

# JoVE: Science Education

## Visualizing Soil Microorganisms via the Contact Slide Assay and Microscopy --Manuscript Draft--

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**PI Name:** Bradley W. Schmitz, Dr. Luisa A. Ikner, Dr. Charles P. Gerba, and Dr. Ian L. Pepper

**Environmental Science Education Title:** Visualizing Soil Microorganisms via the Contact Slide Assay and Microscopy

**Overview:**

Soil comprises the thin layer at the Earth’s surface, containing biotic and abiotic factors that contribute to life. The abiotic portion includes inorganic particles ranging in size and shape that determine the soil’s texture. The biotic portion incorporates plant residues, roots, organic matter, and microorganisms. Soil microbe abundance and diversity is expansive, as one gram of soil contains  $10^{7-8}$  bacteria,  $10^{6-8}$  actinomycetes,  $10^{5-6}$  fungi,  $10^3$  yeast,  $10^{4-6}$  protozoan,  $10^{3-4}$  algae, and  $5^3$  nematodes. Together, the biotic and abiotic factors form architectures around plant roots, known as the rhizosphere, that provide favorable conditions for soil microorganisms.

Biotic and abiotic factors promote life in soils. However, they also contribute stressful dynamics that limit microbes. Biotic stress involves competition amongst life to adapt and survive in environmental conditions. For example, microbes can secrete inhibitory or toxic substances to harm neighboring microorganisms. *Penicillium notatum* is a notorious fungus, as it reduces competition for nutrients by producing an antimicrobial, which humans harvest to create the pharmaceutical penicillin. Abiotic stresses arise from physical or chemical properties limiting microbial survival, such as light, moisture, temperature, pH, nutrients, and texture. Two common microbe groups visualized in the contact slide assay, fungi and actinomycetes, are able to survive low moisture abiotic stresses, as they are the most desiccation resistant groups.

Directly observing the interrelationships between soil organisms, particles, and behaviors within varying soil environments is difficult, but the contact slide assay, also known as the buried-slide technique, developed by Rossi et al. (1936) provides a snapshot viewpoint into soil microbiology. This method is useful for observing soil fungi, actinomycetes, and bacteria via microscopy. Although, it is not intended for microbial quantifications, as it only implies one small portion of a larger heterogeneous environment.

This method is easily performed by burying a glass slide into soil for several days, then fixing the microorganisms on the slide with acetic acid. The microbes are stained with Rose Bengal dye and observed via microscopy using oil immersion on the 100x objective. Three microbial groups can be distinguished, as bacteria appear as small rounded shapes, actinomycetes filaments as thin strings, and fungal hyphae as thick threads. The irregular dark shapes not stained are soil particles. Different nutrient amendments can be added to the soil to provide carbon and glucose sources that promote microbial growth and interactions. This technique allows for easy observation of soil microbiology and helps to identify several organisms present in the environment.

**Procedure:**

Part 1: Soil Slide Microcosm Preparation

1. Weigh 150 g soil into two separate cups.
  - 1.1. If soil has high density of organic matter, weigh 100 g.

2. Label one cup "Treatment" and the other "Control."
3. Calculate amount of water needed to alter moisture content.
  - 3.1. Moisture content is often close to field capacity.

$$\text{Soil Moisture Content} = \frac{\text{Moist Soil (g)} - \text{Dry Soil (g)}}{\text{Dry Soil (g)}} \times 100$$

4. Measure amount of distilled water with a graduated cylinder.
5. Pour amount of distilled water into two vials.
6. Label one vial "Treatment" and the other "Control."
7. Amend the water in the "Treatment" vial with enough glucose for a final soil glucose concentration of one percent, according to a dry weight basis in the "Treatment" soil.
8. Add 200 mg  $\text{NH}_4\text{NO}_3$  into the "Treatment" vial and stir to dissolve the amendments.
9. Do NOT amend the "Control" vial.
10. In small aliquots, mix the contents of the "Treatment" vial into the "Treatment" cup.
  - 10.1. Stir with a spatula after each aliquot addition.
11. In small aliquots, mix the contents of the "Control" vial into the "Control" cup.
  - 11.1. Stir with a spatula after each aliquot addition.
12. Label four clean microscope slides: two "Treatment" and two "Control" slides.
13. Insert the two "Treatment" slides into the "Treatment" soil cup.
  - 13.1. Leave 2 cm of each slide projecting above the soil surface.
  - 13.2. Ensure to leave a gap between the two slides.
14. Insert the two "Control" slides into the "Control" soil cup.
  - 14.1. Leave 2 cm of each slide projecting above the soil surface.
  - 14.2. Ensure to leave a gap between the two slides.
15. Cover the cups with plastic wrap and secure it with a rubber band.
16. Puncture the wrap several times to allow air, but still prevent excessive evaporation.
17. Record the weight of both cups.
18. Incubate the soil-filled cups at room temperature in a designated area/incubator for seven days.

## Part 2: Slide Staining and Microscopy

1. Record the weight of both cups.
2. Calculate the soil moisture at the time of slide removal.
3. Remove the two slides from each cup by pressing each slide to an inclined position and withdrawing so the upper face of the slide is not disturbed.
4. Identify and mark the side of the slide that will be stained and observed.
5. Gently tap the slides on the bench top to remove large soil particles.
6. Using a damp paper towel, clean the lower face of the slides.
  - 6.1. Dry the slides at room temperature.
7. Wearing protective goggles and holding each slide with forceps, immerse the slides into 40% (v/v) acetic acid for 1 – 3 minutes under a fume hood.
8. Wash off the excess acid under a gentle stream of water.
9. Using a dropper bottle, cover the surface of the slides with phenolic Rose Bengal (Figure 1).
  - 9.1. Support the slide on a staining rack over a container to catch the excess stain.
  - 9.2. Be careful, do not wash with force that will remove microorganisms from the surface of the slides.
10. Stain the slides for 5 – 10 minutes.
  - 10.1. *Do not let the slide become dry. Add more stain as needed.*
11. Gently wash the slides to remove excess stain (Figure 2).
12. Let the slides dry at room temperature.
13. Using the oil immersion objective, observe the slide using a microscope (Figure 3).

### Results:

The following observations are made after examination:

Fungi display thick, filamentous hyphae (Figure 4)

Actinomycetes display thin, filamentous hyphae.

Bacteria display small cocci or rod shapes. They're often found in clumps, on soil particles, or lining fungal hyphae.

Soil particles display irregular, dark shapes (Figure 5).

### Applications:

**Comment [DM1]:** This was originally step 14 in the procedure, but I rewrote it as Results. Here was how it originally arrived:

14. Examine the fungi, actinomycetes, bacteria, and soil particles.

14.1 Fungi: thick filamentous hyphae (Figure 4).

14.2 Actinomycetes: thin filamentous hyphae.

14.3 Bacteria: Small cocci or rod shapes. Often found in clumps, on soil particles, or lining fungal hyphae.

14.4 Soil particles: Irregular dark shapes (Figure 5)

We can change it back if preferred.

The contact slide assay, also referred to as the buried-slide, is a simple technique utilized to qualitatively observe soil biota. This assay qualitatively shows the spatial interactions between fungal hyphae, actinomycete filaments, bacteria, and soil particles. Individuals or industry can employ this assay to gather knowledge on a particular soil's health in regards to agriculture, gardening, composting (Figure 6), teaching, and studying. However, this technique does not quantify soil micro biota, as it only encompasses a small portrait of a larger heterogeneous environment.

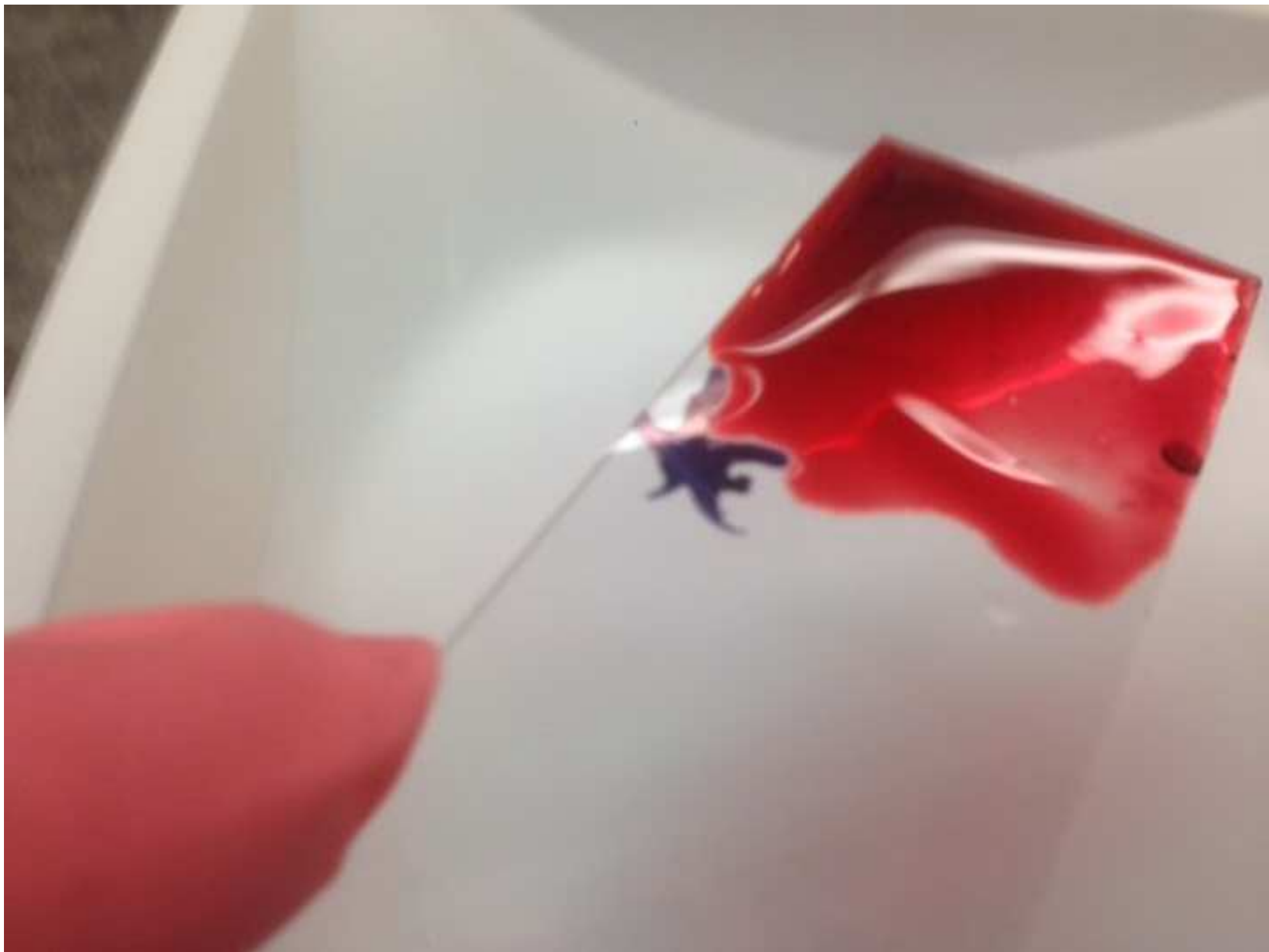
Soil organism relationships can be observed by performing the contact slide assay and viewing the results through 100x oil immersion microscopy (Figures 4 and 5). The simplicity and ease to performing this assay makes it a great starting technique for those who have never been exposed to microbiology and may be viewing microorganisms through a microscope for the first time.

#### **References:**

- Pepper, Ian L., and Charles P. Gerba. "Contact Slide Assay." *Environmental Microbiology A Laboratory Manual*. 2nd ed. N.p.: Elsevier, 2004. 19-25. Print.
- Pepper, Ian L., Charles P. Gerba, and Terry J. Gentry. "Earth Environments." *Environmental Microbiology*. 3rd ed. N.p.: Elsevier, 2014. 59-88. Print.
- Rossi, G., Ricardo, S., Gesue, G., Stanganelli, M., and Want, T.K. (1936) Direct Microscopic and bacteriological investigations of the soil. *Soil Science* **41**, 52 – 66.

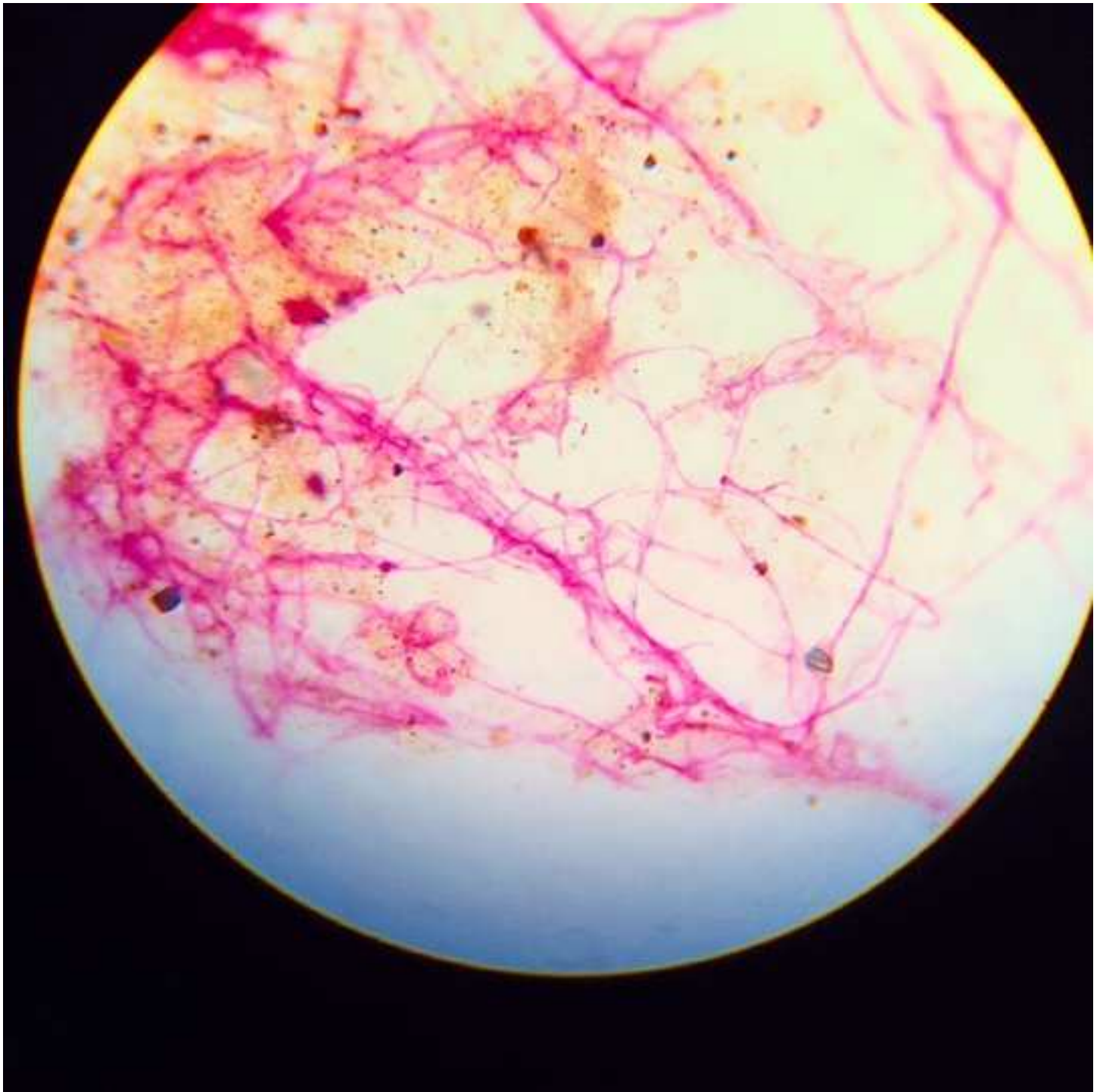
#### **Legend:**

- Figure 1: Cover the surface of the slide with phenolic Rose Bengal.
- Figure 2: Gently wash the slide to remove excess stain.
- Figure 3: A slide under a microscope.
- Figure 4: Contact slide image using 100x objective lens.  
Photo courtesy W.H. Fuller.
- Figure 5: Contact slide image using 100x objective lens.  
Photo courtesy W.H. Fuller.
- Figure 6: Composting with composted earth.  
Photo by Marina Lohrbach.

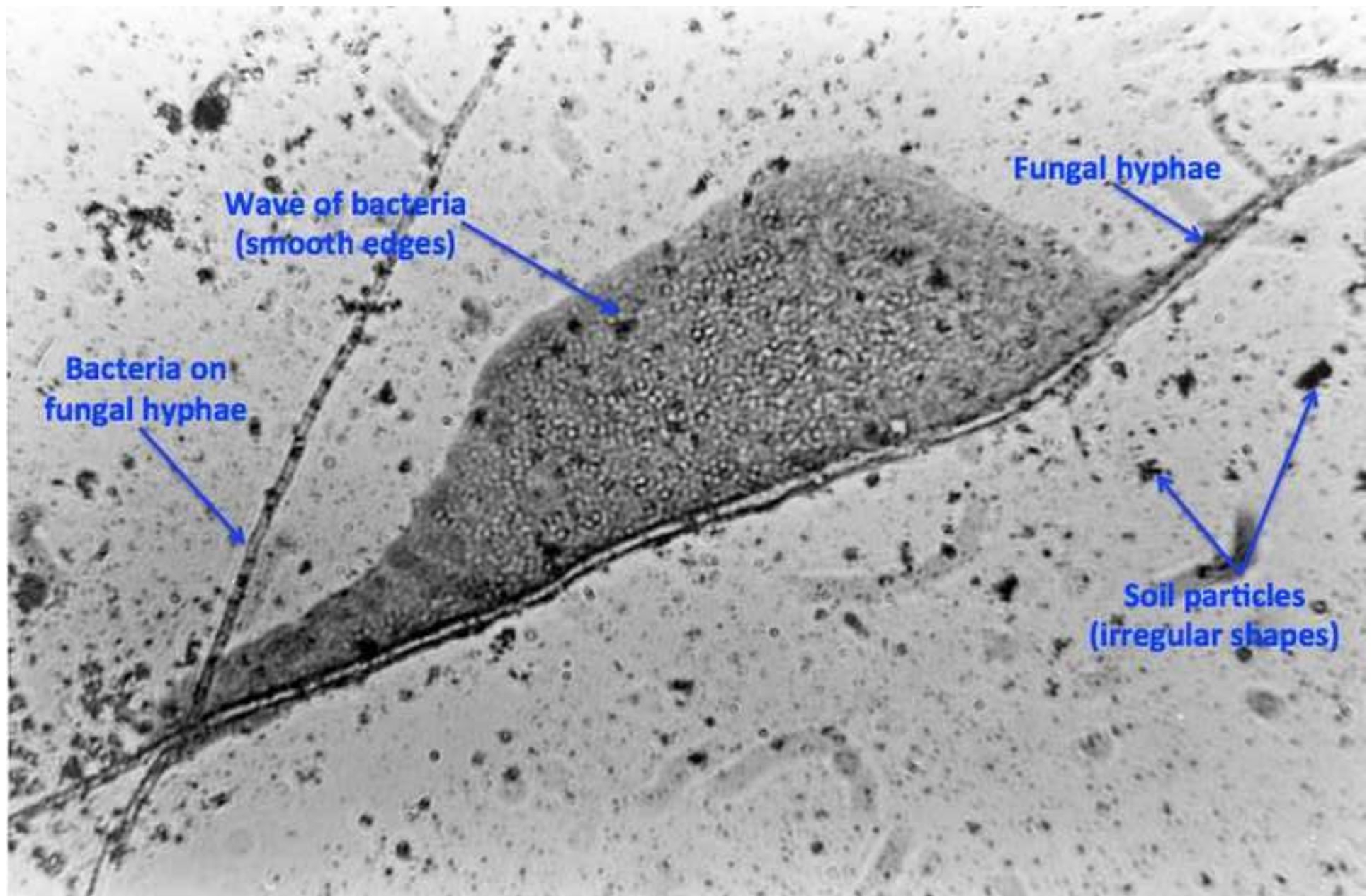


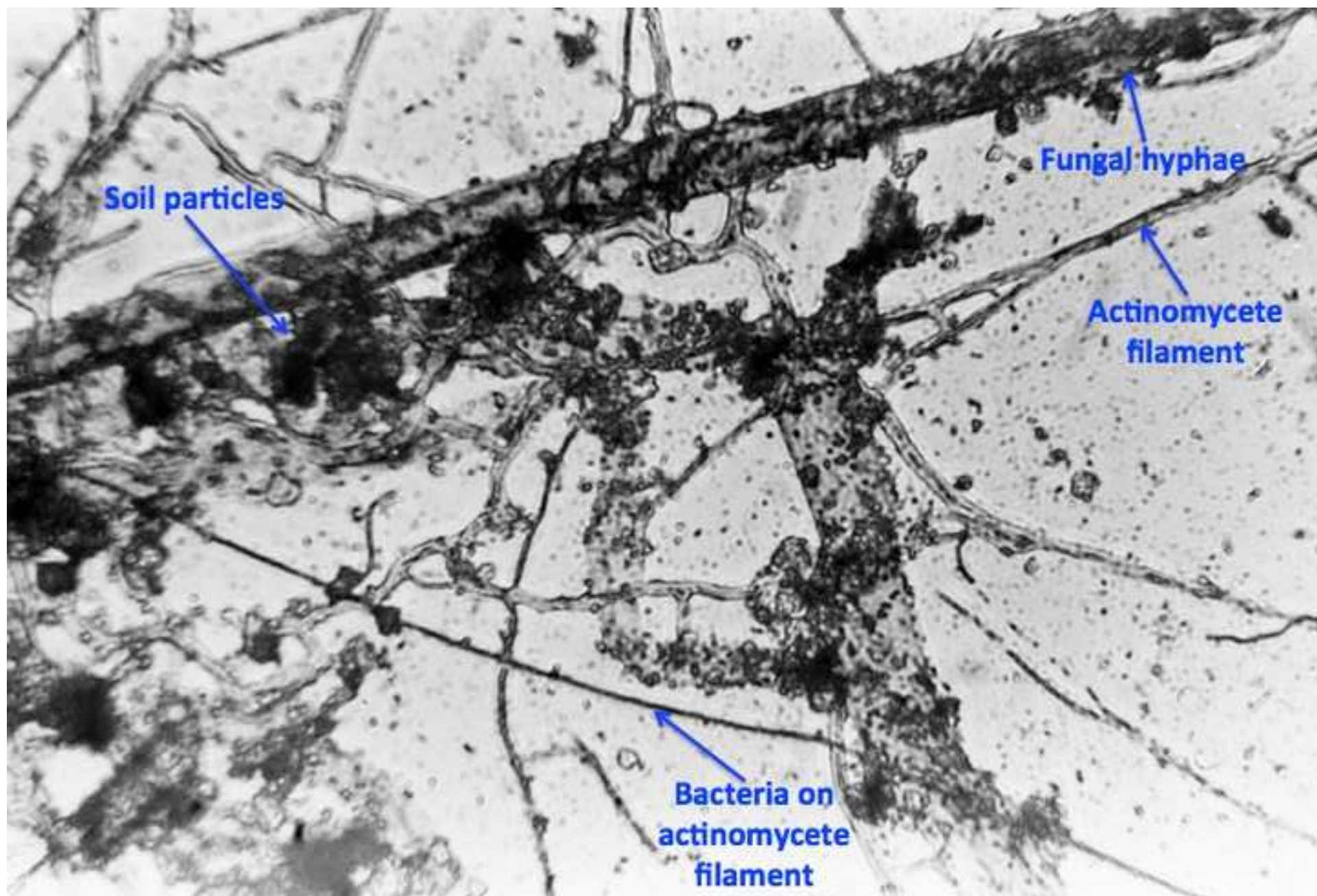
















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This method is easily performed by burying a glass slide into soil for several days, then fixing the microorganisms on the slide with acetic acid. The microbes are stained with Rose Bengal dye and observed via microscopy using oil immersion on the 100X objective. Three microbial groups can be distinguished, as bacteria appear as small rounded shapes, actinomycetes filaments as thin strings, and fungal hyphae as thick threads. In soil, almost all bacteria are smaller and rounder than those in pure culture due to nutritional stress, which causes shrinking and rounding for a more favorable surface: volume ratio. The irregular dark shapes not stained are soil particles. Different nutrient amendments can be added to the soil to provide carbon and glucose sources that promote microbial growth and interactions. This technique allows for easy observation of soil microbiology and helps to identify several organisms present in the environment.

- Comment [A1]:** Provide more information about this dye. What does it stain? What does it not?
- Comment [ILP2]:** The dye stains all microbes.
- Comment [A3]:** What about non-cocci bacteria?

## Procedure:

### Part 1: Soil Slide Microcosm Preparation

1. Collect garden soil from the surface (0-6" depth), and weigh 150 g soil into two separate cups. A good soil to use would be a garden soil collected from the surface (0-6" depth).

**Comment [A4]:** Provide more info on the soil you will be testing: Is it from the rhizosphere? What should we say about sample collection?

1.1. If soil has high density of organic matter, weigh 100 g.

2. Label one cup "Treatment" and the other "Control."

3. Calculate amount of water needed to alter moisture content.

3.1. Moisture content is often close to field capacity.

$$\text{Soil Moisture Content} = \frac{\text{Moist Soil (g)} - \text{Dry Soil (g)}}{\text{Dry Soil (g)}} \times 100$$

4. Measure amount of distilled water with a graduated cylinder.

5. Pour amount of distilled water into two vials.

6. Label one vial "Treatment" and the other "Control."

7. Amend the water in the "Treatment" vial with enough glucose for a final soil glucose concentration of 1%, according to a dry weight basis in the "Treatment" soil.

8. Add 200 mg  $\text{NH}_4\text{NO}_3$  into the "Treatment" vial and stir to dissolve the amendments. The nitrate serves as a nitrogen source of nutrients for the soil microbes.

**Comment [A5]:** What is the ammonium nitrate used for?

9. Do not amend the "Control" vial.

10. In small aliquots of approximately 50 mg, mix the contents of the "Treatment" vial into the "Treatment" cup.

**Comment [A6]:** Roughly how much is each aliquot?

10.1. Stir with a spatula after each aliquot addition.

11. In small aliquots, mix the contents of the "Control" vial into the "Control" cup.

11.1. Stir with a spatula after each aliquot addition.

12. Label four clean microscope slides: two "Treatment" and two "Control" slides.

13. Insert the two "Treatment" slides into the "Treatment" soil cup.

- 13.1. Leave 2 cm of each slide projecting above the soil surface.
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16. Puncture the wrap several times to allow air, but still prevent excessive evaporation.
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8. Wash off the excess acid under a gentle stream of water.
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  - 9.1. Support the slide on a staining rack over a container to catch the excess stain.



9.2. Be careful, do not wash with force that may remove microorganisms from the surface of the slides.

10. Stain the slides for 5 – 10 min.

10.1. *Do not let the slide become dry. Add more stain as needed.*

11. Gently wash the slides to remove excess stain (**Figure 2**).

12. Let the slides dry at room temperature.

13. Using the oil immersion objective, observe the slide using a microscope (**Figure 3**).

### **Results:**

The following observations are made after examination:

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### **Applications:**

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Figure 1: Cover the surface of the slide with phenolic Rose Bengal.

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Figure 3: A slide under a microscope.

Figure 4: Contact slide image using 100x objective lens.

Photo courtesy W.H. Fuller.

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Figure 6: Composting with composted earth.

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