Chapter 15

Arabidopsis Root Microbiome Microfluidic (ARMM) Device for Imaging Bacterial Colonization and Morphogenesis of Arabidopsis Roots

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Abstract

Imaging the spatiotemporal dynamics of host-microbiota interactions is of particular interest for augmenting our understanding of these complex systems. This is especially true of plant–microbe interactions happening around, on, and inside plant roots where relatively little is understood about the dynamics of these systems. Over the past decade, a number of microfluidic devices have been developed to grow plants hydroponically in gnotobiotic conditions and image morphogenesis of the root and/or dynamics with fluorescently labeled bacteria from the plant root microbiome. Here we describe the construction and use of our Arabidopsis Root Microbiome Microfluidic (ARMM) device for imaging fluorescent protein expressing bacteria and their colonization of Arabidopsis roots. In contrast to other plant root imaging devices, we designed this device to have a larger chamber for observing Arabidopsis root elongation and plant–microbe interactions with older seedlings (between 1.5 and 4 weeks after germination) and a 200 μm chamber depth to specifically maintain thin Arabidopsis roots within the focal distance of the confocal microscope. Our device incorporates a new approach to growing Arabidopsis seedlings in screw-top tube caps for simplified germination and transfer to the device. We present representative images from the ARMM device including high resolution cross section images of bacterial colonization at the root surface.

Key words Plant–microbe interactions, Microfluidics, Microscopy, Arabidopsis, Microbiome

1 Introduction

The plant microbiome affects many aspects of plant growth, development, health, and productivity. Over the last 10 years, a wealth of information has been generated about specific interactions between Arabidopsis and its rhizosphere microbiome through the curation and sequencing of microbiome collections [1–3], development of synthetic community systems [4, 5], systems biology studies utilizing -omics [6–8], and careful mechanistic dissection using plant and/or microbial genetics [9–11]. Yet, much is still unknown

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about the spatiotemporal dynamics of microbial colonization, interactions in the rhizosphere, and effects on root tissue morphogenesis. This has led to the development of microfluidic devices [12–20] in which plants can be grown gnotobiotically (or in nearly gnotobiotically) and live-imaged using minimally invasive methods [21].

Each plant imaging microfluidic device consists of a chamber made by covalently attaching a PDMS cast to a glass surface which allows a stable surface for support on the microscope for visualization. Each device's chamber also has a least one inlet and outlet for media exchange and a site to hold the growing plant. The chamber geometry and inlet/outlet features of the individual devices are changed depending on the goals of the device. The "plant on a chip" system [12] was a pioneer in the area of root imaging devices. It uses a special set of inlets to create laminar flow across the chamber and allow flow of a stimulant across a particular cross section of the growing root. The RootChip system [17, 19] allows control of many different parallel chambers and demonstrated root visualization incorporating an engineered Arabidopsis line with fluorescent sensor genetic circuit for glucose and galactose detection. The dual-flow-RootChip [14] developed subsequently features laminar flow past each side of a single root creating asymmetric environments on either side of the same root allowing measurement of root hair phenotypes in these different environments. The Tracking Root Interactions System (TRIS) devices [13] have two different designs to allow nine parallel single chambers for imaging individual seedlings or six parallel dual chambers for imaging pairs of seedlings in the same conditions. The TRIS device is very small and accommodates only very young Arabidopsis seedlings (imaging performed 7–9 days after germination). EcoFAB devices [15, 16] developed a larger chamber for use with Arabidopsis and plants of larger stature (switchgrass, *Brachypodium*, etc.) with the goal of having a standardized, controllable, and reproducible [22] "fabricated ecosystem" (from which the name EcoFAB is derived). While Arabidopsis can be grown in the EcoFAB, the large open design and deep chamber are not ideal for supporting and imaging the thin roots of Arabidopsis.

Here we describe the construction and use of our Arabidopsis Root Microbiome Microfluidic (ARMM) device (Fig. 1) for imaging bacteria and Arabidopsis roots. This device has a 200 μm chamber depth to maintain the thin Arabidopsis roots within the focal distance of the microscope and a large chamber (4.4 cm length × 1.5 cm width) for observing Arabidopsis root elongation and plant–microbe interactions at points between 1.5 and 4 weeks after germination.

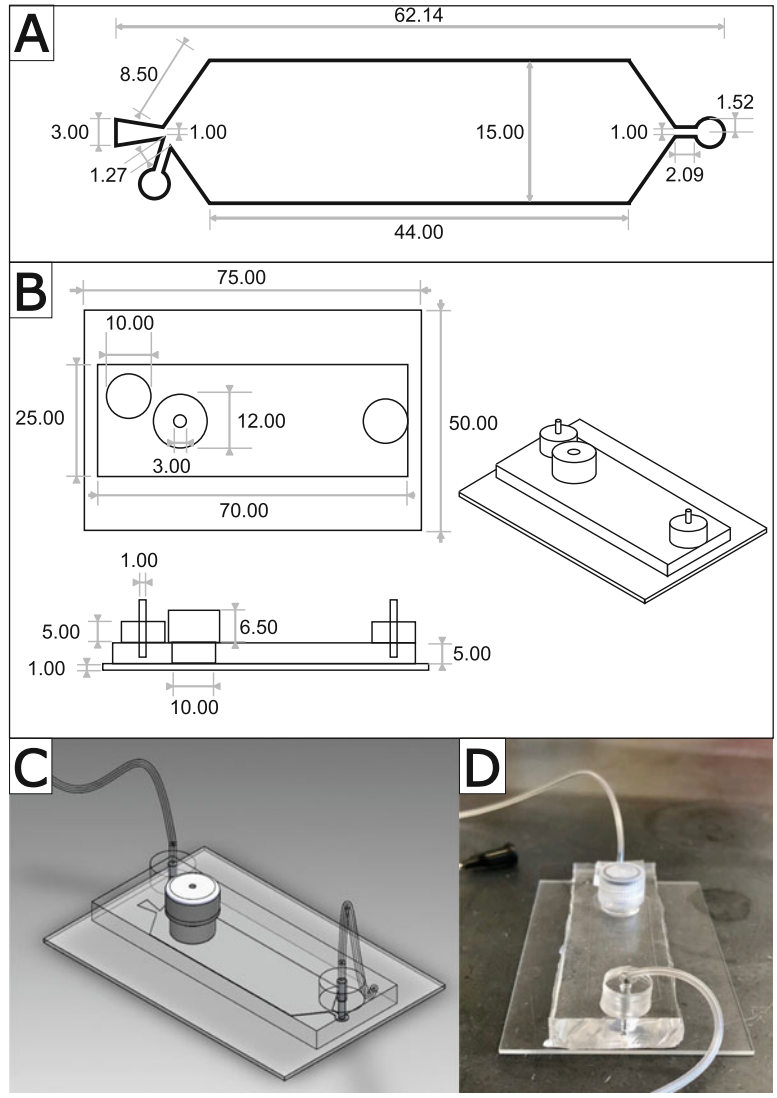

Fig. 1 Schematics of the Arabidopsis Root Microbiome Microfluidic (ARMM) chamber and device. (**a**) Schematic of a single ARMM chamber with dimensions in mm. The inlet is to the left and outlet to the right. (**b**) Schematics of the assembled ARMM device with dimensions in mm. Rendering (**c**) and photo (**d**) of an assembled ARMM device

2 Materials

2.1 Photolithography and PDMS Casting

1. Roland 24″ vinyl cutter (Camm-1 Servo, GX-24).
2. Vinyl with adhesive backing.
3. Clear transparency sheets.

4. Silicone wafers.
5. 10% hydrofluoric acid.
6. SU-8 2050 photoresist.
7. SU-8 developer.
8. Spin coater (Laurell Technologies Corporation WS-650-23B or similar).
9. Heating plate which can be set at: 65, 95, and 200 °C.
10. Karl Suss MA6/BA6 Mask Aligner with UV light.
11. KLA Tencor P-6 Stylus Profiler.

2.2 Microfluidic Device

1. Sylgard 184 PDMS (Dow Corning).
2. Dish and stirrer for mixing PDMS.
3. Balance to weight PDMS components.
4. Vacuum desiccator connected to a vacuum pump.
5. Dish to hold device mold and cast the PDMS (petri dish, aluminum weigh boat, aluminum pie plate).
6. 60 °C oven.
7. Razor blade or scalpel.
8. PDC-001 plasma cleaner (Harrick Plasma) connected to vacuum pump.
9. Glass slides.
10. Glass cover slip.
11. 10 mm circular punch.
12. 14-Gauge blunt needles.
13. Tygon tubing (0.020 inch inner diameter, 0.060 inch outer diameter—Cole Palmer).
14. 22.5-Gauge stainless steel tubing cut in ~1 cm lengths.
15. 22.5-Gauge blunt needles with luer lock connection.
16. Sterile syringes with luer lock connection (1, 10 mL).
17. 2 mL screw cap tubes (USA Scientific #1420-8700) separated: tube body cut off by a machine shop 0.5–1 cm below the cap threading, caps punched with a 1 mm hold punch.
18. 1 mm hole punch.
19. Magenta GA-7 boxes.
20. Aluminum foil.
21. Autoclave.
22. NE-1800 syringe pump (New Era Pump Systems Inc.).

2.3 Seedling Germination and Growth

1. *Arabidopsis thaliana* ecotype Col-0 seeds.
2. 0.5× MS medium (2.22 g/L Murashige and Skoog Modified Basal Medium with Gamborg vitamins (PhyoTechnology Laboratories M404), 0.5 g/L MES hydrate, pH 5.7 with KOH).
3. Agar.
4. Seed sterilization solution (70% bleach, 0.2% Tween 20) made fresh.
5. Vortex mixer.
6. Sterile petri dishes or tissue culture dishes with lids.
7. Forceps and method for sterilization (alcohol lamp, electric heater, etc).
8. BioSafety Cabinet for maintaining gnotobiotic cultures and plants.
9. Sterile aluminum foil sealing film.
10. Breathe-Easy sealing film (Diversified Biotech).
11. Plant growth chambers (Conviron) set at long day (16 h light at 21 °C, 8 h dark at 18 °C) and short day (9 h light at 22 °C, 15 h dark at 18 °C) conditions.

2.4 Bacteria

1. Plant-associated bacteria strains (can be engineered to express fluorescent protein markers).
2. 2× YT medium (16 g/L tryptone, 10 g/L yeast extract, and 5 g/L NaCl).
3. Sterile culture tubes.
4. Biosafety Cabinet.
5. 28 °C incubator.
6. Spectrophotometer for measuring bacterial density OD600.

2.5 Imaging

1. Zeiss LSM 880 confocal microscope and analysis software.
2. Propidium iodide solution (1 mg/mL stock for dilution).

3 Methods

3.1 Photolithography

1. A mask for photolithography was created by designing the device shape (*see* **Note 1**) (Fig. 1) as a vector image and using the Roland 24″ vinyl cutter (Camm-1 Servo, GX-24) to cut the shape out of a large (~10 × 10 inch) piece of black vinyl with adhesive backing. Multiple chambers of the same device can be designed on the same mask to optimize space on the silicone wafer used during photolithography (*see* **Note 2**). The cut device shape was removed and the negative was adhered to a clear plastic transparency sheet to create the mask (Fig. 2a).

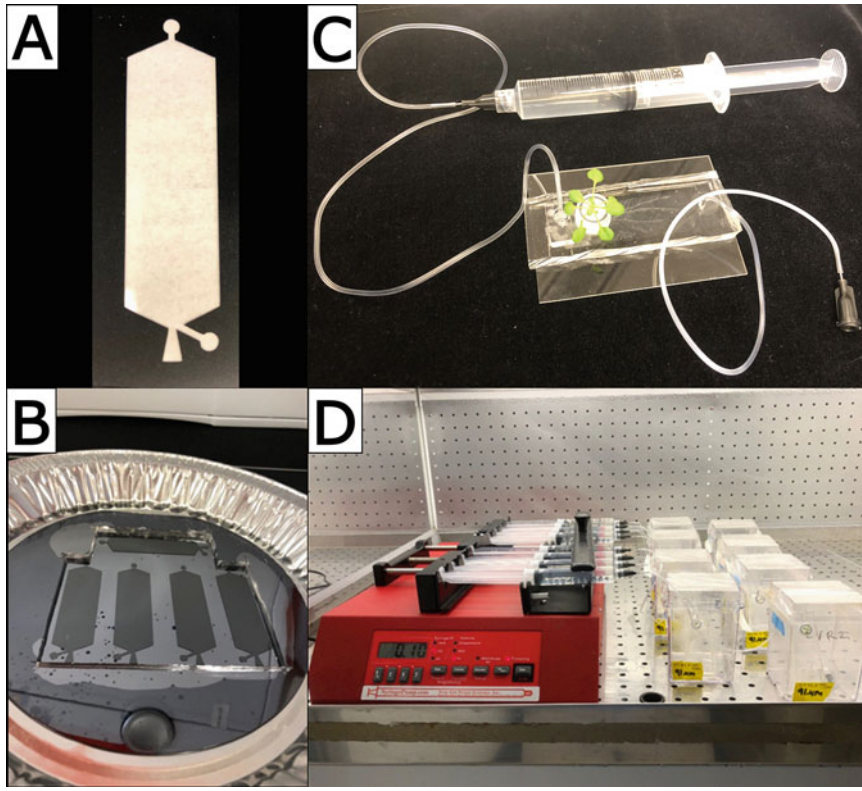

Fig. 2 Photos from fabrication and use of the ARMM device. (**a**) A single chamber mask cut from vinyl and adhered to a transparency as a photolithography mask. (**b**) Mold for five ARMM devices on a 6 inch silicone wafer embedded in PDMS. Only the area around the chambers on the mold needs to be cut and then this area can be recast in PDMS again and again. (**c**) A fully assembled ARMM device containing a 4-week-old Arabidopsis seedling. (**d**) Eight parallel ARMM devices in Magenta GA-7 boxes attached to a syringe pump in the plant growth chamber

2. Under clean room conditions (*see* **Note 3**), wash a silicone wafer of appropriate size (6 inch was used here) with 10% hydrofluoric acid, rinse with distilled water, and bake at 200 °C for 7 min to dry the wafer.
3. Spin coat the wafer using SU-8 2050 photoresist for 1 min at 1000 RPM to achieve a photoresist thickness of ~100 μm.
4. Soft bake the coated wafer at 95 °C for 30 min.
5. Spin coat the wafer again using SU-8 2050 photoresist for 1 min at 1000 RPM to achieve a total thickness of ~200 μm.
6. Soft bake the photoresist at 95 °C for 60 min.
7. Cool the wafer to room temperature and place the transparency mask on top of the wafer and expose the photoresist to UV light through the mask for 60 s in a Karl Suss MA6/BA6 Mask Aligner.

8. After exposure, bake the wafer at 65 °C for 5 min, then 95 °C for 15 min.
9. Cool the wafer to room temperature and place in SU8 developer and on a rotary shaker at low speed until photoresist that was not exposed to the UV light is removed from the silicone wafer.
10. Wash the wafer with isopropanol and allow to dry.
11. Confirm the height of the remaining photoresist on the mold (which will become the microfluidic chamber height) using a KLA Tencor P-6 Stylus Profiler.

3.2 Casting the Device in PDMS

1. Thoroughly mix Sylgard 184 PDMS (Dow Corning) silicone elastomer and curing agent according to the manufacturer's instructions (10:1 ratio of silicone elastomer and curing agent).
2. Degas the PDMS for 10–20 min in a vacuum desiccator hooked to a vacuum pump to remove air bubbles.
3. Place the silicone wafer with the device mold in an appropriate dish and pour the prepared PDMS on top. Enough PDMS should be prepared to cover the mold with about 0.5 cm of PDMS.
4. Cure the PDMS completely by placing the dish containing the mold and PDMS at 60 °C for several hours to overnight. Curing can be done at room temperature, but will take longer.
5. Once the PDMS is completely cured, cut out the PDMS around the device mold using a razor blade or scalpel (Fig. 2b) being sure to leave enough border around the device chamber to give enough PDMS to adhere to the glass slide. Remove the cut PDMS from the mold.
6. Additional areas of cast PDMS can be cut out as ~1 cm × ~1 cm "blocks" to assemble as extra support of the inlet and outlet tubing (*see* **Note 5**).

3.3 Device Assembly

1. Place the PDMS containing the cast of the chamber and two PDMS support "blocks" in a PDC-001 plasma cleaner. The sides to be attached should be facing up to be exposed to the plasma.
2. Close the plasma cleaner chamber and use a vacuum pump to decrease the chamber pressure to below 0.1 torr. Open the inlet to allow air into the chamber until the pressure stabilizes at ~0.9 to 1 torr. Then turn the plasma setting to the "High" setting for 3 min.
3. After 3 min, turn the plasma setting to "off," close the inlet valve, and maintain vacuum for an additional minute.

4. Then, turn the vacuum off and slowly open the inlet valve to return the chamber to atmospheric pressure.
5. Open the plasma cleaner chamber and remove the PDMS pieces and immediately press the sides that were facing up and exposed to the plasma together to bond them. Press the pieces together firmly.
6. Once the PDMS chamber and PDMS inlet and outlet supports are assembled, use a 10 mm diameter circular punch to punch a hole at one end of the chamber for insertion of the cut-off screw cap tube body which will hold the plant. Use a 14-gauge blunt needle to punch one hole at each end of the chamber through both the support "block" and chamber PDMS pieces which are now attached for insertion of the inlet and outlet tubing.
7. Next, repeat **steps 1–5** to attach this PDMS piece to a glass slide or glass cover slip (depending on the imaging application) (*see* **Note 8**). When pressing the PDMS with the chamber to the glass after plasma cleaning, be careful not to press in the middle of the chamber to avoid adhering it to the glass (*see* **Note 4**).
8. Attach one end of a 10–20 cm piece of Tygon tubing (Cole-Palmer, 0.020 inch inner diameter, 0.060 inch outer diameter) to a 1 cm piece of 22.5-gauge stainless steel tubing. Attach the other end of the Tygon tubing to a 22.5 gauge blunt needle with luer lock connection. Repeat this to make two identical pieces of tubing to serve as the inlet and outlet.
9. Insert the 1 cm stainless steel piece of tubing attached to the Tygon tubing into the 14-gauge hole punched in the PDMS support block to create the tubing inlet and outlet of the device.
10. Place the tube portion of the cut-off 2.0 mL screw cap tube which will hold the plant into the 10 mm hole in the PDMS.
11. Place the fully assembled PDMS device (Fig. 1d) in a magenta GA-7 plant culture box, cover with aluminum foil, and autoclave for 20 min on a dry cycle to sterilize.

3.4 Plant Germination

1. Sterilize Arabidopsis seeds by placing them in a 2 mL microcentrifuge tube with a 70% bleach, 0.2% Tween 20 solution. Mix the seeds in this solution in a vortex mixer for 10 min.
2. In a biosafety cabinet, wash the seeds five to ten times with sterile distilled water to remove the bleach solution. For each wash, add distilled water, vortex, centrifuge briefly, and remove the wash solution. After the final wash, add distilled water to the tube containing the seeds.

3. Cover the tube containing the sterile seeds in distilled water with aluminum foil to protect them from light and stratify the seeds by placing the tube at 4 °C for 24–72 h.
4. Punch 1 mm holes in the screw cap tube lids and sterilize them by autoclaving.
5. Prepare 0.5× Murashige and Skoog Modified Basal Medium with Gamborg vitamins (PhyoTechnology Laboratories M404) (henceforth, 0.5× MS medium) with 1% agar and sterilize by autoclaving.
6. In a biosafety cabinet, use sterilized forceps to place the screw caps upside down on sterile aluminum sealing film (top of the cap stuck to the sealing film). Add a small amount of 0.5× MS medium with agar to the underside of each cap and allow it to solidify. Pour additional media in a sterile petri dish and allow it to solidify.
7. When the media in the caps and plate have solidified, use sterile forceps to place the caps onto the media plate with the agar filled underside facing down.
8. Use a 20 μL pipette to place a single sterile Arabidopsis seed in the 1 mm punched hole on top of the agar plug filling the underside of the cap (*see* **Note 6**).
9. Close the petri dish containing the caps with seeds and seal with medical tape.
10. Incubate the seeds in long day conditions (16 h light at 21 °C, 8 h dark at 18 °C) for 7 days to germinate the seedlings.

3.5 Transferring Plants to the Device and Plant Growth

1. Prepare 0.5× MS liquid medium and sterilize by filtration or autoclaving.
2. Working in a biosafety cabinet with good sterile technique, fill a 10 mL luer lock syringe with 0.5× MS medium and flush the sterilized chamber with ~1–2 mL of medium.
3. Using sterilized forceps, transfer one cap containing one healthy germinated seedling to the screw cap tube in the sterilized device and screw it in place.
4. Push an additional ~1–2 mL of 0.5× MS medium through the device to ensure no air bubbles remain and the device is completely full and not leaking (*see* **Note 7**). Refill the 10 mL inlet syringe with media.
5. Return the device, now containing the plant, to the Magenta GA-7 box. The device should be propped at an angle with the inlet and plant at the top and the outlet at the bottom. This will allow the root to grow downward by gravitropism. The inlet tubing should be draped over the edge of the box so the syringe is outside the box. The outlet tubing can remain in

the box or be attached to a tube or syringe for media collection. Finally, seal the box containing the device with a Breathe-Easy sealing film (Diversified Biotech).

6. The devices containing plants were placed in a plant growth chamber operating under short day conditions (9 h light at 22 °C, 15 h dark at 18 °C). The 10 mL syringes containing fresh media attached to the inlet were attached to a NE-1800 syringe pump (New Era Pump Systems Inc.), which was set to inject at 0.1 mL/h. *See* Fig. 2d.
7. Syringes were refilled as necessary by transferring the devices to a biosafety cabinet and attaching a new syringe with fresh media to the inlet, and then returning the devices to the syringe pump in the plant growth chamber. Plants can be maintained with refilling of the inlet media to 4 weeks after germination (Fig. 2c).

3.6 Bacteria Preparation

1. Grow the bacteria strains from the Arabidopsis rhizosphere individually to early stationary phase in 2× YT medium at 28 °C with 200 rpm shaking (*see* **Note 9**).
2. Wash the bacteria three times in sterile 0.5× MS medium. Do this by centrifuging 1 mL of culture at 7000 × *g* for 5 min, removing the medium, resuspending the cells in 0.5× MS medium, and repeating three times.
3. Take the OD600 of the final washed cultures. If a mixture of bacteria is being used, they can be mixed at this step by normalizing the density of each member in the mixture.
4. To inoculate the devices in the biosafety cabinet, inject 0.5 mL of bacteria (either individual strain or normalized mixture) at OD600 = 0.001 in 0.5× MS medium into the device. Replace the 10 mL syringe containing 0.5× MS medium and return to the syringe pump in the plant growth chamber. Inoculation can be timed according to experimental goals. Typically, plants were inoculated 2 days after transferring seedlings into the device when the main root had grown into the chamber.

3.7 Imaging

1. Image the root and bacteria on a Zeiss LSM880 confocal microscope by removing the device from the GA-7 box and carefully transferring the device to the microscope (*see* **Note 10**).
2. Devices with glass slide backing can be imaged with the 10× objective. Tiled images can be captured to obtain an image over a section of root (Fig. 3c, d). Using our device, we collected 16 images at the root tip which could be assembled into a tiled image of 8 × 2 individual images covering 6.8 mm × 1.7 mm.

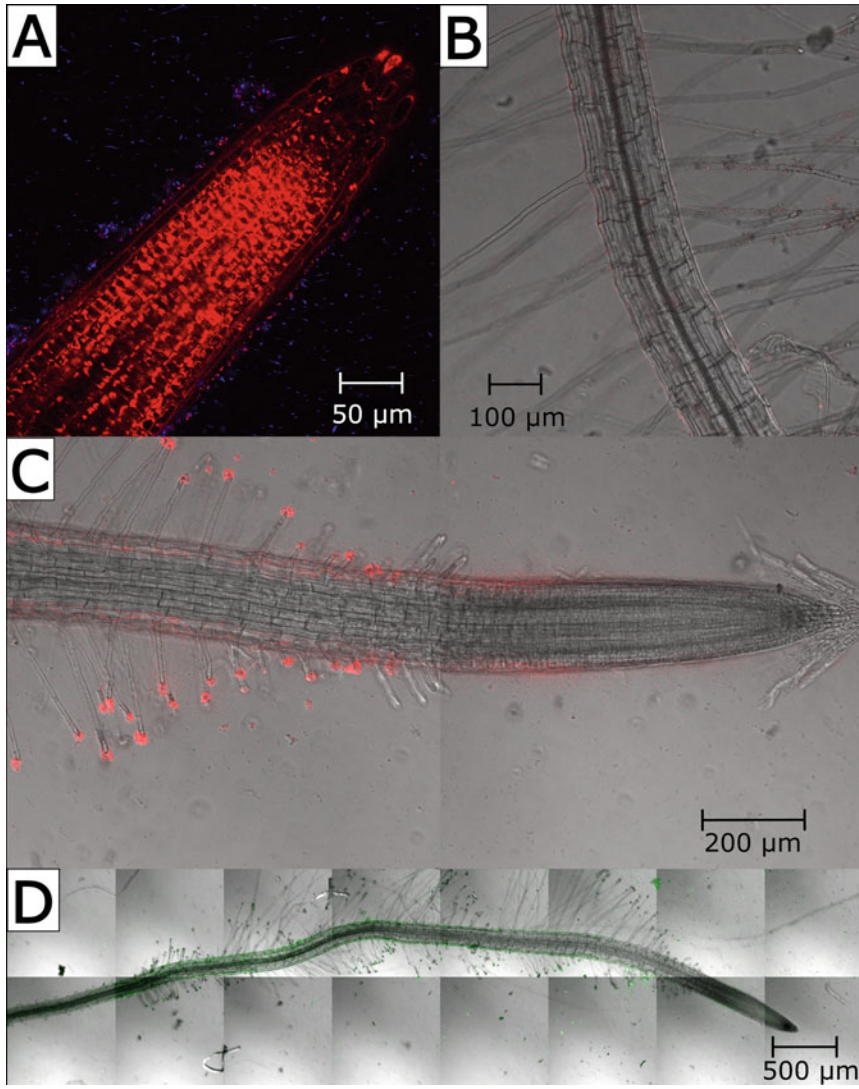

Fig. 3 Representative images obtained using the ARMM device. (**a**) High resolution image of the root tip and fluorescent protein expressing *Pseudomonas* sp. MF397 (blue) stained with propidium iodide to stain root cell walls. This image was taken in an ARMM device with cover slip thickness glass backing with the 40×/1.20 water immersion objective. Scale bar 50 μm. (**b**) Root hairs extending from the main root in the presence of fluorescent protein expressing *Pseudomonas simiae* WCS417r (red). Scale bar 100 μm. (**c**) Tiled image at the main root tip with fluorescent protein expressing *Ralstonia* sp. UNC404CL21Col (red). Scale bar 200 μm. (**d**) Large tiled image showing approximately 6 mm back from the main root tip of a root in the ARMM device with fluorescent protein expressing *Brevundimonas* sp. MF374 (green). Scale bar 500 μm

3. Devices with a glass cover slip backing can be imaged using the C-Apochromat 40×/1.20 water immersion objective to obtain higher resolution images of bacteria (Figs. 3a and 4).
4. Root cell walls can optionally be stained by injecting a small amount of propidium iodide (0.002 mg/mL in 0.5× MS medium) into the device prior to imaging (Figs. 3a and 4).

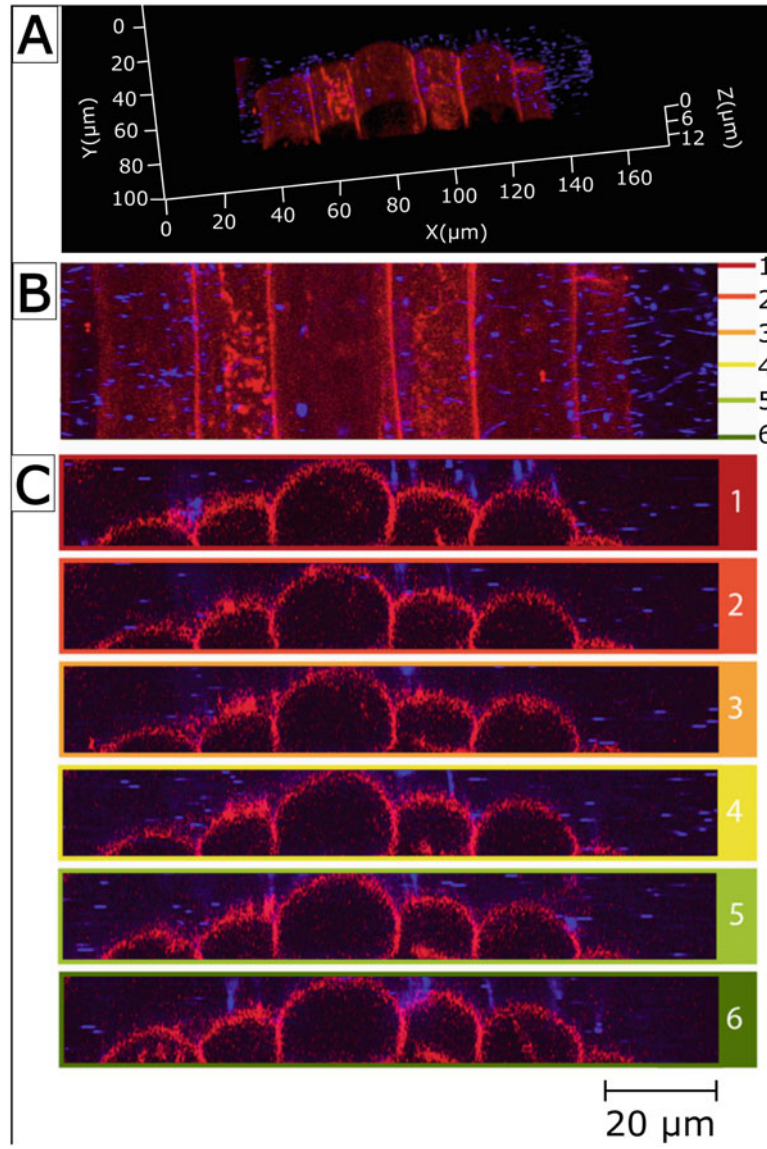

Fig. 4 High resolution imaging of plant microbe interactions in the glass cover slip backed ARMM device. Fluorescent protein expressing *Pseudomonas* sp. MF397 are visualized in blue and root cell walls are visualized by propidium iodide staining. (**a**) 3D reconstruction from a *z*-stack image taken through the main root surface. Scale shown in image. (**b**) *X*–*Y* plane maximum intensity projection from the *z*-stack through the root surface. Numbered at right are the *Y* location of the *X*–*Z* plane slices show in (**c**). (**c**) Visualization of six *X*–*Z* planes through the root surface. Cross sections of cells are shown in this plane as the red circles. *Pseudomonas* sp. MF397 can be seen very closely associated on the root surface in several cross-sections

5. To capture temporal dynamics, a single device can be imaged at intervals over fairly long periods of time (several hours). Alternatively, a device can be imaged over days by carefully returning the device to the Magenta GA-7 box after imaging, resealing it with a Breathe-Easy sealing film, and returning it to the incubator to be imaged again on subsequent days.
6. Another imaging mode for capturing spatiotemporal dynamics over minutes to hours of initial colonization would be to inject bacteria after setting up a device containing a sterile plant seedling on the microscope.

4 Notes (Tips and Tricks to Methods)

1. The device shape and dimensions can be easily manipulated by making a mask of different shape and spin coating the silicone wafer with a different photoresist thickness prior to UV exposure. Devices with increased depth and/or larger chambers could be constructed for imaging plants with thicker roots or plants at a longer time after germination. Additionally, geometries which allow for laminar flow across a cross section of the root [12] or along each side of a root [14] could be constructed.
2. We attempted to make a mold for our device by printing it on an Ultimaker 3 3D printer using Ultimaker CPE filament. We found that the 3D printed mold did not achieve a surface smoothness that was amenable to casting in PDMS, especially for a device with the 200 μm depth we were attempting to achieve. Thus, we proceeded with photolithography. Device molds made via photolithography are extremely smooth. This make the PDMS easy to remove from the mold and the smoothness of the PDMS surface improves the attachment and seal to the glass slide or cover slip device backing. The silicone wafer molds, however, are fragile, so be careful manipulating them because they can shatter if bent.
3. Photolithography is best carried out in a clean room. But if clean room conditions are not achievable, the photolithography here could likely be carried out under normal laboratory conditions with care to attempt to avoid dust, pollen, etc.
4. When using the plasma cleaner to attach the PDMS to the glass slide backing, be careful not to press the PDMS to the glass in the middle of the chamber. The entire PDMS surface is activated and doing this will attach the upper wall of the chamber to the glass backing resulting in a misshapen chamber geometry.

5. When multiple devices are made, the PDMS removed for insertion of the screw cap tube can be used for the inlet and outlet supporting blocks. These blocks are slightly smaller, but give sufficient support if completely bonded to the PDMS housing. Circular supports can be seen in Fig. 1d.
6. Always sow approximately double the number of seeds in caps as will be required for the experiment. Seeds will dry out or germinate poorly, so having extra seedlings is important for experimental timing. This also allows the healthiest looking seedlings to be advanced into the device and used for the experiment.
7. It is helpful to have a few spare sterilized devices as backup during experiment setup. The seal of the PDMS to the glass backing might not be complete and a leaking device could be discarded and substituted for a spare in an experiment. Leaking devices will most likely be discovered during transfer of the plants to the devices.
8. Using cover slip thickness glass as the ARMM device backing is much more fragile than glass slides. Yet the thin cover slip glass can be imaged through using higher magnification objectives resulting in higher resolution images. Cover slip glass should be used when these high magnification images are essential to answering the desired experimental question. When using cover slip thickness glass take extra care assembling the device. Do not press too hard when adhering the PDMS after plasma cleaning. It may be helpful to assemble the entire upper PDMS portion of the device including the inlet and outlet tubing before using the plasma cleaner to attach it to the cover slip glass. This minimizes manipulations of the device with the glass attached and thus minimizes chances to break the glass. If tubing is not inserted prior to attachment to the glass, maintain support of the cover slip on a hard and flat surface while inserting the inlet and outlet tubing, and be gentle when transferring the device to the Magenta GA-7 Box and onto the microscope stage to avoid breakage.
9. The bacteria injected into the device can be changed depending on the goals of the experiment. If observing root morphogenesis is the goal, strains from the Arabidopsis rhizosphere can be used without modification. To visualize the dynamics of bacterial movement and colonization, strains can be engineered to express a fluorescent protein markers as was done for *Pseudomonas simiae* WCS417r [23] and other strains shown in Figs. 3 and 4. Synthetic communities of any number of strains, which are fluorescent and/or non-fluorescent, could also be used.
10. Several different imaging modes can take advantage of the ARMM device. Devices can be prepared and bacteria can be

injected at the microscope to visualize early colonization dynamics. Devices can also be inoculated ~2 days after transfer of plants to the device and visualized periodically over the next 3 weeks.

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Competing Interest Declaration J.L.D. is a co-founder of, and shareholder in, AgBiome LLC, a corporation with the goal of using plant-associated microorganisms to improve plant productivity.

References

1. Levy A, Conway JM, Dangl JL, Woyke T (2018) Elucidating bacterial gene functions in the plant microbiome. Cell Host Microbe 24: 475–485
2. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, Del Rio TG, Edgar RC, Eickhorst T, Ley RE, Hugenholtz P, Tringe SG, Dangl JL (2012) Defining the core *Arabidopsis thaliana* root microbiome. Nature 488:86–90
3. Bai Y, Muller DB, Srinivas G, Garrido-Oter R, Potthoff E, Rott M, Dombrowski N, Munch PC, Spaepen S, Remus-Emsermann M, Huttel B, McHardy AC, Vorholt JA, Schulze-Lefert P (2015) Functional overlap of the Arabidopsis leaf and root microbiota. Nature 528: 364–369
4. Castrillo G, Teixeira PJ, Paredes SH, Law TF, de Lorenzo L, Feltcher ME, Finkel OM, Breakfield NW, Mieczkowski P, Jones CD, Paz-Ares J, Dangl JL (2017) Root microbiota drive direct integration of phosphate stress and immunity. Nature 543:513–518
5. Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, Malfatti S, Glavina del Rio T, Jones CD, Tringe SG, Dangl JL (2015) Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science 349:860–864
6. Muller DB, Schubert OT, Rost H, Aebersold R, Vorholt JA (2016) Systems-level proteomics of two ubiquitous leaf commensals reveals complementary adaptive traits for phyllosphere colonization. Mol Cell Proteomics 15:3256–3269
7. Ma K-W, Niu Y, Jia Y, Ordon J, Copeland C, Emonet A, Geldner N, Guan R, Stolze SC, Nakagami H, Garrido-Oter R, Schulze-Lefert P (2021) Coordination of microbe–host homeostasis by crosstalk with plant innate immunity. Nat Plants 7:814–825
8. Teixeira P, Colaianni NR, Law TF, Conway JM, Gilbert S, Li H, Salas-Gonzalez I, Panda D, Del Risco NM, Finkel OM,

Castrillo G, Mieczkowski P, Jones CD, Dangl JL (2021) Specific modulation of the root immune system by a community of commensal bacteria. Proc Natl Acad Sci USA 118: e2100678118

9. Voges MJEEE, Bai Y, Schulze-Lefert P, Sattely ES (2019) Plant-derived coumarins shape the composition of an *Arabidopsis* synthetic root microbiome. Proc Natl Acad Sci USA 116: 12558–12565
10. Finkel OM, Salas-Gonzalez I, Castrillo G, Conway JM, Law TF, Teixeira P, Wilson ED, Fitzpatrick CR, Jones CD, Dangl JL (2020) A single bacterial genus maintains root growth in a complex microbiome. Nature 557:103–108
11. Colaianni NR, Parys K, Lee HS, Conway JM, Kim NH, Edelbacher N, Mucyn TS, Madalinski M, Law TF, Jones CD, Belkhadir Y, Dangl JL (2021) A complex immune response to flagellin epitope variation in commensal communities. Cell Host Microbe 29:635–649.e9
12. Meier M, Lucchetta EM, Ismagilov RF (2010) Chemical stimulation of the Arabidopsis thaliana root using multi-laminar flow on a microfluidic chip. Lab Chip 10:2147
13. Massalha H, Korenblum E, Malitsky S, Shapiro OH, Aharoni A (2017) Live imaging of root-bacteria interactions in a microfluidics setup. Proc Natl Acad Sci USA 114:4549–4554
14. Stanley CE, Shrivastava J, Brugman R, Heinzelmann E, van Swaay D, Grossmann G (2018) Dual-flow-RootChip reveals local adaptations of roots towards environmental asymmetry at the physiological and genetic levels. New Phytol 217:1357–1369
15. Zengler K, Hofmockel K, Baliga NS, Behie SW, Bernstein HC, Brown JB, Dinneny JR, Floge SA, Forry SP, Hess M, Jackson SA, Jansson C, Lindemann SR, Pett-Ridge J, Maranas C, Venturelli OS, Wallenstein MD, Shank EA, Northen TR (2019) EcoFABs: advancing microbiome science through standardized fabricated ecosystems. Nat Methods 16:567–571
16. Gao J, Sasse J, Lewald KM, Zhalnina K, Cornmesser LT, Duncombe TA, Yoshikuni Y, Vogel JP, Firestone MK, Northen TR (2018) Ecosystem fabrication (EcoFAB) protocols for the construction of laboratory ecosystems designed to study plant-microbe interactions. J Vis Exp (134):e57170. https://doi.org/10.3791/57170
17. Grossmann G, Guo W-J, Ehrhardt DW, Frommer WB, Sit RV, Quake SR, Meier M (2011) The RootChip: an integrated microfluidic chip for plant science. Plant Cell 23:4234–4240
18. Yanagisawa N, Sugimoto N, Arata H, Higashiyama T, Sato Y (2017) Capability of tip-growing plant cells to penetrate into extremely narrow gaps. Sci Rep 7:1403
19. Grossmann G, Meier M, Cartwright HN, Sosso D, Quake SR, Ehrhardt DW, Frommer WB (2012) Time-lapse fluorescence imaging of Arabidopsis root growth with rapid manipulation of the root environment using the RootChip. J Vis Exp (65):4290. https://doi.org/10.3791/4290
20. Parashar A, Pandey S (2011) Plant-in-chip: microfluidic system for studying root growth and pathogenic interactions in Arabidopsis. Appl Phys Lett 98:263703
21. Grossmann G, Krebs M, Maizel A, Stahl Y, Vermeer JEM, Ott T (2018) Green light for quantitative live-cell imaging in plants. J Cell Sci 131:jcs209270
22. Sasse J, Kant J, Cole BJ, Klein AP, Arsova B, Schlaepfer P, Gao J, Lewald K, Zhalnina K, Kosina S, Bowen BP, Treen D, Vogel J, Visel A, Watt M, Dangl JL, Northen TR (2019) Multilab EcoFAB study shows highly reproducible physiology and depletion of soil metabolites by a model grass. New Phytol 222: 1149–1160
23. Wang B, Zhao Z, Jabusch LK, Chiniquy DM, Ono K, Conway JM, Zhang Z, Wang G, Robinson D, Cheng JF, Dangl JL, Northen TR, Yoshikuni Y (2020) CRAGE-Duet facilitates modular assembly of biological systems for studying plant-microbe interactions. ACS Synth Biol 9:2610–2615