

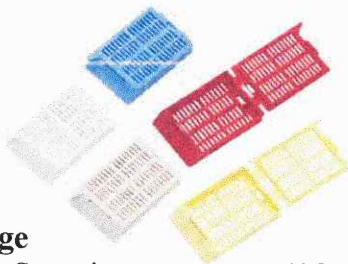
Simple Staining protocol for Arbuscular Mycorrhizal Fungi visualisation

Place the roots (approximately 1 gram) into a staining cartridge. Do not overfill. Roots should not be tightly clumped together. Loosely wrap the roots in cheese cloth to minimize loss from the cartridge. Label with **pencil** (permanent marker will get dissolved in the solutions). Make a solution of 10% w/v (weight / volume) KOH (potassium hydroxide). Make a solution of Ink-vinegar (5% v/v) and preferably use Sheaffer ink. Be sure to wear gloves and a lab coat. KOH will clear the roots, so the stained fungal tissue becomes visible under the microscope.

1. Young soft roots

The key item here is the ink that has to be **black Sheaffer ink** specifically. That was initially proposed by (Vierheilig *et al.*, 1998) but we slightly optimized the protocol as follows.

1. Pre-heat your KOH and ink vinegar solutions at 90 °C.
2. Place your cartridges into a mason jar and fill it with 10 % (w/v) KOH at 90 °C. (100g KOH in 1000 mL H₂O to prepare the solution).
3. Leave them in for 6 min (in 10% KOH at 90 °C.). You can use a water bath to maintain the temperature (or a bain marie method).
 - Alternatively, to avoid heating up the KOH the roots can be placed in KOH for up to 48h at room temperature.
4. Wash the cartridges three times with tap water.
5. Then place the cartridges in a pre-heated ink-vinegar solution and leave for 5 min at 90 °C. Use an entire Sheaffer ink bottle (50ml) and complete up to 1L with vinegar to make the solution.
 - Alternatively, to avoid heating up the ink-vinegar solution you can stain the roots in the solution over night at room temperature.
6. Then empty (but keep) the ink vinegar solution and do not wash the cartridges.
7. Place the cartridges into a jar with water with a few drops of vinegar for 25 min for de-staining. (This step removes the excess ink).
8. Rinse the cartridges until there is no more ink coming out of them.



2. Storage

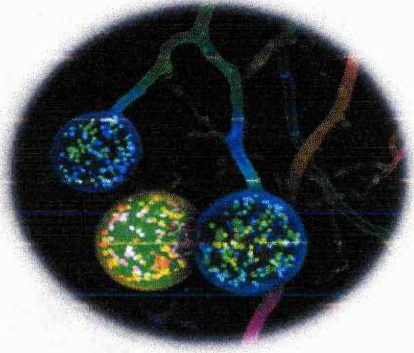
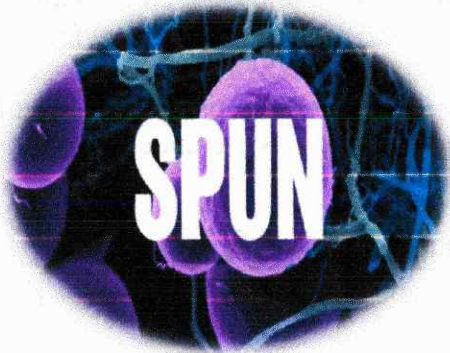
Cartridge: Store in tap water at 4°C.

Ink -vinegar solution: Once cooled, pass the ink through cheese cloth into a storage beaker. The ink solution can be used repeatedly until the solution becomes translucent. It can be stored at room temperature.

Bibliography

Vierheilig H, Coughlan AP, Wyss U, Piche Y. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* **64**: 5004–5007.

Useful links with material/contacts that can help you teach about soil microbes and plant-microbe symbiosis.



the living
Soils
workshop





Visualisation Lab

Visualisation Biology



Visualisation meets Biology

At the Visualisation Lab, ideas, concepts and data become alive with the help of computer graphics. The Virtual Reality (VR) experience that you can try out here is the result of a collaboration of the Visualisation Lab, the Communication Department of the UvA and the MiCROP project.

The biological storyline of this VR experience is the brainchild of MiCROP researchers, which has subsequently been given form by an informatics bachelor student as his thesis project, under supervision of the Visualisation Lab.

More information about the Visualisation Lab on their website: visualisationlab.science.uva.nl

What is this about?

Plant(root)s and microbes communicate using molecules that they emit into the soil.

The microbes in the soil use the nutrients that the plant produces. Microbes, in exchange, help the plant by for example protecting it against stressors (e.g., drought, insects) and by facilitating the uptake of nutrients from the ground. These nutrients are, among others, phosphate, nitrogen and potassium; all of which are vital for plants to grow.

Using fertilizers, too much phosphate ends up in the soil. This is bad for the soil and for the environment, and last but not least also for the plants. It limits among others the growth of beneficial microbes and fungi, which makes it more difficult for the plant to take up important other nutrients from the ground.

What are you going to do?

With the help of VR glasses you will dive underground. Look around and see the plant roots and microbes around you!

After a short introduction on how everything works, you will be asked to help out: help clean up the soil and water by catching phosphate molecules with a virtual net!

! Important health- and security remark: Do you have epilepsie or balance problems? Then you cannot participate in this VR unfortunately. If you are in doubt, please do not hesitate to ask on of the attendants at this station.