Electrical signal measurement in plants using blind source separation with independent component analysis

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1. Introduction

In addition to hydraulic and chemical signals, plant cells also produce and transfer bioelectrical signals as extracellular signals under change in environmental conditions (Davies, 1987). The generation and transmission of electrical signals are probably the initial response of the plant to exterior stimulus. In the course of plant growth, electrical signals in plants may display different features due to weak light, high humidity and shortage of potassium (Yuan, 1988). For non-sensitive higher plants, electrical signals also play an important role in physiological activities, e.g., gas exchange, pollination, fertilization and gene expression (Trebacz et al., 2006). Therefore, there is potential for use of these electrical signals in applications indicating the physiological status of the plants for the adjustment and control of a greenhouse. This is similar to the electrocardiograph used by doctors to monitor the physiological potential of the human body, and thereby obtain relevant information about the heart to facilitate correct diagnosis.

Light serves as an essential factor for the photosynthesis of plants. The capability of perceiving the variation of light is of significant physiological importance to plants. It shows that light can induce electrical signals on the leaf tissue of plants. In fact, the responses of guard cells, mesophyll cells and epidermal cells to light are different (see Figs. 1 and 2a and c). Until now, investigation of the membrane electrical activity of a particular type of cells meant that guard cells, mesophyll cells and epidermal cells had to be isolated with a complex treatment procedure before using either the patch-clamp technique on protoplasts or microelectrode measurement on cells. Extracellular measurement using surface recording can non-invasively detect the electrical signals produced by the various kinds of cells, and is applicable to the monitoring of an individual plant. However, the extracellular recording by surface contact electrodes used to measure the membrane potential on a plant leaf can obtain a mixture of signals of the three types of cells; trace (b) in Fig. 2, which shows a mixed signal, was recorded in an epidermal cell of a leaf section with the epidermis in contact with mesophyll cells. This may lead to wrong interpretation of the signals unless signal analysis methods are applied. Using advanced signal processing algorithms (blind source separation, e.g., independent component analysis, ICA), it becomes possible to obtain each of the signals from the three types of cells. In order to extract individual (independent) signals from the mixtures of signals, the blind source separation (BSS) method was used in this work.

BSS is a general signal processing method, which estimates the source signals independently if the unknown signal sources are made by mixing linearly. The independent component analysis (ICA) method is one technique used to solve the blind source separation (BSS) problem. In contrast with conventional measuring methods used to investigate the electrical signals of plant cells with a complex treatment procedure, the ICA method was provided to achieve separation of the mixed electrical signals to recover the individual signals of each type of cells non-invasively. The proposed method has been tested using simulated signals and real plant electrical signal recordings. The results showed that ICA algorithms provided an efficient tool for the identification of the independent signal components from surface electrode recordings.
a priori knowledge of the sources (Flexer et al., 2005). BSS methods have been successfully used in many fields of applied science and engineering, including medicine, telecommunications, audio processing, noise reduction and data processing (Deville, 1999).

The independent component analysis technique was developed for solving blind source separation (BSS) problems (Stone, 2002). ICA is an algorithm that separates source signals from observed signals by maximizing the statistical independence between the estimated source signals based on various statistical indexes (using higher order statistics information). ICA has already been applied to many engineering fields, e.g., character recognition, audio-signal processing, and image processing. In addition, many researchers have reported on the application of ICA to various multi-channel bio-signals, e.g., ECG, EEG, MEG, for noise reduction and feature extraction (Ikedaa and Toyama, 2000; Vigario, 1997).

The aim of our study is to develop a new method to extract independent signals of particular types of cells from multi-sensor signals from the plant leaf based on non-invasive surface recording by the application of ICA. In the following section, the details of implementation of the ICA algorithm are described. To examine the possible applicability of this technique, the separation quality is estimated using simulation data. Then, the real electrical signal recordings of coexisting signals from different kinds of cells are treated by means of this method and show the successful separation of these signals.

2. Method

2.1. The model of independent component analysis

The ICA model assumes a set of observed signals which are mixtures of some underlying unknown sources. ICA allows recovery of \( n \) independent source electrical signals of different cells \( s = \{s_1(t), s_2(t), \ldots, s_n(t)\} \) from \( n \) mixed signals measured by \( n \) sensors, \( x = \{x_1(t), x_2(t), \ldots, x_n(t)\} \), modeled as the result of multiplying the matrix of source activity waveforms, \( s \), by an unknown square matrix \( A \), as shown in Eq. (1). An illustration of ICA is shown in Fig. 3. Here, individual source components are assumed to have zero–mean and unit variance (Igual et al., 2003):

\[
x = As
\]  

where \( x \) is an \( n \) dimensional observation vector, \( A \) is a non-singular \( n \times n \) mixing matrix, and \( s \) is expressed by an \( n \) dimensional original source vector having independent components.

To recover individual components of the original source vector \( s \), a full rank \( n \times n \) matrix \( W \) is described in Eq. (2); \( W \) is called the separation matrix, which inverts the mixing process linearly:

\[
y = Wx
\]  

In this ICA implementation, as in many other ICA algorithms, we use the fourth-order cumulant, also called the kurtosis. For the \( i \)th source signal, the kurtosis is defined as

\[
\text{Kurt}(s_i) = E[s_i^4] - 3[E[s_i^2]^2]
\]  

where \( E\{\cdot\} \) denotes the mathematical expectation value. Maximizing the norm of the kurtosis leads to the identification of the independent sources.

The only assumptions that are needed in ICA are described as follows (Hyvarinen et al., 2001; Funaro et al., 2003): (1) the sources are statistically independent; (2) the probability densities of the sources are non-Gaussian (at most, one of them is allowed to be Gaussian); (3) the mixing of the sources into the observations is...
linear; (4) the number of observations is larger than or equal to the number of sources.

2.2. The ICA algorithm and software

Considering the ICA model and the assumptions described above, the steps of the ICA algorithm are explained as follows:

1. The first step in ICA processing is to center x, by subtracting its mean vector \( \mathbf{m} \) in order to make \( \mathbf{x}(t) \) a zero-mean variable.

2. Whitening (sphering) removes the second-order relationship between these vectors. A new vector \( \mathbf{v} = [v_1, v_2, \ldots, v_n] \) was obtained with uncorrelated components:

\[ \mathbf{v} = \mathbf{Vx} \quad (4) \]

where \( \mathbf{V} \) is the whitening matrix.

3. Calculation of \( \mathbf{v} \) takes place in this step:

\[ \mathbf{v} = \mathbf{VAs} \quad (5) \]

4. The estimated signals are written as

\[ \mathbf{y} = \mathbf{Wv} \quad (6) \]

where \( \mathbf{W} \) is the separating matrix. In this step, one of the columns of the separating matrix \( \mathbf{W} \) is established and one independent component at a time is identified (see Fig. 4).

In this work, the rows of the input matrix \( \mathbf{x} \) are three signals recorded in a leaf of a bean at different positions. All studies reported in this paper were carried out using MATLAB code, based on the FastICA package. The Matlab code of the FastICA is available on the World Wide Web free of charge (URL, 2005).

2.3. Data analysis

The separation quality is considered to be the performance evaluation measure of the ICA algorithms. The pre-processing in the ICA procedure with whitening and centering means that loss of the original amplitude of the sources produces the difference between the original sources and the independent components. In this work, quality is defined as the accuracy of the algorithm in the separation of the sources in terms of the signal shape (or trend). To investigate separation quality of the ICA algorithms, statistical analysis is performed and acceptability criteria are defined in the following.

The Spearman correlation coefficient (Klemm et al., 2009) is used to compare the original source and the independent component because it is not dependent on the absolute amplitude but instead on the (relative) shape of the signal. Spearman’s rank correlation coefficient is defined by

\[ R_s = 1 - \frac{6 \sum d_i^2}{n(n^2 - n)} \quad (7) \]

where \( d_i \) is the difference between each rank of corresponding values (original source and source recovered), and \( n \) is the number of pairs of values.

3. Materials and the experimental protocol

3.1. Plant materials

Plants of a type of bean (Vicia faba L.) were grown from seeds in 0.5 L plastic pots containing all the nutrients essential for the plants with 70% vermiculite and 30% humus soil. Growth conditions were 16 h/8 h light/dark with temperature ranging from 15°C (dark) to 22°C (light). Watering was four times per week with tap water. Plants were used for measurements after 24 days.

3.2. Experimental protocol

The multi-channel monitoring system consisted of hardware and software with the functions of data acquisition and processing. According to the experimental demands, various environmental factors in the greenhouse, including temperature, illumination, humidity, and supplements of CO2, were taken into account. The electrical signals and leaf temperature were also recorded. All analog signals from the amplifiers were converted to digital and transferred using a KH-9250H data acquisition board (Ke Hai Corporation), allowing 32-channel recording. The KH-9250H board provided high resolution, a wide gain range and each channel was sampled at 100 kps/s. The characteristics of the measuring and reference electrodes, the pre-amplifier (impedance: 10^{12} \Omega), the details of other sensors and the A/D D/A card have been described in a previous paper (Wang et al., 2007.)

Electrical signals in plants were detected using a cotton thread soaked in an experimental solution (KCl 0.1 mM, MgCl2 0.1 mM, CaCl2 0.5 mM, Na2SO4 0.05 mM) at room temperature (22–24°C). One end of the cotton thread with a 5–6 mm² tip was attached to the leaf surface, while the other end was soaked in a 25 ml volume chamber of the electrode supported with an adjustable holder, and the calomel electrode was also inserted into a chamber con-
Fig. 5. (a) Experimental setup for detecting electrical signals in plants and (b) the overall measurement profile for the physiological signals using the monitoring system.

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Fig. 6. Three simulated mixed signals $x_1(t)$, $x_2(t)$ and $x_3(t)$ were calculated from $s_1(t)$, $s_2(t)$ and $s_3(t)$ by the mixing matrix $A$ (generated randomly); amplitude of the three signals normalized.

Fig. 7. Separated independent signals by using ICA and amplitudes of the three signals normalized.

4. Results and discussion

4.1. Simulation study

To verify the effectiveness of the proposed ICA method for analysis of electrical signals in plants induced by light/dark stimuli, the following experiments were performed as essential examples for further confirmation. The standard response modes as original independent signals used in this section were taken from the studies (Dietrich et al., 2001; Elzenga et al., 1995), shown in Figs. 1 and 2a and c, and were assigned as $s_1(t)$, $s_2(t)$, and $s_3(t)$, respectively. Three simulated mixed signals, $x_1(t)$, $x_2(t)$ and $x_3(t)$ in Fig. 6, were calculated from $s_1(t)$, $s_2(t)$ and $s_3(t)$ by the mixing matrix $A$ (generated randomly). Fig. 7 shows the output components obtained by applying FastICA to the mixed signals of Fig. 6. Indeed, the shapes of the separated signals (estimated signals) shown in Fig. 6 are close to those of the original signals ($s_1(t)$, $s_2(t)$, $s_3(t)$).
Table 1 indicates the Spearman correlation coefficient values between the recovered signals and the original source signals. The correlations were found to be significant ($P<0.01$). These results suggest that independent components can be easily and feasibly identified. Although there are differences in the coefficients between $A$ and $W$ due to the use of normalization processing, the extracted signals demonstrate the success of the ICA in separating and reconstructing these independent sources.

### 4.2. Experimental study

We used this ICA algorithm to separate real-world plant electrical signals. The experimental protocol is discussed in Section 3.2. As an illustration of the separation abilities of ICA algorithms in practical analysis for mixed electrical signals in plants, three recorded signals from a bean leaf induced by light/dark stimuli were plotted in Fig. 8. The traces of normalized measured electrical signals are shown in Fig. 9. Fig. 10 shows the results of the independent component analysis performed by FastICA. Table 2 shows that the correlation coefficients between the recovered electrical signals (independent components) and the standard responses (i.e., $s_1(t)$, $s_2(t)$, and $s_3(t)$) are higher. The 'reconstructed' independent signals of the guard cells, epidermal cells and mesophyll cells.
ophyll cells, clearly free from the recorded signals, are plotted. Also, it is known that the extracted source signals under light stimuli all have clear physiological interpretation (Elzenga et al., 1995; Shabala and Newman, 1999; Dietrich et al., 2001). As a consequence, the proposed ICA method is useful for measuring particular types of cells without damaging the plant. Beside this application, due to the potential use of bioelectrical phenomena for indicating the physiological condition of plants in agricultural fields, e.g., in our previous work recording and analyzing electrical signals in water-stressed plants, the ICA may be used to extract independent components to avoid wrong interpretation and provide a remarkable improvement in the source estimation.

Although ICA appears to be generally useful for analysis for electrical signals in plants, it also has some inherent limitations. First, ICA decomposes at most N sources from data at N sensors. Second, the assumption of temporal independence used by ICA cannot be satisfied when the training set is small. In our application, the main potential hurdle is that ICA can only extract as many independent sources as there are input signals.

### 5. Conclusions

This work indicates the feasibility and discusses the potential of using ICA in measurement of mixed electrical signals in plants. Also, we presented an application of ICA to extract the individual signals from different cells (e.g., guard cells, epidermal cells and mesophyll cells) in the recorded signals. The method is also useful in situations where accurate indications of power and frequency content of electrical signals in plants are needed. Unlike intracellular measurement, which involves inserting a glass micro-electrode to observe the electrical activity of a single type of cell, surface non-invasive measurement is performed with the possibility of monitoring different kinds of cell activities by using ICA to deal with the BSS problem. From an engineering perspective, there is indeed substantial progress in acquiring plant electrical signals.

### Table 2

<table>
<thead>
<tr>
<th>Spearman correlation coefficient (P&lt;0.01)</th>
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<tr>
<td>IC1-S3(t)</td>
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<tr>
<td>0.91</td>
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IC denotes independent component; si(t), i=1,2,3, denote standard responses.

### Conflict of interest

No conflict of interest.

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### References


