Development of a Low-Cost Micro-Environment Device for Root-Nutrient Interaction

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Proposal Title	Development of a Low-Cost Micro-Environment Device for Root-Nutrient Interaction
The Idea	Standard lab conditions for plant growth typically involve homogeneous nutrient conditions, but actual field conditions are rarely homogeneous. Interesting patterns in root architecture arise from heterogeneous conditions, or even dynamic conditions through time. Such patterning calls into question the underlying, likely non-linear processes among root cells that can generate diverse, plastic architecture. Indeed, understanding such phenomenon is critical for development of true synthetic plant systems. I propose development of a low-cost microfluidic device that can finely control rapid changes in the micro-environment surrounding the root structure. A prototype of such a device could easily be tested with cut vinyl molds for PDMS rather than soft-lithography. The device would produce heterogeneous nutrient conditions along the root structure, either by laminar flow or gradient generation. The goal is to build a proof-of-concept device, and use it in conjunction with fluorescence imaging for a preliminary test of a well-documented growth response to heterogeneous nutrient conditions.
Who We Are	Dr. W. Tyler McCleery (Project Leader) Postdoctoral Scientist, Computational and Systems Biology, John Innes Centre Experience in building low-cost microfluidic devices; bioimaging analysis; has developed testable hypotheses and computer simulations for plant-environment interaction; and has helped design experiments probing nitrate-dependent root architecture response in Arabidopsis. tyler.mccleery@jic.ac.uk Dr. Ziyi Yu Postdoctoral Scientist, Department of Chemistry, Cambridge University Experience in microfluidic design and fabrication zy251@cam.ac.uk Dr. Zhijun Meng Postdoctoral Scientist, Department of Chemistry, Cambridge University Experience in microfluidic design and fabrication zm286@cam.ac.uk Dr. Veronica Grieneisen (Project Sponsor) Project Leader, Computational and Systems Biology, John Innes Centre Experience in plant system dynamics and plant development; biophysics and bioimaging analysis veronica.grieneisen@jic.ac.uk

Implementation	Our specific aims are as follows: 1. We aim to produce a proof-of-concept microfluidic device that is capable of creating heterogeneous and dynamic nutrient environments for a developing Arabidopsis root. Iterations of this device will be prototyped using standard soft lithography techniques by W.T. McCleery, Z. Yu, and Z. Meng. These prototypes will be tested using time-lapse imaging equipment to characterize the sensitivity of the device to Arabidopsis root physiology and its precision of control. Control of flow rates will be a critical component of the success of this device to ensure temporal as well as spatial control of the nutrient micro-environment. Effort will be put forth to determine cost-effective means of pumping and flow control. 2. We aim to replicate the viable proof-of-concept device in a low-cost manner. The working plan is to use xurography (razor cutting) methods to create a mold out of vinyl. This mold will then be used to cast PDMS into the appropriate shape. Due to the large channel sizes needed (~300x150 microns in crossection), it will not be necessary to fabricate the device in a clean room setting. W.T. McCleery has previous experience in similar low-cost fabrication techniques and will lead Z. Yu and Z. Meng in developing this version of the device. 3. The device will be used for preliminary testing on a fluorescently-tagged root system to measure the effect of heterogeneous and dynamic nutrient conditions on growth. We are particularly interested in tracking auxin and cytokinin expression profiles along the root axis in response to nitrate availability. A heterogeneous mixture of low-high-low nitrate will be applied to a 3 day old root system tagged for auxin and/or cytokinin response, e.g. D2-Venus (auxin response) or TCS-Venus (cytokinin response). Prolonged fluorescence imaging (~2 days) of this system will provide valuable data for analyzing root-environment interactions. Moreover, developmental changes will be monitored as well, such as lateral root initiation and emergence in response to the micro-environmental changes. This testing will be carried out by W.T. McCleery with assistance from the Grieneisen lab. As a fail-safe for this proposal, Aim 3 does not rely on the success of Aim 2; but comparative testing of the soft lithography and low-cost devices will provide justification to the viability of the low-cost design and fabrication technique.
Benefits and outcomes	This project aims to deliver the plans and characterization of a novel microfluidic device that is capable of modulating the micro-environment around a plant root in a controlled manner. Specifically, we will focus on essential nutrients, in particular nitrate and, time permitting, iron and boron. Moreover, if successful, this project will provide a low-cost method of fabricating a device that can be implemented by an inexperienced plant biology lab. Achieving this goal will provide a critical tool in the toolbox of future synthetic biology work as understanding plant-environment interaction dynamics will be key for testing the viability of engineered plant biology systems. All plans and techniques developed in this project will be made publicly available for dissemination to the synthetic biology community. The aims listed above will rely on close collaboration of team members in Chemistry at Cambridge and Computational and Systems Biology at Norwich. In both cases these techniques and studies provide novel interdisciplinary interactions – Norwich members will share their expertise in plant biology and low-cost microfluidic fabrication, which Cambridge members will share their experience with microfluidic design and soft lithography fabrication.

Sponsor for the research and cost centre	Dr. Veronica Grieneisen Project Leader, Computational and Systems Biology, John Innes Centre veronica.grieneisen@jic.ac.uk
Budget	Soft Lithography Materials 1. A4 film mask, £105 * 3 = £315 2. Silicon wafer, £300 3. SU8 2025 photoresist, £500 4. Sylgard® 184 Silicone Elastomer, £200 5. Tubing, £150 6. Other consumables including glass slides, scalpel blades, chemicals, £300 Subtotal: £1,765 Xurography Materials 1. Silhouette Vinyl Cutter, £200 2. Vinyl, £10 3. Additional PDMS + Curing agent (Sylgard 185 kit), £90 4. IV Bag (x2), £20 5. Additional tubing connectors and miscellaneous materials, £26.20 Subtotal: £346.20 Plant Growth Media and Nutrients 1. Murashige and Skoog Basal Salt Micronutrient Solution (10×, 1L), £25 * 2 = £50 2. Gamborg's Vitamin Solution (1000×, 50mL), £15 * 2 = £30 Subtotal: £80 Microscope Time 1. Wide-field Fluorescence Microscope (JIC), £10.60/hr * 48hr = £508.80 2. Confocal Fluorescence Microscope (JIC), £50/hr * 24hr = £1200 Subtotal: £1,708.80 Miscelaneous Expenses 1. Travel between Norwich and Cambridge £20/return trip * 5 = £100 Subtotal: £100 Total: £4,000