

# Using Transparent Media and Time-Lapse Photography to Observe Root Growth in a Research-Focused Educational Laboratory Exercise

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Roots typically grow in the unseen depths of soil; consequently, lab students are rarely able to directly observe important structures or physiological responses such as root hairs, lateral root growth, tropism, or rhizosphere dynamics. In this lab, students will design and implement a research project that tests the effects of an environmental variable on the growth and development of root systems of *Raphanus raphanistrum* (radish). By growing study plants in transparent media, the students are able to witness root system development in real time. Students experience the process of science at all levels, from question formation to research presentation. They are guided through the manufacture of custom-made equipment, review basic concepts of photography, and create a time-lapse video of their root systems as they develop.

**Keywords: roots, botany, prototype, time-lapse, collaboration, guided inquiry, design thinking**

## Introduction

A concrete understanding of the diverse biological functions of roots is essential for a comprehensive education in plant sciences, but their use in educational laboratories is often underutilized. One reason for this is the inherent difficulty in making direct observations of roots grown in the ground or in potting soil. To observe or model the complexity of living root systems, the roots are typically excavated from their substrate (Jastrow and Miller, 1993; Neumann *et al.*, 2009). This process damages root hairs and fine roots and greatly alters the shape and dynamics of the rhizosphere. Hydroponic or aeroponic culture systems, whereby the roots are submerged in or sprayed with a liquid nutrient solution (Bucher, 2006), can be used to allow easier observation, but the natural rhizosphere is still substantially altered due to the absence of substrate (Neumann *et al.*, 2009). As a result, most lab students only get to examine roots using microscope slides. Moreover, those slides are often used as part of a larger discussion on plant tissue types or as exemplars of the stages of mitosis; not for use in studying roots, themselves. Protocols that better allow direct observation of roots can be integrated into educational laboratories in ways that allow students to study a diverse range of topics such as root morphology, meristems, water potential, nutrient uptake, tropism, mycorrhizal

associations, and much more. This lab provides students with an active, collaborative, and inquiry-based learning activity that demonstrates how roots grow and respond to environmental factors. Root growth is directly observed because plants are grown in clear glass tanks, called rhizoboxes, which are filled with Phytigel™ media, a translucent gelling agent that is commonly used in plant tissue culture (Glocke *et al.*, 2006; Sansberro, 1999; Zhao *et al.*, 2013). The activities apply elements of design thinking (Razzouk and Shute, 2012) and provide opportunities for the students to experiment, create, prototype research equipment, gather feedback, reflect on their progress, and redesign.

Initially designed for a junior-level, small-sized (<15) college course in Plant Anatomy and Physiology, this lab can be modified to work in Introductory Botany labs, major's or non-major's Introductory Biology labs, or in high school Biology classes. It is conducted as a guided inquiry research project, where an overview of the equipment and topic is presented to the students, but they must identify a specific question, formulate a hypothesis, design a methodology for testing that hypothesis, construct the equipment necessary for performing the research, and record and analyze the data. The students may then develop a presentation that culminates in their time-lapse video, they may write a paper, or both.

## Student Outline

# Testing and Observing the Effects of Environmental Variables on Root System Development

### Prelab Reading:

Review sections in your textbook and other resources that relate to root anatomy and growth. Be prepared to define and discuss the following terms: *Border cells*, *Columella*, *Gravitropism*, *Ground meristem*, *Hydrotropism*, *Lateral roots*, *Mucilage*, *Promeristem*, *Quiescent center*, *Region of cell division*, *Region of elongation*, *Region of maturation*, *Rhizosphere*, *Root cap*, *Root hairs*.

### Objectives:

- Describe and identify basic root anatomical structures
- Describe multiple environmental and biological factors that are necessary for root growth
- Predict how roots would respond to changes in environmental or biological factors
- Design an experiment testing the effects of environmental factors on root growth
- Explain why prototyping can be a vital step in the scientific process

### Skills Acquired:

- Prototyping
- Glass working
- Photography
- Videography

In this lab you will design and conduct an experiment that explores the effects of an environmental factor on the root growth of *Raphanus raphanistrum* (radish). You will also acquire a basic understanding of time-lapse photography and create a video of a radish root system growing. You will grow radishes in a translucent, nutrient-rich media (Phytage<sup>TM</sup>) that, unlike soil, will allow you to easily observe the roots as they grow and extend throughout the plant's substrate. Each group will design and construct two (one for the experimental group and one for the control group) glass observation tanks (called rhizoboxes), will mix its own Phytage<sup>TM</sup> substrate, and will record data at the end of the experiment. Ultimately, each group will pool its data with the rest of the class; therefore, between-group communication will be necessary to ensure replicability. The groups will also take turns maintaining the equipment necessary for capturing time-lapse photography of the roots as they grow, and each group will use the images to construct a final video. Depending on the growth rate of *R. raphanistrum*, this experiment may last up to six weeks, but the majority of the work will occur during the first and second week.

## Part I. Experimental Design

### *Selecting a Variable*

Discuss, in your groups, several types of environmental variables that potentially affect how roots grow. List some of your ideas in table 1, and put asterisks next to the two ideas your group has the most interest in pursuing. Also, discuss how you could manipulate these variables in the lab to measure their effects. After sufficient group discussion has occurred, decide on a single variable to be measured by the whole class and what type of methodology would be necessary to explore their effects. For example, if you wanted to study how a root responds to a barrier (such as a rock) in the substrate, the methodology would involve partially filling the rhizobox with substrate to the desired barrier depth, letting it solidify, adding the barrier, finish filling the rhizobox, then growing the plant above the barrier in the tank.

### *Data Analysis*

Review in your groups, and then with your instructor, the most appropriate way to collect and analyze your data. The methods you choose will be dependent upon what variable you manipulate and the overall methodology of your project. You may choose to simply weigh dry mass, or you may want to incorporate some morphometric analyses that help you model the shape and magnitude of root growth. As you answer the questions below, provide a rationale for the decisions you make regarding your methodology.

**Table 1.** Environmental variables that could potentially affect root growth.

Growth Variables		
1.	5.	9.
2.	6.	10.
3.	7.	11.
4.	8.	12.

Questions:

1. What variable did your class decide to study? Explain why you selected that variable, and include some rationale addressed during the class discussion.

2. State your class’s research question.

3. State your class’s hypothesis and include a short discussion of your rationale.

4. Briefly describe your methodology. Include in your description how you will set up both your control and your experimental rhizoboxes.

5. How will you collect data on this experiment? Be specific and provide a clear rationale for your approach.

6. What types of analyses will you use and why?



## Part II. Preparing the Experiment

Now that you have a working prototype, it is time to make your two rhizoboxes.

### *Building the Rhizoboxes*

1. Using a thin-tipped marker and a ruler, draw lines on the glass panels to mark places where cuts should occur. Each cut should run from one edge of the glass to the other, so plan the cuts accordingly. You cannot make cuts that are 90° angles.
2. Lay a straight edge close to the line and position your cutter over the line so you know where to position the straight edge as a guide. Have one group member hold the straight edge securely in place while another member prepares to score the glass.
3. Dip the cutter in the oil. The oil creates a smoother score line.
4. Holding the cutter as you would a pencil, apply pressure to the glass as you score the line along the glass sheet. Listen for a smooth, light sound; not a gritty, rough sound. A gritty sound means that you are either applying too much pressure or that you need more oil, and usually results in poorly cut edges or shards that break off when snapping the glass. Also, make the cut in one smooth motion. Do not stop or run the knife backward and forward.
5. Check your score line. It should resemble a small scratch, barely visible, and it should run continuously from one edge to another.
6. Position the line that you just scored along the edge of your desk. The glass that you want to break off should be hanging off the desk. The rest of the glass should be secured on the desk by one of your hands or by other group members.
7. Break the glass along the scored edge by gently pushing down on the glass hanging over the edge. Apply light pressure at first, and then increase the pressure slowly until the glass breaks along the line. If you do this too quickly or with too much initial force, it could result in an uneven break.
8. If the glass didn't break smoothly, you can straighten the edges using the glass-cutting tool or sandpaper.
9. Be careful of any small glass shards that may have broken off. Make sure you sweep them into a dustpan and dispose of them appropriately in the glass disposal box.
10. Continue this process until you have cut all the glass panels for your rhizobox.
11. Glue the panels together using the glass epoxy.
12. Use books or other items to help hold the panels in place while the epoxy dries.
13. Repeat the procedure to make the second rhizobox, and let both sit overnight to allow the epoxy to dry.
14. Once the epoxy has dried, you need to run additional epoxy along each seam to seal the junctions. Do this by running a bead of epoxy on the inside of the tanks along each seam and gently spreading it out using a gloved finger. Dispose of glove when finished.
15. Allow the rhizoboxes to dry.
16. Fill with water to test for leaks. If leaks are found, reseal using the method above. If no leaks are found, pour water out and let the rhizoboxes dry. They are now complete.

*Making the Substrate*

1. Based on the dimensions of your two rhizoboxes, calculate the total volume of substrate solution you will need, as if you were going to completely fill both tanks.
2. Add this volume of water to a large beaker.
3. Make a 0.43% solution of Murashige and Skoog nutrient water by multiplying the volume of water you used in the first step by 0.0043 and adding that mass, in grams, of Murashige and Skoog powder to the beaker containing your water. Students must do your own calculations, though you may confirm them with your instructor.
4. Adjust to pH 5.7 using 1M KOH or 1M HCl, accordingly.
5. Make a 2% Phytigel™ solution using the nutrient water from step 3. Multiply the volume of water you calculated in the step 1 by 0.02 and add that weight, in grams, of Phytigel™ powder to the beaker containing your nutrient water.
6. Heat to boiling and stir until all the Phytigel™ powder has liquefied. Alternatively, you can autoclave for 20 minutes at 121 °C, 15 PSI, or microwave until it is at a rolling boil.
7. Allow to cool until container can be held with bare hands. Note that if you pour hot solution into the tanks before it has sufficiently cooled it may melt the adhesive causing leaks, or worse.
8. Set up your control rhizobox by filling it with substrate solution to about two centimeters from the top of the tank and allow it to solidify at room temperature.
9. Set up your experimental rhizobox by adjusting the variable under consideration according to your methodology in Part I. Add substrate to the tank and allow it to solidify at room temperature.

**Part III. Executing the Experiment**

Now you are ready to grow your radish plants. The only thing you will need to worry about is providing them sufficient light, as the substrate will supply the water and nutrients necessary for growth.

*Setting Up the Experiment*

1. Place three *R. raphanistrum* seeds onto the surface of the substrate near or against one of the edges of the rhizoboxes.
2. Place the rhizoboxes under grow lights. If your grow lights have a timer, make sure you set it to 24 hours of light or it will cause problems for the exposure of your images when the lights turn off.
3. Once seeds have germinated, thin to one seedling.

Watching your seedlings grow in a transparent media will allow you to see, firsthand, characteristics of root growth. However, watching it using time-lapse photography may allow you to recognize patterns that you likely would have missed, otherwise. For this component of the lab, review basic concepts of digital photography such as aperture, shutter speed, and ISO, or listen as your instructor teaches you how these attributes of photography work together to achieve the correct exposure. Having a working knowledge of photography will provide endless benefits throughout your biological careers.

*Preparing the Time-lapse Photography*

1. Attach the camera to the tripod and position it in front of the rhizobox you wish to photograph.
2. Make sure all settings are on manual (manual focus, manual aperture, manual shutter speed, etc.).
3. Activate **Live View** and frame your picture. Make sure the camera is not too close or too far away. You want to be as close as possible to capture as much detail as you can, but as the plant grows you don't want the root system to grow off the frame of the picture. Make sure the camera is back far enough that you won't have to move it while

photographing, or the resulting video will not be as smooth. During video processing you can zoom into select regions of the images to get a closer look at the root as it grows, but you can't zoom out farther than the field of view you set during this step.

4. With the room lights off (but grow lights on), take a few trial pictures to test your exposure and focus. Zoom into one of the pictures so you can see, precisely, where the focal point is and verify that it is set where you want it. Continue to manually fine tune the camera's exposure settings and focus until you are happy with the resulting images. Make sure you delete these photographs afterward so they don't inadvertently get added to the video later.
5. Turn off **Live View**. Interval Timer Shooting isn't available for Live View photography, and it must be turned off in order to continue through the next series of steps.
6. Press the **Menu** button on the camera and select **Photo Shooting Menu**.
7. Highlight **Interval Timer Shooting** and press the **OK** button.
8. Under **Start options** select **Now**.
9. Under **Interval** change the middle number to read **00 : 05' 00"**.
10. Set **No. of Intervals x shots/interval** to some high number such as, **1152 X 1**. You have now told the camera to take a new picture every five minutes until it has taken 1152 pictures.
11. Under **Exposure smoothing** select **OFF**.
12. Scroll back up to the **Start** option and press the **OK** button to begin taking pictures.
13. Periodically, you will need to turn the camera off while you either charge the battery or while you transfer all the pictures to a hard drive to make room for more. Try to accomplish both activities at the same time to minimize interruption of the time-lapse sequence. Repeat steps 6 through 12 to return to photographing the roots. Note: it is important that the camera not be moved from its position as you remove the data card or the battery, or it may result in a choppy video if you fail to get it precisely back in its initial position.
14. Groups should take turns recharging and resetting the cameras needed, depending on the camera's battery life and storage capacity. Plan ahead for weekends.

### *Constructing the Video*

1. Make sure all the images are stored in one folder.
2. Open Adobe Bridge, navigate to the folder, and choose **Edit>>Select All**.
3. Right click on any of them, and select **Batch Rename** from the drop down menu
4. Select **Rename in same folder**.
5. Under the **New Filenames** section, click on the first drop down menu and select **Text**. In the box to the right, type in text according to how you want to name your images (ex. "timelapse" or "root").
6. Click on the next drop down menu below **Text**, and select **Sequence Number**. To the right of this box type in the numeral "1" and then select **Five Digits** from the drop down menu to the right of that.
7. Delete any other boxes in this section by clicking on the minus sign on the far right of the boxes.
8. Click on **Rename** in the upper right corner of that window. You just batch-renamed all your files so that their file-names appear with a numerical sequence corresponding to the order in which they were created.
9. Open Photoshop and choose **File>>Open**.





## Materials

### For Group of 4 Students

Gloves  
 Safety goggles  
 Forceps  
 Paper  
 Tape  
 Scissors  
 Sharpie  
 Ruler  
 (2) Glass sheets (11 x 14 inch; Home Depot)  
 Glass cutting oil  
 Glass cutting knife  
 Sandpaper  
 Glass epoxy (PC Super Epoxy from Home Depot)  
 Large beaker (at least 2 L)  
 1M KOH  
 1M HCl  
 Hotplate with stir capabilities, or a separate stir box  
 Magnetic stir bar  
 Radish seeds

### Shared by Class

Phytigel™ powder (20 g/L of substrate; can be purchased in 100 g container or larger)  
 Murashige and Skoog Basal Medium (4.3 g/L of substrate; can be purchased in 21.5 g container or larger)  
 Autoclave (optional)  
 Microwave (optional)  
 Camera with Interval Shooting capabilities  
 Sturdy tripod  
 Set of grow lights with enough space to accommodate experiment  
 Computer with Adobe Photoshop and Adobe Bridge

## Notes for the Instructor

### Logistics

Though the majority of the work will be accomplished during the first two weeks, the activities presented in the lab as a whole require approximately six weeks, depending on the growth rate of the plants. However, this can be tailored to a shorter or longer period of time to accommodate the level of detail desired by the instructor or the methodology developed by the students. For example, some instructors may wish to spend an entire lab period teaching basic photographic technique or video editing; and some students may require longer periods of time be devoted to data collection, such as when tissues need to be desiccated for use in measuring dry weights.

Also note that the sophistication of the analytical techniques will depend on the breadth of knowledge of the

instructor, but don't be too hesitant to require the students to explore methods other scientists have used to measure similar types of data, even if they are not within your sphere of experience. For additional reading, Ennos (1985), French *et al.* (2009), Karl and Doescher (1991), Richards (1984), and William *et al.* (2001) are all good articles that may be of use to the instructor or to the students while developing methodology for data collection and analysis.

Below is an overview of the activities that correspond with the four parts of the lab (table 2), and a review of the timeline used by the author.

**Table 2.** Overview of activities for root growth investigation.

Part #	Activities
I. Experimental design	Deciding on a variable
	Building a prototype
II. Preparing the Experiment	Building the rhizoboxes
	Making the substrate
III. Executing the Experiment	Setting up the experiment
	Preparing the time-lapse photography
	Constructing the video
IV. Data collection and presentation	Collecting data
	Making the presentation

### *Week One: Design and Build*

The instructor summarizes the nature of the research project and informs the students that they will be designing and maintaining the projects themselves. Though it is summarized in the student handout, the instructor may also wish to explain that they will be building and using rhizoboxes, and that they will be growing plants in transparent media containing all the nutrients needed for plant growth (Murashige and Skoog, 1962) so that they can observe and record root growth as it occurs. Students then work on developing their research design, manufacturing their prototypes, reflecting on the preferred dimensions, and constructing their rhizoboxes. They will need to return to the lab at least two additional times this week to seal and test the rhizoboxes, respectively. These activities do not take more than ten minutes each, and the students are typically excited about checking on the progress of their work.

### *Week Two: Learn Photography and Deploy Experiment*

After the students get their substrate poured into the rhizoboxes, the instructor may spend time reviewing basic photographic technique. The author shows a PowerPoint presentation that teaches the students the basic anatomy of a digital single-lens reflex (DSLR) camera and how to get the correct exposure by adjusting aperture,

shutter speed, and ISO. The author invites students to bring their own DSLR's this week to practice what they learn.

### *Week Three and Beyond: Executing the Experiment*

At the beginning of class on the third week the students plant their seeds into the rhizoboxes and initiate the time-lapse protocol for the camera. The students also need to schedule when and which groups will be responsible for maintaining the photographic equipment. This does not take a whole lab period, so be prepared to start whatever lab you have planned next in your sequence. At least one more lab at the very end of the project will be devoted to data collection and analysis.

### **Pooling Class Data**

For classes of moderate to small size, it is necessary that the groups pool their data to be better positioned to conduct meaningful analyses. The instructor can use this as an opportunity to review sources and types of statistical error as well as the need for replicates. However, in classes of larger size, or classes that have multiple sections, it may be advantageous to allow different groups to study different aspects of root growth by testing different variables. All the students in one lab may not agree on what constitutes the most interesting variable to test, and this would allow for more flexibility in researching to their own interests. This would likely be more interesting for the instructor, as well.

### **Potential Technical Issues**

#### *Camera Equipment*

The protocols above are for use with a Nikon camera that has Interval Timer Shooting capability. Most other mainstream camera brands have something similar, though their menu items are different. The respective user's manuals will identify the necessary steps to use those cameras. Special note: many cameras have a devoted time-lapse program. However, the author finds them too limiting, as they only allow the user to make short videos that are taken over the scope of a few minutes to hours, not over the scope necessary for this project.

#### *Resetting the Camera*

Pausing the time-lapse while the battery charges can be problematic for the resulting video, particularly if it takes much time. This can be eliminated by using an extra battery or by plugging the camera directly into an outlet. Some camera models can also be tethered directly to a computer, eliminating the need to clear the storage disk.

#### *Different Photoshop Versions*

The protocols above are for use with Photoshop CC, but the ability to edit video was introduced in Photoshop CS6 and has carried over into all new versions. The instructions should be quite similar. Also, there are many

other programs (some free) that allow you to stitch images together to make a video, and those would likely be preferable when Photoshop is unavailable to an institution. One highly popular program that can do all of these things is Image J, which is developed and distributed for free by the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation.

#### *Problems with Narrow Tanks*

If a rhizobox is too narrow to reliably stand up on its own, have the group engineer a wider base or glue a wider piece of glass to the base to eliminate this issue. There is usually enough scrap glass left over to build a base.

#### *Contamination*

The media that is used in this project supplies everything a plant needs to grow, but it also supplies everything bacteria, fungi, and algae need as well. Because of the nature of the lab, contamination is not of great concern and it has never been an issue, particularly if you cover the tops of the tanks with a glass cover or petri dish until the plants outgrow the tops of the rhizoboxes. Additionally, the instructor may discuss this concern with the students, and may even incorporate a smaller experiment determining an optimal seed-sterilization technique before planting.

#### *Safety*

Working with glass does present a safety hazard, and a few precautions should be emphasized to the students.

1. Safety goggles and appropriate foot wear must be worn at all times
2. Students must verify that their glass is scored before they attempt to snap the glass. They will see a thin line stretching the length of the glass
3. Never force the glass to break. If it is properly scored, the glass will cleanly snap with minimal effort. If too much force is being used it means the glass was not scored appropriately, and when the glass does finally break it will result in sharp edges and may even stab the student. The instructor will get an idea of how much force is necessary very quickly. It doesn't take much.

#### *Alternatives to Building Rhizoboxes in the Lab*

Prototyping and building of the rhizoboxes is a fundamental element of this laboratory exercise, as it immerses the students in design thinking (Razzouk and Shute, 2012) and cooperative methodologies. However, there are some situations where safety may play an even greater concern than with traditional undergraduate students, or when there is simply not enough time to have the students do this part of the

exercise. In these cases, you can provide your rhizobox dimensions to any glass maker and they will gladly (and with extreme precision) build the boxes for your class. A week is usually more than enough time for one or two boxes, but for larger numbers give the glass worker additional preparation time.

## Glossary

*Border cells* – rootcap cells that have separated from the rootcap but remain in the rhizosphere. They may remain alive for several weeks and begin producing and secreting specific proteins that benefit the growing root.

*Columella* – the central region of cells in a rootcap

*Gravitropism* – the response of a shoot or root to a gravity field

*Ground meristem* – the primary meristem that gives rise to the ground tissue

*Hydrotropism* – the response of a shoot or root to water

*Lateral roots* – a root that develops out of another root. Sometimes referred to as secondary roots

*Mucilage* – a polysaccharide-rich secretion of the rootcap

*Promeristem* – the least differentiated precursor cells in an apical meristem that give rise to all other cells

*Quiescent center* – a region of the apical meristem that is relatively inactive. Functions as a set of reserve cells if the promeristem is damaged

*Region of cell division* – section of cells along a root where mitosis is most active

*Region of elongation* – section of cells along a root where cell division has slowed, but cell size continues to increase

*Region of maturation* – section of cells along a root where cell size and remains stable, but other specialization (such as root hairs) begin to develop

*Rhizosphere* – the area of soil around a root that is affected by the root

*Root cap* – a mass of cells that covers the growing tip of a root

*Root hairs* – outgrowths of root epidermal cells that significantly increase the surface area and absorptive capacity of the root

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