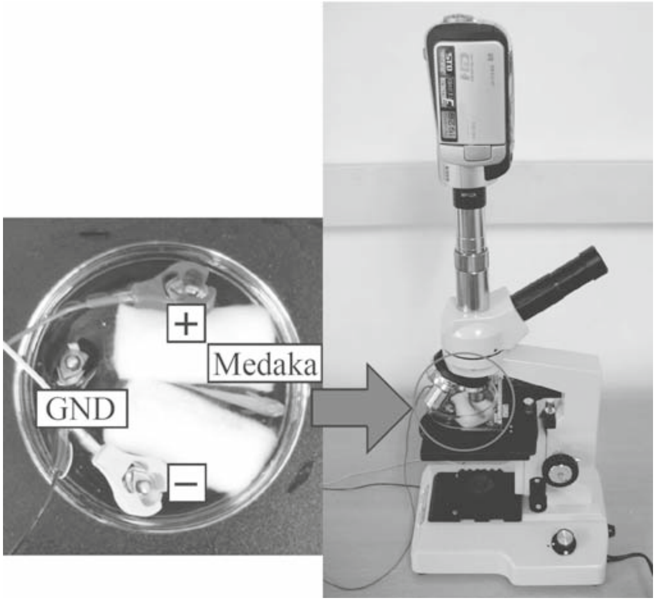

Fig. 3. Experimental apparatus for the verification experiment

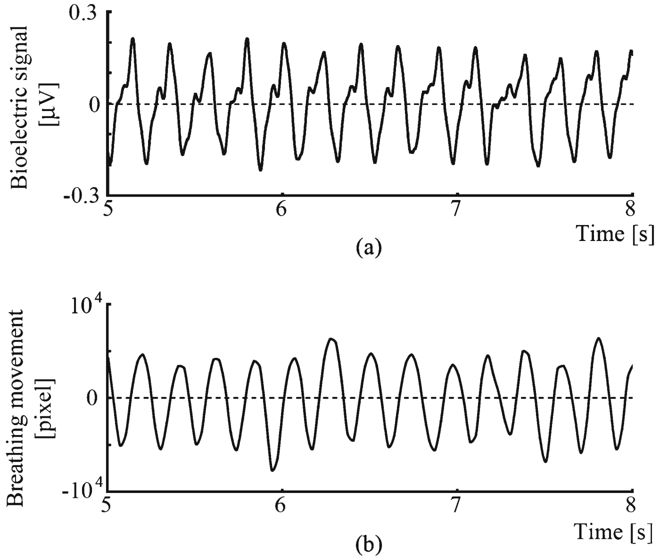

Fig. 4. Examples of the experimental verification results

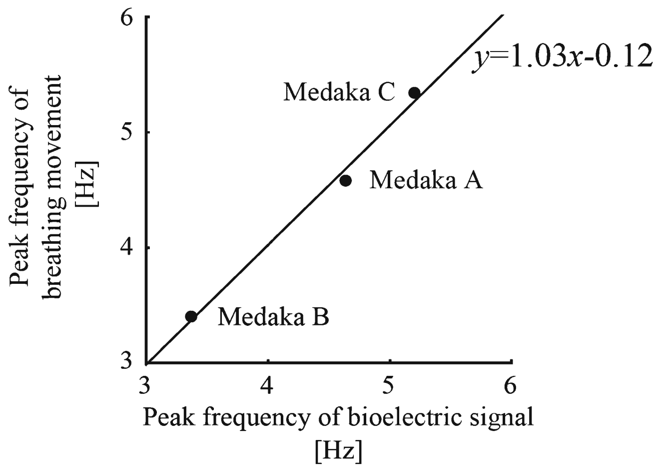

Fig. 5. Relation between signal frequency and ventilatory frequency

zation between measured signals and breathing was confirmed. Then the influence of the electrode distance on the signal quality was examined to allow remote sensing. The water used for the experiment was dechlorinated ahead of time. The temperature and electrical conductivity were also measured before the experiment, and the temperature was kept constant during the test period.

4.1 Experiment to verify ventilatory signals

The correlation between the measured signals and gill cover movement was examined to verify that the measured signals are synchronized with breathing. First, a medaka was placed on a petri dish, and its range of movement was limited using absorbent cotton, as shown in Fig. 3. Then a pair of active electrodes (+, −) and a reference electrode (GND) were placed in the petri dish for signal measurements. At the same time, the gill cover movements were recorded using a video camera (frame rate 29.97 fps) mounted on a microscope to quantify the movements by image analysis.

The video images were analyzed using Cosmos32 image analysis software (Library). The picture was converted into a binary image, and the area of gill cover corresponding to the number of black pixels was calculated. The same process was performed on all frames of the video, and the number of black pixels in each frame was obtained. Then the numerical data were processed using a band-pass filter (low cutoff frequency 1 Hz; high cutoff frequency 10 Hz), and the data thus obtained were used to define the breathing movement.

Figure 4 shows an example of the experimental results obtained from three subjects. Figure 4a shows a measured signal. The horizontal axis represents time, and the vertical axis is the electric potential. Figure 4b shows the breathing movement. The horizontal axis represents time, and the vertical axis is the number of black pixels. Both the measured signal and the gill cover movements showed a periodic wave pattern, as seen in Fig. 4.

Figure 5 shows the correlation between the peak frequencies of the signals and gill cover movements as calculated using data from a 60-s period. The result for the correlation coefficient of each point shows a value of 0.995. This high correlation verified that the measured signals were synchronized with breathing.

4.2 Measuring experiment with different inter-electrode distances

Next, we conducted a measuring experiment with different inter-electrode distances to confirm the influence of this distance with medaka. The fish was constrained using absorbent cotton to confine the motion of its fins. The size of the aquarium used for the measurement was 500 mm

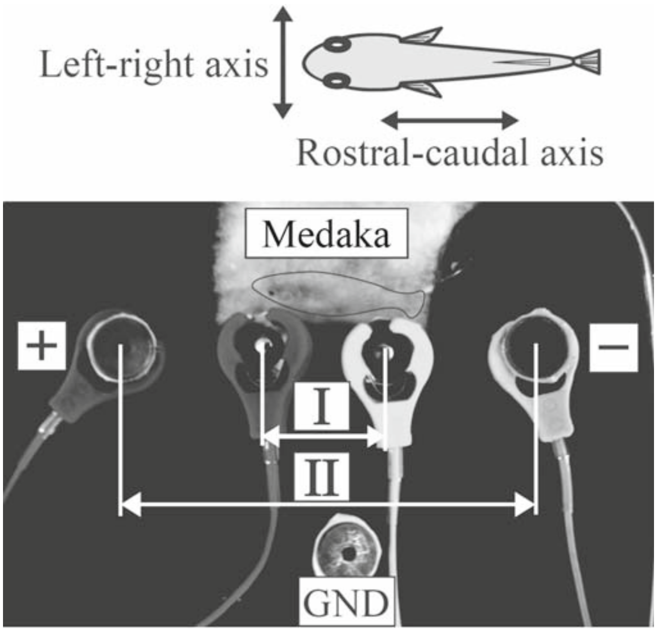

Fig. 6. Electrode arrangement for the measuring experiment with different inter-electrode distances

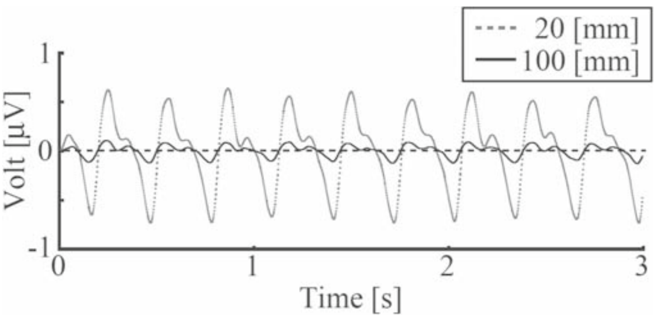

Fig. 7. An example of the experimental results under constrained conditions

(width) × 350 mm (depth) × 200 mm (height), and two pairs of active electrodes were used, as shown in Fig. 6. Electrode pair I was attached to the fish to measure the low-noise standard signal with an inter-electrode distance of 20 mm. The inter-electrode distance of electrode pair II was changed from 40 mm to 300 mm in increments of 20 mm. The experiments were conducted under two sets of conditions: one with the direction of the electrode pair along the rostral–caudal axis, and the other along the left–right axis against the axis of the fish's body.

Experiments were conducted with four subjects. Figures 7–9 show the results when the electrode pairs were placed along the rostral–caudal axis. Figure 7 shows an example of the ventilatory signals measured when the inter-electrode distance of electrode pair II was 100 mm. The relationship between the inter-electrode distance and the signals is shown in Fig. 8. The vertical axis denotes the ratio of the effective value (V_2/V_1). V_1 and V_2 are the effective values of electrode pairs I and II, respectively. Figure 9 shows the relationship between the inter-electrode distance and the

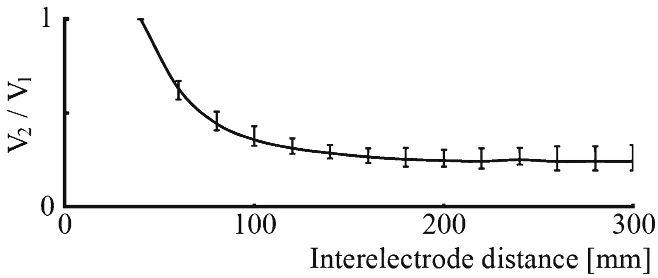

Fig. 8. Effective value of measured bioelectric signals with different inter-electrode distances

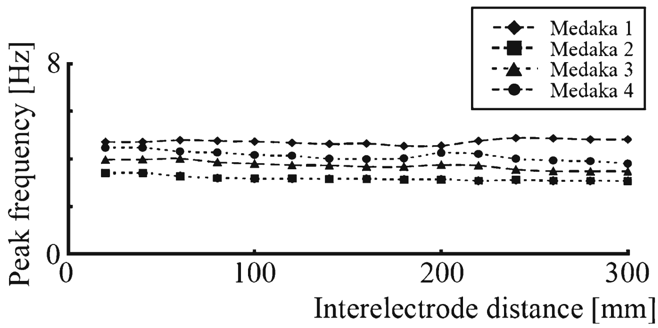

Fig. 9. Peak frequency with different inter-electrode distances

peak frequency of the signals. The differences in the signal amplitude were confirmed by the differences in distance, as shown in Fig. 7, and the amplitude decreased as the electrode distance increased, as shown in Fig. 8. This is due to increased electrical resistance between the subject and the electrodes. In contrast, the peak frequency bore no relation to the distance and remained almost constant, as shown in Fig. 9. Similar results were obtained under the condition in which the electrode pairs were placed on the left–right axis. From the results described above, it can be considered that the information on signal frequency is more suitable for evaluating the changes in the conditions of fish than the amplitude in unconstrained and noninvasive conditions, because the medaka would move freely around the aquarium causing constant changes in amplitude information.

5 Measuring experiment under free-swimming conditions

It is necessary to measure ventilatory signals under no-stress conditions from the viewpoint of developing a practical bioassay system. Accordingly, we conducted an experiment under free-swimming conditions using a medaka in a polyethylene resin measurement aquarium with dimensions 150 mm (W) × 100 mm (D) × 50 mm (H) (Fig. 10). The size was determined based on the measurement results described in the previous section and further preliminary experimentation. Electrodes were placed in the four lower corners of the aquarium to keep the medaka between the active electrodes (+, −). The other experimental conditions were the same as those described in Sect. 4.2. The water

Fig. 10. Measuring aquarium for the experiment under free-swimming conditions

temperature and electrical conductivity were 20.2°C and 12.08 μS/mm, respectively.

5.1 Ventilatory signals under free-swimming conditions

We performed an experiment to verify the feasibility of measuring ventilatory signals from medaka allowed to swim freely using the proposed system. Figure 11 shows an example of the experimental results. Figure 11a shows the signals measured in the period between 300 and 310 s. Figure 11b is the spectrum variation of the signals from 290 to 320 s. The magnitude of PSD is expressed in gray-scale. Figure 11c shows the trajectory of the medaka from 290 to 320 s. It was confirmed that ventilatory signals could be measured from medaka even under free-swimming conditions, as shown in Fig. 11. It was also confirmed that the amplitude of signals changed according to the position of the medaka, as shown in Fig. 11a, but the signal frequency was almost constant during swimming, as shown in Fig. 11b. An unexpected change in frequency was recorded when the medaka rushed to the aquarium wall (310–320 s). These results imply that the signal frequency can be affected by a mechanical stimulus, and can possibly be utilized to monitor abnormal behavior in the subject.

5.2 Response of signals to bleach exposure

A water contamination experiment was performed to confirm the response of ventilatory signals to chemical irritation. The experiment started with the same conditions as those described in the previous subsection. Then 4 ml of household bleach, mainly consisting of NaClO, was poured into the aquarium at 180 s.

Figure 12 shows an example of the experimental results after the exposure. Figure 12a shows the signals measured from 330 to 340 s, and Fig. 12b shows the spectrum variation of the signals from 320 to 350 s. Figure 12c shows the trajectory of the medaka from 320 to 350 s. In

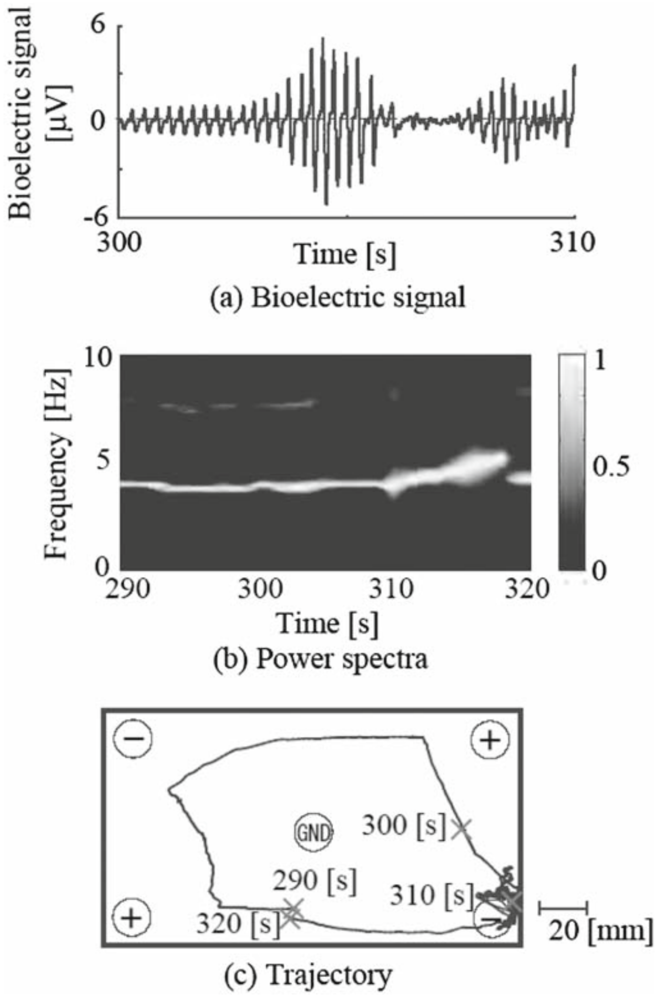

Fig. 11. An example of the experimental results under free-swimming conditions

comparison with Fig. 11, it can be seen that both the spectrum and the amplitude of the signals showed a marked change after exposure. Note that the signal changes were not evoked by movement; the medaka stayed in the same position, as shown in Fig. 12 c. It is therefore suggested that aquatic contamination can be detected using ventilatory signals.

6 Conclusions

In this article, we have proposed a system to measure the ventilatory signals of medaka, and report on an experiment conducted to measure these signals. The results confirm that the system can successfully measure signals from medaka in unconstrained and noninvasive conditions. Further, it was suggested that signal frequency could be an important factor in estimating levels of water contamination. In the future, we plan to analyze changes in the patterns of bioelectric signals in line with exposure to different toxic substances, and to develop a system that can distinguish aquatic contamination from bioelectric signals.

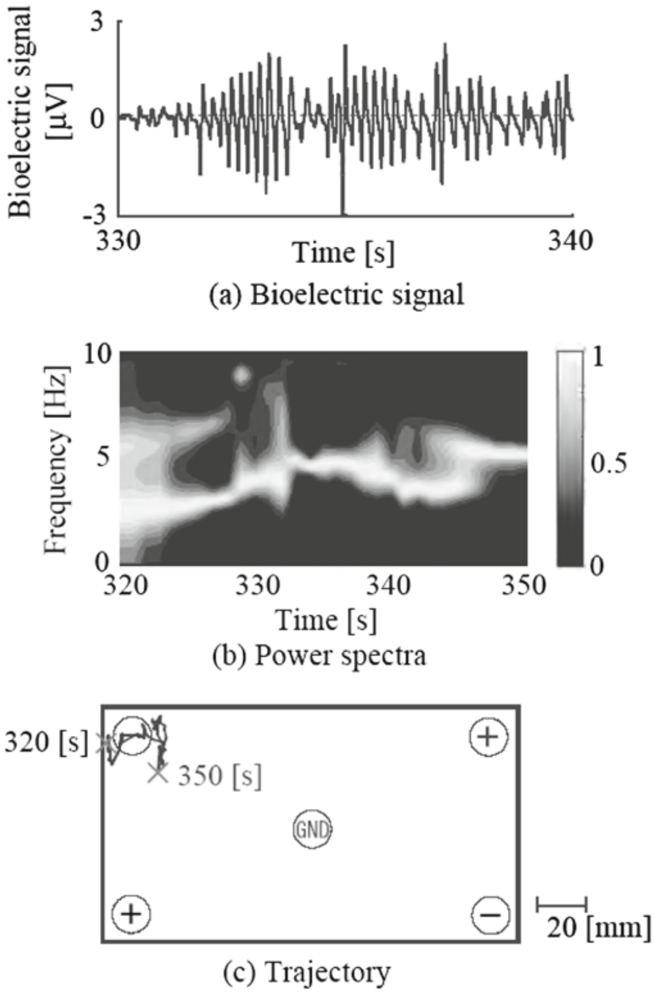

Fig. 12. An example of the results from the bleach exposure experiment

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