

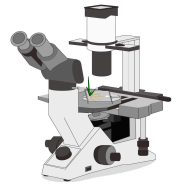


Live imaging of bacterial colonization of the plant root

(g) Bacteria-root hair interaction o
under an inverted optical micr

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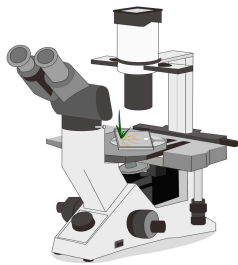
Keywords: EcoFAB, EcoFAB-N, Brachypodium, gnotobiotic chamber, phenotyping, *Herbaspirillum seropedicae*, image analysis, Roots, microscopy, bacterial colonization, live imaging, imaging steps of bacterial colonization, live imaging of bacterial colonization, plant root in the ecofab, plant root, plant root this protocol, bacteria *herbaspirillum*, root growth, bacteria, dependent dynamics of root growth, cereal model brachypodium distachyon, imaging step, root, lapse microscope, lapse microscope after removal, imaging, ammonium uptake

Abstract

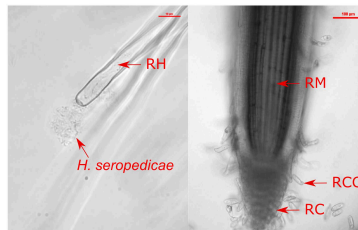
This protocol describes the live-imaging steps of bacterial colonization of the plant root in the EcoFAB-N chamber published in the study Kuang et al., "N-dependent dynamics of root growth and nitrate and ammonium uptake are altered by bacteria *Herbaspirillum seropedicae* in cereal model *Brachypodium distachyon*"

The Eco-FAB-N was imaged under an inverted time-lapse microscope after removal from the plastic enclosure that ensures sterility, and therefore although bacteria and roots can be imaged over time, this procedure is invasive.

(g) Bacteria-root hair interaction observed under an inverted optical microscope



(h) Root and bacteria cells



(g) Cellular level imaging was done on EcoFABs mounted on an optical inverted microscope. (h) *Herbaspirillum seropedicae* associated with a *B. distachyon* root hair (RH, left), and root cap cells (RCC) around a root cap (RC, right) were observed through the bases of the EcoFABs.



Materials

- EcoFAB-N bonded to a cover microscopy slide(Ted Pella, Cat No. 260462)
- Inverted time-lapse microscope (Eclipse Ti-E, Nikon) coupled with oil objective lens (Apo TIRF 100 × Oil DIC N, Nikon, Japan).
- Microscope incubation chamber, set to 24°C (Temp-Controller 2000-2, Pecon).
- CCD color camera (DS-Fi, Nikon, Japan)
- Nikon software NIS Elements AR 4.40.02.

- 1 Transfer the EcoFAB-N bonded with a cover microscopy slide to the slide holder in a microscope incubation chamber at 24°C (Temp-Controller 2000-2, Pecon).
- 2 Inoculate bacteria into root solution (here inoculation occurred at 4 days after transfer to EcoFAB-N).
- 3 Image with CCD color and software. Initially focus on bacteria then and then adjust to field shared by the bacteria and root.
- 4 Image / Record root-bacterial view over time (here recorded for 4 hours, Fig 1(h), Kuang et al.).