Wireless, portable, low cost, open source hardware for monitoring plant electrophysiology

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The Idea

Cell-cell communication is the hallmark of multicellularity. Our understanding of how individual cells communicate and give rise to organismal-level behaviors are thus critical for engineering complex multicellular systems such as animals or plants. In animals, cell-cell communication via electrical signalling plays important roles in nervous systems, enabling fast responses to environmental stimuli. Previous studies indicate that all higher plant may also utilise electrical signalling to regulate a wide-range of physiological functions including stress responses to drought, light condition and wounding. Tools for recording and controlling plant electrophysiology could open up promising applications in diagnosis of plant pathology and modulation of plant adaption. Nonetheless, existing setups for monitoring plant electrophysiology often require the uses of cumbersome, expensive and specialised equipment on only small areas of plants in well-controlled laboratory environment. In practice, one would prefer to have low-cost measurement tools that can function robustly in the field, capture overall electrical activities of the whole plant and easily be reconfigured for various applications. Here we plan to prototype a plant electrophysiology measurement device based on open source Programmable System on the Chip (PSoC) and wireless communication system. The device should be able to operate outside laboratory environment, collect and store data for at least several days without human intervention. The measurement setup procedure should be simple enough for a layperson to learn within an hour. The design of the device should be simple and cheap enough to be mass produced for whole plant / whole field measurement. In addition, we plan to use our device for community outreach: as a tool for learning about interfacing engineering to biology and as a platform for building up a database of plant electrophysiological profile through citizen science projects. An ability to reduce cost, lower down barrier of entry and scale up plant electrophysiological research would bring us closer to realising the full application potential of this field.

Who We Are

Pakpoom Subsoontorn (ps690@cam.ac.uk) received his B.S. degree in Biology and Computer science from California Institute of Technology. He finished his Ph.D. in Bioengineering from Stanford university and currently works as a postdoctoral researcher at department of plant science, University of Cambridge. His previous research projects cover diverse topics in synthetic biology ranging from in vitro DNA nanotechnology, biophysics of gene expression and synthetic gene networks. His current research focuses on cell-cell communication and emergent properties of multicellular systems.

Sakonwan Kuhaudomlarp (Sakonwan.Kuhaudomlarp@jic.ac.uk) received her B.A. and M.Sci. degree in Biochemistry from University of Cambridge. She has research experiences in plant cell wall synthesis under guidance of professor Paul Dupree and microfluidics platform as diagnostic tools under the supervision of professor Florian Hollfelder. She currently pursues her Ph.D. in Plant Sciences and Microbiology at the John Innes Centre, under the supervision of professor Robert A. Field, investigating beta glucan metabolism in Euglena gracilis.

Kyle Lopin (kvl6@case.edu) received his B.S. and M.S. degree in Electrical and Computer Engineering from the University of California at Santa Barbara. He received a Ph.D. in Physiology and Biophysics from Case Western Reserve University for working on research of electrophysiology and computer modeling of ion channel permeation and gating. He worked as a Research Fellow at the Cleveland Clinic Foundation in the BioMedical Engineering department developing an electrical device to measure physiological properties of organs-on-a-chip as part of the microphysiology research program by the United States Defense Advanced Research Projects Agency (DARPA). He is currently a faculty member at Naresuan University, Thailand, working on computational biology, and biomedical device development.

Settha Tangkawanit (setthzer@hotmail.com) received the B.Eng. degree in Computer Engineering and M.Eng. degree in Electrical Engineering from Naresuan University. He currently works at the Department of Electrical and Computer Engineering, Faculty of Engineering, Naresuan University, Thailand. His research interests are in the area of embedded system, computer vision and wireless sensor network. He is also the founder and the owner of Settzer Lab, a school of electronic engineering for kids.

Implementation

Aims

1.To design and build a robust, portable, low cost, and open source device for measuring and recording plant electrical potential. Our primary goal is to focus on extracellular electrical potential recording from woody plants. The device should be able to stably operate in open environment, collecting and storing data for several days without human intervention. The device should be modular enough to be scaled up for collecting data from multiple locations on plant simultaneously without the need for major hardware changes.
2.To provide preliminary results on plant electrophysiological activities in natural habitats. Thus far, electrophysiological studies in plants have be done in isolated, well-controlled laboratory environment. While plant electrophysiological responses to several environmental stimuli have been reported, it is not clear to what extent such responses really occur in natural environment where plants are constantly exposed to multiple noisy stimuli. This aim would help future researchers to re-evaluate possible applications of plant electrophysiology measurement in a more realistic context.
3.To create a platform for community engagement in plant electrophysiology research. The platform will consist of self-contained tutorial for operating the device, standard protocols for depositing data and open forum for sharing problems and application ideas. This platform would not only expand our workforce for exploring plant electrophysiology in natural environment, but also serve as educational tools for students who are interested the interface between biology and engineering.

Methods

1. Possible sensor/controller implementation. A Programmable System on a Chip (PSoC) from Cypress Semiconductor will be used to develop the open-source device to measure extracellular potentials of plants. We plan to use the CY8KIT-059 PSoC®

5LP Prototyping Kit by Cypress semiconductor (**http://www.cypress.com/?rid=108038**). The use of such prototyping kits manufactured in large batches would allow us to lower the cost (~ £10 total cost per kit) and standardise device quality. The PSoC is a novel programmable electrical chip that has both programmable analog and digital components. This will allow the use of just one integrated circuit to make the device. Devices used in previous plant electrophysiology studies usually have separate digital and analog boards, resulting in higher cost and complexity. This is especially true for the analog part where equipments in previous studies need to be custom-made, an expensive and difficult undertaking for researchers who are not experts in electrical engineering. The proposed device will make it easier for biologist to study plant electrophysiology. To verify this device, we will compare the results of our device and a device that uses a higher-end instrumentation amplifier. It could be a problem that the electrical potentials of the plants need an amplifier with a lower input bias current and higher gain than that can be provided by the PSoC 5LP. Compared to other devices in the literature, the PSoC 5LP has better current and gain characteristics. To confirm that the PSoC is adequate, an external instrumentation amplifier with lower input current and higher gain will be used for comparison. If it is found that an instrumentation amplifier is needed to adequately record plant electrical activity, it will be incorporated into our design.

2. Possible wireless network implementation. Our whole-plant or multiple-plant electrophysiology setup consists of three main components: wireless sensor, coordinator and analyser. The wireless sensor will be installed on plant(s) in order to measure electrical potential(s). The coordinator collects data from each wireless sensor and transfers to the analyser, which can be a personal computer used for storing, visualising and postprocessing the data. Each wireless sensor has a pair of Ag/AgCl electrodes (Mousavi et al., 2014): one for measuring electrode (positive terminal) and another as a reference electrode (negative terminal). The electrodes are connected to voltage **shifter** which amplifies and maps measured voltage data to a positive range. The signal will then be sent to ZigBee module and further transmitted to the coordinator. Wireless transmission distance of 10-100 meter should be sufficient for whole tree or whole plot measurement. We would also need 9 V battery to power the circuit and possibly timer/relay components to reduce power consumption. The coordinator consists of microcontroller (Arduino UNO) and ZigBee module (XBee board). For simplicity, the code in the microcontroller is minimized by doing only checking address and data (as a string) extraction from the receiving signal (from ZigBee module). Standard libraries will be used. APIs for both recording data from the sensor node and retrieving/showing data for the user will be developed by using simple c code. For the scope of this project, we will simply use personal computers and scripts developed in house to store and visualise data.

3. Reference plants and test conditions. To test whether our device function as expected, we will use a selection of plants of which electrophysiology have been well-characterised as references. Here are some tentative examples:

a) Venus Fly trap (respond to touch) is available for ordering online at **http://littleshopofhorrors.co.uk/** for £18 per plant with shipping fee. For reference, we will use measurement data from Backyard Brain (**https://backyardbrains.com/experiments/plants**).
b) *Mimosa pudica* (respond to touch) is available for ordering online at **http://www.touchmenotplant.co.uk/buy-sensitive-plant.html** for £12 with shipping fee. For reference, we will use measurement data from Volkov *et al* 2010 (**http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3115031/**)
c) *Arabidopsis thaliana* (respond to wounding) is available at Plant Science Department, University of Cambridge, for example, from Alex Web's lab (**http://www.plantsci.cam.ac.uk/research/alexwebb**) and can be readily ordered from Horticultural service at JIC. We can use measurement from Mousavi *et al* 2013 (**http://www.ncbi.nlm.nih.gov/pubmed/23969459**) as a reference.
d) Poplar trees (daily cyclical variation) — we can find them around Cambridge**http://www.cperc.org.uk/downloads/Black%20Poplar_report_final.pdf** .We can use measurement result from Gilbert *et al* 2006 (**http://www.ipgp.fr/~gibert/PDF_Files/48.pdf**) as a reference.
e) Willow tree (electrical stimulus) is available for online ordering at **http://www.trees-online.co.uk/Common-Osier-Willow.html** for £20 per 12 trees) We can use measurement result from Fromm & Spanswick 1993 as a reference (**http://jxb.oxfordjournals.org/content/44/7/1119**)

4. In order to calibrate our device, we will employ one of the following electrophysiology equipments that have been well-characterised as our standard measures:
   a) We could use a commercialised backyardbrain kit as a standard device, which costs approximately £130 (**https://backyardbrains.com/products/plantspikershield**).
   b) Alternatively, we could follow the protocol from Mousavi *et al*., 2013. For this setup, we would need high impedance amplifier, e.g. FD223A (World Precision Instrument) and data acquisition interface and software, e.g., LabTrax-4/16 with Datatrax2 or Labscribe software (World Precision Instrument). **http://www.nature.com/nprot/journal/v9/n8/full/nprot.2014.136.html**

5. Community outreach implementation. We will create a website to publish a step-by-step detail on how to assemble our hardware, source code and installation guide. We will host tutorial session at University of Cambridge, JIC in the UK and Naresuan University in Thailand. We hope to hold our first exhibition at Fascination of Plants Day, which is held annually at JIC where school children around Norwich are welcome to participate and learn about plant electrophysiology through our device demonstration. We also plan to collaborate with local high schools near University of Cambridge, JIC and Naresuan University to demonstrate our devices in science classroom. In the future, we aim to reach out to rural areas in Thailand where shifting cultivation is one of the contributing factors to deforestation and use our

device to educate and raise awareness to the local people about plants as "living" organisms.

Outcomes
1. Deliverable hardware prototype for outdoor, portable, and wireless plant electrophysiology together with publishable research article describing the work.

2. Online database collection for electrical response in plants. We will create an online website for database deposition in a similar fashion to PhysioBank, which is an archive of well-characterized digital recordings of human cardiopulmonary, neural and other biomedical signals, which can be freely downloaded for use by the biomedical research **http://www.physionet.org/physiobank/**

3. Educational outreach: to promote study of electrical signal in plant and in the larger sense and expose students to the synergy between hardware and biological engineering.

Who will be involved
1. Pakpoom/Kyle have leading roles in electrophysiology and hardware engineering.
2. Sakonwan will be responsible for hardware validation on plant subjects and feedback to hardware developers.
3. Settha will be a design advisor on microcontroller and wireless communication devices.

Benefits and outcomes

1. The project involves the collaborative efforts of researchers from the John Innes Centre (JIC), the department of Plant Sciences, University of Cambridge and Naresuan University, Thailand. Not only will it complement the existing collaboration between JIC and Cambridge, but also expand the work into international setting, which could substantially enhance the progression of the project.

2. The protocol for device construction and data interpretation will be published via an online interface, which will be easily accessible by plant research communities for scientific research purposes and also available for non-scientific communities for public engagement and outreach opportunities. The online platform will also be used for information sharing between plant researchers, technical support for instrument set-up, and article collections providing background to plant electrophysiology and neurobiology fields.

3. The portability and user-friendly design of the device will enable the collection of plant electrical signals from various plant species, both in well-controlled and natural environments. The data collection process could be easily adopted by plant research communities and non-scientist citizen, which will aid data acquisition and creation of an unprecedented "plant electrical signal database" that could be shared on our online platform. The database could provide a stepping stone for addressing further biological questions related to plant electrophysiology.

4. Regarding the educational outreach aspect of this project, we hope to demonstrate our device to children from schools around Norfolk at Fascination of Plants Day event, which is

held annually at JIC. We plan to engage in outreach activities by holding classroom demonstrationห of our project in schools around University of Cambridge, Naresuan University and JIC in order to raise awareness in plant electrical perception and the interface between the engineering of living matter and electrical circuit.

Sponsor for the cost-centre

1. Haseloff Lab, University of Cambridge: sponsoring research facility and laboratory space
2. Field Lab, John Innes Centre, Norwich: : sponsoring research facility and laboratory space
3. Naresuan University, Thailand: sponsoring research facility, laboratory space and matching fund (~£4000) if our proposed project received OpenPlant Fund

Budget

** If our OpenPlant fund application is successful, there will be a match funding provided by Naresuan University, Thailand, as aforementioned in the previous section.

	Budget category	**Requested amount (£)**	**Sub-totals (£)**
a	**Hardware (cost cover up to 40 prototypes)**		1300
	Programmable System on the Chip (PSoC)	400	
	Zigbee module	400	
	Ag/AgCl electrode	300	
	Protective boxes for electrical circuit containment	200	
b	**Biological materials**		800
	• Venus fly trap	200	
	• *Mimosa pudica*	200	
	• *Arabidopsis thaliana*	200	
	• Willow	200	
c	**Outreach activities**	300	300
d	**Web design, data deposition**	500	500
f	**Other costs (eg. electronic tools, facility, waste disposal etc.)**	500	500
	Total project expenses		3400