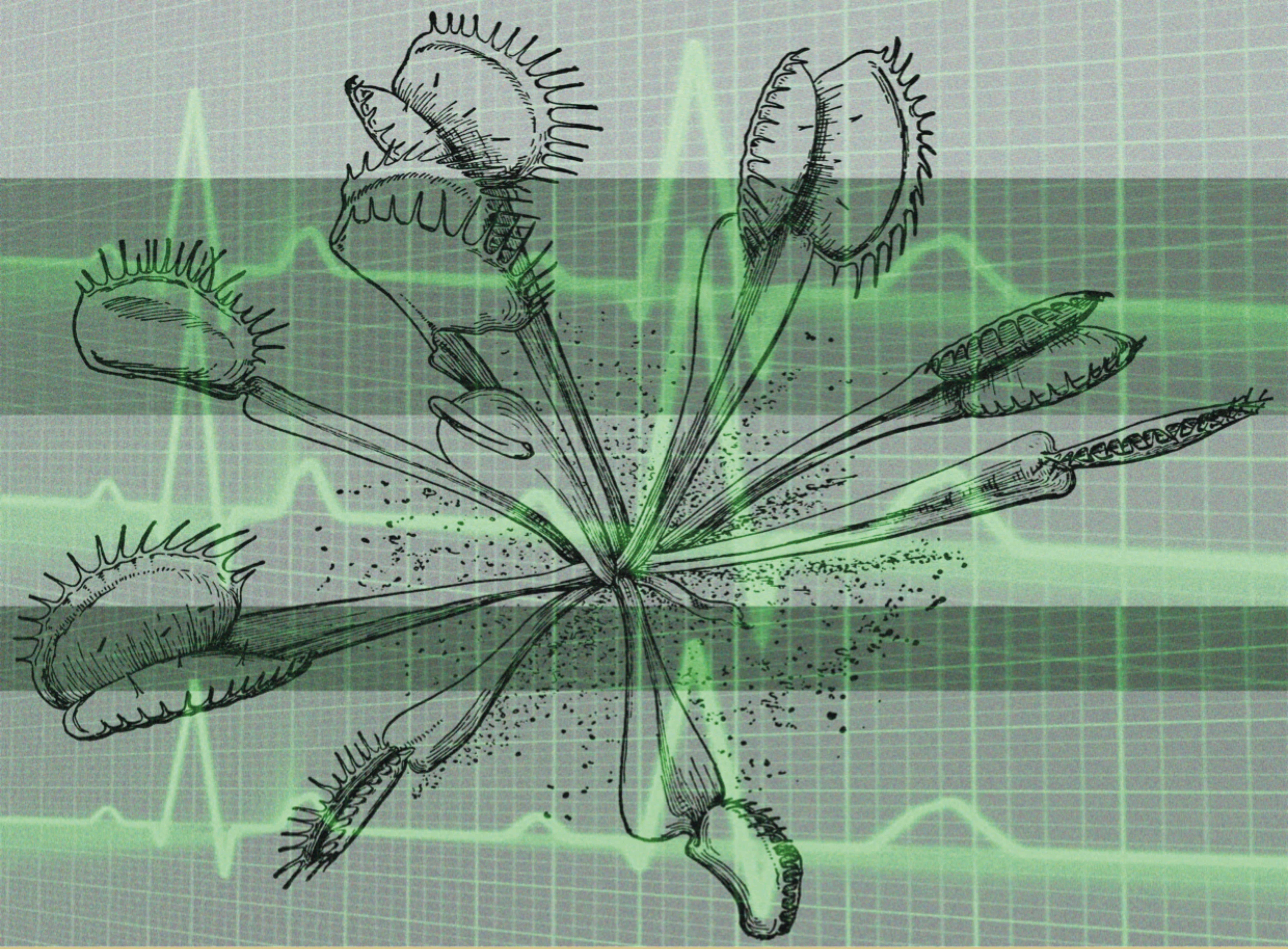


Ricardo de Abreu Silvério Cabrita

Developing an Acquisiton System to Study Plant Electrophysiology

Dissertação de Mestrado em
Engenharia Electrotécnica e de Computadores

Fevereiro de 2016

Universidade de Coimbra

• U C •

Developing An Acquisition System To Study Plant Electrophysiology

Ricardo de Abreu Silvério Cabrita

Dissertação para obtenção do Grau de Mestre em

Engenharia Electrotécnica e de Computadores

Orientador:	Doutor Lino José Forte Marques
Co-Orientador:	Doutora Sabrina de Almeida Carvalho

Júri

Presidente:	Doutor Pedro Manuel Gens de Azevedo de Matos Faia
Orientador:	Doutor Lino José Forte Marques
Vogais:	Doutor Mário João Simões Ferreira dos Santos

Fevereiro de 2016

Agradecimentos

Gostaria de começar por agradecer aos meus orientadores, Doutor Lino Marques e Doutora Sabrina Carvalho por toda a disponibilidade e ajuda prestadas, sem as quais este trabalho não teria sido possível. Agradecer também a todos os membros da Coolfarm por todo o apoio e abertura.

Ao Centro de Ecologia Funcional (CEF) da Universidade de Coimbra, pelo espaço cedido e a abertura com que me receberam. Aos colegas de laboratório, em especial ao João, pela ajuda e partilha de conhecimentos. Ao Doutor Alexander G. Volkov pela ajuda prestada. A todos muito obrigado.

Aos colegas da ”Crew” do Curso. Ao pessoal da ”Nár(n)ia”. Aos colegas do Aikido e Kendo. Agradecer por terem estado tão presentes durante todo o percurso académico e na esperança que assim continuem no que quer que se siga.

Agradecer ao Pedro por estar presente nos momentos cruciais, ao Carlos pela paciência, à Catarina pela amizade, à Filipa pelo apoio e companhia. Também a todos os outros, felizmente em número demasiado grande para nomear, que de uma forma ou de outra, estão próximos. Finalmente, estou agradecido à Sofia, aos meus pais pelo apoio incondicional e, ao meu irmão, por tudo.

Ricardo Cabrita

Nature Is Orderly. That which appears to be chaotic in nature
is only a more complex kind of order - Gary Snyder

Abstract

Plants are constantly tapped to the environment in order to maintain homeostasis, synchronising internal functions to external phenomena. There is great interest in advancing in the field of plant electrophysiology so as to employ plants as biosensors in the future. A custom-made acquisition system to study plant electrophysiology was developed, including a three channel signal conditioning circuit and ADC with dynamic sampling rate. Also, air temperature and humidity, soil temperature and humidity and irradiance are monitored. To test the system an experiment was conduced to study lettuce, Batavia Amari Paris, response to acid. For this two groups of 4 lettuce each, one used as control the other subject to fertilising treatment, were studied in the absence of stimuli and in response to acid deposition. Feature selection was made using discrete wavelet analysis. The fertilised lettuce showed little response to acid, but fairly strong correlations between signals in the leaf zone. Control group showed high incidence of high frequency fast transients in response to acid, especially in the root zone. Both groups showed higher variance of DC values during day time than night time.

Keywords

Plant Electrophysiology, Electrical Potentials, Biosensors, Data Acquisition, Signal Conditioning, Compressive Sensing, Wavelets

Resumo

As plantas estão em permanente contacto com o meio envolvente de forma a manter homeoestase, sincronizando funções internas com fenómenos externos. Há um grande interesse em fazer avanços no campo da electrofisiologia das plantas de forma a, no futuro, possibilitar o uso de plantas como biosensores. Desenvolveu-se um sistema de aquisição para estudar a electrofisiologia das plantas. O sistema inclui um circuito de condicionamento de sinal de três canais e um ADC com aquisição dinãmica. Também são monitorizadas a temperatura do ar e do solo, humidade do ar e do solo e irradiãncia. Para testar o sistema realizou-se uma experiência com alfaces da qualidade Batavia Amari Paris, para estudar a resposta deste ao ácido. Nesse sentido, dois grupos de 4 alfaces cada, um o grupo de controlo o outro sujeito a um tratamento com fertilizante, foram estudados na ausência de estímulos e na resposta a ácido. A selecção de features foi feita através de análise com Wavelets. O grupo com fertilizante não mostrou grande resposta ao ácido, tendo no entanto mostrado uma correlação razoavelmente forte nos sinais das folhas. O grupo de controlo mostrou alta incidência de transientes rápidos em resposta ao ácido, especialmente na raiz. Ambos os grupos mostraram maior variãncia dos valores DC durante o dia que durante a noite.

Palavras-Chave

Electrofisiologia das Plantas, Potenciais Eléctricos, Biosensores, Aquisição de Dados, Condicionamento de Sinal, Compressive Sensing, Wavelets

Contents

List of Figures

List of Tables

List of Acronyms

AP Action Potential

VP Variation Potential

A/D Analog to Digital

ECG Electrocardiography

VI Vegetation Indice

PAR Photosynthetically Active Region

CS Compressive Sensing

AER Adress-Event Representations

1

Introduction

1.1 Electric Signalling in Plants

Plants continually gather information about their environment in order to maintain homeostasis. Environmental changes elicit various biological responses, synchronizing internal functions according to external phenomena thanks to electrochemical excitability in plant cells [1]. “About 95% of living matter existing on Earth is the biomass of plants” [2]. Plants are the basis of many food webs, producing oxygen, influencing climate and soil characteristics. They capture solar energy, convert it into forms accessible to biological systems, and synthesize all of the material from which organic substances of the biosphere are built [2]. Plants are in constant interaction with the surrounding environment playing a major role in the maintenance and sustenance of most life forms. It is, therefore, of interest to explore the potential of plants as biosensors tapping into their electrochemical activity.

Electrical signals in plants have been known and described at least since 1783 (Pierre Bertholon) [3] and object of study ever since [4, 5]. In 1873 on Charles Darwin’s request Burdon-Sanderson conducted experiments with the carnivorous plant *Dionaea muscipula*, Venus flytrap. The speed of action of the Venus flytrap when entrapping its prey suggested that electrical signalling might be essential to the process. The normal chemical signalling wouldn’t prove fast enough for such fast response of the Venus flytrap. Still, for the best part of the 20th century it was thought that only a handful of plants requiring rapid action would possess such signalling. Also, the possibility of a neuronal system in plants, similar to that found in animals, was dismissed under the assumption that there was no real need for rapid signalling in organisms with apparently little movement or action [6].

In truth, even though apparently a plant might not make use of rapid action it still needs a mechanism to respond rapidly to biotic and abiotic stress factors, such as herbivore attack, diseases or temperature shifts. Not only external stimuli , however, evoke the need for rapid signalling. For example, it has been shown that plants can have very rapid systemic responses on fundamental processes, such as gene expression [7]. Thus, in the last decades research on plants electrophysiology has resurfaced to find plants possess most of the chemistry of neuromotoric systems present in animals, albeit in a smaller scale. With their specific neurotransmitters, cellular messengers, cellular motors and voltage-gated ion channels a simple neural network takes form within the phloem, making long distance electric signalling within the plant a reality [8].

In fact almost all of plant’s metabolism can be linked to ”electrical” activity since any flux of ions across the plasma membrane of any given cell will generate a membrane potential [8]. Therefore, it is possible that virtually all relevant information with respect to the plant might be contained in the electrical signals being transmitted along its vascular system. To study plants' electrophysiological responses one cannot solely rely on recorded electrical signals but also on all environmental factors relevant to plants' metabolism. After analysing the challenges of plant signal acquisition and processing and the relevant external factors we developed and tested a multi-sensor set-up to conduce plant electrophysiology studies.

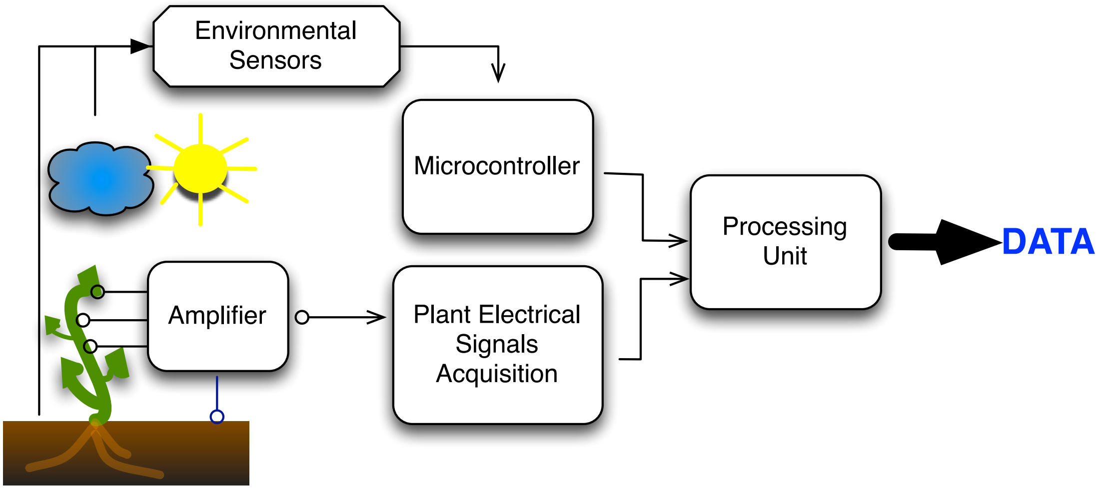


Figure 1.1: General diagram of proposed acquisition system.

1.2 Motivation and Main Contributions

Recently interest in plant electrophysiology has spiked. Plants play a crucial role in maintenance and sustenance of most life forms, humans included, and interact with virtually every environmental phenomena. Nowadays, due to pressing environmental concerns, there is great interest in the possibility of using plants as environmental biosensors to monitor atmospheric, meteorological, geographic and even geomagnetic phenomena.

Also, virtually all information concerning a plant's metabolism may be contained in the electrical activity arising from membrane potentials [8], thus many efforts have been made to try and understand the mechanisms behind electrical signalling in plants. Plants could even be used as sensors of themselves to monitor health, growth, nutritional value etc. Sustainability and food storage and management is one of the great challenges of the 21st century and this understanding of electrical signalling may play a big role in this effort.

Despite recent interest and advances in plant electrophysiology studies, there is still very little knowledge of the underlying mechanics behind electrical signalling in plants and its electrophysiological reasoning. Most studies focus on the internal mechanisms of plants relating them to observed signals, however, due to the complexity of bio-systems, progress has been slow. Also, there has yet to be reached some kind of standard as to how to record and process these signals. Technical information on methodologies used is often amiss and most results come from heterogeneous experimental set-ups which hinders comparisons and interchangeability of data and results.

Another problem with electrophysiology studies is that when using high sampling rates, because of fast transients, long duration recordings result in huge amounts of data. This amount of data being most of the times totally disproportionate to the amount of high frequency information present. This becomes an issue not only in data storage but more importantly in data analysis.

With this in mind we propose the development of an extracellular acquisition system to study plant electrophysiology. A dynamic acquisition algorithm with variable sampling rate is proposed and tested.The system also monitors several environmental factors. Namely air temperature and humidity, soil temperature and humidity and irradiance.

The system is tested with a small, yet suitable, experiment to prove its significance in plant electrophysiology studies. It is known that membrane potentials are regulated by calcium (Ca^{2+}) ion channels as well as potassium (K^{+}) and chlorine (Cl^{-}) [9], indicating that nutrient levels may influence signal transmission in plants. For the test, two groups of 4 lettuce each were grown, one group as control, the other subjected to nutrient fertilising treatment. All plants were studied in the absence of stimuli for long periods of time (over 24 hours) and in response to acid deposition on the leaves. Electrical potentials and environmental data is cross analysed. Discrete Wavelet Transform is used to analyse plant signals and select relevant features.

From this work resulted a functioning custom-made acquisition system with several environmental sensors and dynamic sampling for the plant signals. Also wavelet analysis is established as a crucial tool for feature selection of plant signals. These features such as signal correlation, dc variation and incidence of fast transients across different channels not only may have use in separation/classification but are also intuitively connected with physiological traits of plants, giving important information about their state and characteristics.

1.3 Dissertation Outline

This thesis is organized in six chapters. Succeeding the introduction, Chapter 2 provides the knowledge and tools that form the basis of this work. First a review of the literature concerning Plant Electrophysiology, starting with an overview of the subject, than a review of findings in relation to plant potentials, followed by a review of employed methods for plant potential measuring and finally the latest in signal processing and classification of these signals. Than, a brief review of the main abiotic factors concerning plant growth and development and how to measure them is presented. Finally some useful concepts are discussed, compressive sensing and the wavelet transform, which will be important in the following chapters.

Chapter 3 concerns the steps taken to arrive at the acquisition system. Describes the signal conditioning circuits developed, a preliminary experiment not only to test the concept but also to develop a dynamic acquisition algorithm later used in the system.

Chapter 4 describes the final functioning acquisition system, the experiment conduced to test it and the analysis of the experiment's results using Wavelet analysis.

Chapter 5 discusses the results presented on chapter 4 and explores what can be learned from them. Chapter 6 presents the conclusions of this work and gives some thought to future Work.

The appendixes present extra information, images, graphs and schematics for consultation.

2

Literature Review and Useful Concepts

2.1 Review: Electrophysiology of Plants

Study of the electrical signals in non-vascular plants, such as the giant *characean* algae, have served as important models and stepping stones in the development of electrophysiology methods for plants, especially in regard to intracellular measurements and the inner workings of membrane potentials [10]. We will however, focus on higher (vascular) plants.

Vascular plants, or higher plants, is the large group of land plants that have conducting tissues, the xylem and phloem, which transport fluids and nutrients internally. Higher plants are composed of two main structural axes: the stem and the root. From the stem, petioles branch and connect to leaves. The root is mainly (but not only) responsible for the absorption of water and inorganic nutrients, anchoring and supporting the plant body. The xylem, composed mainly of dead cells, transports water and soluble mineral nutrients from the roots throughout the plant. Its flow is caused by transpirational pull and root pressure. The phloem, composed mainly of living cells, transports organic nutrients resulting from photosynthesis, in particular sucrose. Conducing cells in phloem are known as sieve tube elements [11].

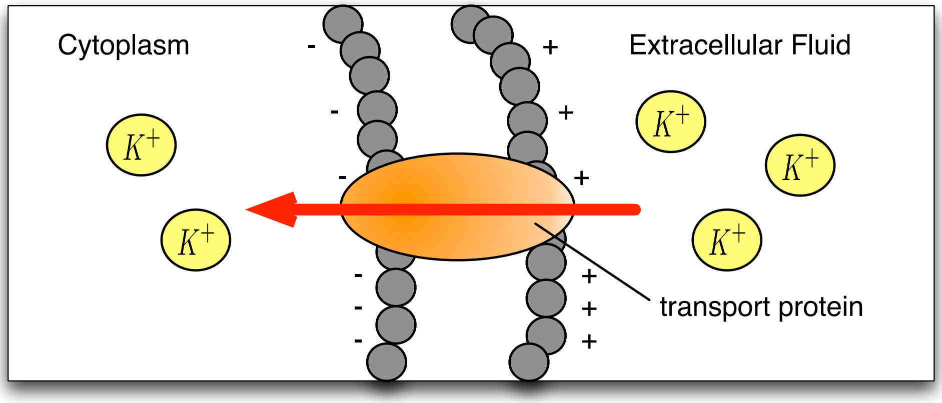


Figure 2.1: Example of a cell's plasma membrane and cation distributions'(K^+ in this case) effect on membrane potential.

The source of signal generation in plants is at the plasma membrane level, which plays a role in many essential cell biological processes, such as nutrient acquisition, pH and ionic homeostasis, energy transduction and signalling [12]. Figure 2.1 illustrates an example of such membranes and corresponding potential. Membrane potentials are regulated by calcium (Ca^{2+}) ion channels as well as potassium (K^+) and chlorine (Cl^-) [9]. This was verified in trees, showing that calcium influx as well as potassium and chlorine efflux are involved in the generation of action potentials [8]. Different stimuli evoke specific responses in the ion flux across a cell's plasma membrane eliciting an electric membrane potential and thus originating a signal in the cell. These signals are in turn transmitted to other cells via the plasmodesmata[1], or even to the phloem, where they can travel long distances along the sieve tube [13].

There have been identified many types of electrical signals in plants, the two most relevant are: i) Variation Potentials, Graded or Electrotonic Potentials (VP), and ii) Action Potentials (AP). VP's are waves of electrical excitation proportional to the size of the stimulus propagating with exponentially decreasing amplitude [14]. They are thought to be local responses to stimuli, caused by chemical reaction in the xylem [6, 15] and don't seem to propagate too far away from the zone subject to said stimuli. AP's, however, are thought to be evoked in response to stimuli functioning as informative signals travelling along phytoneural channels [16]. They behave similarly to the action potentials found in animal cells albeit slower [17]. They are also the only long distance signal that can be said to be "genuinely" an electrical signal [18]. It is self-propagating and obeys the all-or-nothing law, it is transmitted with, practically, constant velocity and amplitude [19] and it suffers from a refractory period. The primary location for its transmission is, in vascular plants, the phloem, especially the sieve tubes. Nevertheless, its spread can occur through any other cell types containing voltage-gated-channels. In this way, all cells with symplastic connection to the phloem will not go unnoticed [8]. This fact suggests that extracellular measurements can record such signals possibly carrying useful information regarding the plant's internal functioning and its relation to its surroundings.

2.1.1 Electrophysiological Responses of Plants

After introducing the basic principles behind bioelectrical signalling in plants, we will now review what is known of the impact of various factors (stimuli and environmental conditions) on the electric potential of plants. Electrical responses have been found in response to mechanical wounding, heating or chilling, night and day cycles, gas pollution, acidity and salinity [1, 20–24] . Electric signalling has also been related to physiological responses such as respiration, photosynthesis, stomatal opening[2] and closing, and gene expression [15, 20, 25].

Heating (and also mechanical wounding in general) is generally associated with the generation of VP's [20, 22, 26]. For example, Mancuso [22] tested the wounding response of *vitis vinifera*, the common grapevine, when subjected to a flaming match for 3 seconds.

[1]plasmodesmata are microscopic channels transversing the cell walls of plant cells enabling transport and communication between them.

[2]stomata are typically in the outer leaf skin layeer (epidermis) and regulate the exchange gases such as carbon dioxide, water vapor and oxygen in and out of the leaf.

An electrical response in the form of VP's with multiple AP occurrences was observed. The same experience repeated across a dead zone of tissue showed the VP's without the occurrence of AP's. At saturating humidity, VP's were completely suppressed but the presence of AP's was evident. Under hypoxia (deficiency of oxygen reaching the tissues) AP's were not apparent and the average amplitude of the VP approximately halved. Also, Stankovic investigated into the possibility of VP's being the result of hydraulic responses. It was observed that the signals in response to flame-wounding were very similar to those resulting from the application of non damaging pressure in *Helianthus annuus* (sunflower), which suggests that these signals might result from the activation of mechanosensory pumps or channels [27].

Other environmental factors have been shown to elicit electric responses in plants. Volkov observed that acid (sprayed or added to the soil) induced fast action potentials in soybean, decreased variation potential, and inhibited flow of sap in xylem. Aluminium salts, whose concentration in mineral earth increase sharply as the soil's pH decreases, also induced fast action potentials, but did not affect variation potential or flow of sap in xylem [23]. Addition of proton translocators PCP, DNP and FCCP [3] induced fast action potentials and reduced the variation potential to zero in soybean. All of these are used as pesticides, and PCP is known as a pollutant [24]. In the context of the PLEASED ("PLants Employed As SEnsor Devices") project, addition of sulfuric acid to the soil elicited local electrical potentials in tomatos, although no action potentials were identified [4]. Also, atmospheric ozone treatment resulted in clear variations in the electrical potential in tomatoes plants, while salt treatment resulted in arbitrary responses, difficult to reproduce [21].

In soybean, electric potentials and phototropism (growth of organisms in according to light stimulus) appeared to be related since AP's were observed in response to the change of direction of incident radiation, for various wavelengths. In this instance, no response was observed due to light-dark cycles [1]. However, electric potential variations have been observed in response to light-dark cycles, in olive tree, avocado tree and maple tree [28] [16]. When studying the characteristics of spontaneous action potentials in sunflowers, a 24 hour rhythm was identified, adding to the suspection of a relation between electric potentials and the circadian rhythm of plants [29].

Not only it is of interest to study the electrical activity in plants, but also to study their responses to electrical stimulation itself. For example, a classic stimuli that has been

[3]Proton translocators facilitate the transport of protons across biological membranes. Abbreviations: PCP - Pentachlorophenol, DNP - 2,4- Dinitrophenol, FCCP - Carbonylcyanide-4-trifluoromethoxyphenyl hydrazone

[4]Note that sampling rate was 10 samples per second, while in Volkov's experiment it was 10k samples per second

known to produce AP's, for example in sunflower, is electrostimulation [6]. It is now known that electrostimulation can influence plant growth, for example, Mizuguchi [30] with the application of 1V to the culturing bath accelerated the growth of bean sprouts by 30%. Also, Goldsworthy [31] in his review of the effects of electrical and electromagnetic fields on plants, suggests that plants appear to be using the electrostatic fields associated with thunderstorms to make the best of the coming rain. In the same review, the surge of *electroculture* is described, after Karl Lemstrom theorised that the electrical currents from aurora borealis were responsible for the green and healthy vegetation in the Arctic. To test his theory he conducted a series of experiments in several European countries, exposing various crops to high voltages from wires suspended above them. Treated plants appeared to be in general greener and healthier, and often there was a significant increase in yield, in average around 45%.

It is still uncertain what mechanisms underlie this electric signalling, but even more elusive is what mechanisms are regulated by them. In poplar [15], electric responses to flaming were linked, to some extent, to photosynthetic responses according to chlorophyll fluorescence and gas exchange assessments. Also, electrical activity seems to be related to gas exchanges in plants. A significant increase in respiration following stimulation was observed in *Conocephalum conicum* (great scented liverwort) thallus by Dziubinska [32]. Provided an AP had been generated, both cutting a thallus edge and non-damaging electrical stimuli elicited a burst of respiration. When excitability was blocked, no significant respiration rate changes were observed in spite of wounding. Changes in the respiration rate related to AP generation were also registered in *Cucurbita pepo* (pumpkin) and *Vicia faba* (fava bean).

Due to the complexity of bio-systems, understanding the mechanics behind electrical signalling and its relation to plants' physiology is a complex task. Recent research sheds some understanding on this matters but it also raises many questions. Is it possible to develop a standardised theory behind the electrical signalling in plants? Is it possible to employ plants as biosensors? And what are the steps and methods that will take us there?

2.1.2 Extracellular Methods of Plant Electrophysiology

Two major types of electrical signals in plants are considered: intracellular and extracellular. Intracellular measurements involve the use of microelectrodes and their introduction into the cytoplasm of an individual cell. Although many methods have been developed for this kind of study, we will focus on extracellular measurements since our aim is to develop a sensor for use in the field.

Electrodes

Extracellular measurements involve the use of at least two electrodes to complete a circuit, one of them being the reference electrode. Although extracellular electrodes are not in direct contact with the phloem or the cytoplasm of an individual cell, they still provide readable and useful data. This is especially valid when concerning AP's [16] as it propagates through any cell with voltage-gated-channels. These electrodes can be invasive (by use of piercing silver or platinum electrodes) or non-invasive surface contact electrodes. Surface contact electrodes do not cause tissue damage, however they contain KCl and tend to dry out after a few hours [6]. Probably the most used electrodes are Ag/AgCl connected to the plant by means of a conductive aqueous gel. Non-invasive electrodes are generally sensitive to temperature and have been found to be physically unstable for prolonged recording sessions [21].

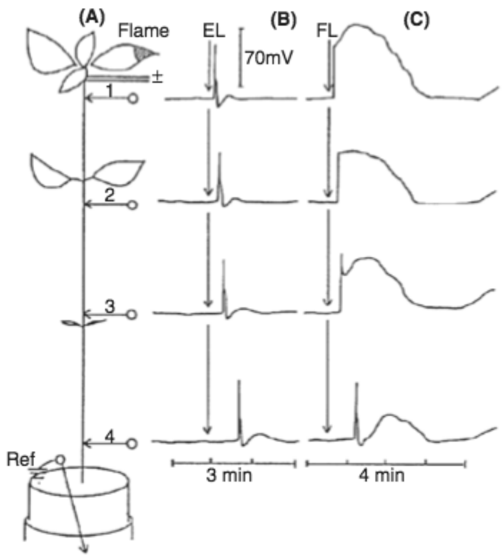


Figure 2.2: "shows typical action potentials and variation potentials measured in a sunflower. The plant diagrammed to the left (A) was stimulated electrically (5 V for 1 s) at a point about 5 cm below the lowest petiole or heat wounded with a gentle flame applied to the tip of a leaf (W). Measuring electrodes (inserted silver wires) were placed along the stem, and a reference electrode was placed in the pot. The electrical responses to electrical stimulus are action potential that are shown in the middle (B). The electrical responses to the heat wounding stimulus are variation potentials that are shown to the right (C)" [6].

Piercing electrodes have the disadvantage of inflicting damage which can be circumvented by allowing some time for the tissue to recover [6]. The most commonly used are silver wire electrodes, although platinum and steel are also viable options. The PLEASED project compared the use of both silver and stainless steel electrodes and found the latter more physically stable for prolonged recording sessions [21]. Piercing electrodes have the advantage of being useable for days, and according to Davies, even weeks [6]. In experimenting with spontaneous generation of action potentials in *Helianthus annuus* (sunflower), silver wire electrodes were seen to provide "constant and stable contact" for up to 7 days [29]. Nonetheless, in terms of signal responses, different types of electrodes produce similar results [21]. Generally, the reference electrode is positioned either in the soil or at the base of the stem [6, 21], and several electrodes are placed along the plant to measure potentials' variation (Figure 2.2).

When studying the trans-root potential and the method of collecting plant/tree electricity, steel needles (length: 9.78cm, diameter: 3.45mm) were nailed to the trunk of subject trees, and steel probes pierced to the soil (length: 4.3cm). The various trees experimented on, were at a lower potential then the surrounding soil. No relation was found between the height of the electrodes and the corresponding electric potential. Also no relation was found between the electrode spacing in the soil and corresponding electric potentials [33]. When studying the effects of electrode material on the potentials measured in trees in respect to the ground, it was found that while metal reactivity had no significant effect on measured voltage, passivation of the electrode materials visibly reduced the voltage. Also metals with higher conductivity result in higher voltage levels. [34].

Signal Conditioning and Acquisition

Besides the electrodes, "some kind of recording device" [6] is also needed. Not only recording devices, but also good signal conditioning, due to the nature of the signals: small in amplitude and prone to noise, since the plants themselves work as antennas. The PLEASED project used in one experiment, for studying responses to acid and gases, a 2 channels DUO-773 electrometer with 10^{15} and 10^{11} Ω input resistance from World Precision Instruments and a HiZ-223, 10^{15} Ω input resistance electrometer from Warner Instruments. Data acquisition was made through Lab-Trax 4-Channel DAQ. In the other experiment, mainly studying lighting stimuli, an EI-1040 instrumentation amplifier connected to a NI-USB 6008 board [21] was used. When measuring the spontaneous Action Potentials of the sunflower silver wire electrodes were interfaced with an 8-channel A/D converter with 10MΩ input resistance [29]. Also in sunflower measuring of action and variation potentials, a custom-made Op-Amp of $10^{12}\Omega$ input impedance, operating as a voltage follower, was used, connected to either a chart-recorder or a 16 channel A/D converter [26]. When studying wound-induced signals in *Vitis vinifera* a 4-channel $10^{14}\Omega$ impedance electrometer (built from an AD 645 JN op-amp) was used. The electrometer signals were low-pass filtered, amplified and connected to a multi-channel A/D converter (Lab-PC-1200, National Instruments) [22]. Volkov and Markin [35] in a series of experiments recording electrical signals in green plants used a setup which included a SC-2040 signal conditioning board, PXI-4110 - DC Power Suply, PXI-4071 Digital Multimeter, PXI-6115 Data Acquisition Card and a PXI-1042 Microcomputer, all from National Instruments.

Most endeavours into the study of plant electrical signals rely on top grade acquisition equipment to acquire good readings. However, there are already some custom approaches to the problem. A transducer unit was developed to transmit plant electrical signals to an acquisition system [36]. Ag/AgCl electrodes connected to two very high impedance Op-Amps and the difference of the signals amplified (40 db gain). The resulting signal then goes through a Butterworth Filter, with pass-band at 5kHz and a signal-to-noise ratio of 72db. Finally to connect to the acquisition system, the signal goes through a voltage to current converter. The transducer proved to be very little affected by the noise of an external radio-frequency source. Amplified signals, from *Chrysantemum morifolium* were found to be no great than 300mV. BackyardBrains which is a company that makes educational kits to teach neuroscience based on off-the-shelf electronics, also has an amplifier circuit for reading signals in plants, the Plant SpikerShield, an Arduino Shield. It uses an instrumentation amplifier to remove the common mode between the electrode in the plant and an electrode on the earth of the vase, connected to the ground of the system. The signal is then filtered and amplified through two stages using Op-Amps. The filter's bandpass is 0.2-130Hz. The Plant SpikerShield has been successfully used to record action potentials in the Venus flytrap and *Mimosa pudica*. The PLEASED project also has made a custom acquisition board, PLANTSENSE, running on a MSP430F559 chip from Texas Instruments with 8 input channels and 1 input reference, 1Hz second order analog filter and 12 bit ADC resolution sequential conversion, up to 30 samples/second/channel.

Sampling

When transmitting the plant signal, analog in nature, to a computer, the signal needs to be sampled. If the sampling rate is too slow, higher frequencies of the analog signal can be misrepresented as lower frequencies. This is known as aliasing. The Nyquist Criterion tells us that to avoid aliasing the sampling rate must be at least twice the bandwidth of the original signal. Bretschneider F. [37] identifies the frequency band of plant action potentials as 0-1Hz. Accordingly, many setups used a sampling time of 1 second, since plant action potentials have been found to be significantly slower than animal action potentials [6], as seen in [21, 29]. However, in more recent research, fast action potentials, or simply fast transients, have been found in plants and much higher sampling rates have been used, from 5000 up to 50000 samples per second [1, 23, 24].

Many experiments used faraday cages to avoid electromagnetic interference (eg. [36],

[21]), also in the experiments using the Plant SpikerShield the plant vase appeared to be wrapped in aluminium foil. However, many experiments have been conducted without the use of faraday cages, at least it has not been reported, such as in [29], [26] and [22]. Apart from faraday cages and other types of shielding, other aspects of signal acquisition, like signal conditioning and filtering can account for electromagnetic interference.

2.1.3 Analysis of Bio-Signals

Despite the large number of literature reporting electrical signals in higher plants, there is very little about predicting the time-course and shapes of plant signals. There is a great interest in developing mathematical models and tools in order to further develop our knowledge of the mechanics behind electrical signals in plants. Because bio-signals share some common characteristics, reviewed literature also included recent developments in neural and health sciences, besides plant electrophysiology.

Recently, the wavelet transform has seen a rise in interest as a powerful pre-processing method in the context of bio signals such as ECG's, neural signals and even plant electrical signals, showing promising results for feature detection and extraction [38, 39]. The interest in this transform lies in its compressing abilities (in the case of the discrete transform) and time-frequency localization for feature extraction [39]. The wavelet transform is especially appropriate for analysing non-stationary signals, making it an obvious choice for bio-signals.

More specifically in the case of ECG's and neural signals, it has been used for the detection and classification of AP's. Quotb [39] uses the discrete Wavelet transform for real-time detection of AP's in neural signals, experimenting with various types of mother wavelets: Symlet 2, Haar, Biorthogonal 1.3 and Daubechies 4. Best correct detection rate was reported for Biorthogonal 1.3, closely followed by the Haar wavelet. Wavelets have also been used coupled with Support Vector Machines for heartbeat classification with very good classification accuracy results [38]. The mother wavelet used was of the Daubechies family, and 4 level-decomposition was used, based on the frequencies of interest. Autoregressive Modelling coefficients were also used to complete the feature vector. The Kernel used in the SVM was the Gaussian Radial basis function (RBF) and its parameters were chosen using cross-validation.

In the context of plant electrical signals, studies on feature extraction and classification are more scarce. However, related work is currently surfacing. The approximation coefficients of the discrete wavelet transform of plant signals have been studied for similarity to the original signal. The aim was to study the possibility of using it as a compressed representation of the signal, fit for signal processing. Reportedly, 5 level decomposition with Daubechies 3 mother wavelet appeared to be the best representation [40]. *Aloe* *vera* and *Scindpsus aureus* were analysed under time, frequency and time-frequency domains. Classical spectrum analysis was deemed inappropriate for plant signal processing and some indications of the utility of discrete wavelet decomposition were made [41]. An original method using discrete wavelet decomposition and back propagation neural networks showed promising results in plant signal classification [42]. The method used 5-level wavelet decomposition and the feature vector was comprised of the calculated energy of both approximation and detail coefficients, for every decomposition layer. The PLEASED project also delved into plant signal extraction and classification. Characteristic parameters of the signals were extracted using second-order polynomial fitting following a least squares approach. For classification, a feed-forward neural network was used, trained with back propagation. Training errors were very low and classification results were very satisfactory [43].

Although the first steps in feature extraction and classification have been taken, there is still a long way to go in the matter of understanding and processing all the information contained in the electrical signals of plants.

2.2 Monitoring Abiotic Factors

Plants respond to the environment in which they live. This interaction can be divided into biotic (living) and abiotic (non-living) components of an habitat. Examples of important abiotic factors are soil composition, air pollution, water availability and quality, solar insolation and humidity, temperature and precipitation [44]. When conducing electrophysiology experiments with plants monitoring these factors is of adamant importance in order to discern what motivates electrical activity in plants. Abiotic factors are easier to monitor with current sensors and, since abiotic factors influence the distribution of organisms and ecosystem functioning [45], controlling them might give some insight into biotic factors also.

Radiation

Radiation is the energy source for photosynthesis, the process through which plants and other photosynthetic organisms process light energy into chemical energy. Not only the intensity of radiation is of relevance, but also the wavelengths made available and their duration [46]. This means that it is as important to know the amount of light reaching a plant as knowing the spectral composition of that light. It is then useful to speak in terms of irradiance (W/m^2). Deficient levels of irradiation can lead to anomalies such as smaller leaves with a greater length-to-width ratio, reduced concentration of chlorophyll, and less dry weight in various stages of growth. High levels of irradiance can also be detrimental to the plant, possibly resulting in photodestruction of chlorophyll (bleaching), increased anthocyanin production and increased water loss due to leaf heating, which can lead to desiccation and eventually necrosis [46].

Radiation spectrum can be divided into four important regions for plants: the visible region(450-nm-700nm), the red-edge (700-800nm), the near-infrared (700-1300nm) and the mid-infrared (1300-2500nm) [47]. Photosynthetic pigments are mostly influenced by the visible region. Photosynthesis is driven by radiation in this spectral region and as such it is also known as the photosynthetically active radiation (PAR) region. Photons in different wavelengths of this region are not equally efficient in photosynthesis stimulation [46]. Most of the radiation absorbed is around the wavelengths of 400-450 nm and 650nm [48]. This is why, when it comes to photosynthesis, it is of interest to monitor the irradiance.

The red-edge is the shifting region between the pigment absorption areas and the infra-red area linked to water and cell structure. It has been correlated to characteristics such as chlorophyll content of leaves, water stress, pesticides and ozone damage. The near infra-red and the mid-infrared regions are both related to water content in plants and the surrounding atmosphere, while the mid-infrared is related to molecular vibration (changes of state in molecules) [48]. This spectral features can be related to proteins, sugars and cellulose [47].

Many recent studies in applied agriculture have used remote spectral observations in relation to crop characteristics. This functional multispectral relationships have been transformed into various vegetation indices (VI) and the most used of this indices focus on the red and infrared wavelengths. Correlations have been found between these indices and various vegetation features, such as green leaf area, standing biomass, photosynthetic activity among others [49].

Spectroscopy and hyperspectral imaging have been successfully applied to plant studies. For example, Carvalho (2013) [48] identifies chemical variations in the spectral reflectance of *Jacobaea Vulgaris* relating these to differences in soil communities and estimating defence compounds. Zhang [50] develops hyperspectral VI's for salinity monitoring, based on plants' spectral response to salinity. Hyperspectral data has also been sucessfully used in recording critical plant features (leaf pigment, water content and chemical compostion) and in discriminating between different species [51].

Temperature

Temperature is an indicator of the thermal energy content in a system. Thermal energy in plants influences multiple physiological processes and controls the rate of chemical reactions [46] [45]. In a broader sense, temperature is one of the dominating factors in animal and plant distribution. Physiological activities have a temperature range outside which they cease to function, and an optimum point at which reactions occur at a maximum rate [45]. Temperature not only affects plants directly but also other surrounding factors, like the soil. It affects the activity of the soil biota by controlling the rate of enzyme activity which in turn has an effect on diffusion and solubility of nutrients, evaporation etc. [52].

To monitor ambient temperature there is a vast array of sensors available: thermoresistive, thermoelectric, semiconductor P-N junction sensors and more. We will focus on thermoresistive temperature sensors. The advantage of these sensors is their simplicity of interface circuits, sensitivity and long-term stability. One example of this kind of sensors are thermistors, which are resistors sensitive to temperature exhibiting a visible and predictable change in electrical resistance when subject to a change in temperature. A thermistor is considered an absolute-temperature sensor since its measurements are referenced to an absolute-temperature scale. All thermistors are divided in two groups: NTC (negative temperature coefficient, where resistance decreases as temperature increases) and PTC (positive temperature coefficient). Only the first are used for precision temperature sensors. Also very common are Resistance Temperature Detectors (RTD) which exploit the temperature dependence of resistivity of a wire or thin film of a metal for temperature sensing. Platinum is the metal chosen almost exclusively because of its predictable response, durability and long-term stability. A favourite in electronic equipment are Silicone bandgap temperature sensors due to their ease of integration in integrated circuits and at very low cost. The forward voltage of a silicon diode is temperature dependent, this fact in conjunction with bandgap voltage reference circuits makes the silicone bandgap temperature possible [53].

To measure temperature directly in plants thermography can be used. Thermography allows the visualisation of differences in surface temperatures of leaves, canopies or plants, operating in wavelengths as long as 14000 nm. Infrared radiation can be detected by thermographic cameras where each pixel corresponds to a temperature value. Infrared thermography is widely used for the detection of pathogen-induced changes in plant transpiration and water status [54].

Humidity and Moisture

Humidity designates the water vapour content of a gas and its most direct and significant effect on plants is transpiration. Generally a decrease in humidity results in an increase on transpiration. However, the integrated effect of other various multiple abiotic factors also have an increasing effect on transpiration. Excessive or deficient transpiration rates may affect the translocation of water and nutrients from roots to shoot, mineral nutrient imbalance and plant growth regulator imbalance [45]. Generally air humidity is measured as relative humidity and ideally it is around 60%, while it should not go below 20%. Since moisture in the atmosphere changes air electrical permittivity, an air-filled capacitor may be used as a relative humidity sensor. This forms the base for relative humidity capacitive sensors [53].

Moisture is the mechanically mixed water content of any solid, liquid or gas [46]. Water soil availability is crucial for the germination of seeds and root development. Soil moisture is critical to soil chemical processes being a solvent for biological nutrients and other chemicals. For example, nitrogen fixation is highly dependent on water availability in the soil. Nitrogen fixation is a requisite to biosynthesize basic building blocks of plants (and other life forms) such as amino acids. Also, water is essential for enzyme activity and metabolism and its distribution in the soil influences movement and predation of microorganisms in soil, which are critical to nutrient synthesis [52]. Three regions of soil moisture are considered in what concers plants' well-being. At the lowest there's the wilting point, which is the level at which a plants starts to wilt. Irrigation start point where one should start irrigation to avoid reaching wilting point. And field capacity where soil is saturated with water. Moisture levels depend on type of soil, and can range from 4% (on wilting sand) to around 45% (clay at field capacity). An easy and very common way to measure soil moisture is using two probes to pass current through the soil and measure resulting resistance. As moisture increases so does current and the resistance value decreases.

pH

Soil pH is very important for plant growth because it has a great effect on the solubility of minerals and nutrients. In fact, most plants prefer a slightly acidic soil, since many nutrients are more readily available in this kind of environments. The more acidic the soil the greater the uptake of some cations by the plant. So much so, that in some cases compounds that weren't supposed to be absorbed find their way into the root of the plant. Such is the case of aluminium which when absorbed in high quantities may be toxic [23]. Natural rainwater generally has a pH of 5.6 and is considered acid water below this value. Acid water has been found to have pH as low as 1.7 in the USA. Between 5 and 5.6 pH there appears to be no impact on plants' electrical potentials, however, from 4.9 and below potentials are disturbed and responses in the form of action potentials have been recorded [23].

2.3 Useful Concepts

2.3.1 Compressive and Asynchronous Sensing

With the advent of digital technology and the booming processing capacity all kinds of sensing systems have been thriving with ever-increasing resolution and fidelity. This digital take over is founded on the Nyquist-Shannon sampling criterion that states that any data in the form of a continuous signal can be exactly recovered from a set of uniformly spaced samples taken at the *Nyquist rate* of twice the highest frequency present in the continuous signals. Riding the wave of Moore's law, digital systems took over the technological world enabling the creation of more robust, flexible and cheaper sensing and processing systems. As a result of this success we are now flooded with data. In fact, in many applications the Nyquist rate is so high that acquisition or processing is technically not feasible, either because of the costs or physical constraints involved. In areas such as imaging, video, spectroscopy, medical imaging, and genomic data analysis, where data easily reaches enormous quantities and complexity, acquisition and processing is still a tremendous challenge [55].

To address this issue we mostly lean on compression, aiming at the most concise representation of a signal, granted an acceptable level of distortion. One of the most common compression technique is known as transform coding, which looks for a basis or frame able to provide a sparse or compressible representation for a signal. A sparse representation is one such that a signal of length n can be represented by $k << n$ non-zero coefficients. A compressible representation is one such that a signal is well approximated (with acceptable error) by $k < n$ non-zero coefficients. The process of preserving only the values and locations of the largest coefficients is known as sparse approximation and is the foundation of transform coding schemes such as the JPEG, MPEG and MP3 standards. Thus emerges compressive sensing (CS) as a new framework for signal acquisition and sensor design. Instead of compressing large amounts of acquired data, CS is interested in directly acquiring data in an already compressed form [55].

Typically, CS focuses on finite-dimensional vectors in $\mathbb{R}^n$, as opposed to classical sampling which considers infinite-length continuous-time signals. Also, generally, CS systems acquire measurements in the form of inner products between the signal and general test functions. Often, randomness is key in the generation of such test functions. Signal recovery is generally based on highly non-linear methods, while in the case of classical sampling, reconstruction is achieved simply through sinc interpolation. A simple example of a sparse model application is the use of wavelet thresholding in image compression and denoising. The wavelet transform recursively decomposes the original signal into its high and low frequency components. The low-frequency coefficients correspond to a coarse scale representation, while the high-frequency coefficients correspond to fine details and edges in an image (often, noise in a signal). For example, computing the wavelet transform of an image with little number of sharp edges will result in a set of coefficients where the majority will be small in amplitude. Hence, thresholding the coefficients (those under a threshold are set to zero) results in a *k-sparse* representation of the image. This is the basis for nonlinear approximation, and the nonlinearity is in the choice of the threshold, which depends on the signal itself [55].

Related to this topic is asynchronous sensing and adress-event representations (AER) circuits. Surging from this interest in directly acquiring data in an already compressed form, and taking advantage of asynchronous acquisition, new sensors are appearing, especially vision sensors. Inspiration comes from biology that teaches us that the retina outputs through the optic nerve a stream of asynchronous spikes corresponding to only relevant information for vision, functioning somewhat like an edge detector. The first commercially available of this kind of sensors is the Dynamic Vision Sensor, which, instead of sending entire images at fixed rates, sends only the local pixel-level changes (caused by movement) at the moment they occur. Its output is then a sequence of asynchronous events produced individually by pixels when perceived illuminance increases and decreases under a certain threshold. Each event is time-stamped as with any asynchronous transmission. This computation is done through an analog circuit, an AER circuit, whose biases can be tuned to change pixel sensitivity and other properties [56].

2.3.2 The Wavelet Transform

Any signal x, belonging to a space S, can be described in terms of elementary signals $\Phi(i)$ (complete in S) such as $x = \sum \alpha_i \Phi_i$. Preferably α_i coefficients should be sparse and characterize the signal with as few coefficients as possible. This is the basic idea behind signals transforms like Fourier and Wavelet transform, and why they have interesting applications in signal compression and feature extraction.

The wavelet transform can be applied to signals, functions or operators, with varied applications in multiple areas of interest. We will however, focus on the signal analysis framework. The wavelet transform of a signal evolving in time depends on two variables: scale (closely related to frequency) and time. It is then a tool for time-frequency localization, making it especially interesting when studying non-stationary signals [57]. Time-frequency localization is however not an easy feat to achieve: the Heisenberg's uncertainty principle, in signal processing terms, states that it is impossible to exactly represent a signal in time-frequency space [58]. Many techniques have been tried to get as good time-frequency localization as possible, one such technique is the windowed Fourier transform, precursor of the Wavelet transform . A signal f is first windowed into well-localized slices of f and then it's Fourier transform is taken (in its continuos and discrete form) [57]:

$$(T^{win}f)(\omega,t) = \int dsf(s)g(s-t)e^{-2\omega s}. \tag{2.1}$$

$$(T^{win}_{m,n}(f) = \int dsf(s)g(s-t_0)e^{-2m\omega_0 s}. \tag{2.2}$$

The wavelet transform can be understood in a similar way, with a few important differences. Akin to the windowed Fourier transform, the following equations describe the wavelet transform in its continuous (equation 2.3) and discrete (equation 2.4) forms respectively:

$$(T^{wav}f)(a,b) = |a|^{(-1/2)} \int dtf(t)\psi(t-b/a). \tag{2.3}$$

$$T^{wav}_{m,n}(f) = a_0^{-m/2} \int dtf(t)\psi(a_0^{-m}t - nb_0). \tag{2.4}$$

$$\int dt\psi(t) = 0 \tag{2.5}$$

Variables a and b are the dilation/scaling and translation parameters respectively. In the discrete form, equation 2.4, a and b are restricted to only discrete values: $a = a_0^m$, $b = nb_0a_0^m$. The function ψ is known as the mother wavelet because varying a,b spans different $\psi^{a,b}$ wavelets, dilated in frequency and translated in time. Large values of a correspond to small frequencies and a coarse scale, broader $\psi^{a,b}$, while small values correspond to high frequencies and a very fine scale, much narrower $\psi^{a,b}$. Changing the translation parameter b allows us to change the time localization center of the wavelet. The main difference between the wavelet and windowed Fourier transforms lies in the choice of the analyzing functions $g^{w,t}$ and $\psi^{a,b}$. While $g^{\omega,t}$ envelopes have the same width regardless of ω, $\psi^{a,b}$ has variable widths, depending on frequency (figure 2.3). As we have seen high frequency $\psi^{a,b}$ have very narrow time-widths, and low frequency have much broader time-widths. We can see now that the wavelet transform is very capable of either "zooming in" on very short-lived high frequency phenomena, or focus on the coarser aspects of a signal, thus being closer to an ideal time-frequency representation [57].

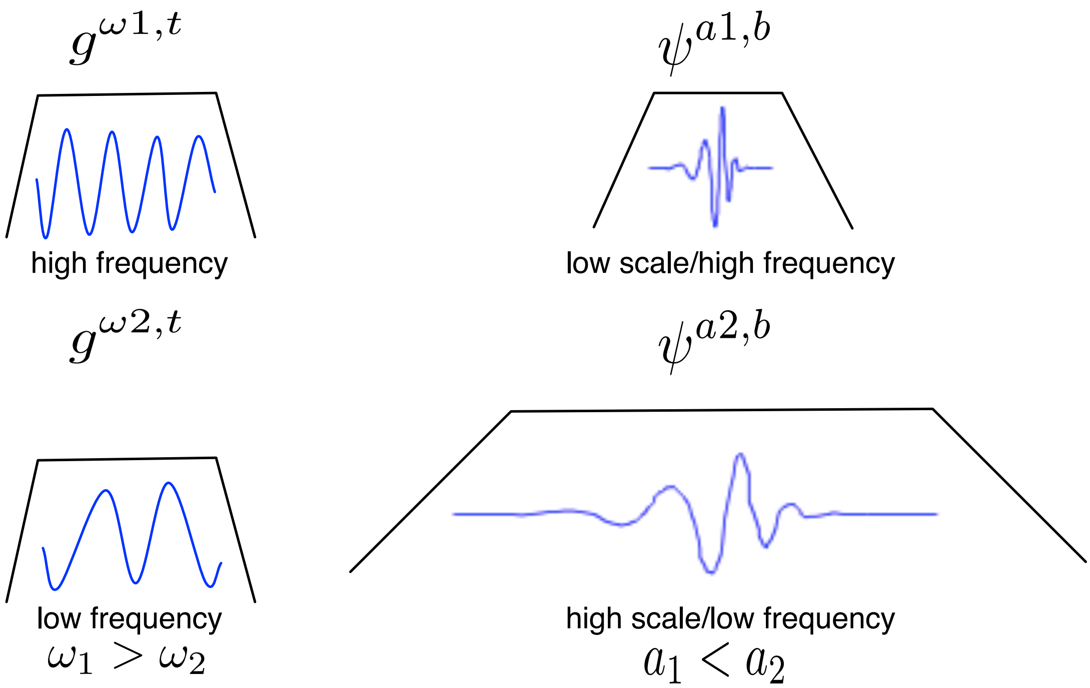


Figure 2.3: Analyzing functions $g^{\omega,t}$ and $\psi^{a,b}$ time-width in windowed Fourier transform and Wavelet transform.

In a more intuitive way, each wavelet coefficient $C_{a,b}$ corresponds to a likeness coefficient between a window n of the signal and the scaled wavelet for each b translation. This is exemplified in figure 2.4:

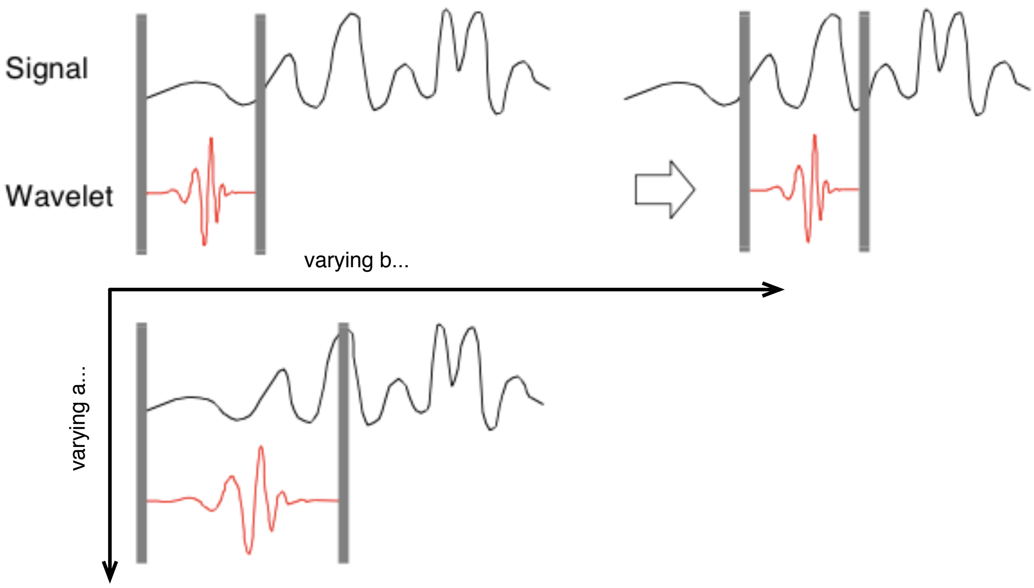


Figure 2.4: Example wavelet of varying scale a being shifted in time. Image is adapted from Matlab Wavelet Toolbox User Guide [59].

While the continuous wavelet transform has the scaling and translation parameters a, b varying continuously over $\mathbb{R}$ ($a \neq 0$), in the discrete case we choose a subset of scales and translations from which to compute the transform. This discrete sampling of the a, b parameters is done based on powers of two, so we get dyadic scales and positions, which still result in an accurate analysis of the signal and is a natural choice for computers, the human ear and music for instance [58].

Mallat [60] developed one of the most reliable digital implementations of the discrete wavelet transform, which is still in use today and known as the DWT. It is an iterated filter bank decomposition on an orthogonal base. As we have seen wavelets allow us to build orthogonal bases on $L^2(R^n)$ which can be used to calculate a complete multiresolutional representation of an original signal. This can be done efficiently using a pyramid architecture using quadrature mirror filters. Put simply, an original signal is convoluted with two filters, high-pass (G) and low pass (H) the resulting signals are reduced by a factor of 2 giving a two-signal representation of the original signal. In the case of wavelets, the high-pass signal is generally known as the detailed signal (short-lived phenomena) and the low-pass as the approximation signal (coarse representation), as seen before. The approximation and detail coefficients are given by the following equations [39]:

$$d^j[n] = \sum_{k=0}^{L-1} g[k] * a^{j-1}[2n-k]. \tag{2.6}$$

$$a^j[n] = \sum_{k=0}^{L-1} h[k] * a^{j-1}[2n-k]. \tag{2.7}$$

Note that L represents the order of the filters, which depends on the choice of mother wavelet, j the decomposition iteration and n the sample number. Both filters make up the mother wavelet, which shape depends on the filter coefficients ($g[k]$ and $h[k]$), in accordance to what we've already seen: unlike the case of the Fourier transform where the convolution function is fixed, wavelets width vary with frequency, or in this case, with varying sub-bands. To move along the scales, the signal goes through this process multiple times, functioning like an iterated filter bank. Note that, both approximation and detail signals only depend on the previous approximation signal, the previous detail signal is discarded (fig 2.5).

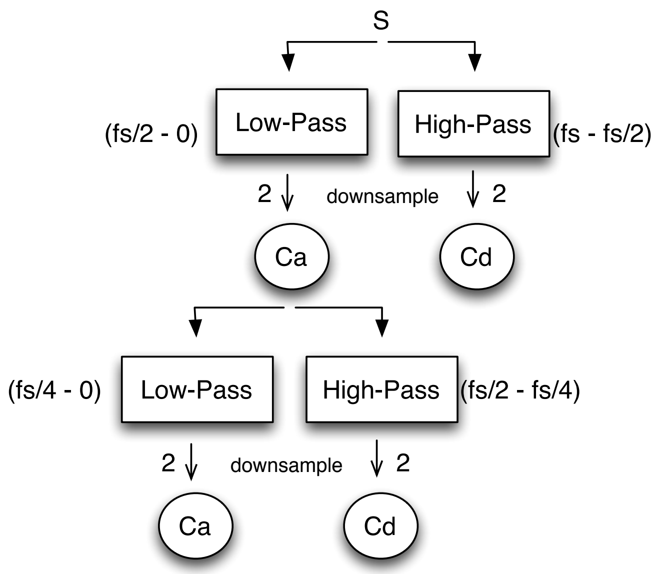


Figure 2.5: Visual representation of digital implementation of the Discrete Wavelet Transform.

This is one of the most commonly used implementations of the discrete wavelet transform, and is the one used in this work, from Matlab signal processing toolbox.

3

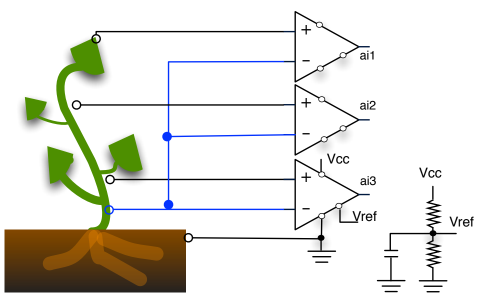
Measuring Electrical Signals in Plants

3.1 Signal Conditioning

To read electrical signals from a plant and to send the received data to a computer a signal conditioning circuit is necessary. Because plants have a high impedance as a whole (and output very low currents), the circuit used to condition the signals needs to have an even higher input impedance to correctly measure potentials. For this reason no input decoupling was used, so as not to reduce input impedance and also so that signals suffered from no *a-priori* filtering. Plant electrical signals have been known to be both very slow [6] and very fast [1]: filtering the signals, in such a way that hides the high-frequency components, is not interesting for our purposes. As we have seen in chapter one, there are different kinds of electrical signals in plants, with different characteristics. For example, AP's propagate with practically constant velocity and amplitude [19] and the amplitude of VP's decreases exponentially with distance [14]. For this reason we decided on three acquisiton channels in order to appropriately evaluate the propagation of signals in the plants.

Amplifier Circuit 1:

In a first approach instrumentation amplifiers were chosen to provide with high-impedance differential readings with common-noise reduction (common-mode rejection ratio, CMRR). An electrode connected to the base of the plant is connected to the negative input of three instrumentation amplifiers. While three electrodes can then be pierced to the plant in any desired configuration and connect to positive input of the instrumentation amplifiers (fig. 3.1).



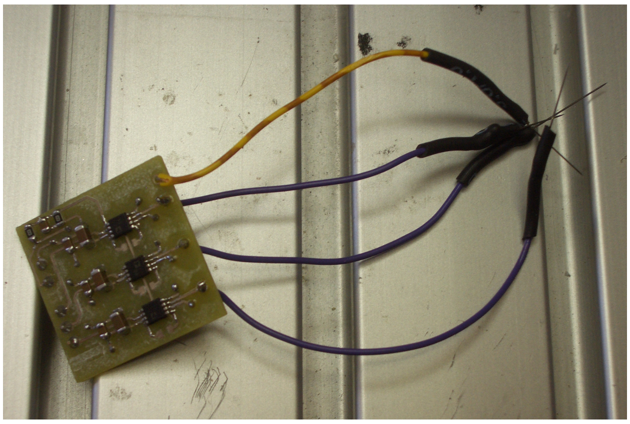


Figure 3.1: Simplified view of circuit 1 and board implementation.

Instrumentation amplifiers are useful for small signals with comparably high common mode values. The amplifier of choice was the AD623 from Analog Devices. It has a

0.35% gain accuracy and 0.10% with unit gain, set with a single external resistance. Provides a minimum of 70dB CMRR with unit gain, and 90dB CMRR with gain = 10. In our case, unitary gain was used. It has output rail-to-rail swing and allows for single and dual supply operation. Input resistance is in the order of $10^9\Omega$.

Because polarity of plant signals varies, reference was set to Vcc/2 by connecting a voltage divider to the reference node of the amplifier. The signals are then shifted by Vcc/2 but can have now ample room to swing in either polarity.

Normally the Vref input of the instrumentation amplifiers would be connected to the plant to make up for the fact that the plant doesn't share a common ground with the acquisition system. However, since it is being used for rail-to-rail swing, the ground of the system needs to be connected to the plant or to the earth of the vase (results are similar either way).

Amplifier Circuit 2:

However, if our purpose is to measure potential variations in three channels in respect to a given reference, maybe another design would be better suited. With this in mind a circuit using operational amplifiers was used (fig. 3.2).

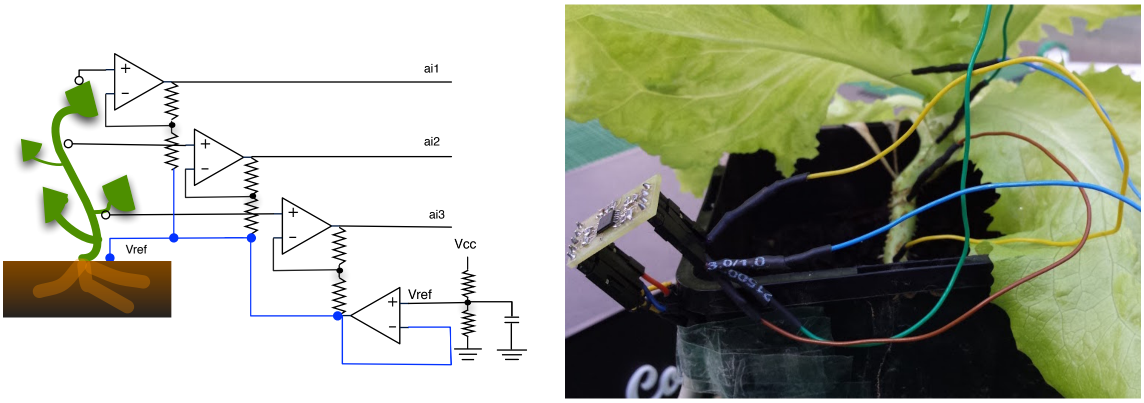


Figure 3.2: Simplified view of circuit 2 and board implementation.

Instead of grounding the acquisition system on an unknown system (the plant) it might prove more stable to directly reference the plant itself. In this way, three electrodes connect to three op-amps with feedback net gain connected to Vref (Vcc/2) to allow potentials to swing around this value. At the same time one fourth op-amp is used to feed Vref to the plant through its very low-impedance output, functioning as a voltage follower/source. The circuit uses the high impedance LMV774 Quad Operational Amplifier from Texas Instruments with 250pA maximum input bias current and maximum offset voltage,Vos of

$850\mu V$. The gain used with this ensemble was 2. Another advantage of this circuit is its lower cost. Obviously in both cases care was taken to source and IC decoupling.

Both amplifier circuits interface with the plant through piercing electrodes, more stable for prolonged readings. To make the electrodes, acupuncture needles of stainless steel with silver coating and 0.3mm of diameter were used. Both circuits were preliminarily tested on a *Scheflera sp*.

3.2 Preliminary Tests

In order to test the amplifier circuit 1 and to get an idea of what kind of electrical signals to expect, amplitude range, slow variations over time and fast transients, a battery of real plant tests was conduced (fig. 3.3).

Figure 3.3: Example set-up for the tests on lettuce using Amplifier Circuit 1 and NI USB-6009.

To this purpose 6 lettuce of the summer wonder variety were germinated in an hydroponic set-up and later changed to soil vases. For each case data was recorded after insertion of the electrodes, and the signals were monitored until apparent stabilisation was reached. Then, 0.1 *ml* of sulphuric acid (pH 0.7) was deposited on the leaf of the lettuce being monitored while the signals were being recorded. Duration of recordings was 10 to 15 minutes at most.

For signal conditioning Amplifier Circuit 1 was used with unitary gain and interfaced with NI USB-6009. The USB-6009 board's analog input provides 14 bits of resolution in single-ended mode and 40 $ksps$ of maximum sample rate (either single channel or aggregate). Eight single-ended analog input (AI) channels are provided, four if differential mode is used. The computer was interfaced with NI USB-6009 with a Python script using pyDAQmx driver running on a Windows 7 virtual machine (fig. 3.3).

Sampling rate was 5 $ksps$ for each of the 3 channels. Because the signals are already previously conditioned (Amplifier Circuit 1), referenced single-ended mode was used instead of the differential mode. To connect the amplifier circuit to the USB-6009 shielded cables were used to avoid antennas and stray capacitance between output signals and power-supply.

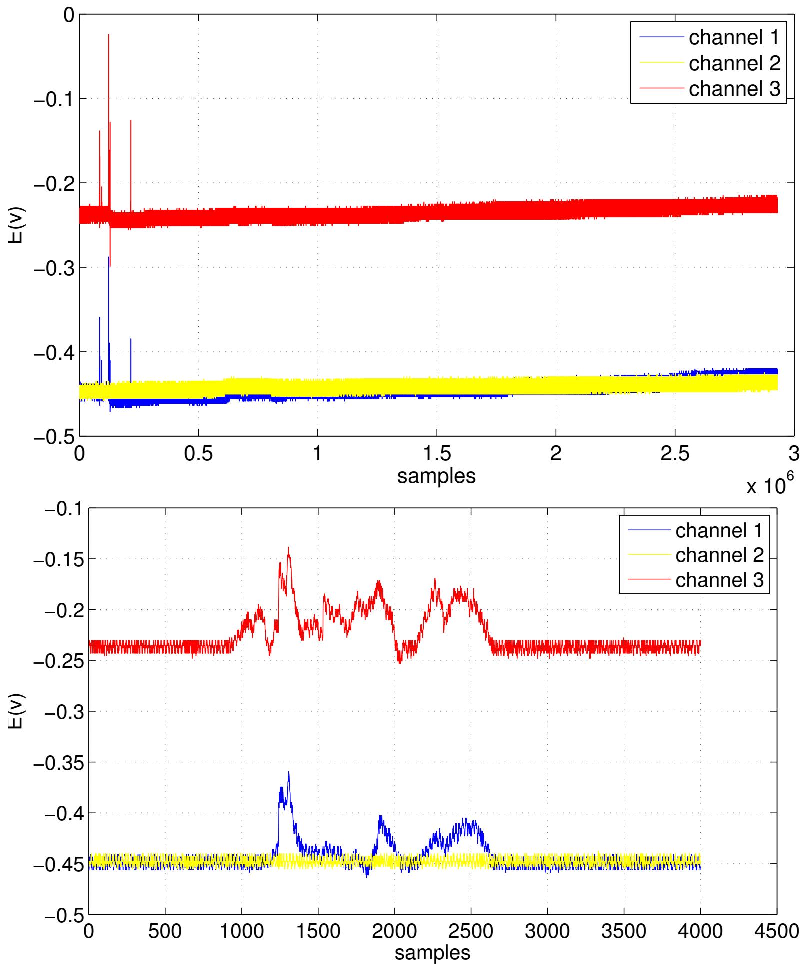


Figure 3.4: Example of Lettuce electrical response to acid and example detail of fast transient response, sampling rate: 5$ksps$.

Fast transient response shortly after deposition of acid was observed in four test cases, while in one instance it consisted of only one isolated peak. The response presented (fig. 3.4) was the most significant. In the following hours varying kinds of slow variations in the potentials were sometimes observed.

In response to the electrode insertion itself, a characteristic response was observed, in the form of an abrupt fall in potential followed by a slow recovery. Always with varying amplitudes and recovery times. Only in two test cases was this not observed, and never in response to acid, only to electrode insertion. The response presented (fig. 3.5) shows the characteristic variation in potential described in all three channels, when in the rest of the cases it occurred isolated in only one of the three channels.

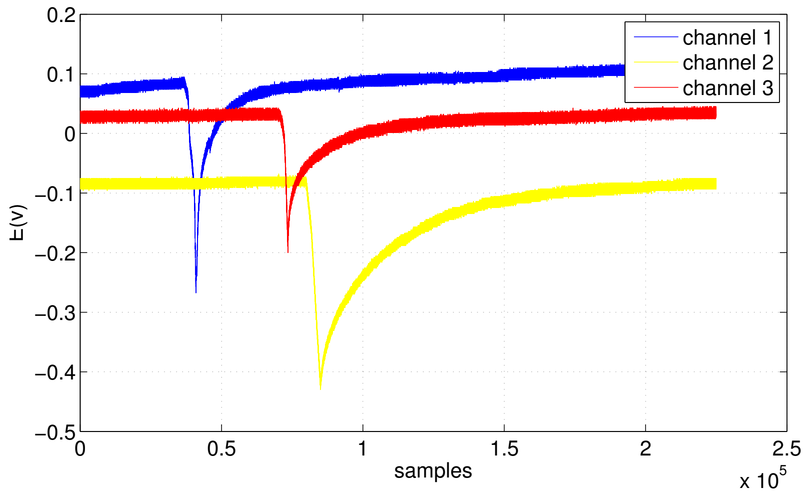


Figure 3.5: Example of Lettuce electrical response after insertion of electrodes, sampling rate: $5ksps$.

It is obvious from looking at these signals that the information contained in them is not much when compared to the number of data points, especially in the case of the higher frequencies. In figure 3.4 , over almost 10 minutes, only about 0.65% of the signal contains relevant information, or fast transients. Sparsity of plant electrical signals is evident, and while high sampling rates are crucial not to miss high frequency activity, in long recording sessions storing such high amounts of data with comparatively such little information in them, is highly undesirable.

3.3 Dynamic Acquisition

Looking at how plant signals generally evolve in time, apart from very slow transients [16], little happens of relevance in between responses to stimuli, some of them being fast [13, 23, 24], others much slower [14, 26, 29]. This can be observed not only from literature but from our own data, obtained with the NI USB-6009 acquisition set-up. Furthermore, if one is interested in prolonged recording sessions (over 24hrs) at high sampling rates, the size of data sets recorded become unnecessary hurdles to processing, especially when compared to the actual relevant information contained in them. This is why so many times high grade electrometers and other expensive acquisition equipment are used in plant electrophysiology experiments. We are then faced with the problem of how to efficiently acquire electrical signals from plants, from a data management point of view. Backed by the evident sparsity of plant electrical signals and taking inspiration from the Dynamic Vision Sensor, a dynamic acquisition algorithm was developed and tested on the signals obtained from the preliminary experiments with the USB-6009 set-up and finally implemented on the Tiva C Series TM4C123GHSPM.

The DVS described before can only sense motion, no events are generated if there's no movement in the scene or if the camera is stationary [56]. In our case, motion corresponds to variations in the electrical signal being measured. However, these variations may be fast or slow, and thresholding alone cannot account for both efficiently. Efficiency here means acquiring the signal with the lowest number of data points as possible, while still being able to reconstruct the original signal within an acceptable error margin. With one threshold, however, it is very difficult to obtain both a good approximation of fast transients and a good overall compression ratio. To solve this, the algorithm samples at a very slow fixed rate, capable of accounting for the slow variations, while running an asynchronous sampling based on a threshold tuned for the fast transients alone.

In the case of the DVS, the threshold value depends on illuminance range and desired sensitivity. In the case of plant signals it will depend on the range of the signal, noise, and desired sensitivity. The slowly varying nature of the DC value of the signals poses a problem for the use of a fixed threshold. A relative thresholding approach was used to address this issue. Each acquired data point n is subtracted to the exponential moving average of all data points up to $n-1$ and if the modulus of this value is greater than a constant (proportional to noise), then said value is saved along with its corresponding time-stamp. We finally arrive at a dynamic acquisition algorithm, with both static and asynchronous acquisition. Tuning the threshold value and the exponential moving average coefficients allows for an optimum compromise between quality approximation/reconstruction and compressibility. Algorithm 1 shows pseudo code for this implementation.

To have maximum sensitivity the exponential moving average filter has $\alpha = 0.1$, behaving very nearly to a low-pass filter. There are some subtleties to the algorithm, for example, data-points corresponding to an event do not count for the moving average, consequently increasing the sensitivity and accounting for extremities/far away peaks. Also, note that the algorithm saves the filtered data instead of the actual data points, unless values are above the threshold *and* corresponding time-stamps under 100. The aim is to save the filtered signal except for the case of fast transients, further smoothing the output, so not only is the signal compressed,it is also filtered to some extent. All these details were played with in Matlab, while testing the algorithm.

Algorithm 1 DynAQ

init:
  update = 1 $ExpAvg = adcData_{init}$ $\alpha = 0.1$

loop:
1: sub = adcData-ExpAvg
2: **if** $||sub|| > threshold$:
    if $timeStamps > 100$
      save ExpAvg
      save timeStamps
    else
      save adcData
      save timeStamps
    timeStamps = 0
    update = 0
  else if $timeStamps > 1000$:
    save ExpAvg
    save timeStamps
    timeStamps = 0
3: **if** update:
    $ExpAvg = (1-\alpha) * ExpAvg + \alpha * adcData$
4: update = 1
5: timeStamps = timeStamps+1

Absolute time-stamps are the sum of all previous relative time-stamps up to that point. To plot data, simply plot the received values against absolute time-stamps. To evaluate the performance of the algorithm two characteristics were considered: compression ratio and correlation in relation to the original signal. Correlation was calculated by taking the compressed signals and the absolute time-stamps and stuffing all the values in between time-stamps with the last value and then calculating the correlation to the original signal. The algorithm behaves differently with different kinds of signals, so when representing the correlation results, signals were divided in three groups (fig: 3.6). Those represented by red circles are signals with negligible slow variations, and with a small percentage of fast transients or none at all. Those represented by blue circles are signals where slow variations predominate. Those represented by green circles are smaller signals consisting solely of fast transients. In this way we can observe what to expect from the algorithm for different kind of signal variation situations.

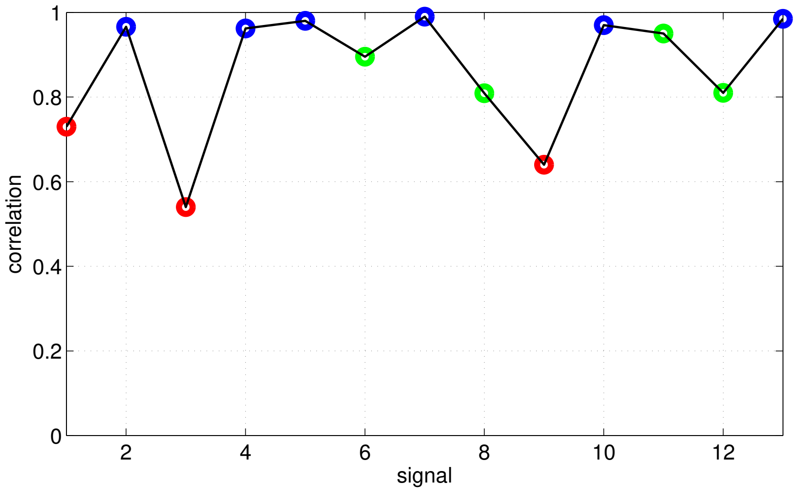


Figure 3.6: Correlation of varying compressed signals with the DynAQ algorithm in relation to the corresponding original signal.

Normally the data compression ratio (CR) is represented as $CR = \frac{UncompressedSize}{CompressedSize}$. Here since we are interested in transmitted data, we have to take into consideration the number of time-stamps also, and so: $CR = \frac{UncompressedSize}{CompressedSize+TimeStampsSize}$. Furthermore if we consider the size in bits, since data points are 12bits sized, and relative time-stamps are 10 bits sized, we get: $CR_{bits} = \frac{UncompressedSize*12}{CompressedSize*12+TimeStampsSize*10} = \frac{UncompressedSize}{CompressedSize+0.833*TimeStampsSize}$.

Table 3.1: Compression Ratio and Correlation to the original signal of the various test signals compressed with DynAQ algorithm.

Signal	CR_{bits}	Corr	Signal	CR_{bits}	Corr	Signal	CR_{bits}	Corr
2	40.21	0.985	6	5.96	0.895	1	105.71	0.738
4	53.97	0.995	8	27.55	0.809	3	55.95	0.544
5	42.24	0.966	11	2.72	0.951	9	86.81	0.640
7	48.91	0.966	12	8.09	0.815	-	-	-
10	45.02	0.962	-	-	-	-	-	-
13	45.02	0.985	-	-	-	-	-	-

Inspecting table **??** it can be seen that compression is maximum for red type of signals, where almost nothing happens. Correlation values, however, are the lowest. Correlation is computed in respect to the range of signals, because variation range is small, noise has a more negative effect on correlation. Compression is at a minimum for the green signals, the details of fast transients, where we are interested in more data points so as to correctly describe the signal. In blue signals correlation is at a peak, since slow varying signals are easy to follow and signal variation range is high. Compression is also very high as desired. Visual representation of original signals and compressed signals is presented in annex B for consultation. The algorithm functions as desired varying the compromise between compression and accuracy as need be.

The algorithm was later implemented in a Tiva C series micro-controller. In the micro-controller implementation, each time one of the channels saves a data point, the other 2 channels also save the corresponding data points, along with the relative time-stamp for all 3 channels. This will obviously decrease actual compression ratios. Note, however, that while in these tests time-stamps represent about 42% of the size of compressed data, in the Tiva implementation, it only stands for about 21% of the size of compressed data. This happens since for each transmission of three data points (one for each channel), only one time-stamp is transmitted.

Finally, note that in truth, acquisition is made at full rate ($6\,ksps$) by the Tiva micro-controller. It is the transmission which is compressed. However, considering the Amplifier Circuit + Tiva a sensing device, from the stand-point of the Olimex, sensed data is arriving in an already compressed form.

4

An acquisition system to study Plant Electrophysiology

4.1 The System

There are some features which a suitable experimental design should take into consideration. Set-up conditions should be well controlled so that the impact of unexpected external factors on the results is minimised or taken into account. In the case of plant signals, data must be exempt from the heterogeneous conditions in which it was gathered. Experiments must be reproducible so that other investigators may verify the results. Also, most often, results need to be replicated several times in order to be analysed mathematically. Essential to reproducibility is replicating not only the experiment but also the conditions in which it was first conduced.

When designing our acquisition system to monitor and study plant electrical signals we tried to address this issues having developed a multi-sensor set-up.

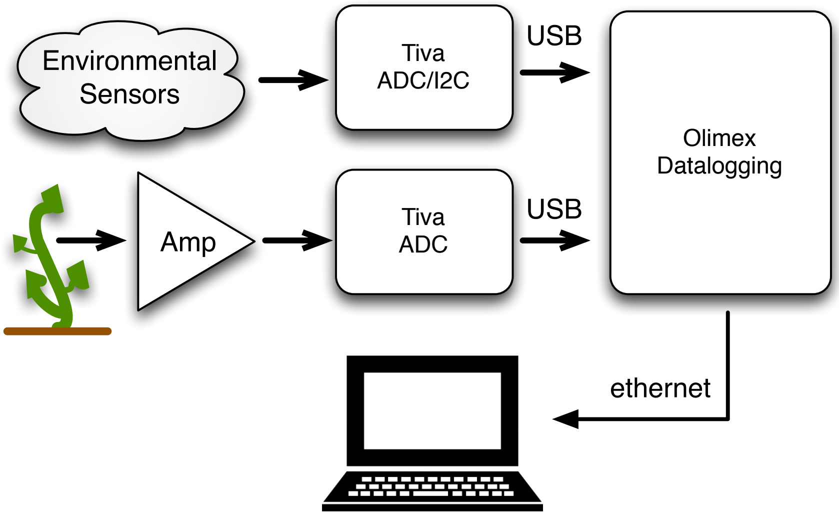


Figure 4.1: Diagram of Designed Acquisition System for Plant Electrophysiology studies

Plant signal conditioning was made through Amplifier Circuit 2 interfaced with a Tiva C series Microcontroller through a shielded cable.

The Tiva C series TM4C123GHSPM uses a 32-bit, 80MHz, ARM Cortex-M4. It has a wide range of peripherals, PWM's, eight UART's, four SPI's, four I2C's, USB, 27 timers and a 1M samples per second ADC with 12 shared input channels of 12-bit precision allowing for both single-ended and differential-input configurations. ADC also features four sample conversion sequencers: Sequencer 0 with 8 entries, sequencers 1 and 2 with 4 entries each and sequencer 3 with 1 entry, all with corresponding FIFO depth. Ground and power for the analog circuitry are separated from the digital power and ground. The micro-controller is interfaced with an A20-OLinuXino-Lime from Olimex through UART connected to the USB port of the Olimex. The Olimex is powered through a medical grade power source plugged to the electrical grid. It is also prepared to run with a backup battery in the case of power grid failures. In regards to long term robustness, the system ran for six consecutive days without any software or hardware problems.

Once again, acupuncture needle electrodes were connected to amplifier circuit 2 which was connected to the Tiva's three ADC channels. The channels connected to sequencers 0, 1 and 2 respectively, using 4 slots in each sequencer's FIFO. Each channel was over-sampled at 24kHz with an interrupt triggered by Timer0 being issued at a rate of 6kHz, after 4 samples of each channel had been acquired. The 4 samples of each channel are then averaged, smoothing the signal, resulting in an actual sampling rate of $6k\,sps$. In a 4-point averaging filter, most attenuation is above $f_s/4$, 6kHz in this case . Data is then buffered to a circular buffer which is read by a routine which implements the algorithm described in section 3.3. Time-stamps are relative, representing the number of samples since last trigger. Since there is a fixed rate, these never go above 1001, so 10 bits suffice to transmit the time-stamps.

A FTDI working at 1Mbps was used for transmission of data points. A simple UART transmission protocol was devised to avoid corrupted data, which could be misinterpreted as plant activity. First a start byte is sent, then 12 bits for each ADC channel, followed by the relative time-stamp and a checksum of 10 bits, cramming everything in 8 bytes (fig. 4.2).

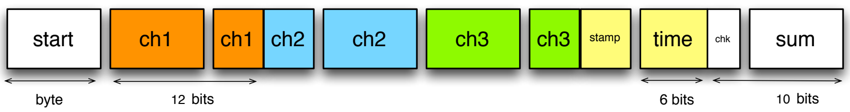


Figure 4.2: Simple UART communication protocol.

On the Olimex end side, a python script using a typical producer/consumer double thread was used to receive data. Producer thread reads the serial port continuously and writes all bytes to a queue. Consumer thread pulls the bytes from the queue and processes them, checking for the start byte, rebuilding data and finally running the checksum. If no errors are found, data is written to a file. Queue python module implements multi-producer, multi-consumer synchronised queues, useful for exchanging data safely between multiple threads.

Environmental Sensors were also interfaced with another Tiva through both ADC and I^2C. Every one minute the Tiva would wake from hibernation module and acquire data from sensors.

Ambient Light

The BHT1750 is a digital Ambient Light Sensor integral circuit for I^2C interface. Spectral response is close to that of the human eye, and also close to the PAR region. It also shows little dependency on light source (Sun light, LEDs or different kind of lamps). Data comes in lux ($lumen/m^2$) with a wide range and high resolution, from 1 to 65535 lux. For small measurements accuracy is +/- 20%. Since one lumen (or lux) corresponds to different values of irradiance depending on wavelength, to convert lux to irradiance, the light source must be taken into consideration. In this case, the light source is the Sun light itself. Knowing the Sun's spectrum and it's function of luminosity, one can calculate the average irradiance corresponding to one lux of sunlight. Langhans (1997) gives an approximated conversion ratio for sunlight, $lux * 4.02 = irradiance$, according to the Sun's spectral characteristics in the PAR region [46].

Air temperature and Humidity

The SHT21 is the standard version of the SHT2x series from Sensirion [1] . This sensor containing a capacitive type humidity sensor and a band gap temperature sensor has become an industry standard and popular in a wide array of applications on various platforms. Communication with the sensor is made by I^2C. Relative humidity operating range is 0 - 100% RH (+/-2% accuracy, 12 bit resolution) and temperature operating range is -40 to +125 Celsius (+/- 0.25 accuracy at 25 Celsius, 14 bit resolution).

Soil Moisture and Temperature

The moisture sensor simply consists of two conducting pads that act as a variable resistor. The more water is in the soil, the more conductivity between the pads and the smaller the resistance, resulting in a higher signal. All it is needed is to connect the GND and Vcc, and the output to an ADC. A known problem of these low cost sensors is their short lifespan due to depolarisation resulting from the moist environment in which they are used. The polarisation of the conducting pads distorts the signals read by the amplifier circuit. A second vase of another lettuce had to be used to measure the soil humidity. Since all plants were always watered with the same water and the same time, the measurement should be reasonably accurate.

The epoxy coated thermistor from Adafruit has 1% precision and is prepared for damped environments. A $10k\Omega$ resistor in series with the thermistor is used for a voltage divider in order to derive the value of the resistance of the thermistor for a given temperature.

[1] `http://www.sensirion.com`

4.2 Testing the System - Lettuce response to acid

As discussed before different ion channels play a role in the electrochemical signalling in plants, suggesting that nutritional levels of plants may have an impact on the electrical signals registered [9]. For this experiment we will once again study the electric response of lettuce to the application of acid. This time 8 lettuce of the Batavia Amari Paris were germinated in an hydroponic set-up and later changed to soil vases. At this time, 4 of them were set apart as the control group (A), and the other received a nutrient fertilising treatment from Advanced Nutrients in the following 5 weeks of growth (group B). Treatment consisted of the application of 4ml/L of each Grow, Micro and Bloom formulas.

Figure 4.3: Example set-up for the test experiments on lettuce using the developed acquisition system.

Plants of both groups were tested for potentials in the absence of stimuli and over long periods (24 and 48 hours) covering day/night transitions and in response to acid. More specifically, the deposition of 0.1ml of sulphuric acid (pH 2.5) on the leaf of the lettuce being monitored (fig. 4.3). One electrode was pierced to the root zone, another in the base of the leaf and the other in middle vein of the leaf so as to observe the varying nature of electrical potentials across different sections of the lettuce (fig. 4.4). Potentials were higher than expected, sometimes even saturating the amplifier circuit, with the root potential going below -800mV, for which reason not all 8 lettuce were tested for acid response.

Figure 4.4: Electrode placement in lettuce, from bottom up: reference, root, base of the leaf and leaf.

Dynamic Acquisition

Knowing the number of samples at full rate acquisition and the actual number of points we can calculate the actual compression ratio of the implemented dynamic acquisition routine as: $CR = \frac{samples_{fullrate}*3}{datapoints*4}$. It is the ratio of the total number of sample points at full rate for all three channels and the actual number of acquired data points, plus the same number of timestamps. Mean CR calculated was 742 times, and CR was never below 700 times. Looking at the relative time stamps, we see that when higher frequencies were present the full rate was used since some relative timestamps were as low as 1.

Electrical Responses

By observation of the electrical potentials measured a different response to acid is apparent between lettuce of both groups (fig. 4.5). Lettuce of group A show significant increase in high frequency activity, especially in the root channel. Lettuce of group B however, did not show such glaringly visible reactions to the same stimuli. In both example cases of fig. 4.5) environmental characteristics were similar (fig. 4.6). Mean soil humidity were 64,36% (group A) and 64,60% (group B) of soil capacity [2], and mean soil temperature were 14 (group A) and 16°C (group B).

[2]For example if soil capacity is 50%, registered mean humidity would be around 32%. We considered 3.3V = 100% of soil capacity.

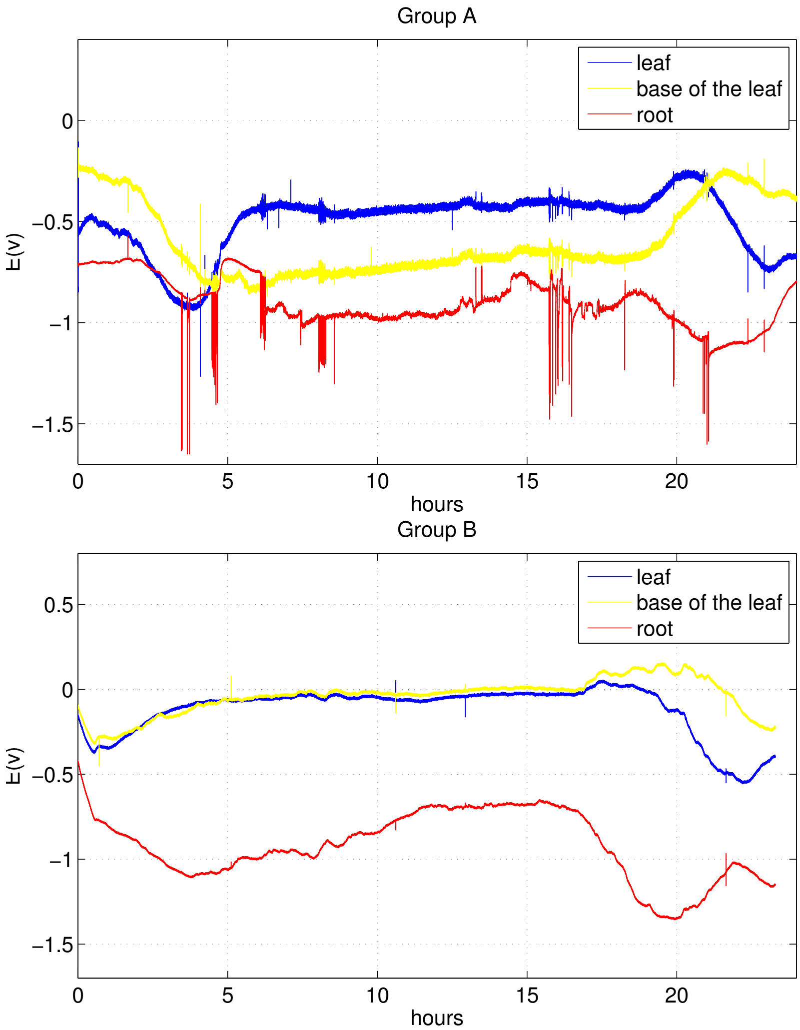


Figure 4.5: Example of electrical response to acid of normal lettuce (group A) and lettuce subject to nutrient treatment (group B). Deposition of 0.1ml of acid on leaf occurred 30 seconds in.

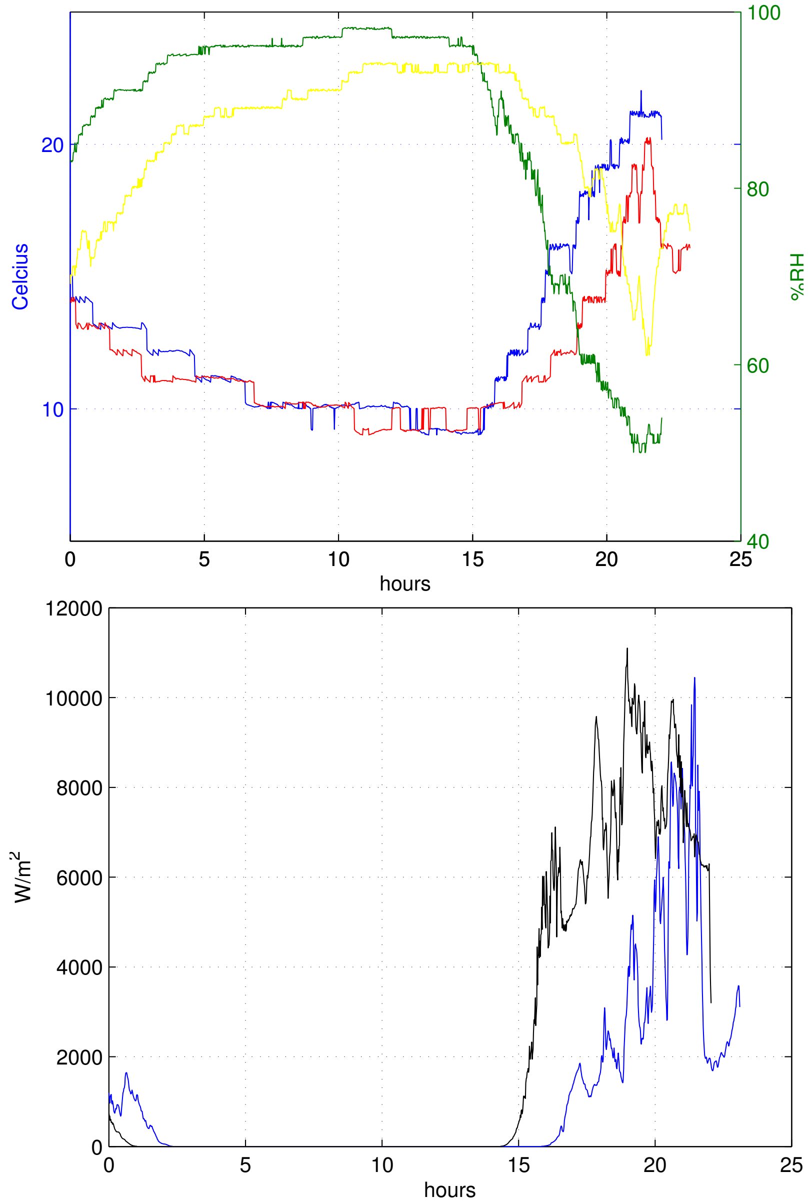


Figure 4.6: Example of environmental conditions during tests of fig. 4.5. In the first plot red (group A) and blue (group B) are temperature, green (A) and yellow (B) are relative air humidity. In the second we have irradiance values, black is group A and blue is group B lettuce.

4.3 Feature Selection using Wavelets

Due to the non-stationarity and time-varying nature of plant signals, classical spectrum analysis is not suitable to characterise these signals. Typically, time-domain analysis is employed, looking for features such as number of zero-crossings, mean and variance, amplitude histograms and so on [41]. Lately, there has been interest in time-frequency domain analysis, with the use of wavelets which has been quite promising. We propose an approach based on discrete wavelet decomposition to analyse the different signals and look for fundamental distinguishing characteristics, with regard to both dc variation and high frequency transient activity.

To quantify high frequency transient activity in the plant electrical signals we used discrete wavelet decomposition. As we have seen, the DWT divides the signal into two sub-bands: $fs - \frac{fs}{2}$, the detail coefficients , with noise and details of the signal and $\frac{fs}{2} - 0$, the approximation coefficients, with the coarse aspects, or the outline of the signal. Normally in compressing applications the approximation coefficients are used, and details are thresholded for noise reduction. In this analysis however, since we are interested in the transient activity, focus was on the detail coefficients. We performed six wavelet decompositions for each root, base of the leaf and leaf signals. First one level decomposition (6000-3000Hz band), then a two level decomposition (3000-1500Hz band) and so forth. To have a numerical indicator of the detail coefficients we computed the energy of the detailed signal for each sub-band. Table 4.1 shows the mean energy of detail signals (ED) for root,base of the leaf and leaf signals in response to acid of lettuce of group A and B. Mother wavelet used was haar. The same was made to the signals of both groups in the absence of stimuli (table 4.2).

$$ED = \sum_{i=0}^{N} C_i^2 \tag{4.1}$$

Table 4.1: Mean High Frequency Indicators of root, base of the leaf and leaf channels in lettuce of groups A and B in response to acid.

	Group A			Group B		
frequency (Hz)	root ED	baseleaf ED	leaf ED	root ED	baseleaf ED	leaf ED
6000-3000	37.54	6.50	6.93	0.38	1.29	1.32
3000-1500	25.39	3.90	4.33	0.20	0.62	0.73
1500-750	23.74	2.83	2.99	0.10	0.35	0.50
750 - 375	24.05	3.38	2.91	0.07	0.23	0.26
375-187.5	28.90	7.62	6.37	0.13	0.30	0.35
187.5-93.75	43.76	14.56	11.25	0.39	0.82	0.59

Table 4.2: Mean High Frequency Indicators of root, base of the leaf and leaf channels in lettuce of groups A and B in the absence of stimuli.

	Group A			Group B		
frequency (Hz)	root ED	baseleaf ED	leaf ED	root ED	baseleaf ED	leaf ED
6000-3000	7.70	3.85	4.13	3.12	8.33	9.04
3000-1500	8.00	2.12	2.21	1.55	4.37	4.44
1500-750	7.60	1.19	1.10	0.78	2.02	2.39
750 - 375	4.59	0.95	0.81	0.40	1.11	1.23
375-187.5	3.95	0.87	0.71	0.25	0.64	0.69
187.5-93.75	2.56	1.25	0.85	0.21	0.46	0.47

Looking at the irradiance data the signals can be divided into day and night time, considering night time the period when irradiance is 0. Using a 6 level DWT with haar mother wavelet, the signals are filtered of the high frequency components and a coarse representation of the signal in the low-pass band of 93.75 - 0 Hz is obtained. This was made to night time an day time sections of each channel's signal, and their variance was computed. This allowed looking into the DC variance of all channels during night and day time (table 4.3).

Table 4.3: Mean variance of root, base of the leaf and leaf DC components during both night and day time.

channels	Variance	
	Day	Night
root	3.70	1.06
baseleaf	4.73	0.39
leaf	2.01	0.25

To quantify correlation between the signals along the lettuce we used the same DWT to filter the higher frequencies and leave only the DC characteristic of the, now complete, signals. Correlation is calculated between the approximation coefficients in the low-pass band of 93.75 - 0 Hz of all three channels. Table 4.4 shows the mean correlations of group A and group B channels in the absence of stimuli and in response to acid.

Electric potentials in the different channels

Over long periods of time the DC value of signals was not constant, often changing very slowly, only perceptible in long duration readings. The root signal was generally below the leaf signals, especially on plants subject to the nutrient treatment. As such, it is possible to distinguish between root potentials and leaf potentials. Rarely the root signal crossed the leaf signals, and only in lettuce not subject to nutrient treatment. In the case of group A (no treatment), the root signal showed consistently an higher incidence

Table 4.4: Mean correlation of root, base of the leaf and leaf channels in lettuce of groups A and B in response to acid and in the absence of stimuli.

	Group A		Group B	
channels	no stimuli	acid	no stimuli	acid
leaf-baseleaf	0.50	0.28	0.75	0.72
leaf-root	0.24	0.48	0.40	0.61
baseleaf-root	0.13	0.27	0.51	0.53

of higher frequencies than the leaf channels (tables 4.1 and 4.2). In both groups the DC value of signals was found to vary more during day time than night time (table 4.3). In the case of group B lettuce (subject to treatment) the two leaf channels consistently showed higher correlation coefficients between each other than in relation to the root channel. Also group B lettuce showed overall significantly higher correlations between the signals from different channels (table 4.4).

Response to acid

There were significant differences in the response to acid by lettuce from the control group (group A) and those subject to nutrient treatment (group B). Lettuce of group A showed very significant increase in high frequency transients after deposition of acid, especially in the root potential. In this case most predominant transient activity was seen in sub-bands 6000-3000Hz and 187.5-83.75Hz across all three channels (table 4.1). In the absence of stimuli transient activity mainly manifested istelf in the 6000-3000Hz sub-band for both groups (table: 4.2).

5

Discussion

5.1 Discussion

Plants are in constant interaction with the environment and their physiological responses and interaction are as complex as expected from bio-systems. Because of this, up until now, most experiments in electrophysiology have focused on specific stimuli, however trying to isolate the response of a living organism to solely one stimuli is tremendously difficult to say the least. In this respect we believe environmental data to be adamant to cross-correlate with plant electrical signals.

Physiology processes may have long periods (24 hours for example) so long duration recordings are a necessity, and in this regard data management and processing often becomes an issue. Especially when a reasonable slice of the data is redundant,due to the sparsity of plant electrical signals. Decreasing sampling rate could be an answer, however, high incidence of transients in the 6000-3000Hz sub-band justify the need for higher sampling rates. Taking inspiration from the concepts of compressive sensing, we devised a simple, yet proved efficient, asynchronous acquisition algorithm, in accordance to signal variation. In terms of compression the implementation of the algorithm was higher than previously tested, on average a CR of 742 times was obtained. These can be owed to the lower noise of signals in the acquisitions with Tiva in comparison with the earlier acquisitions with the USB-6009 and the choice of threshold. Also, in this implementation, the weight of time-stamps transmission was only 21% (as it was transmitted along with 3 channels), opposed to 42% in the Matlab tests, transmitted along with only 1 channel.

Straying away from the high-grade-high-cost acquisition equipment commercially available we were able to develop and build a competent custom acquisition system. It presents fairly low noise in a controlled situation, dynamic acquisition with low latency without loosing the finer details of the electrical potentials, and environmental monitoring. Very easily with a wifi module it can be accessed through the internet instead of ethernet, or send the data directly to an online database. There are however still some improvements to be made. Signals were sometimes found to have larger amplitudes than expected [6] so it is important to power the amplifier circuit with at least 5V instead of 3.3V. Also, using multiple circuits in various plants at the same time could surely be important in many plant electrophysiology experiments. As for the signal conditioning, while in this case the purpose was to measure how potentials varied along the plant, in other cases, a differential approach might be appropriate. For example specific electrophysiological sensors might target the potential difference in a specific plant zone. In this case, a dual-supply line and connecting the reference to the plant could be more suitable.

In the simple experiment made to test how the system faired some observations were possible with the help of signal processing. Although no linear relationship between electrode positioning along the plants and electrical potentials was found, it is clear that different sections of the plant, with different physiological functions, behaved differently. In the case of nutrient treated lettuce, potentials in the root zone were distinguishable from potentials in the leaf, having almost always a lower value. Additionally, the leaf channels appeared to be fairly correlated. Lettuce not subject to treatment did not show such clear patterns. It is possible that higher nutrient levels result in healthier cation channels [9], hence the differentiation between nutrient and control lettuce groups. Also, in both groups, DC signal variation was higher during the day then during the night. Taking in consideration that plants have circadian rhythm [16], it is possible these variations may be related to mainly diurnal physiological activities. In response to acid, non-treated lettuce showed significant increase of high frequency transients (particularly in 6000-3000Hz and 183.5-93.75Hz sub-bands), especially predominant in the root signal. Nutrient treated lettuce took longer to visibly show sings of damage from acid (personal observation), which might be related to the lack of fast transient response from this group. Note however, that the tests were too few to establish conclusions about lettuce electrical signal properties. This study's aim was to show the feature extraction possibilities with wavelet analysis and how it can be used not only to separate/classify but also help to understand the underlying mechanisms behind electrical signalling in plants.

Wavelets intuitively lend themselves to address plant signals, succeeding where classical frequency analysis fails [41]: scrutinising variations along the spectrum of these non-stationary, time-varying signals. The ability of the discrete wavelet transform to divide a signal into a coarse and a detailed representation along varying scales is a feature most appropriate for plant signals that present sparse high frequency transients and very slow changing dc values. We believe the wavelet transform will play a big role in feature extraction of plant signals in future developments. With advances in Big Data processing and with large enough data sets plant electrophysiology secrets have the potential to be unlocked in the near future.

6

Conclusions and Future Work

6.1 Conclusions

A summary of the conclusions discussed in the previous chapters is presented:

• A custom-made acquisition system to study plant electrophysiology was developed and tested. It includes an amplifier circuit and dynamic sampling rate ADC (up to $6\,ksps$) and various environmental sensors: air temperature and humidity, soil temperature, soil humidity and irradiance.

• The dynamic acquisition algorithm developed functioned properly and with higher compressive ratio than predicted, an average compression of 742 times.

• Signal analysis based on Discrete Wavelet Decomposition was successfully used in the extraction of various characteristic features of plant signals: identifying sub-bands of predominant fast transient activity, both in the presence and absence of stimuli, signal correlations between different channels and dc variation of signals.

• Some observations in regards to the lettuce response to acid experiment:

◦ Lettuce subject to nutrient treatment had little apparent electric response to acid deposition. Also, root potential was practically always below the leaf channels. Leaf channels appeared to be fairly correlated.

◦ Lettuce non-subject to nutrient treatment had a significant increase in fast transient activity, especially in the 6000-3000Hz and 187.5-93.75Hz sub-bands, particularly in the root channel.

◦ In both groups during day time signals showed more DC variation than during night time.

◦ Observed potentials were higher than expected, even sometimes saturating the amplifier circuit with root channel potentials below -800mV.

6.2 Future Work

Before anything else, there are somethings in the acquisition system that should be improved. More important is the change from 3.3V to at least 5V source of the signal conditioning circuit and ADC sections, to give more swing room to plant signals. Secondly, adding the possibility to read from at least two, possibly three, plants at the same time. In terms of environmental sensors there are some promising sensors that would be interesting to co-relate with plant signals, namely IR sensors and hyper-spectral imaging. In both cases many recent developments have been made and it would be of interest to see how they fare alongside plant potentials. Also, connecting the system directly to an online database would be more than a commodity, but an important step to start creating solid electrophysiology data-sets to work on.

Repeating the lettuce experience with control and fertilised groups with a larger number of subject lettuce could support the interesting observations made in our small test and provide a deeper understanding of plant potentials. More broadly, experiments with larger numbers of plants and carefully thought procedures, are crucial if we are to start applying feature extraction techniques and classification to further our knowledge of electrical signalling in plants and start developing practical sensors based on plant electrophysiology.

Bibliography

[1] A. G. Volkov, "Electrophysiology and phototropism," in *Communication in Plants*. Springer, 2006, pp. 351–367.

[2] O. S. Ksenzhek and A. G. Volkov, *Plant energetics, Chpt Introduction*. Academic Press, 1998.

[3] N. Bertholon, *De L'Électricité Des Végétaux (etc.)*. Bernuset, 1783.

[4] J. B. Sanderson, "Note on the electrical phenomena which accompany irritation of the leaf of dionaea muscipula," *Proceedings of the Royal Society of London*, vol. 21, no. 139-147, pp. 495–496, 1872.

[5] C. Darwin, "Insectivorous plants. 1875," *London Murray*, 2011.

[6] E. Davies, "Electrical signals in plants: facts and hypotheses," in *Plant electrophysiology*. Springer, 2006, pp. 407–422.

[7] E. Davies and A. Schuster, "Intercellular communication in plants: evidence for a rapidly generated, bidirectionally transmitted wound signal," *Proceedings of the National Academy of Sciences*, vol. 78, no. 4, pp. 2422–2426, 1981.

[8] J. Fromm, "Long-distance electrical signaling and physiological functions in higher plants," in *Plant Electrophysiology*. Springer, 2006, pp. 269–285.

[9] V. Demidchik, "Characterisation of root plasma membrane ca^{2+} - permeable cation channels: Techniques and basic concepts," in *Plant Electrophysiology: Signalling and Responses*. Springer, 2012, pp. 339–369.

[10] R. Stahlberg, "Historical introduction to plant electrophysiology," in *Plant Electrophysiology*. Springer, 2006, pp. 3–14.

[11] F. de Oliveira, *Fundamentos de farmacobotânica*. Atheneu, 2000.

[12] S. Shabala, "Non-invasive microelectrode ion flux measurements in plant stress physiology," in *Plant Electrophysiology*. Springer, 2006, pp. 35–71.

[13] A. G. Volkov and C. L. Brown, "Electrochemistry of plant life," in *Plant Electrophysiology*. Springer, 2006, pp. 437–459.

[14] A. G. Volkov, C. L. Vilfranc, V. A. Murphy, C. M. Mitchell, M. I. Volkova, L. O'Neal, and V. S. Markin, "Electrotonic and action potentials in the venus flytrap," *Journal of plant physiology*, vol. 170, no. 9, pp. 838–846, 2013.

[15] S. Lautner, T. E. E. Grams, R. Matyssek, and J. Fromm, "Characteristics of electrical signals in poplar and responses in photosynthesis," *Plant Physiology*, vol. 138, no. 4, pp. 2200–2209, 2005.

[16] P. W. Barlow, "Moon and cosmos: plant growth and plant bioelectricity," in *Plant Electrophysiology: Signalling and Responses*. Springer, 2012, pp. 249–280.

[17] M. R. Sussman, "Shaking arabidopsis thaliana," *Science*, vol. 256, no. 5057, p. 619, 1992.

[18] E. Davies, "New functions for electrical signals in plants," *New Phytologist*, vol. 161, no. 3, pp. 607–610, 2004.

[19] H. Dziubińska, "Ways of signal transmission and physiological role of electrical potentials in plants," *Acta societatis botanicorum Poloniae*, vol. 72, no. 4, pp. 309–318, 2011.

[20] X. Yan, Z. Wang, L. Huang, C. Wang, R. Hou, Z. Xu, and X. Qiao, "Research progress on electrical signals in higher plants," *Progress in Natural Science*, vol. 19, no. 5, pp. 531–541, 2009.

[21] "Report of the electrical activity of single plants grown in the ad-hoc setup," *Plants Employed As SEnsor Devices*, 2011.

[22] S. Mancuso, "Hydraulic and electrical transmission of wound-induced signals in vitis vinifera," *Functional Plant Biology*, vol. 26, no. 1, pp. 55–61, 1999.

[23] T. Shvetsova, J. Mwesigwa, A. Labady, S. Kelly, D. Thomas, K. Lewis, and A. G. Volkov, "Soybean electrophysiology: effects of acid rain," *Plant Science*, vol. 162, no. 5, pp. 723–731, 2002.

[24] A. G. Volkov and J. Mwesigwa, "Electrochemistry of soybean: effects of uncouplers, pollutants, and pesticides," *Journal of Electroanalytical Chemistry*, vol. 496, no. 1, pp. 153–157, 2001.

[25] B. Stanković and E. Davies, "Both action potentials and variation potentials induce proteinase inhibitor gene expression in tomato," *Febs Letters*, vol. 390, no. 3, pp. 275–279, 1996.

[26] B. Stanković, D. L. Witters, T. Zawadzki, and E. Davies, "Action potentials and variation potentials in sunflower: an analysis of their relationships and distinguishing characteristics," *Physiologia Plantarum*, vol. 103, no. 1, pp. 51–58, 1998.

[27] B. Stankovic, T. Zawadzki, and E. Davies, "Characterization of the variation potential in sunflower," *Plant physiology*, vol. 115, no. 3, pp. 1083–1088, 1997.

[28] L. A. Gurovich, *Electrophysiology of woody plants*. INTECH Open Access Publisher, 2012.

[29] T. Zawadzki, H. Dziubińska, and E. Davies, "Characteristics of action potentials generated spontaneously in helianthus annuus," *Physiologia Plantarum*, vol. 93, no. 2, pp. 291–297, 1995.

[30] Y. Mizuguchi, Y. Watanabe, H. Matsuzaki, Y. Ikezawa, and T. Takamura, "Growth acceleration of bean sprouts by the application of electrochemical voltage in a culturing bath (e)," *Denki Kagaku Oyoki Kogyo Butsuri Kagaku*, vol. 62, no. 11, pp. 1083–1085, 1994.

[31] A. Goldsworthy, "Effects of electrical and electromagnetic fields on plants and related topics," in *Plant Electrophysiology*. Springer, 2006, pp. 247–267.

[32] K. Trebacz, H. Dziubinska, and E. Krol, "Electrical signals in long-distance communication in plants," in *Communication in Plants*. Springer, 2006, pp. 277–290.

[33] X. Li, L. Gao, W. Li, and W. Yu, "Exploration on trans-root potential of living trees?" *J. Inform. Comput. Sci*, vol. 10, pp. 6131–6138, 2013.

[34] Z. Hao, G. Wang, W. Li, J. Zhang, and J. Kan, "Effects of electrode material on the voltage of a tree-based energy generator," *PloS one*, vol. 10, no. 8, p. e0136639, 2015.

[35] A. G. Volkov and V. S. Markin, "Phytosensors and phytoactuators," in *Plant Electrophysiology*. Springer, 2012, pp. 173–206.

[36] K. Kalovrektis, G. Th, J. Antonopoulos, A. Gotsinas, and N. A. Shammas, "Development of transducer unit to transmit electrical action potential of plants to a data acquisition system," *American Journal of Bioinformatics Research*, vol. 3, no. 2, pp. 21–24, 2013.

[37] F. Bretschneider and J. R. De Weille, *Introduction to electrophysiological methods and instrumentation*. Academic Press, 2006.

[38] Q. Zhao and L. Zhang, "Ecg feature extraction and classification using wavelet transform and support vector machines," in *Neural Networks and Brain, 2005. ICNN&B'05. International Conference on*, vol. 2. IEEE, 2005, pp. 1089–1092.

[39] A. Quotb, Y. Bornat, and S. Renaud, "Wavelet transform for real-time detection of action potentials in neural signals," *Frontiers in neuroengineering*, vol. 4, 2011.

[40] L. Wang and L. LI HX, "Studies on the plant electric wave signal by the wavelet analysis," in *Journal of Physics: Conference Series, 4th International Symposium on Instrumentation Science and Technology (ISIST'06). Bristol & Philadelphia: IOP Publishing*, 2006, pp. 520–525.

[41] L. Jingxia and D. Weimin, "Study and evaluation of plant electrical signal processing method," in *Image and Signal Processing (CISP), 2011 4th International Congress on*, vol. 5. IEEE, 2011, pp. 2788–2791.

[42] Z. Xiaohui, Z. Haihe *et al.*, "Feature extraction of plant electrical signals based on wavelet transform," *International Journal of Control and Automation*, vol. 8, no. 7, pp. 37–46, 2015.

[43] "Documentation and tool development for local classifier (addendum)," *Plants Employed As SEnsor Devices*, 2011.

[44] C. M. Hogan, "Abiotic factor," *Retrieved from http://www.eoearth.org/view/article/149786*, 2011.

[45] J. Muoghalu, "Priority parameters: abiotic and biotic components," *environmental monitoring (edited by Inyang, H. and Daniels, J.). Encyclopaedia of Life Support Systems (EOLSS)*, pp. 1–8, 2003.

[46] R. W. Langhans, T. Tibbitts *et al.*, "Plant growth chamber handbook," 1997.

[47] L. Kumar, K. Schmidt, S. Dury, and A. Skidmore, "Imaging spectrometry and vegetation science," in *Imaging spectrometry*. Springer, 2001, pp. 111–155.

[48] S. Almeida de Carvalho, *Jacobaea through the eyes of spectroscopy: identifying plant interactions with the (a) biotic environment by chemical variation effects on spectral reflectance patterns*, 2013.

[49] F. Baret and G. Guyot, "Potentials and limits of vegetation indices for lai and apar assessment," *Remote sensing of environment*, vol. 35, no. 2, pp. 161–173, 1991.

[50] T.-T. Zhang, S.-L. Zeng, Y. Gao, Z.-T. Ouyang, B. Li, C.-M. Fang, and B. Zhao, "Using hyperspectral vegetation indices as a proxy to monitor soil salinity," *Ecological Indicators*, vol. 11, no. 6, pp. 1552–1562, 2011.

[51] K. Wang, S. E. Franklin, X. Guo, and M. Cattet, "Remote sensing of ecology, biodiversity and conservation: A review from the perspective of remote sensing specialists," *Sensors*, vol. 10, no. 11, pp. 9647–9667, 2010.

[52] K. Killham, *Soil ecology*. Cambridge University Press, 1994.

[53] J. Fraden, "Handbook of modern sensors, 4th edition," 2010.

[54] A.-K. Mahlein, E.-C. Oerke, U. Steiner, and H.-W. Dehne, "Recent advances in sensing plant diseases for precision crop protection," *European Journal of Plant Pathology*, vol. 133, no. 1, pp. 197–209, 2012.

[55] M. A. Davenport, M. F. Duarte, Y. C. Eldar, and G. Kutyniok, "Introduction to compressed sensing," *Preprint*, vol. 93, no. 1, pp. 1–11, 2011.

[56] A. Censi and D. Scaramuzza, "Low-latency event-based visual odometry," in *Robotics and Automation (ICRA), 2014 IEEE International Conference on*. IEEE, 2014, pp. 703–710.

[57] I. Daubechies *et al.*, *Ten lectures on wavelets*. SIAM, 1992, vol. 61.

[58] C. Valens, "A really friendly guide to wavelets," *Retrieved From: http://perso. wanadoo. fr/polyvalens/clemens/wavelets/wavelets. html*, 1999.

[59] M. Misiti, Y. Misiti, G. Oppenheim, and J.-M. Poggi, "Matlab wavelet toolbox user\'s guide. version 3." 2004.

[60] S. G. Mallat, "A theory for multiresolution signal decomposition: the wavelet representation," *Pattern Analysis and Machine Intelligence, IEEE Transactions on*, vol. 11, no. 7, pp. 674–693, 1989.

[61] R. G. Lyons, *Understanding digital signal processing, 3rd Edition*. Pearson Education, 2010.

A

Signal Averaging and Digital Filtering

A common problem when acquiring measurements from natural phenomena is to distinguish between the true vale of the signal being measured and fluctuations resulting from the measuring set-up or any other unknown random factors. The later can be understood as disturbances, and in signal processing are known as noise. One of the most basic ways of minimising noise is through averaging.

If we consider the standard deviation a measure of uncertainty in measurements, we can see how averaging can affect said uncertainty. Considering an original signal sequence $s[n]$ with standard deviation σ it has been shown that the standard deviation, σ_{ave}, of the N-point averaged signal, $s_{ave}[k]$ can be given by:

$$\sigma_{ave} = \frac{\sigma}{\sqrt{N}}. \tag{A.1}$$

This equation tells us that N-point averaging reduces the variation by a factor of $\sqrt{N}$, hence, reducing the effective uncertainty in measurements [61]. Another way to quantify noise is to look at signal to noise ratio, which can be defines as $SNR = \frac{A}{\sigma}$, where A is the amplitude of a signal and σ the standard deviation for said amplitude. Likewise, for the averaged signal comes $SNR_{ave} = \frac{A}{\sigma_{ave}}$. Now if we define the signal-to-noise ration gain as $SNR_{gain} = \frac{SNR_{ave}}{SNR}$ it can be seen that:

$$SNR_{gain} = \frac{\sigma}{\sigma_{ave}} = \frac{\sigma}{\frac{\sigma}{\sqrt{N}}} = \sqrt{N}. \tag{A.2}$$

Signal-to-noise ratio improvement is then proportional to the square root of the number of samples being averaged. Note that these results only apply to coherent (linear) averaging [61].

Interestingly it can be seen that time-domain averaging indeed performs low-pass filtering. Actually, sucessive outputs of an N-point averager are identical to the output of an (N-1)-tap FIR filter, with coefficients equal to $\frac{1}{N}$, equation A.3. Finite impulse response (FIR) filters are so called because their impulse response settles to zero in finite time, having no internal feedback. Meaning the output results only from current and previous input samples and none of the past output samples.

$$y(n) = \sum_{i=0}^{N} b_i x(n-i) = \frac{1}{N}\sum_{i=0}^{N} x(n-i). \tag{A.3}$$

Something of interest is the frequency response of this N-point averaging filter, and we can do that studying the frequency response of the corresponding FIR filter. To do that the Discrete Fourier Transform (DFT) can be applied to the filter's coefficients to give the frequency response. Lyons in *Understanding Digital Signal Processing* does this with a 128-point DFT for some examples of N, the result is showed in figure A.1.

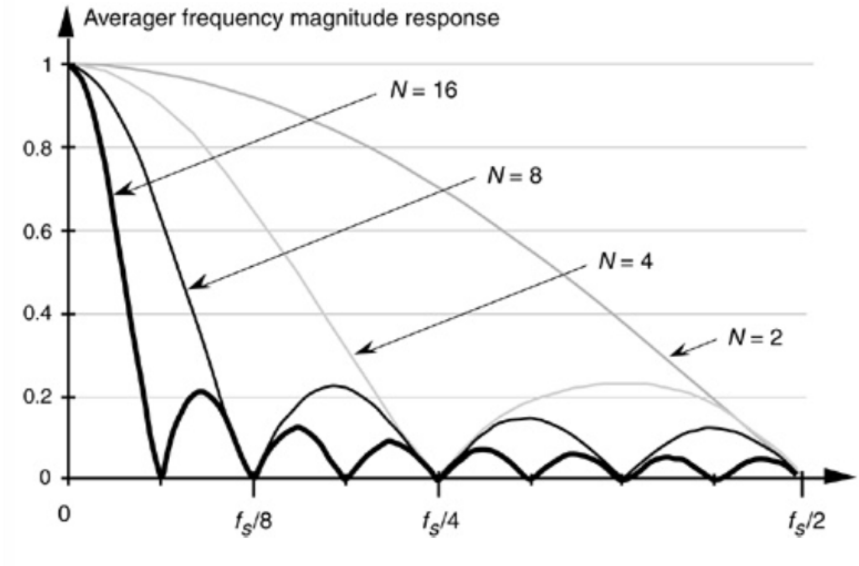


Figure A.1: Frequency response of N-point averager for various values of N. f_s indicates sampling frequency. Image taken from Lyons' (2010) Understanding Digital Signal Processing, Chapter 11 [61].

Another kind of averaging of great practical use is exponential averaging. It consists of multiplying an input sample by a constant and adding that product to the same constant's one complement multiplied by the averager's output, this is illustrated in equation A.4.

$$y(n) = \alpha x(n) + (1-\alpha)y(n-1). \tag{A.4}$$

This kind of averaging is practical because it is very light to implement, only one past value is needed ($y[n-1]$) and the current value $x[n]$. It is called exponential due to its time-domain impulse response which falls to zero exponentially over time, according to the chosen α. Varying the weighting factor α controls the amount of noise reduction. A weighting value of 1 gives no attenuation and output equals input. As α decreases attenuation increases and the output takes more time to respond to changes in the input. There is then a tradeoff between noise reduction and response time of the filter. Equation A.5 shows the SNR reduction of the exponential averager as function of α [61].

$$SNR_{exp} = \frac{outputnoisevar}{inputnoisevar} = \frac{\alpha}{2\alpha}. \tag{A.5}$$

Exponential averaging filters behave as one-tap infinite impulse response (IIR) filter. IIR filters are distinguished by their response which never settles exactly at zero, varying indefinitely, even if in practice, past a certain point this value can be neglected. Also (as is the case with the exponential averager), previous output samples are used to calculate the current output. Its governing equation differs from that of FIR filters, having two sets of coefficients: feedback ($a(k)$) and feedforward ($b(k)$), as shown in equation A.6.

$$y(n) = \frac{1}{a_0}(\sum_{i=0}^{P} b_i x(n-i) - \sum_{j=0}^{Q} a_j y(n-j)). \tag{A.6}$$

Considering the exponential averager is infact an IIR filter with $a_0 = 1$, $b_0 = \alpha$ and $a_1 = 1 - \alpha$, its frequency response can be derived from the general expression of the frequency response of an IIR filters. The resulting expression is shown in equation A.7 [61].

$$H_{exp}(\omega) = \frac{\alpha}{1-(1-\alpha)cos(\omega)+j(1-\alpha)sin(\omega)}. \tag{A.7}$$

Lyons in *Understanding Digital Signal Processing* plots the module and phase response of an exponential averaging filter for multiple values of α. The magnitude response is shown in figure A.2. It can be seen that as α decreases its response gets closer to that of a low-pass filter.

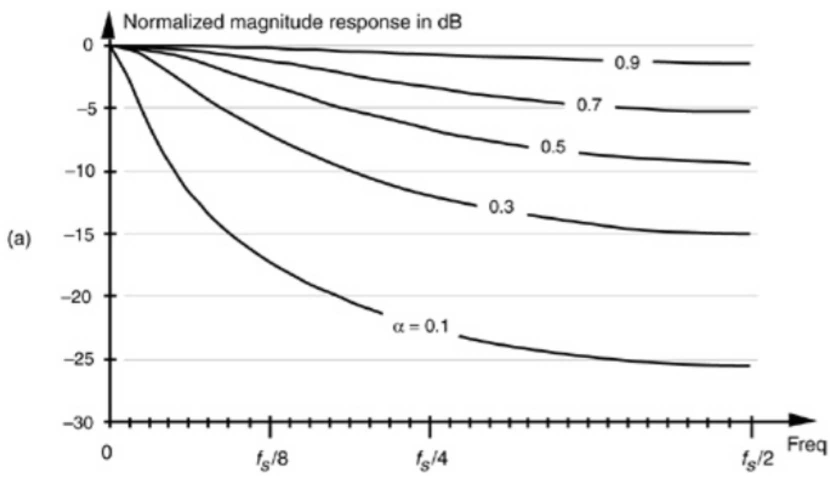


Figure A.2: Normalized magnitude response in dB of exponential averager for various values of α. Image taken from Lyons' (1997) Understanding Digital Signal Processing, Chapter 11 [61].

B

Testing DynAQ Algorithm: Example Graphs

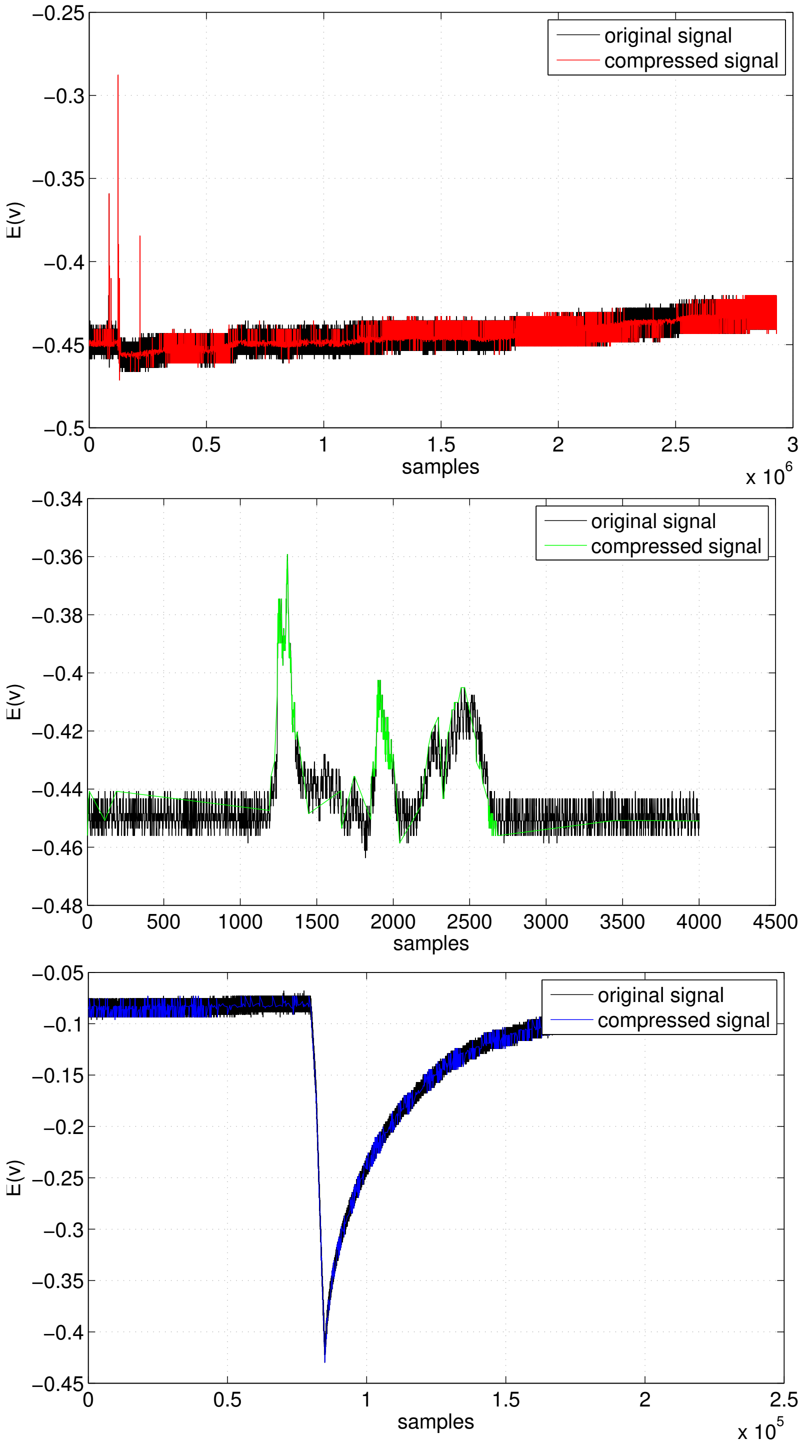


Figure B.1: Example of visual representation of compressed signals versus original signal.

C

Sensors and Schematics

Environmental Sensors

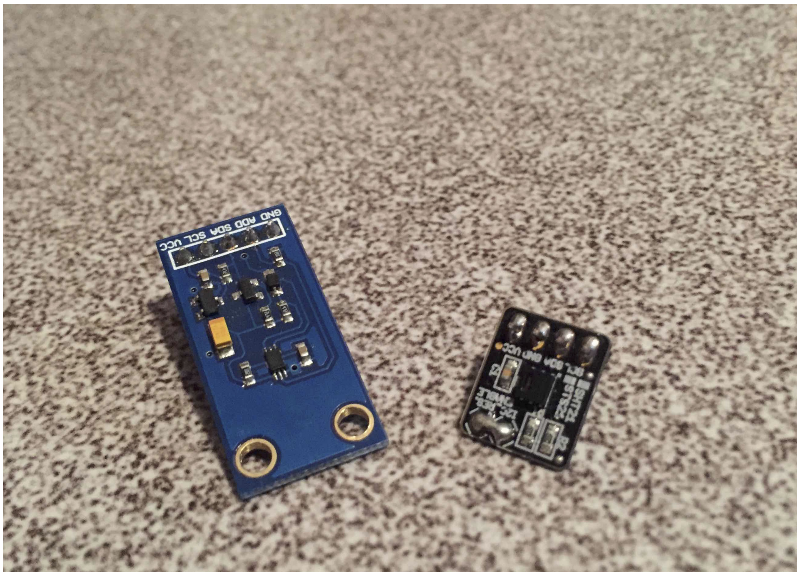


Figure C.1: BHT1750 ambient light sensor on the Left, SHT21 air temperature and humidity sensor on the right.

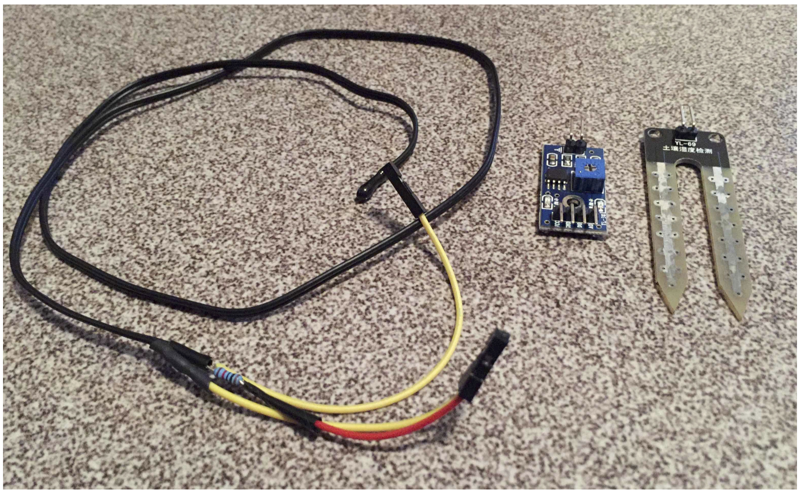


Figure C.2: Thermistor 3950-NTC on the left and moisture sensor on the right.

Sensors were interfaced with the Tiva Launchpad, shielded cables were used for the I^2C communications. The thermistor needs a series resistor, $10k\Omega$ as recommended. The voltage divider is connected to the ADC. The moisture sensors comes with a signal conditioning circuit. The probes however are prone to damage because of electrolysis and should have a protective shell (which is lacking).

Amplifier Circuit 1

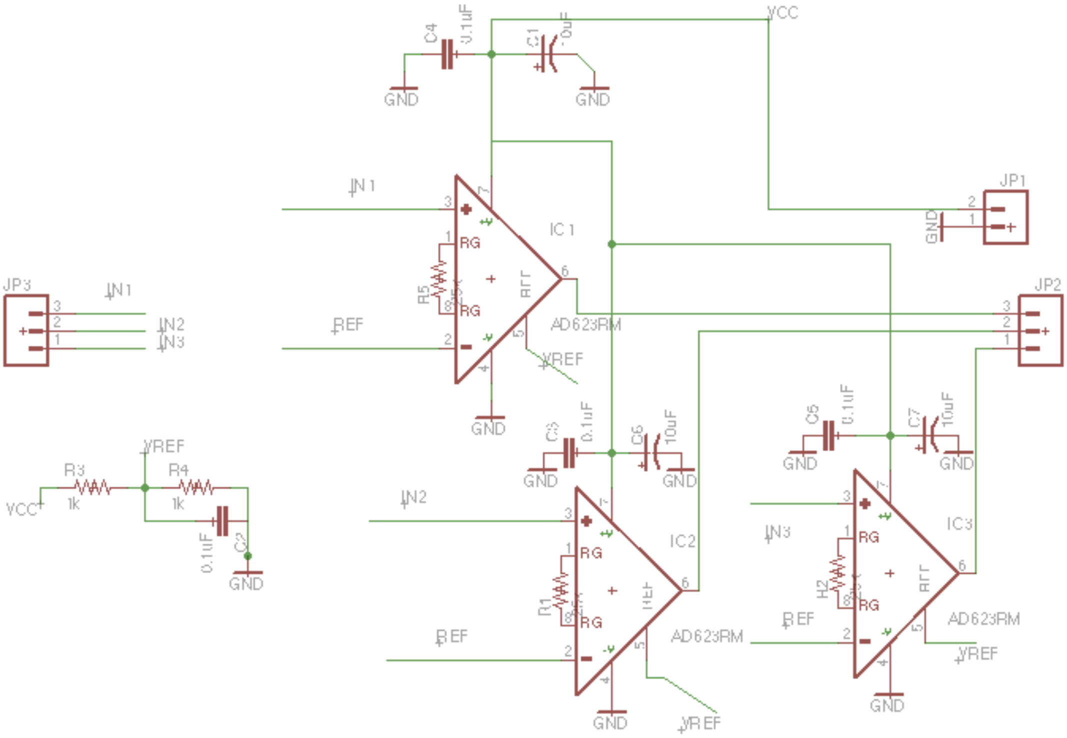


Figure C.3: Schematic of Amplifier Circuit 1.

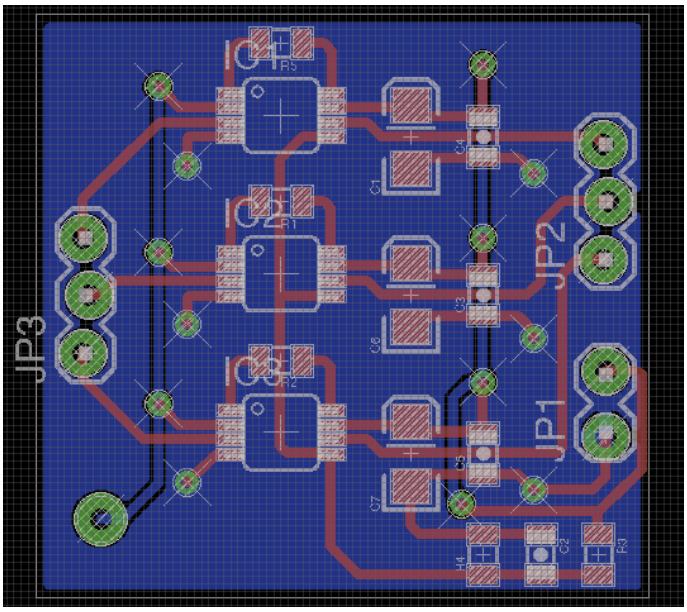


Figure C.4: PCB board of Amplifier Circuit 1.

Amplifier Circuit 2

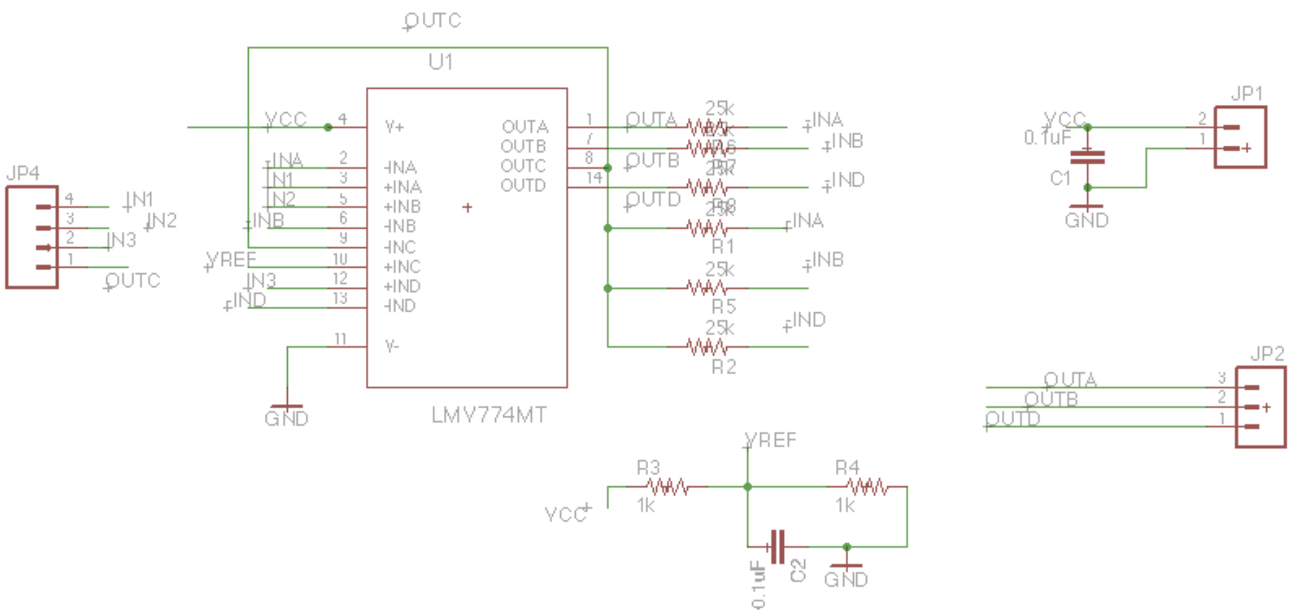


Figure C.5: Schematic of Amplifier Circuit 1.

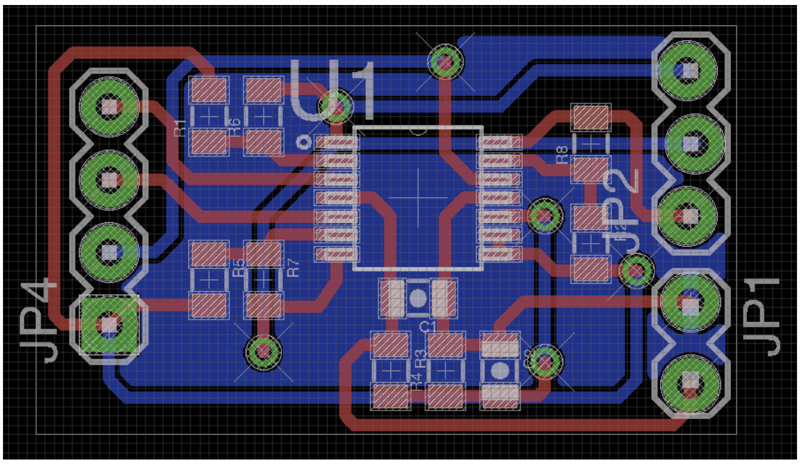


Figure C.6: PCB board of Amplifier Circuit 1.

Both circuits have header pins to connect the electrode cables. Cables were made using silver coated acupuncture needles with 0.3mm of diameter (fig. C.7).

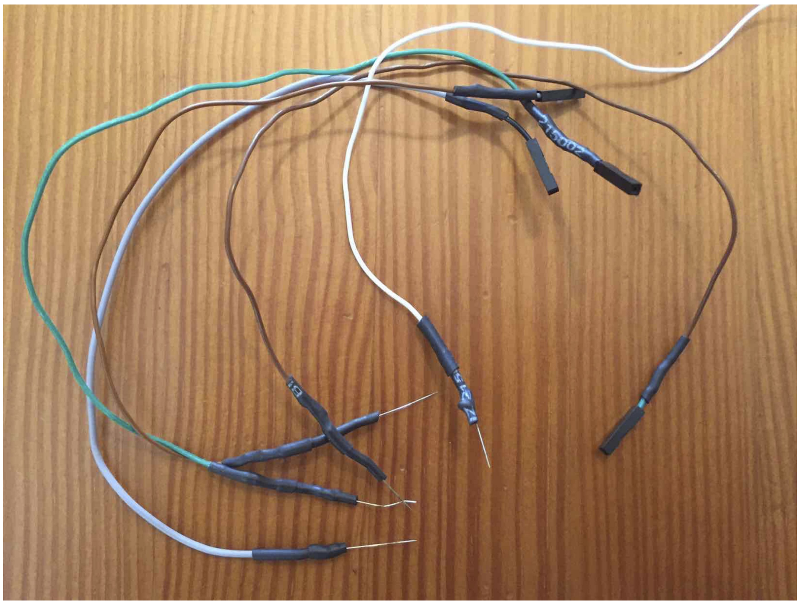


Figure C.7: Some electrode cables made out of acupuncture needles.

D

Python Scripts

```
###     DAQ123.py     ###
# Python Script to acquire continuosly from NI-USB 6009,
# 3 single-ended channels
# Uses PyDAQmx
# Ctrl+C to stop acquisition (safe exit)

from PyDAQmx import *
from PyDAQmx.DAQmxCallBack import *
from numpy import zeros
import matplotlib.pyplot as plt
import signal

#choose newline char according to OS:
nl = '\n\r' #windows
#nl = '\n' #linux

#Sampling Rate
srate = 5000.0
taskHandle = TaskHandle(0)

### clear text files ###
with open('ai1.txt','w'): pass
with open('ai2.txt','w'): pass
with open('ai3.txt','w'): pass

class MultiChannelAnalogInput():
    def __init__(self):
   self.sampbundle = int(srate)
   self.data = zeros(self.sampbundle*3)
   self.task = taskHandle
   self.counter = 0
   #time
   self.timesys = zeros(self.sampbundle)
   for i in range(0,self.sampbundle):
          self.timesys[i] = i

    def configure(self):
   #Create one task handle for all 3 channels
   DAQmxCreateTask("",byref(taskHandle))
   DAQmxCreateAIVoltageChan(taskHandle,'Dev1/ai1:3',"",DAQmx_Val_RSE,
         -5.0,5.0,DAQmx_Val_Volts,None)
   DAQmxCfgSampClkTiming(taskHandle,"",srate,DAQmx_Val_Rising,DAQmx_Val_ContSamps,
                                            self.sampbundle*3)
   DAQmxStartTask(taskHandle)
   print "Data Acquisition Task Created: 3 channels being used.."
   return 0
```

```
 def read(self):
read = int32()
DAQmxReadAnalogF64(taskHandle,self.sampbundle,10.0,DAQmx_Val_GroupByChannel,
self.data,self.sampbundle*3,byref(read),None)

#  print "Acquired {} points" .format(read.value)
#  print self.data
   return self.data

 def saveData(self):
j = 0
data1 = []
data2 = []
data3 = []
while True:
    miscdata = self.read()
    for i in range(0,self.sampbundle*3):
   if j < self.sampbundle:
       data1.append(miscdata[i])
            j = j+1
      elif j >= self.sampbundle and j < self.sampbundle*2:
       data2.append(miscdata[i])
            j = j+1
      elif j >= self.sampbundle*2 and j < self.sampbundle*3:
       data3.append(miscdata[i])
       j = j+1
   #print "ai1: {}\n\rai2: {}\n\rai3: {}" .format(data1,data2,data3)
    j = 0
    with open('ai1.txt','a') as fh:
      for i in range(0,self.sampbundle):
       fh.write("{}
           {}\n\r".format(data1[i],self.timesys[i]+self.sampbundle*self.counter))
    with open('ai2.txt','a') as fh:
      for i in range(0,self.sampbundle):
       fh.write("{}
           {}\n\r".format(data2[i],self.timesys[i]+self.sampbundle*self.counter))
    with open('ai3.txt','a') as fh:
      for i in range(0,self.sampbundle):
       fh.write("{}
           {}\n\r".format(data3[i],self.timesys[i]+self.sampbundle*self.counter))
    self.counter = self.counter+1
    data1 = []
    data2 = []
    data3 = []

 def halt(self,signal,frame):
print "Stopping Acquisition"
DAQmxStopTask(taskHandle)
DAQmxClearTask(taskHandle)
```

```
    sys.exit(0)
    return 0

if __name__ == '__main__':
     data = zeros(3)
     multipleAI = MultiChannelAnalogInput()
     multipleAI.configure()

     signal.signal(signal.SIGINT,multipleAI.halt)

     multipleAI.saveData()
```

```
###     plant_link2.py   ###
# Python Script to read serial port for ADC data from
# Tiva Micro-controller
# Receives bytes, unpacks according to protocol and
# saves data to a file if no errors are found (checksum)
# Ctrl+C to stop acquisition (safe exit)

import time
import serial
import signal
import sys
from threading import Thread
from Queue import Queue

exit = False

#port = '/dev/ttyUSB0' #linux
port = 5 #windows
baudrate = 1000000

Lmask = 15 # 0b00001111 #low mask
Hmask = 240 # 0b11110000 #high mask
mask6 = 63 # 0b00111111
mask2 = 192 # 0b11000000

error1_count = 0
error2_count = 0

queue_size = 120000

queue = Queue(queue_size)

class ProducerThread(Thread):
    def run(self):
   global exit
        global queue
   points = 0
        serial_port = serial.Serial()
   serial_port.port = port
   serial_port.baudrate = baudrate
        serial_port.open()
        if not serial_port.isOpen():
            print ' [x] Failed to open serial port %s:%d' % (port,
                baudrate)
            return
        while not exit:
            bytes_to_read = serial_port.inWaiting()
```

```
            bytes = serial_port.read(bytes_to_read)
            for b in bytes:
                queue.put(b)
      points += 1
        serial_port.close
   print 'Closing Producer Thread, %d bytes received..\n' % (points)
   return

class ConsumerThread(Thread):
    def run(self):
        global queue
   global error1_count
   global error2_count
   global exit
   points = 0
        try:
            file_name = 'plantdata' + time.strftime("%Y%m%d-%H%M%S") +
                '.csv'
            file_handler = open(file_name, 'a')
        except:
            print ' [x] Failed to open file %s' % (file_name)
            return
   while not exit:
            high_byte = queue.get()
            queue.task_done() #!!
            if ord(high_byte) == 122: #if it catches start byte
      #get chan1
                high_byte = queue.get()
               queue.task_done() #!!
      temp1 = ord(high_byte) #least sig byte of chan1
      low_byte = queue.get()
               queue.task_done() #!!
      chan1 = ((Lmask&ord(low_byte))<<8) + temp1 #chan1 assembled
      #print chan1
      #get chan2
      high_byte = queue.get()
               queue.task_done() #!!
      chan2 = (ord(high_byte) << 4)+ ((Hmask&ord(low_byte)) >> 4)
          #chan2 assembled
      #print chan2
      #get chan3
      high_byte = queue.get()
               queue.task_done() #!!
      temp3 = ord(high_byte) #least sig byte of chan3
      low_byte = queue.get()
               queue.task_done() #!!
      chan3 = ((Lmask&ord(low_byte))<<8) + temp3 #chan3 assembled
      #print chan3
      #get timestamps
```

```
        high_byte = queue.get()
        queue.task_done() #!!
        timestamps = ((mask6&ord(high_byte)) << 4) +
            ((Hmask&ord(low_byte)) >> 4) #timestamps assembled
        #print timestamps
        #get checksum
        last_byte = queue.get()
                queue.task_done() #!!
        checksum = (ord(last_byte) << 2) + ((mask2&ord(high_byte)) >> 6)
            #checksum assembled
        #print checksum
        #print '\n'
        #print
            chan1,chan2,chan3,timestamps,checksum,((chan1^chan2^chan3)>>2)
        #check for errors
        if ((chan1^chan2^chan3)>>2)^checksum == 0:
            file_handler.write('%d,%d,%d,%d\n' % (chan1, chan2, chan3,
                timestamps)) #write to file
            points += 1
             else:
            error2_count += 1
              else:
                  error1_count += 1
    file_handler.close()
    print 'Closing Consumer Thread, %d type 1 errors and %d type 2
        errors. %d data points gathered.\n' %
        (error1_count,error2_count,points)
    return

if __name__ == '__main__':
     print ' [*][PlantLink] Waiting for data from plant sensor. Press
         Ctrl+C to exit.'
     ProducerThread().start()
     ConsumerThread().start()
     try:
         while True:
              pass
     except KeyboardInterrupt:
         exit = True
    raise
```