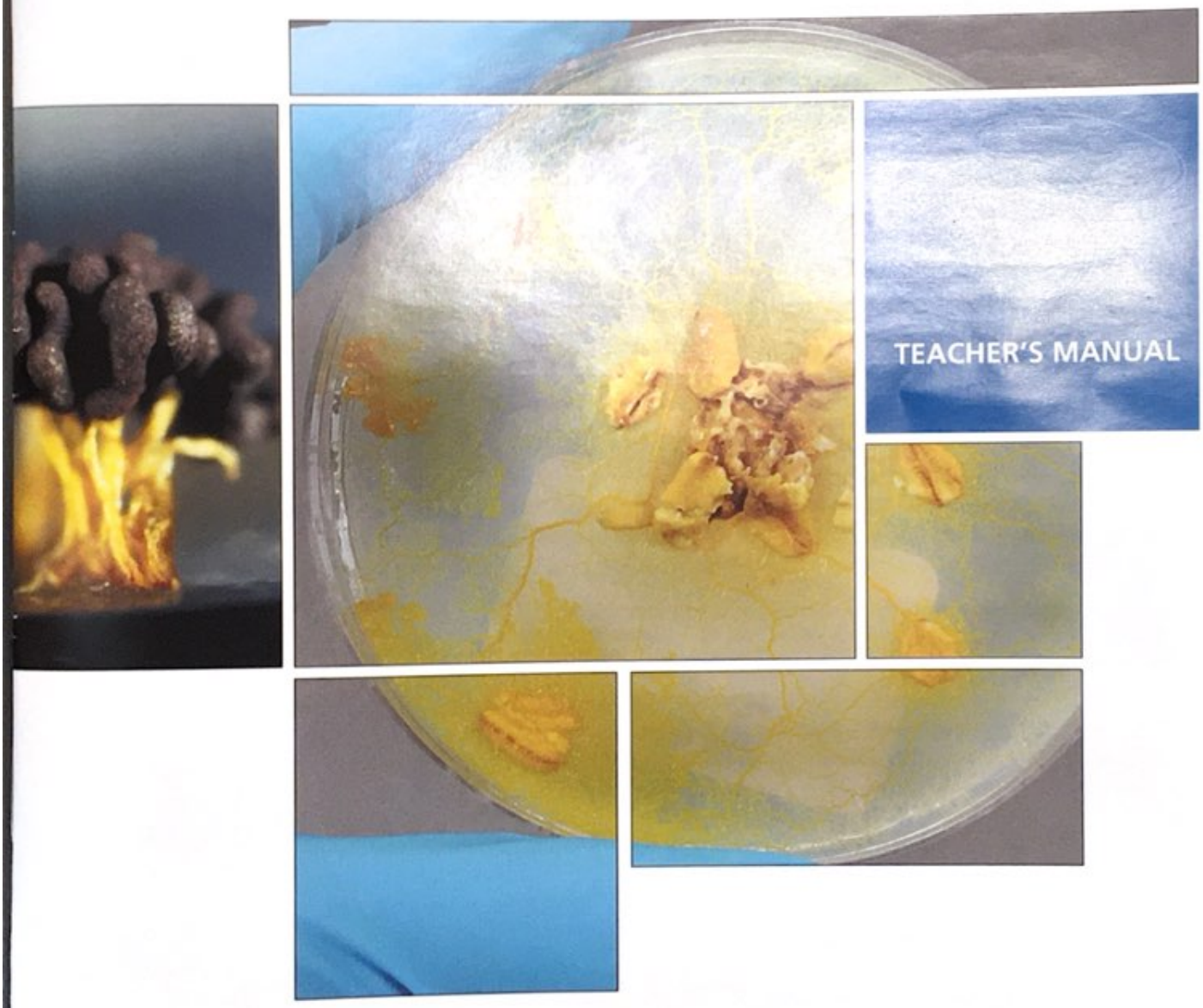
Carolina™ Physarum Manual



CAROLINA®

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TEACHER'S MANUAL

Photocopy portions as needed for use in your classroom.

Carolina™ Physarum Manual

Overview

The slime mold *Physarum polycephalum* is an intriguing organism that can be used for hands-on introduction of a number of subjects or for students' independent investigations. This manual accompanies four different Carolina *Physarum* products and includes instructions for the preparation of plates, science background on *Physarum polycephalum*, ideas for student investigations, questions, and an appendix regarding sterile technique. Your background, your students' background, and your intended use of the materials will determine whether and how you use the information provided.

Prerequisite Knowledge and Skills

- working knowledge of sterile technique
- basic knowledge of cell structure
- microscope skills
- mitosis and meiosis
- familiarity with the concept of cytoskeletal structure
- familiarity with the concept of a life cycle

Safety

Use this kit only in accordance with established laboratory safety practices, including appropriate personal protective equipment (PPE) such as gloves, chemical splash goggles, and lab coats or aprons. Ensure that students understand and adhere to these practices. Know and follow all federal, state, and local regulations as well as school district guidelines for the disposal of laboratory wastes. Students should not eat, drink, or chew gum in the lab and should wash their hands after entering and before exiting the lab.

Download Safety Data Sheets (SDS) at **carolina.com/sds** or scan this code:

Melted agar can be extremely hot, and will stick to skin and other surfaces. Avoid thermal burns by avoiding contact with melted materials. When pouring plates, use a heat-resistant glove.

Physarum polycephalum is not pathogenic under normal circumstances. However, treat all microorganisms as potential pathogens. Sterilize any used plates and other materials that have come into contact with the *Physarum* by autoclaving (40 minutes at 121°C and 15 lb per square inch of pressure) or disinfecting by covering in a solution of 1 part household bleach to 9 parts water and soaking for 2 hours. Disinfect all work surfaces and wash hands before and after working with *Physarum*.

Digital Resources

The Physarum Culture Kit (155825) and the Physarum Review Set (155774) include a digital Teacher's Manual with hyperlinks to the following resources. Additional resources may be available. To use these resources, log on to the website below and enter your access code. See the Digital Resource Instruction Card for more information.

http://www.carolinascienceonline.com

RESOURCE	DESCRIPTION
Fill-in Answer Sheet	A PDF that can be printed out or assigned digitally, with spaces for students to record their answers.
Physarum Life Cycle Sheet	Physarum Life Cycle Sheet PDF for printing
Appendix on Sterile Technique	Information on Sterile Technique as PDF for printing
Video	Video of cytoplasmic streaming in *Physarum polycephalum*
Editable Questions	The questions as a Microsoft® Word document
Whiteboard Resources	Color graphics and photos for use with whiteboards

Materials

If the product you purchased includes a digital Teacher's Manual, see the Digital Resource Instruction Card for more information.

Included in the kit:	*Physarum* Culture Kit (155825)	*Physarum polycephalum* Sclerotium (156190)	*Physarum polycephalum* Plasmodium Plate (156193)	*Physarum* Review Set (155774)
plate culture of *Physarum polycephalum* (plasmodium)	1		1	1
box of 5 *Physarum* sclerotia	1	1		
bottle of sterile 2% agar	4			
package of oat flakes*	2	1		
sterile petri dishes	20			
disposable sterile scalpel	5			
autoclave bag with instructions and twist tie	1			
sterile water (rehydration medium)	1			
Digital Resource Instruction Card	1			1
Teacher's Manual	1	1	1	1

Needed, but not supplied:	*Physarum* Culture Kit (155825)	*Physarum polycephalum* sclerotium (156190)	*Physarum polycephalum* Plasmodium Plate (156193)	*Physarum* Review Set (155774)
Bunsen burner	X	X	X	X
ethanol, isopropyl alcohol, or other disinfectant for cleaning work surfaces and items	X	X	X	X
sterile oat flakes* or other sterile food source			X	X
sterile petri dishes or other sterile growth chamber		X	X	X
sterile distilled or deionized water		X		
sterile 2% agar or other sterile growth media		X	X	X
autoclave bag	X	X	X	X
scalpels or spatulas that can be sterilized		X	X	X
stereomicroscopes	X	X	X	X
heat-resistant glove for handling 2% agar	X	X	X	X

*Use "old-fashioned" oats. "Instant" or "quick-cooking" oats will not work.

Preparation

1. Photocopy any instruction sheets needed for your students.
2. Gather the materials that are needed but not supplied.
3. The *Physarum* Culture Kit includes a bottle of prepared, sterile 2% agar and 100-mm sterile petri dishes for making 2% agar plates. Pour the 2% agar plates as described in the following steps. Use sterile technique while handling the agar and pouring the plates.

   Note: To prepare your own 2% agar, add 2 g bacteriological agar to 100 mL distilled or deionized water and autoclave for 20 minutes at 121°C under 15 pounds pressure per square inch (psi).

   a. To melt the 2% agar that came with the Culture Kit, use either a boiling water bath or a microwave oven as follows:

      To melt the agar in a hot water bath, slightly loosen the cap and set the bottle of 2% agar into a beaker of boiling water on a hot plate or in a boiling water bath. Make sure that the boiling water stays at or above the level of the agar in the bottle. Hasten melting by occasionally swirling the agar as it melts. The agar should melt within about 30 minutes. This time does not include the amount of time it takes to boil the water. Do not place cold bottles into the water bath. To completely eliminate the chance of the glass cracking, place the bottles in the water bath or beaker of water before heating the water.

      To melt the agar in a microwave oven, slightly loosen the cap before placing the bottle in the microwave. To prevent boiling over watch carefully and swirl the bottle every minute or so as it heats. Depending upon the power of the microwave it will take 5–15 minutes to melt the agar using this method.

   b. Allow the agar to cool to approximately 55°C either by allowing the boiling water to cool or by letting the bottle sit for 5–10 minutes at room temperature. If you cool the bottle at room temperature swirl the bottle

occasionally as it cools. At around 55°C the bottle should feel comfortably hot to the touch, but you will still need a heat-resistant glove to protect your hand when holding the bottle for any length of time.

c. While you are melting and cooling the agar, prepare a work surface away from drafts or breezes. Wipe the surface with a disinfectant such as 10% bleach or 70% alcohol.

d. Wash your hands thoroughly. Open the petri dish sleeve, being careful not to open the lids of the plates and thus introduce contamination. Align the sterile plates along the edge of your work surface.

e. Once the agar has cooled, pour the plates. Lift the lid of each petri dish just enough to pour in the melted agar. Pour each plate to a depth of about 5 mm. Replace the lid immediately after pouring to prevent contamination. Each plate requires 22–25 mL. Thus, each bottle will make approximately five plates.

f. Allow at least 30 minutes for the plates to solidify before moving them.

g. Store the plates in a plastic bag or sleeve at room temperature or in the refrigerator.

Science Background

Introduction

The slime mold *Physarum polycephalum* is found in nature in a variety of environments, but most commonly in cool, humid, dark places such as leaf litter or other organic debris in forests. Classification of *Physarum polycephalum* has long challenged scientists because slime molds possess characteristics that cross typical taxonomic boundaries. Thus, depending on the reference, you may find *Physarum polycephalum* placed in the *Amoebozoa*, or the *Mycetozoa*, or, at the class level, as a myxomycete.

Physarum is an intriguing organism that can be used to introduce, discuss, or reinforce many subjects, including life cycles, **mitosis**, **meiosis**, **sexual** and **asexual reproduction**, **cytoplasmic streaming**, **chemotaxis**, basic navigation, simple decision making, mechanisms for survival in stressful environments, cell structure, and developmental biology.

Physarum has been used as a model organism for studying many processes, including **cell differentiation**, **cell cycle regulation**, **mitosis**, **meiosis**, **cytoskeletal rearrangement**, and **cytoplasmic streaming**. *Physarum*'s peculiar life cycle includes several different forms. Its transformations involve processes similar to those seen during development in more complex organisms. Because of this, *Physarum* interests developmental biologists. One area of investigation currently is the analysis of the specific genes that are transcribed as the organism changes from one form into another.

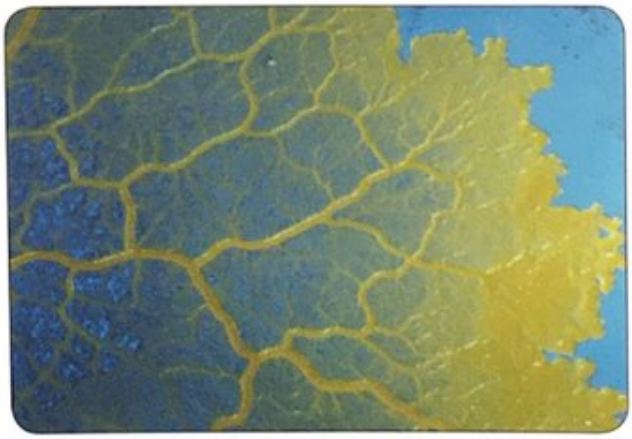
In addition, *Physarum*'s ability to navigate using relatively complex strategies to find food and to form networks between different sources of food has drawn the attention of people outside traditional areas of biological research, including those who study networks and artificial intelligence.

Life Cycle

During its life cycle, *Physarum polycephalum* changes into different forms, some of them **haploid** and others, **diploid**. Some forms are specialized adaptations to survive harsh conditions. The organism's transition from one form to another is most often triggered by the conditions of its environment, including the presence or absence of other *Physarum*. A *Physarum* Life Cycle sheet is included as an appendix to this manual.

Plasmodial Form

Physarum is most often used in science classes in its diploid, **plasmodial** form. A plasmodium exists as a single large cell containing multiple diploid nuclei that replicate their DNA and divide synchronously. A single **plasmodium** may contain more than 10^{10} nuclei. Genetically identical plasmodia are capable of fusing together. In the laboratory, plasmodia have been grown to a diameter of more than 30 cm. A plasmodium moves using a flowing motion and feeds by means of **phagocytosis**. *Physarum polycephalum* and other plasmodial slime molds should not be confused with the **cellular slime molds**, which exist as individual cells that come together in response to certain chemical signals to behave as a single organism.



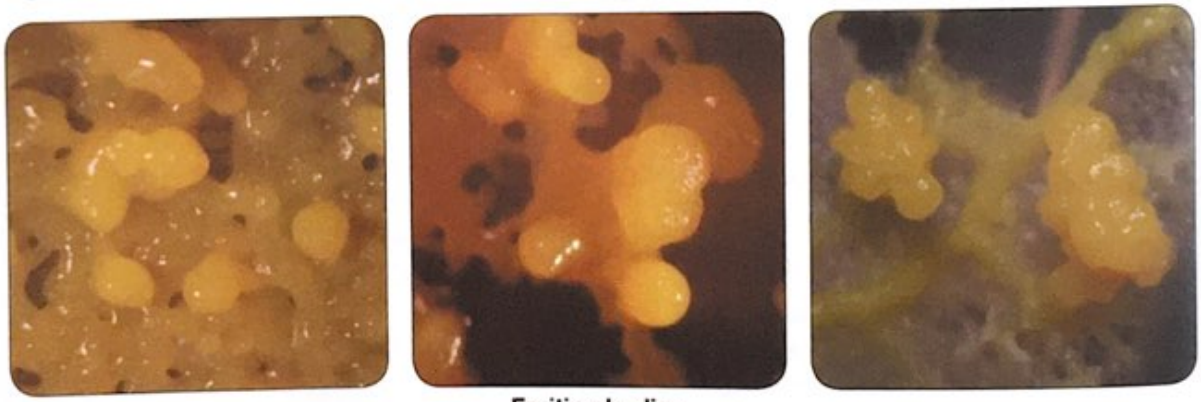
Sporulation

When a plasmodium is starved and then exposed to light it may **sporulate** (produce spores). In nature, sporulation has been observed after a plasmodium climbs out of leaf litter. **Starvation** and subsequent light exposure alone are not sufficient to trigger sporulation. Also required is the presence of niacin or niacinamide, along with other factors,

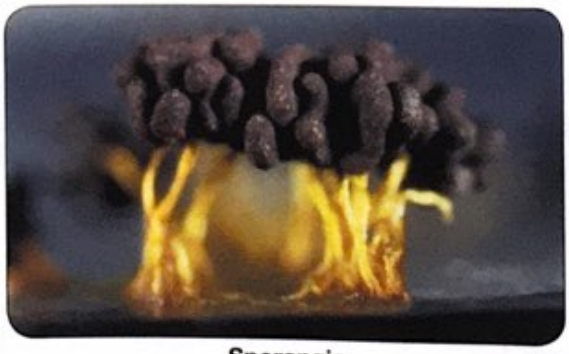
some known and some unknown. The **spores** can survive for many years. Thus, the formation of spores is one way the slime mold survives harsh conditions until more favorable living conditions return. Sporulation is also the first step in sexual reproduction, described later in more detail. With the unaided eye, spores look like smaller, darker poppy seeds. Under a microscope, their multilobed structure is apparent.

Spores

Although starvation induces changes that make the plasmodium able to sporulate, it is light that triggers the actual process of sporulation. During early sporulation, the organism forms **fruiting bodies** that first appear as bumps on the plasmodium. The bumps develop into stalked sporangia that project from the surface. During spore formation, one diploid nucleus is incorporated into each spore. However, as the spore develops, meiosis occurs, so that each spore initially contains four haploid nuclei. Later, three of the four break down, resulting in a fully developed spore that contains a single haploid nucleus.



Fruiting bodies

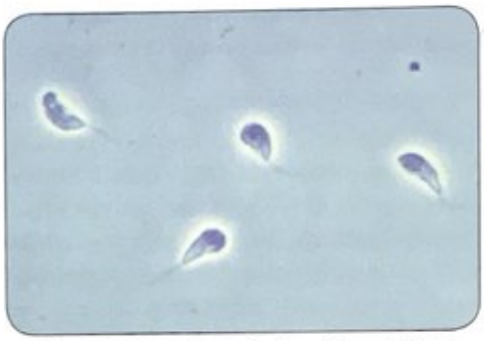


Sporangia

Amoebas and Flagellates

When there is sufficient moisture, a haploid, single-nucleated **amoeba** emerges from a spore. The amoeba feeds on bacteria and other microorganisms by phagocytosis and moves using typical amoeboid locomotion. Unlike mitosis in the plasmodial form, mitosis in the amoebal form is accompanied by cell division. Repeated division of the *Physarum* amoeba results in a colony of amoebas that are genetically the same.

The amoebas can change into two different forms: **flagellates** or **cysts**. Transformation from amoeba to flagellate and transformation from amoeba to cyst are both reversible. If an amoeba runs out of food or encounters excessively dry or certain other adverse conditions, it forms a cyst with protective walls. When conditions become more favorable again, an amoebal cell reemerges from the cyst. Transformation to a flagellate occurs under wet conditions. Suspension in liquid is not enough to trigger the transformation; other factors still not completely understood appear to be involved. Flagellates are unable to feed or perform mitosis and will transform back into amoebas under drier conditions. The amoeba's ability to transform allows *Physarum* to survive a broader range of conditions than it would otherwise.



***Physarum polycephalum* flagellates**

Sexual Reproduction

Genetic diversity is beneficial to the long-term survival of a species. Sexual reproduction is one of the mechanisms generating **genetic diversity** in *Physarum polycephalum*. The second half of sexual reproduction, the formation of haploid spores by a diploid plasmodium, has been discussed. The earlier half of the process, the combining of genetic material from two different organisms, occurs when two haploid amoebas fuse to form a single organism with a single diploid nucleus. This single diploid cell then goes on to develop into a plasmodium. Usually, the mitochondria from one of the original amoebas are degraded. Sometimes, depending upon the two **mating types** fusing, mitochondria from both parents are retained in the diploid plasmodium.

The mating of the two amoebas is controlled by multiple, **multi-allele mating loci**. One locus, the **matA locus**, controls the development of the organism from a zygote to a plasmodium. Generally, the two fusing amoebas must have two different alleles at the matA locus in order for development into a plasmodium to occur. The selection of which amoeba's mitochondria become degraded is one of the processes controlled by the matA locus. The different matA alleles have a different "status," and this status determines which individual's mitochondria are degraded and which one's are retained. Two other loci, **matB** and **matC** are involved in controlling the probability that the two amoebas will fuse. Amoebas with different alleles at these loci are more likely to fuse than amoebas carrying the same alleles.

There are some strains in which a haploid amoeba can develop into a haploid plasmodium. This process is referred to as **apogamy**. Many of the strains that are able to perform apogamy are lab strains. How often a haploid plasmodium develops from a haploid amoeba in nature is unclear.

Sclerotium

As discussed, *Physarum polycephalum* when in the amoebal form can respond to stressful conditions by forming a cyst. When in the plasmodial form, *Physarum* responds to starvation and light by sporulating. However, if the stressful conditions occur in the absence of light the plasmodium will form a **sclerotium**, a collection of **macrocysts** (also called **spherules**) surrounded by a hard dry protective layer. **Sclerotia** can form in response to a number of stimuli such as starvation, dryness, cold, low pH, solutions with high osmotic pressure, and some heavy metals. Early in the formation of the sclerotium, **streaming** slows and the substance of the plasmodium begins to congeal. In addition, the nuclei become more evenly distributed throughout the organism. Strands appear, which eventually become the

walls of the macrocysts. Macrocysts, unlike spores, are multinucleate, and the number of nuclei they contain varies. A second membrane forms around each macrocyst and the nuclei shrink. Depending upon the conditions of its environment, a sclerotium can remain for months and then be revived to the active form of the plasmodium. By comparison, though, spores can survive for many years and then produce an active amoeba.

The substantial morphological changes between *Physarum*'s different forms are accompanied by changes in gene expression. For example, the cytoskeleton, including both the **microtubule** and **actomyosin networks**, is reorganized. Some of this reorganization involves changes in the expression of the genes coding for the proteins composing these networks.

Foraging and Streaming

Physarum in the plasmodial form uses phagocytosis to ingest its food, which consists of small particles of organic material, bacteria, and other microorganisms, including myxomycete amoebae. It also secretes enzymes to break down materials, which are then absorbed by **pinocytosis**. As it forages, the plasmodial form of *Physarum* flows slowly across a substrate. The leading edge has a fanlike configuration. As the organism searches for and takes in food, the cell contents stream back and forth at intervals of approximately 60 seconds through a network of vein-like tubes. A single vein may be up to 1 mm in diameter, and streaming is easily observed using a stereomicroscope. Absorbed material is distributed throughout the cell by means of this streaming. The network of tubes is reorganized as the organism moves in search of food.

The **periodic streaming** is accomplished through the creation of **hydraulic pressure gradients**. Contraction of the actomyosin network within the plasmodium creates these pressure gradients. The actomyosin network is part of an extensive network of **microfilaments** that exists throughout the entire plasmodia. A layer of **actin** lies just inside and in close contact with the cell membrane. In addition, bundles of **actin filaments** run parallel to and wind around the veins. **Myosin**, a protein that, like actin, is found in the muscles of other organisms, is also present in this network. In *Physarum*, however, the proportion of myosin molecules to actin molecules is much lower than that found in the skeletal muscle of other organisms. The myosin forms bridges from one actin filament to another—hence the term, the actomyosin network.

Multiple other proteins are associated with this network. As a group, these proteins are referred to as actin-binding proteins and play a role in carrying out the functions of the actomyosin network, including its reorganization.

Foraging Strategies

The strategies used by the plasmodial form of *Physarum* to find food are complex. The organism senses food at a distance and detects when it has moved closer to or further from the food. It adjusts its movement according to the input it receives, exhibiting simple **chemotaxis**. In addition, the organism has at least one additional strategy for optimizing how it searches an environment for food. The plasmodium avoids any areas it has already explored until it has covered all the unexplored areas in its vicinity. This behavior is thought to increase the efficiency of foraging. This type of foraging strategy has long been observed and studied in more complex organisms that have internal neurologic memory and can remember where they have been. However, *Physarum polycephalum* is a unicellular organism without a neural network for remembering. In the absence of a neurological memory, the organism creates a type of **external memory** by leaving a slime trail in areas from which it withdraws. The organism then strongly avoids areas with a slime track until it has explored areas without residual slime or unless a new food source is placed in the slime-covered area. The signal from a new food source can override the message for the *Physarum* to avoid its own slime track.

As one of its foraging behaviors, *Physarum* can form a network connecting multiple food sources. A research group interested in the efficiency of human-designed networks hypothesized that an organism such as *Physarum*, which has had its network-forming strategies honed by natural selection over millennia, might create networks that optimally balance cost, efficiency, and resilience. The research group created a small model of the Tokyo area and placed food sources for *Physarum* at the location of major cities along the existing Tokyo rail network. Geographical features that

had constrained the building of rail lines were represented as illuminated areas that would similarly be avoided by network-forming *Physarum*. *Physarum* placed into the model created a network very similar to the existing rail lines.

In addition, *Physarum* can make seemingly complex decisions regarding its diet. Plasmodium with access to patches of food that varied in the ratio of carbohydrate to protein and in the concentrations of carbohydrates and proteins migrated to the patch of food that provided the optimal diet.

The Genome

Sequencing the 250-megabase **genome** of *Physarum polycephalum* has been difficult because of the many regions where a short specific sequence of nucleotides repeats many times in a row and where a single type of nucleotide repeats many times. These features make sequencing a challenge and make assembling a complete genome difficult. To supplement the data obtained from sequencing the genome, researchers created a **transcriptome**. A transcriptome is a collection of the sequences of the RNAs transcribed by a certain cell type. In this case the "cell type" is actually the complete organism. The researchers pooled transcriptome sequence data from multiple life cycle stages. Computer analysis of the data predicted the presence of 34,438 genes. Half of these predicted gene loci could be linked to a **transcript**. Analysis of the data and comparison to similar data from closely related and not so closely related organisms have provided information regarding the evolutionary relationships between the different organisms. For example, two phytochrome genes found in the *Physarum* genome appear to code for proteins that are most similar to bacterial phytochromes. These predicted *Physarum* phytochromes also show some similarity to plant phytochromes. Phytochromes are light receptors that sense light in the red or near red range of the electromagnetic spectrum. The *Physarum* genome also appears to include some photoreceptor genes that show the greatest similarity to sequences in animals. More genetic studies may one day make it clear how *Physarum polycephalum* came to possess characteristics displayed in organisms from very different taxa.

In the Classroom

The plasmodial form of *Physarum polycephalum* is simple and inexpensive to grow in the classroom. Basic procedures are described in the following section of the manual. A section regarding sterile technique has been included as an Appendix in case your students need it. In the Ideas for Investigations section you will find ideas for independent student research. Potential questions to pose to your students have also been included.

Reference

Alim, K., G. Amselem, F. Peaudecerf, M.P. Brenner, A. Pringle. 2013. Random network peristalsis in *Physarum polycephalum* organizes fluid flow across an individual. *Proceedings of the National Academy of Science*, Vol. 110 (33), 13306–11.

Bailey, J. 1995. Plasmodium development in the myxomycete *Physarum polycephalum*: genetic control and cellular events. *Microbiology*, Vol. 141, 2355–65.

Bailey, J. 1997. Building a plasmodium: development in the acellular slime mould *Physarum polycephalum*. *BioEssays*, Vol. 19, 985–92.

Daniel, J.W., H.P. Rusch. 1962. Niacin requirement for sporulation of *Physarum polycephalum*. *Journal of Bacteriology*, Vol. 83, 1244–50.

Dussutour, A., T. Latty, M. Beekman, S.J. Simpson. 2010. Amoeboid organism solves complex nutritional challenges. *Proceedings of the National Academy of Sciences*, Vol. 107 (10), 4607–11.

Jump, J.A. 1954. Studies on sclerotization in *Physarum polycephalum*. *American Journal of Botany*, Vol. 41 (7), 561–7.

Kawano, S., T. Kuroiwa, R.W. Anderson. 1987. A third multiallelic mating-type locus in *Physarum polycephalum*. *Journal of General Microbiology*, Vol. 133, 2539–46.

Moriyama, Y., S. Kawano. 2003. Rapid, selective digestion of mitochondrial DNA in accordance with the matA hierarchy of multiallelic mating types in the mitochondrial inheritance of *Physarum polycephalum*. *Genetics*, Vol. 164, 963–75.

Reid, C.R., T. Latty, A. Dussutour, M. Beekman. 2012. Slime mold uses an externalized spatial "memory" to navigate in complex environment. *Proceedings of the National Academy of Sciences*, Vol. 109 (43), 17490–94.

Reid, C.R., M. Beekman, T. Latty, A. Dussutour. 2013. Amoeboid organism uses extracellular secretions to make smart foraging decisions. *Behavioral Ecology*, Vol. 24(4), 812–18.

Schapp, P., et. al. 2016. The *Physarum polycephalum* genome reveals extensive use of prokaryotic two-component and metazoan-type tyrosine kinase signaling. *Genome Biology and Evolution*, Vol. 8(1), 109–25.

Stockem, W., C. Brix. 1994. Analysis of microfilament organization and contractile activities in *Physarum*. *International Review of Cytology*, Vol. 149, 145–215.

Tero, A., S. Takagi, T. Saigusa, K. Ito, D.P. Bebber, M.D. Fricker, K. Yumiki, R. Kobayashi, T. Nakagaki. 2010. Rules for biologically inspired adaptive network design. *Science*, Vol. 237, 439–42.

Youngman, P.J., R.W. Anderson, C.E. Holt. 1981. Two multiallelic mating compatibility loci separately regulate zygote formation and zygote differentiation in the myxomycete *Physarum polycephalum*. *Genetics*, Vol. 97, 513–30.

Procedures

All procedures should be performed using sterile technique. See the Appendix for more information on sterile technique.

Culturing Physarum from Sclerotia

1. Place a drop of sterile water on a 2% agar plate.
2. Place the filter paper with the sclerotium on it face-down on the water drop.
3. Place approximately 10 oatmeal flakes next to the sclerotium. Ideally, this should be done using sterile forceps or a similar sterile implement. The oat flakes may instead be carefully shaken from their container, but that method increases the chance of contaminating the culture.
4. Place the lid on the 2% agar plate, place it in a sealed plastic bag to retain moisture, and place the bag containing the dish in a dark place with the petri dish lid facing up. Allow 8–16 hours for the sclerotium to become a plasmodium.
5. Once a plasmodium forms, it will flow off the filter paper and onto the oat flakes and agar plate.
6. Continue to add oat flakes to feed the plasmodium. If the plasmodium is not kept sufficiently fed, it will flow out of the petri dish and onto the inside of the plastic bag. If conditions become too dry or the plasmodium does not have enough food, it will form spores or a sclerotium.
7. Subculture the plasmodium to a new plate after the culture completely fills the plate or every 5–7 days, whichever is first.

Subculturing Physarum (culturing Physarum from an Active Culture)

1. Move the *Physarum* to a new plate using the methods described below.
   a. Obtain a scalpel for cutting the agar. Metal forceps are also useful.
   b. Sterilize the scalpel by dipping it in 70% ethanol or isopropanol and then briefly passing it through a flame.
   c. Immediately, using the now sterile scalpel, cut a 1-cm square of plasmodium-containing agar from the plate. Move the square of agar to a new 2% agar plate using the scalpel. Flame-sterilized forceps may also be used. Place the square on the plate with the plasmodium side facing down.

   Note: In the absence of a flame, the scalpel can be sterilized by soaking it in alcohol, shaking off the excess alcohol, and allowing the instrument to air dry for a few seconds just prior to use. Using this method may increase the chance of the culture becoming contaminated.
2. Add approximately 10 oat flakes to the plate. Ideally, this should be done using sterile forceps or another sterile implement. The oat flakes may instead be carefully shaken from their container, but that method increases the chance of contaminating the culture.
3. Place the new plate into a sealed plastic bag with the lid side up and place in a dark location.
4. Continue to add oat flakes to feed the plasmodium. If the plasmodium is not kept sufficiently fed it will flow out of the petri dish and onto the inside of the plastic bag. If conditions become too dry or the plasmodium does not have enough food, it will form spores or a sclerotium.
5. To prevent contamination, subculture the plasmodium to a new plate when the culture completely fills the plate or every 5–7 days, whichever is first.

Inducing Physarum to Form Sclerotia

1. Place a piece of sterile filter paper onto a 100-mm 2% agar plate. Using sterile water, dampen the paper. Then lightly sprinkle the plate with sterile oat flakes.
2. Using the technique described earlier in "Subculturing *Physarum* (culturing *Physarum* from an Active Culture)," move a 1-cm square chunk of agar with plasmodium on it to the filter paper and place it with the plasmodium side down.
3. Put the plate lid-side-up into a plastic bag to keep it moist. Allow the plasmodium to grow until it fills the piece of filter paper.

4. At some point during this period of growth, set up a larger piece of sterile filter paper in a sterile container with a lid that can be sealed shut.
5. Once the plasmodium has filled the smaller piece of filter paper, use sterile water to dampen the larger piece of filter paper in the larger sterile container.
6. With forceps, move the smaller, plasmodium-covered filter paper and the oat flakes that are on it to the center of the larger piece of filter paper. Close the lid on the container to keep the paper moist.
7. Once the plasmodium has spread to the larger piece of filter paper and covered a significant portion, starve the plasmodium by removing the smaller paper containing the oat flakes. In addition, allow the filter paper to dry by opening slightly the lid on the container.
8. As the paper dries, a sclerotium will form.

Sporulation

Inducing sporulation can be difficult in a classroom setting. In a research lab, it is done under carefully controlled conditions. Sporulation occurs with some consistency if these steps are followed:

1. Allow the plasmodium to fill the plate and exhaust its food supply.
2. Place the plate upside down where it will be exposed to daylight, but not directly in a window.
3. Wait. Sporulation may occur in a few days, or it may take longer. If it has not occurred within 2 weeks, it is not likely to happen.

Observing the Culture

Physarum is best viewed using a stereomicroscope with at least 10× to 40× magnification. Many aspects such as streaming and fruiting bodies can be found and identified at 10× but are revealed in much better detail at 40×. Use bottom illumination. The periodic streaming is best observed on an agar plate during a period of plasmodial growth. Streaming is most likely to be found in the vein-like strands near the periphery of plasmodial growth. Should you need it, the digital resources available with this product include a video showing the streaming.

Although it is less effective than a stereomicroscope, a compound scope with a scanning objective (for a total magnification of no more than 40×) can be used to observe *Physarum*. Be sure to set the diaphragm to maximize the amount of light shining through the plate. To protect the higher-power objectives on the microscope, remind students to keep the lid on the culture plate and to not view the *Physarum* with any other than the lowest-power objective.

To facilitate viewing plasmodium with a compound scope, you can also grow plasmodium on a microscope slide coated with 2% agar for a short period of time. To do so, follow these steps:

1. Soak a standard microscope slide in alcohol for at least an hour (e.g., in a sterile petri dish).
2. Place the slide in a dry sterile petri dish to dry. Cover the dish while the slide dries, preferably overnight.
3. Once the slide is dry, use a sterile pipet to carefully coat the upper surface of the slide with a layer of sterile 2% agar. Keep the slide in the sterile petri dish as you do this, and cover the dish while the agar solidifies.
4. One the agar has solidified, seed the slide with *Physarum* by placing an approximately 0.5-cm chunk of agar containing *Physarum* plasmodium onto the slide, plasmodium side down. To maintain sterility and keep the agar moist, keep the slide in a closed petri dish the entire time. If the agar begins to dry, add 1 or 2 drops of sterile water to its surface.
5. Once the plasmodium has moved from the chunk to the thin layer of agar on the slide, the plasmodium can be viewed. Typically, the plasmodium moves onto the slide within 24 hours after placement of the chunk.

Questions and Answers

1. Kinesins are proteins that are involved in intracellular transport, the movement of things within the cell. A domain is a distinct functional and structural part of a protein. Preliminary examination of the *Physarum polycephalum* genome reveals that kinesin domains appear to be present in a larger number of the organism's proteins than in those of other organisms closely related to it. One of these organisms is, like *Physarum*, a slime mold. However, it is a cellular slime mold and does not form a single

giant cell as does *Physarum* but exists as multiple smaller cells. Based solely on consideration of this difference between these two organisms, come up with a reasonable explanation for why *Physarum polycephalum* would have more proteins with kinesin domains than this other slime mold would.

A likely answer might include the following logic. *Physarum polycephalum* is one large cell. These cells can be very large, larger than those found in the cellular slime mold. Molecules need to be moved from one part of the large *Physarum* cell to another, and such movements would be over a great distance. It is logical to expect that the organism would have a larger number of genes devoted to organized movement of materials around the cell.

2. In the absence of an internal memory, *Physarum polycephalum* creates a type of "external memory" to aid it when it is foraging for food. Wherever the organism goes, it leaves a slime track. As it continues to search for food, it avoids areas containing its slime track until it has searched the remainder of the entire area. Explain the logical advantage of this behavior to *Physarum*. Can you think of other organisms that use chemical signals during foraging?

   By avoiding its slime track, *Physarum* does not expend energy to explore an area that it has just explored and that is unlikely to contain food.

   Ants lay down a chemical signal for nest mates when foraging. Students may come up with other examples.

3. *Physarum polycephalum* has long been difficult for scientists to classify, and its classification has changed over time. Pick a period of time during which *Physarum polycephalum* has been studied, and on the basis of what was known about the organism at that time make an argument for classifying *Physarum polycephalum* in a taxon of your choosing. Base your argument on evidence drawn from what was then known about the organism. You will have to explore the science literature to answer this question. Include references to the facts you use in your argument.

   Answers will vary. The strength and logic of a student's argument is what should be evaluated.

Ideas for Investigations

1. Have students construct a maze. Have them make a maze and test a *Physarum* plasmodium's ability to find its way through it, or to connect a source of food at the beginning of the maze with a source of food at the end of the maze using the shortest path. One way to do this is to cut a maze from an acetate sheet. The maze can be cut so that it can be placed on top of a 2% agar dish and can be sterilized by soaking it in alcohol. Be aware that *Physarum* may, under some conditions, cross the acetate. Students may also want to experiment with using different types of material to make the maze, or using different coatings in order to prevent *Physarum* from taking a shortcut.
2. Have students investigate chemotaxis. They can test the ability of different substances to attract or repel a *Physarum* plasmodium. Have them perform a test in multiples and use statistics to measure the significance of their observations.
   The scientific literature includes experiments showing that *Physarum* moves toward some chemicals (e.g., 1% glucose, 1% galactose, and 1% mannose) and away from others (e.g., 1% sucrose and 1% ribose). Students may wish to test *Physarum*'s reaction to other materials such as spices, cedar oil, clove oil, basil, geranium, or vegetable oils.
3. Have students explore the effects of temperature on the plasmodial form of *Physarum*.
4. Have students examine the effect of the slime track that a *Physarum* plasmodium has left behind on the tendency of the same or a different plasmodium to forage in that area.
5. Have students explore the conditions that cause a sclerotium to form. They could test the effect of desiccation, solutions of different metals, cold and hot temperatures, changes in pH, starvation in the dark, etc.

Questions

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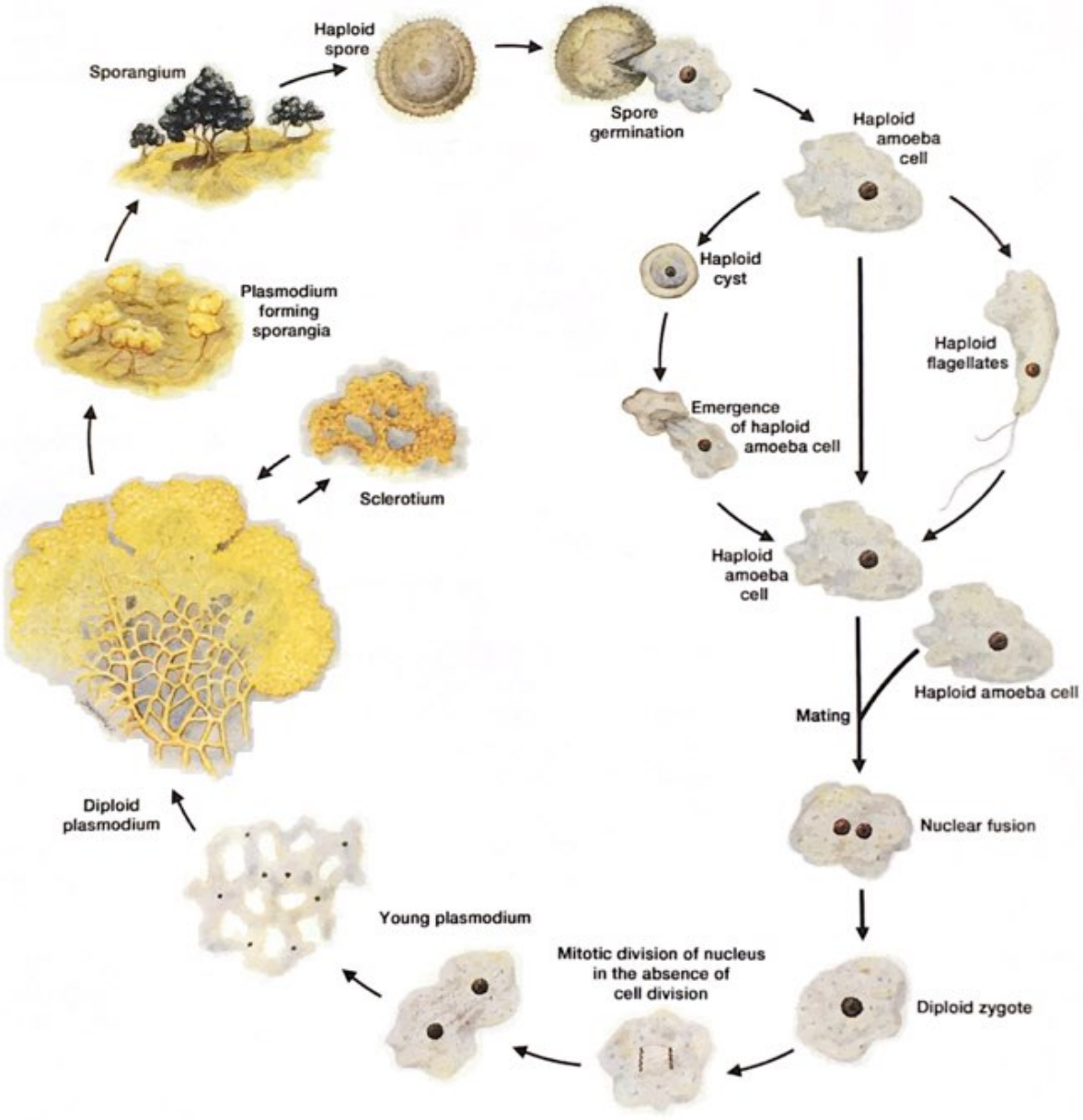
Sterile Technique

In most microbiological procedures, it is necessary to protect instruments, containers, media, and cultures from contamination by microorganisms present in the environment. Sterile technique includes the sterilization of tools, glassware, and media, as well as the use of presterilized plastic ware to prevent the introduction of contaminating microorganisms into a culture. It also involves following specific procedures to minimize the introduction of any contaminating organisms.

1. Wash your hands thoroughly before and after working with cultures.
2. Work in an area free of drafts.
3. Wipe your work area with 10% bleach or 70% ethanol or isopropanol before and after working with cultures.
4. Always open the wrappers of presterilized tools such as sterile pipets, tweezers, and scalpels, so that you do not touch the working end of the implement. Open a pipet wrapper from the bulbous end of the pipet. Open a disposable scalpel from the end opposite the blade, and forceps from the handle end.
5. If you use reusable metal tools, dip them in alcohol and briefly run them through a flame to sterilize them before each use.
6. Once a tool has been removed from its wrapper or passed through a flame to be sterilized, do not place it down or touch it to anything that may contaminate it. Use it immediately.
7. To avoid contaminating your work surface, once materials have come into contact with *Physarum* do not place them onto your work surface but into a waste container.
8. When inserting pipets into bottles, tubes, or vials while transferring material, do not touch the pipet to the side of the container.
9. When removing the cap from a bottle, vial, or tube of sterile reagent, do not drop the cap or put it down, and make sure that you hold the cap with the open side facing down. Also, make sure you keep your fingers away from the cap's rim. Some people remove and hold the cap using the pinkie finger of the hand holding the tool being used. Others are more comfortable removing and holding the cap using the thumb and forefinger of the hand that is holding the bottle, vial, or tube. Regardless of the technique you choose, replace the cap quickly once you have removed the reagent from the container.
10. When adding or removing reagents, oat flakes, cultures, or other items from agar plates, hold the lid over the plate as much as possible to prevent contaminants from falling onto the surface of the plate. Open the plate the minimum amount needed to perform the manipulation.

Physarum Life Cycle



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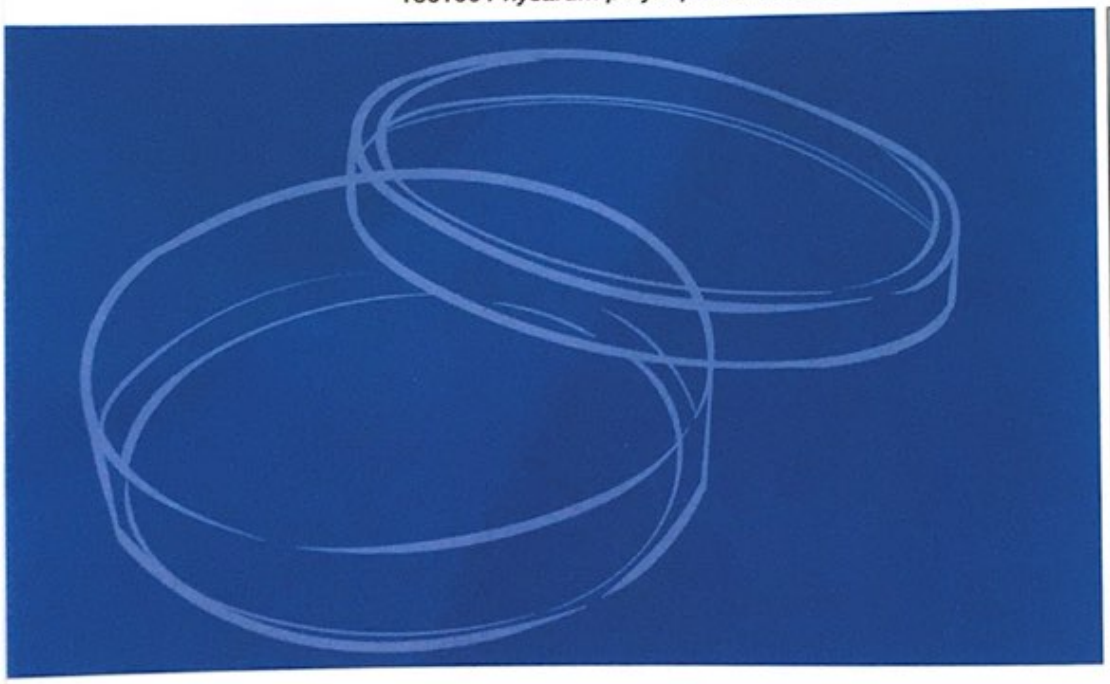
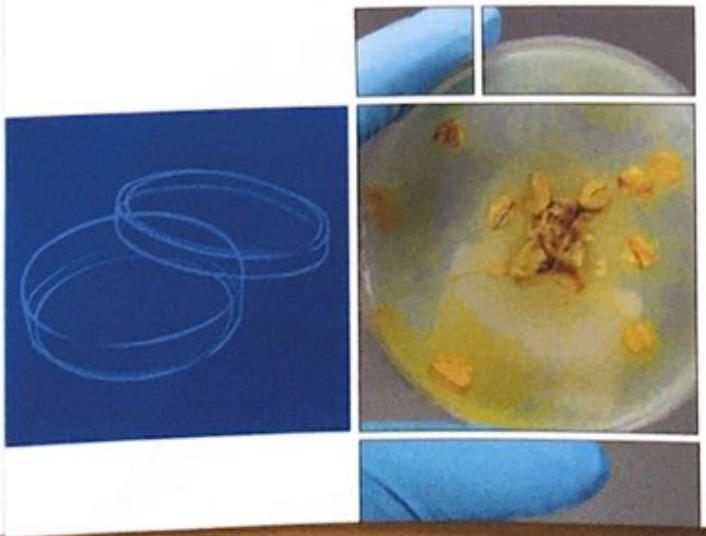
156190 *Physarum polycephalum* Sclerotium, Living, Box





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