Microfluidic Plant, Soil and Nematode Assay Chips for High-throughput Plant Phenotyping and Sustainable Agricultural Management

Liang Dong
Department of Electrical and Computer Engineering
Iowa State University
Ames, Iowa, USA
ldong@iastate.edu

***Abstract*—This invited presentation summarizes our recent research and development of microfluidic plant chips, miniature soil sensors, and microfluidic nematode assay chips, to advance plant science and sustainable agriculture.**

Keywords—Plant chip, soil sensor, electrophoresis, agriculture, plant science

I. INTRODUCTION

High-throughput microarray and next generation sequencing technologies have enabled a systems approach to obtaining and studying information about plant's genotypes [1]. But, the genotype information becomes useful insofar as it allows for efficient prediction of plant's phenotypes (e.g., traits and characteristics). Because genome sizes are so large and genes respond differently to various external and internal stimuli, systematic characterization of plant phenotypes is challenging [1]. For instance, *Arabidopsis thaliana*, a widely used small model plant, contains tens of thousands of genes. In addition, current greenhouse and growth chamber technologies are not able to easily change many different plant growth conditions (e.g., temperature, humidity, light, etc), thus affecting throughput of phenotyping seed germination and plant development and growth under biotic and abiotic stresses [2]. Therefore, rapid and inexpensive plant phenotyping with high spatial and temporal resolution is difficult to achieve.

Second, soil nutrient management is critical to achieve agriculture and environmental sustainability, and meet an increasing need in food, fiber, and natural resources. Precision agriculture has recently attracted considerable attention, aiming at realizing efficient and economic nutrient management in crop fields to reduce environmental impacts and increase agricultural productivity and growth. It is noteworthy that nitrogen, second to water, is the most limiting resource for corn production in the U.S. Corn Belt. Excessive applications of N fertilizer have caused negative effects on the environment, including loss of biodiversity, pollution of ground water, reduction of crop productivity, and climate change. Improving N use efficiency of crops has the potential to reduce these negative externalities. Precise nutrient applications in farming can be made possible only if we are able to frequently monitor nutrient availability in soil [3]. Laboratory tools for detection and quantification of soil nutrients include electrochemical sensors, spectrophotometry, ion chromatography, and ion-selective membrane based sensors [3]. However, field deployable sensors remain limited.

Third, plant-parasitic nematodes have caused significant damage to crop plants and thus large economic losses worldwide. Anthelmintic drugs and nematicides tend to lose their effectiveness owing to the development of drug resistance. Also, there are serious concerns over environmental impacts of chemical treatment in crop fields. Molecular methods for detection of altered genotypes associated with chemical resistance of nematode species are accurate but suffer from high cost and low throughput. Motility assays for nematodes are inexpensive but have drawbacks such as low efficiency, large material consumption, and inadequate information on changes in locomotive behaviors of nematodes.

This presentation gives an overview on our recent research activities on miniaturized devices for plant and soil science and agriculture [1-8], including microfluidic plant chips and modified plates for studying seed germination and plant growth [1, 2], sensors for monitoring nutrient ions in soil to aid in advancing sustainable agriculture [3, 4], and microfluidic chips for detecting drug resistance of nematodes [5, 6].

II. PLANT CHIPS AND MODIFIED MULTIWELL PLATES

A. Vertical Microfluidic Plant Chip [1, 7]

We developed a microfluidic device for high-throughput phenotyping of *Arabidopsis* plants (Fig. 1a-c) [1]. The device allows multiple plants to grow in different growth sites of the chip. A hydrodynamic trapping method is used to load *Arabidopsis* seeds into the seed sites. Seeds are carried by a liquid medium into the chip. Each seed site allows only one seed to come and settle in under a sucking pressure. The seeds are germinated inside the seed sites. The plant roots grow downward into a tapered channel, while the shoots grow upward into a horizontal channel. The plants can grow within the chip for about eleven days. The vertical arrangement of the chip makes it convenient to image seed germination, and

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emergence and growth of root, hypocotyl, cotyledon, and the first true leaves. By opening up the horizontal channel, the plants can grow out of the chip over more than two weeks. This chip facilitates easy and high-quality observation of plant phenotypes at the whole plant level, as well as at the cellular level [1]. Clear visible phenotypes of the *immutans* mutant of *Arabidopsis*, and phenotypic changes at different developmental stages due to plant-pathogen interactions are observed (Fig. 1c). For *Arabidopsis* plants grown in the chips, the phenotypic variations and the timeline for different developmental stages are consistent with *a priori* data and highly comparable to growth in agar plates. Based on this work, we further developed a phenotyping system [7] consisting of improved vertical plant chips, a programmable robotic arm, low-cost gravity driven pumps, and microfluidic concentration gradient generators. We demonstrated the ability of the system in phenotyping mutants of *Arabidopsis* under chemical (e.g., hormone) stresses [7].

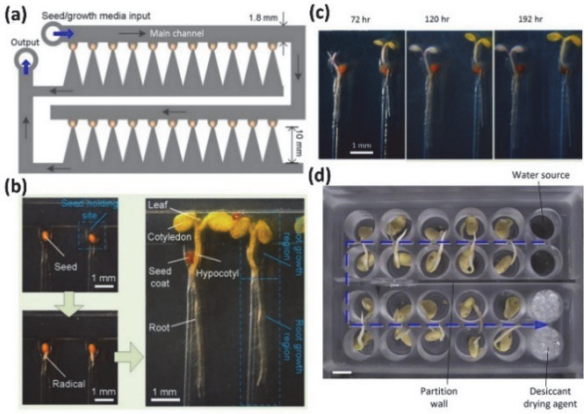

Fig. 1. (a, b) Microfluidic plant chip design [1]. (c) Phenotypic variations of *Arabidopsis* plants [1]. (d) Humidity assay for soybean-pathogen interaction [2].

B. Modified Multiwell Plate for Humidity Assay [2]

The ability to generate controllable humidity conditions is of significant benefit for assaying the role of air water contents in studying plant-pathogen interactions [2]. We developed an economical approach to generate different discrete relative humidity conditions in a modified multi-well plate (Fig. 1d) [2]. The device consists of a freeway channel in the top layer, multiple compartmented wells in the bottom layer, a water source, and a drying agent source [2]. The effects of evaporation, diffusion, and convection are utilized to realize a stable discrete humidity gradient. The humidity gradient can be realized in just a few minutes and maintained over a few days inside the device. The device has been employed to study visible and molecular disease phenotypes of soybean in responses to infection by *Phytophthora sojae*, an oomycete pathogen, under a set of humidity conditions, with two near-isogenic soybean lines, Williams and Williams 82, that differ for a *Phytophthora* resistance gene (Rps1-k) [2]. Our result has showed that at 63% relative humidity, the transcript level of the defense gene GmPR1 was at minimum in the susceptible soybean line Williams and at maximal level in the resistant line Williams 82 following *P. sojae* CC5C infection [2]. The device will benefit laboratories in the area of seed science and plant-microbe biology, where humidity is a key factor that influences disease infection, establishment and development [2].

III. MINIATURIZED SOIL NURITENT SENSORS

A. Portable Electrophoretic Soil Sensor System [3, 8]

Electrophoresis exploits the fact that bioparticles exhibit different mobility characteristics under an applied electric potential [3]. Most commercial electrophoresis instruments are bulky and not suitable for field applications [8]. Recently, many electrophoresis chips have been developed for a variety of applications [3, 8]. However, the field of soil nutrient detection using electrophoresis has not been addressed with the same intensity [8, 9]. A group of researchers at Iowa State University has recently developed a portable electrophoretic soil nutrient sensor system capable of separating and quantifying anions in minute amounts of soil solutions (Fig. 2a-b) [3, 8]. The system includes an active soil water extraction unit, an electrophoresis chip, a high voltage generation and application control unit, and an electrical conductivity measurement unit [3]. Specifically, the soil water suction unit has a porous ceramic tube-based suction cup with one end sealed, a collection chamber, and a mini-vacuum pump (Fig. 2b). The 150 nm mean pore size in the ceramic head allows effective filtration of soil particles and microbes out of the system. The water collection chamber has an embedded sphere as a valve to control vacuum status in the chamber for suction, collection, and loading of soil water into the electrophoresis chip. Different ions are separated as they travel along an electrophoretic channel under the influence of an applied electrical field, owing to their differential electrical mobilities [3]. We have demonstrated that a mixture of anions in the soil water, including chloride, nitrate, sulfate, dihydrogen phosphate, is separated and detected using the system [3]. This sensor has a limit of detection of 7.25 μM for nitrate. Because this sensor required only a minute amount of the extracted soil solution on the order of microliters, it would make a negligible response to the measured environment [3].

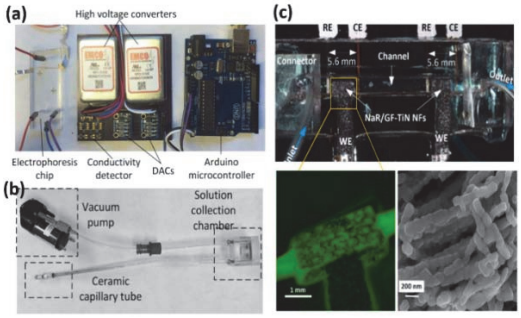

Fig. 2. (a) Microfluidic electrophoresis-based soil nutrient sensor [3, 8]. (b) Soil water suction unit [3]. (c) GF-TiN based microfluidic soil nitrate sensor [4].

B. Enzymatic Soil Nutrient Sensor [4]

We developed an enzymatic microfluidic sensor with a record limit of detection of 0.01 mgL^{-1} for nitrate ions in soil solutions (Fig. 2c) [4]. This electrochemical sensor integrates a graphene foam−titanium nitride (GF−TiN) nanocomposite, specific enzyme molecules, and other electrodes into a channel. The GF−TiN nanocomposite provides high electrochemical reactivity, high electron transfer rate, improved loading capacity of enzyme, and large surface area [4]. GF is realized by chemical vapor deposition of graphene on a metal foam based template, and its networked interior surfaces allow

electrostatic attraction of electrospun TiN nanofibers. The GF−TiN NFs composite is functionalized with nitrate reductase (NaR) enzyme molecules to realize specific recognition of nitrate in soil water [4]. Generally, it is not easy to integrate GF and GF-based materials into microfluidic channels, because their irregular interior pore shape and geometry, rough exterior surface, and large thickness make it difficult to conduct photolithography and etching processes. Therefore, liquid-phase polymerization process is used to overcome this issue. In addition, soil water solutions can flow through and interact with the immobilized NaR enzyme. This device provides a wide dynamic range of nitrate concentration from 0.01 to 442 mg/L. The use of NaR/GF−TiN bioelectrode results in an improved loading capacity of enzyme for catalytic reactions, leading to a higher sensitivity of 683.3 $\mu A mg^{-1} L cm^{-2}$, compared to those using other nanomaterials [4].

IV. Microfluidic Nematode Assay Chips

A. *On-chip Locomotion Tracking Device for Nematodes* [5]

A microscope, along with a camera and tracking software, is a popular setup to detect locomotive parameters of microscopic nematodes. This setup can provide detailed information about the behaviors of nematodes [5]. But, the limited field of view of microscope often makes it challenging to simultaneously monitor multiple experiments in multi-well plates [5]. We developed a lens-less and image-sensor-less approach for real-time monitoring of the locomotion of nematodes (Fig. 3a) [5]. The core of the device is two orthogonally arranged arrays of microelectrode lines. These two electrode arrays are spaced by a microfluidic chamber containing a liquid medium of interest (containing a certain anthelmintic drug). As a worm moves inside the chamber, the resistance profile of the electrode grids (and thus the physical pattern of the worm) can be obtained. A drug resistance screening experiment has been conducted by using this device with a resolution of 30×30 μm^2. Moving speed and oscillation frequency are extracted from reconstructed images [5]. Phenotypic differences between the wild-type and mutant *C. elegans* worms in response to different doses of the anthelmintic drug, levamisole, have been obtained. It is found that the locomotive parameters obtained by the grids agree with those obtained by microscopy [5].

B. *Biomechanical Force Detector* [6]

There is a considerable interest to study how different drugs affect organismal biomechanics of nematode species. Because drug resistance of parasitic nematodes may be associated with changes in signaling-muscle-contraction pathways, it is possible to screen drug resistance of nematodes by directly examining their muscular force changes under different chemical environments [6]. We developed a fiber-optic microfluidic device for measuring muscular forces of nematodes with high sensitivity and data reliability (Fig. 3b) [6]. A moving nematode squeezes through multiple detection points created between a thinned single mode fiber (SMF) cantilever and a sine-wave channel with open troughs. The SMF cantilever is deflected by the normal force imposed by the worm, reducing optical coupling from the SMF to a receiving multimode fiber [6]. Thus, multiple force data can be obtained at multiple detection points to verify with each other, improving data reliability. A noise equivalent force of the device is 143 nN [6]. The workability of the device has been demonstrated to detect muscular normal forces of the parasitic nematodes *Oesophagotomum dentatum* L3 larvae on the SMF cantilever. In addition, this technique has been applied to measure force responses of levamisole-sensitive (SENS) and resistant (LERV) isolates in response to different doses of the anthelmintic drug. The results show that both of the isolates generate a larger muscular normal force when exposed to a higher levamisole concentration. Muscular force phenotype differences between the SENS and LERV are observed [6].

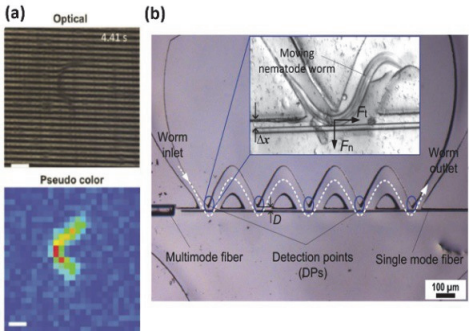

Fig. 3. (a) Microfluidic lens-less locomotion tracking device for nematodes [5]. (b) Nematode muscular force detector [6].

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