

# Quick, Safe, and Simple Silver Staining for Ciliates and Other Protists

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In ciliated protists (Ciliophora), the basal bodies (kinetids) are arranged in species-specific patterns that are maintained from one generation to the next. Kinetids stain strongly when exposed to silver compounds. So does the ciliate “silverline system,” the edges of the alveolar sacs underneath the plasma membrane. Most techniques for silver-staining ciliates are technically difficult and require toxic reagents. We present a simple, quick, inexpensive, and safe stain technique that reveals the complexity of ciliates and some other protists. Our procedure can easily be used in a project-based unit on protist diversity, or in inquiry-driven labs for secondary and post-secondary students.

**Keywords:** ciliates, Protista, staining, basal bodies, silver

## Introduction

Quite possibly the most complex of all eukaryotic cells are the **ciliates** (Ciliophora). These single-celled organisms bear many cilia, usually, although not always, covering the entire cell. Cilia beat back and forth, either to move the cell through the water, or to move water past the cell. They make it possible for a ciliate to move and feed. (Lynn 2008)

The basal body of each cilium is anchored within the outer layer of the cell by a specialized fibril and by ribbons of microtubules. In many species, cilia are linked together in pairs, creating **dikinetids**; occasionally, more than two cilia may be linked to make a **polykinetid**. Whether single or not, the kinetids in turn are arranged in rows called **kineties**. Around the “mouth” (the **cytostome**) of some ciliates, the cilia may be tightly packed into rows—packed so closely together that each row beats as one, making a single unit called a **membranelle**. Some ciliates have cilia “bundled” into bristle-like structures that also beat as one; these are **cirri**. As a ciliate divides, kineties lengthen and then divide along with the rest of the cell, and kinetids may multiply and organize themselves into new membranelles or other key structures. Thus the pattern of cilia is kept constant through the generations; it is an important feature in identifying and classifying ciliates.

Visualizing the arrangement of cilia is not easy with ordinary light microscopy. But kinetids take up silver compounds, and this can be used as the basis for stains. Silver also stains the edges of the **alveolar sacs**

just underneath the plasma membrane, revealing a “network” that resembles the stitching on a quilt. This is significant for understanding eukaryote evolution, because dinoflagellates (including the marine species that cause “red tides”) and apicomplexans (including the parasites that cause malaria, toxoplasmosis, coccidiosis, and so on) also contain alveolar sacs. Despite their seemingly huge differences, ciliates, dinoflagellates, and apicomplexans all share this basic structural feature, and thus all are classified in the supergroup Alveolata.

The basic idea of silver staining is very similar to traditional black and white photography. In traditional photography, film or paper that has been coated with a layer of silver chloride particles is exposed to light. Then the film or paper is placed in a developer solution; this converts the light-sensitized silver chloride to grains of metallic silver, which look black. A solution of sodium thiosulfate (known as “fixer”) removes any remaining unreacted silver chloride, creating a print that won’t darken. This is very much like what we’ll be doing here, except that we use silver nitrate, which is less sensitive to visible light, allowing you to work in normal room light. Silver nitrate is picked up by the kinetids and the alveolar sacs, and then reacts with ultraviolet light to produce silver grains.

The “dry silver” stain procedure that we’ve worked out is a simplification of one developed by Foissner (2014, and references therein), which in turn is based on a discovery by Klein (1958, and references

therein). We've tested multiple variations of the procedure and tried them out on different ciliate species.

## Student Outline

### Put on gloves and lab coats before you begin.

1. Place a *small* drop of your ciliate culture on a clean microscope slide. If you're not sure whether there are ciliates of interest in the drop, check it quickly under a dissecting scope.
2. If the drop is satisfactory, spread the drop around with a clean probe to cover an area smaller than a standard cover slip. Let this drop dry slowly and completely. If the drop "beads up" on the slide as you're trying to spread it, you probably have grease or dirt on the slide. This will make it harder for the ciliates to stick during the staining process; you should probably clean off your slide and start over.
3. Place the slide on a staining rack and cover the dried drop with a few drops of 1% silver nitrate solution. Let sit for 1 minute.
4. Gently rinse the slide for a few seconds with distilled water. Do this by tilting your slide over the waste dish, gently squirting distilled water from a squirt bottle onto the upper part of the slide, and allowing the water to run down over the dried drop. Do not directly squirt the dried drop, as you could rinse your ciliates completely off the slide. When you're finished, blot off excess water from the sides and underside of the slide, without touching the dried drop. (The slide does not have to be completely dry for the next step).
5. Place the slide on a pure white background and expose it to the light of a 60W electric light, about 10 cm away, for 30 seconds. Turn off the light and remove the slide.
6. Place the slide on the rack again and cover the dried drop with several drops of developer solution. Let it sit for 1 minute. You should see areas of the dried drop turn dark brown almost immediately.
7. Gently rinse the developer off for a few seconds into the waste dish, using distilled water as you did before. Again, do not let the stream of water directly hit the ciliates. Instead, tilt the slide and let the stream hit the top of the slide and flow gently over the ciliates.
8. Immerse the slide in a dish of sodium thiosulfate solution for 5 minutes. Occasionally rinse the slide by gently rocking or swirling the dish. This will "fix" the stain by removing any unreacted silver.
9. Remove the slide from the sodium thiosulfate with forceps, and place in a dish of tap water. Gently rinse the slide by rocking or swirling the dish. Then remove the slide, change the water, and place the slide in the dish and rinse it again. Repeat the rinsing at least three times. (If you don't do this, the stain will "bleach" and fade.)
10. Blot off excess water from the back and sides of the slide, without touching the stained area. Let it air-dry and examine it under the microscope. You will have to search the slide carefully to find the best and clearest ciliates. This technique can give rather patchy results; you may find a beautifully stained and nearly undistorted ciliate right next to a badly overstained or severely distorted ciliate. If you had a rich ciliate culture to work with, you may find the ciliates clumped in the center of the drop, with the ciliates in the center staining lightly and the ciliates at the edges staining too darkly or obscured by debris. Somewhere in between, you should be able to find a happy medium.
11. Describe and document what you see. Your instructor may ask you to mount your slide permanently. If so, once your slide is *completely* dry, add a small drop of mounting medium. Then place a coverslip onto the drop, taking care not to get any air bubbles underneath. Once the medium has flattened out completely, let your slide dry undisturbed for several days. Make sure you label it with the ciliate name, your name, the date, and any other pertinent information.
12. When finished, pour the chemical waste in your waste dish into the class waste disposal bottle.  
A well-stained ciliate under high magnification will show rows of black dots. These rows are the **kineties**, and each dot is a **kinetid**. Some species of ciliate have **membranelles**, which are rows of tightly packed cilia that all

beat together as one; or **cirri**, clusters of tightly packed cilia that form a bristle-like structure. You may or may not see the actual membranelles or cirri, but the packed kinetids will look like black bars (membranelles) or black spots (cirri).

You may also see the so-called **silverline system** or **argyrome**, which will look like a network of fine fibers running among and between the kinetids. This is not actually a network of fibers. Rather, ciliates have a set of flattened membrane vacuoles, the **alveoli**, just underneath the plasma membrane. The edges of these alveoli pick up silver and stain dark. (Imagine a quilt. The silverline system is analogous to the stitching that divides the quilted pockets from each other.) The details of the silverline system are useful in identifying species.

Below the alveoli are the various fibers that anchor and connect the basal bodies. You will probably not be able to see these with this staining technique, although it might be possible with luck. Although the silver stain is not the best for staining cell contents in general, by carefully adjusting the fine focus you may be able to spot the macronucleus and vacuoles.

If you don't get good results the first time, don't panic. Practice helps!

## Notes for the Instructor

## Supplies

The instructor should have the following on the main bench:

- culture(s) of ciliates with transfer pipets
- disposal bottle for waste chemicals
- (optional) mounting medium (Permount, euparal, etc.), with cover slips and a dropper

Each student should have access to the following:

- clean microscope slides
- microscope (oil immersion is optional; most pertinent details are visible at 400x)
- method of labeling slides (permanent marker, diamond scribe, etc.)
- tissues for drying slides, blotting excess water
- staining rack
- 1 small squirt bottle with distilled water
- 1 opaque dropper bottle of 1% w/v  $\text{AgNO}_3(\text{aq})$  solution
- 1 opaque dropper bottle of developer solution
- 1 small dish or tray of 5% w/v sodium thiosulfate pentahydrate solution
- 1 small dish or tray of water (tap or distilled)
- 1 empty dish or tray for collecting waste chemicals, clearly labeled as such
- 60 watt electric lamp (other wattages are acceptable but should be tested first)
- white background for exposing the slide to light (a white enamel tray, or in a pinch, a few sheets of plain paper will work fine)
- timer (if necessary—students may prefer to use their smartphones)
- 1 pair forceps
- protective gear: gloves, goggles, and lab coats or aprons

Foissner (2014) originally recommended a developer solution that is a mixture of two commercial German B&W film and print developers, made alkaline with sodium hydroxide. We have found that several American brands of developer give good results “off the shelf”. We prefer a product called Liquidol (Photographers’ Formulary product #02-0200; see <http://stores.photoformulary.com/>), which gives excellent results and is most practical in a laboratory setting. Kodak’s Dektol developer also works well; their D-76 and Polymax-T developers gave us lighter and less satisfactory stains, although this can be compensated for by increasing the light exposure. Anyone who wishes to try other brands, or to tinker with formulas, is quite welcome to do so—Ansell (2008) includes recipes for film and print developers and an overview of the chemical processes involved.

Finding darkroom chemicals was easier in the days before digital photography, but Kodak and other companies still make some. If your area still has a photo supply store, it should at least have Dektol and sodium thiosulfate (“hypo”) in stock. If not, online retailers stock them. (Freestyle Photographic Supplies, <http://www.freestylephoto.biz/>, stocks a wide selection and gave us excellent service.) Alternately, if your area has a group that still practices old-school darkroom photography, such as a camera club or a fine arts department, consider asking them for developer and fixer, as they’ll often have stock solutions on hand.

Liquidol is sold in bottles of stock solution. For printing photos, Liquidol is intended to be diluted, 1 part stock solution to 9 parts water. However, a 1:2 dilution of Liquidol gave us the best results for staining ciliates. A pint bottle of Liquidol stock solution (0.47 liters) costs about \$10. Since each slide requires at most a milliliter or two of Liquidol, one bottle can make enough solution for over a thousand slides. Liquidol has a long shelf life, and since it is sold as a liquid in a capped bottle, it’s easy to mix up only as much as you need. We have seen mixed Liquidol solution last almost a week in an unstoppered flask, and it will last longer if capped. If it turns lemon yellow, it’s time to throw it out.

Dektol is sold in packets of powder; the standard packet is enough to make one gallon (3.8 liters) of stock solution, and costs about \$7-10. (You’d dilute this stock solution further to develop photos—but don’t dilute it for staining ciliates.) Unfortunately, Dektol is prone to oxidizing; unless you have a very large class that makes thousands of slides in a week, you may end up discarding more Dektol than you use. I store dry Dektol in tightly capped opaque bottles, and mix up smaller quantities as needed: dissolve 13.8 g of powder in 100 ml hot tap water, and then top up to 126 ml. If your Dektol turns dark, discard it.

Sodium thiosulfate pentahydrate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) is traditionally known as “hypo”, and costs about \$7/pound from photography supply stores. One pound (453 g) will make about nine liters of 5% w/v solution. Sodium thiosulfate solutions will last for years if capped tightly, and the dry product is also quite stable.

The most expensive reagent is silver nitrate. Current prices from chemical suppliers are over \$100 for 25 grams (ACS grade). A 1% w/v solution (1 g/100 mL) should last for years if kept in opaque bottles away from light. Since each slide requires just a few drops of silver nitrate, 100 ml of solution will be enough for about 300 to 400 slides.

All of the chemicals used here can cause skin and eye irritation, and gloves and eye protection should be worn. That said, none of the chemicals are strongly flammable, explosive, corrosive, or toxic, although some

people are more sensitive to them than others. The room should be well ventilated, but a fume hood is not necessary. Rinsing with soap and tap water should be adequate treatment for limited skin contact. MSDS are available at <http://www.freestylephoto.biz/msds>.

According to Kodak (“Environmental Guidelines,” 1999), individuals may safely discard small amounts of most common B&W photographic chemicals into sewer systems, although not into septic systems. Developers are typically alkaline; if this is a problem, they may be neutralized with mild acids such as vinegar. Chemicals containing silver can be discarded safely, but may be treated before disposal to recover the silver. Please note that the above guidelines apply only to individuals; institutions that produce larger amounts of waste, including most colleges and universities, must follow stricter regulations. Most colleges and universities should have established policies and procedures for chemical disposal. If your institution does not, your local sewer system or landfill, or your state’s government agency that oversees hazardous waste, can provide more information.

### Time

The most time-consuming step is allowing the ciliate culture drop to dry. We seem to get the best results when the drop is allowed to dry at room temperature, without heating. A small drop of ciliate culture, spread out on the slide, will dry in about ten to twenty minutes at room temperature. The instructor may want to have the students set up the slide and then do something else while waiting for it to dry, or she might prefer to set up the slides herself in advance; dried slides can be left for up to 24 hours with no obvious loss in quality that we could detect. With a clearly laid-out bench setup, the stain itself can be completed, except for the final air-drying, in ten minutes.

When we began experimenting, we used very large drops of ciliate cultures, thinking that this would give us many specimens to choose from. However, a small drop of ciliate culture, if it’s fairly rich, yields much more clearly stained specimens. This is an important point emphasized by Klein (1958, p. 99): as the edges of a large drop evaporate, the remaining water (where the ciliates often congregate) increases in ionic concentration so much that the ciliates are damaged before they finally dry out. Furthermore, silver nitrate reacts with chloride ions (found in just about any ciliate habitat or culture medium) to form silver chloride, which precipitates out as black grains when exposed to any light. These grains can blanket the slide and blot out detail on the ciliates. Thus a large drop not only takes a long time to dry—the ciliates look worse when stained.

### Which Ciliates?

It’s a general rule that not every staining method works equally well with every ciliate species. This is certainly true for this method! Since this method does not use chemical fixation of the ciliates, you *will* get some distortion. Details may also be obscured by organic debris, dirt, or silver precipitates. Individual cells of even a “good” species can range from almost undistorted to unrecognizable, often on the same slide. You’ll need to cherry-pick the best specimens to get the clearest view of structure.

All of the ciliates that we have personally tested so far are cultures from biological supply houses, costing about \$8 each—although you can certainly establish your own cultures of any species, or stain samples taken directly from the environment. Of the ciliates that we have personally tried: *Tetrahymena* stains very well. Other small ciliates such as *Colpidium* also stain well, but details may be harder to see. We have had excellent results with *Paramecium caudatum* and *P. multimicronucleatum*, which seem to be firm enough that any given slide will have several relatively undistorted specimens. *Euplotes* keeps its shape very well, and well-stained *Euplotes* can look spectacular, showing both the kinetids of the cirri and membranelles, and the prominent silverline system. (For some reason, *Euplotes* seems to give best results with longer exposure to light: 45 seconds to one minute, instead of the 30 seconds recommended for other ciliates.) Large, “floppy” ciliates such as *Blepharisma* and *Bursaria* usually end up badly distorted; the ciliature is still visible, but it takes time and patience to reconstruct it. The peritrich *Vorticella* and its relatives stain well, but because cilia are only present around the cytostome, there is not much ciliature to see, although the silverline system can be made out.

“Wet” silver staining methods involving chemical fixation give much higher-quality results for most ciliates. Unfortunately, the standard fixatives for ciliates are highly toxic; most call for osmium, cobalt, chromium, and/or mercury, and some require picric acid, a dangerous explosive when dry. Even the relatively safer silver carbonate method involves the toxic chemicals formalin and pyridine. These methods also require more time and expense, more reagents, and greater skill. We would not recommend having students try these in a typical undergraduate course, although they should certainly be used for advanced study or formal taxonomic description. (If you want to try these, begin by consulting Foissner 2014.)

### Uses of the Technique

The senior author began developing this protocol for his invertebrate zoology class, typically taken by

advanced undergraduates and a few masters'-level graduate students. Students use it to demonstrate ciliate anatomy, along with other stains for different cellular structures (nigrosin for surface sculpture, acid methyl green or acetocarmine for nuclei, neutral red for vacuoles, etc.). These stains are carried out after the students have carefully observed living ciliates. Students are asked to observe a ciliate species alive, stain it using multiple techniques, and create a composite diagram of that species using data from all of the methods used.

That said, this stain and others could be used in a wide variety of labs demonstrating more general concepts. In particular, it allows students to gather data to be applied to inquiry-based studies. For example, in a lab demonstrating cladistics, students might observe live ciliates, draw up a data matrix of characters they can see, analyze it to find the most parsimonious tree, and then augment their data matrix with additional characters revealed by staining to test whether their initial tree holds up under further investigation. (For a general introduction to teaching cladistic methods, see Kosinski, 2006.)

Another use that lends itself well to open-ended, inquiry-based labs might be to raise a ciliate species and test the effects of varying environmental parameters, such as temperature or food levels, on the pattern of ciliature and silverlines. These patterns are known to be determined in part by genes (e.g. Générumont et al., 1992), but they also propagate from one generation to the next by cytoplasmic inheritance, in which the kinetics of the parent cell serve as templates for assembling the kinetics of the daughter cells. In some species, the ciliature and silverlines are known to vary considerably with the environment. Still another possibility would be to isolate a clone of a ciliate species by taking one individual and allowing it to reproduce asexually in culture. Ciliates begin to degenerate after a few hundred generations of asexual reproduction—sexual recombination between different mating types is necessary to repair chromosome damage—and it would be interesting to see what effects this lineage senescence has on the ciliature and silverlines.

Yet another possibility is a water-quality lab. Many ciliates are fairly sensitive indicators of water conditions, specifically the degree of organic pollution. Many well-known species such as *Paramecium* and *Vorticella* thrive best in organically rich waters such as sewage effluent (e.g. Laybourn-Parry, 1984, pp. 177-185). A lab might involve analysis of water samples from various sources. Along with other tests, students might identify the ciliate species present, using the silver stain as an aid to identification. (Bick, 1976 provides a useful key.) Note that if the water sample has few ciliates, it may be gently centrifuged to concentrate them; if it is especially rich in dissolved matter, the stain may work

better if the investigator gently centrifuges the sample, pours off the supernatant, and replaces it with clean spring water.

Some ciliates can be made to synchronize cell division in the laboratory, generally by controlling the temperature and applying carefully timed heat shocks (e.g. Williams and Scherbaum, 1959). In a cell biology lab, it might be possible to demonstrate how ciliates assemble new cortical structures, such as the membranelles around the cytostome, by staining samples of synchronised ciliate cultures at different times during the division process. Our attempts to do this with *Tetrahymena* have not been successful thus far, but we certainly encourage further experimentation!

Finally, as we found out in the course of writing this paper, the staining process itself can be the subject of open-ended experimentation. There are many experimental variables to manipulate—length of silver impregnation, light intensity and duration, developing time, and so on. Different developers can also be tested—we discovered that we can get decent results by developing our slides with “Caffenol”, a photographic developer home-brewed from washing soda, vitamin C, and instant coffee! At least for us, the experiments proved almost addictive—with each tweak of the procedure and each stained slide, there was the hope that *this* would be the one that revealed the most perfect detail.

### Variations on Technique

One of the potential problems with this method is keeping the dried ciliates on the slide without washing them off. Foissner's original protocol (2014) begins with spreading an extremely light, thin coat of egg white on the slide and letting it dry before adding the drop of ciliates. Our own experience is that egg white stains fairly strongly itself and can obscure the ciliates. Dried ciliates will stick to the slide without adhesive as long as the washing is done extremely gently. A gentle stream of water from a squirt bottle, applied for about three seconds onto a gently tilted slide (but *not* onto the dried ciliates themselves!), is all that is needed.

For an even simpler alternative to the full staining procedure, Klein's original dry silver stain can give good results (Klein, 1958). Simply put a small drop of ciliates on a slide, let it dry slowly, and cover the dried drop with a drop of silver nitrate. Let this sit for about five minutes, rinse the silver nitrate off with distilled water, and then expose the slide to bright light until the ciliate smear begins to turn brown. (Bright sunlight will develop the slides in about two minutes.) The slide can be examined without using developer. In our experience, the results are not as clear, especially for small ciliates like *Tetrahymena* or densely ciliated ciliates like *Paramecium*. Excessive exposure to light can turn a whole ciliate into a

solid brown lump; you may need to run “test exposures” to get the best results. Nonetheless, we have gotten excellent results with *Euplotes*.

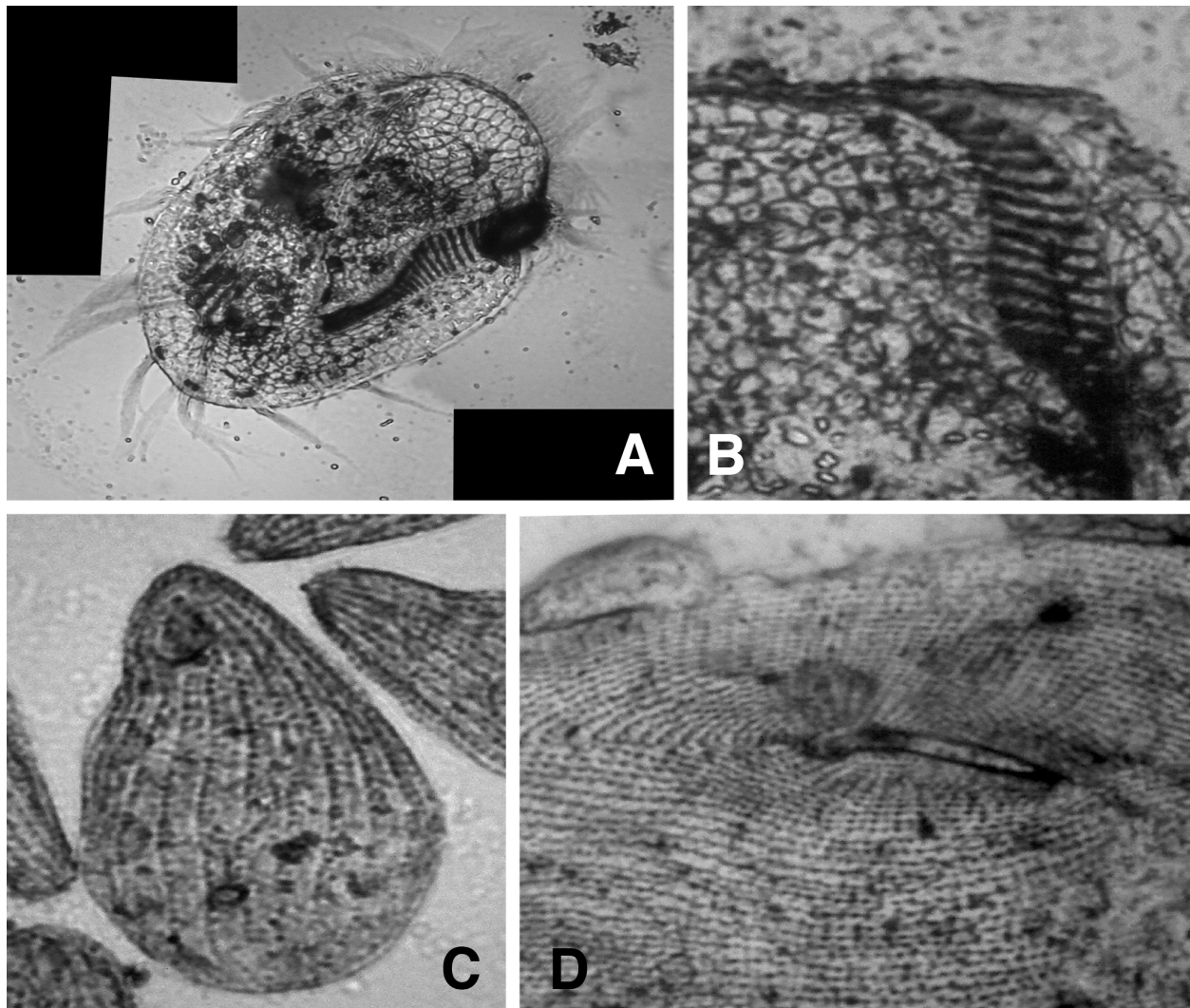
A final note: This method also works on other microorganisms. We discovered serendipitously that endospore-forming bacteria stain well, with the endospores clearly visible as lighter than the rest of the bacterial cells. Foissner (1976) showed that amoebas, flagellates, apicomplexan parasites, and cyanobacteria all have silver-staining structures. Many flagellates look rather nondescript, but euglenoid flagellates can look spectacular: euglenoids have a set of microtubules that form spiraling strips just underneath the plasma membrane, creating a semirigid pellicle. These stain well. The photosynthetic euglenid *Phacus* clearly shows the strips, although the presence of chloroplasts inside the cell makes it harder to see these. Unfed specimens of the colorless euglenoid *Peranema* clearly showed both the spiraling pellicle and the small ejective organelles known as mucocysts, although in well-fed individuals the numerous vacuoles obscured these details.

*Conclusions.* Protists have been used as model organisms for decades. Interest in protists as model organisms is currently resurging: many of them are easy to culture, and they are exceptionally versatile, having uses in research areas ranging from molecular biology to ecology (Montagnes et al., 2012; Simon and Plattner, 2014). Thus it seems timely to present a method of demonstrating protist complexity that can be incorporated into many different classes and areas of research. Self-driven inquiry allows each participating student to have a unique experience. The personalization of the produced results may drive the students’ interest further. If students are aware they can generate authentic and exquisite results themselves, they will be more motivated to put wholehearted effort into the inquiry.

### Literature Cited

- Ansell, S. G. 2008. *The Darkroom Cookbook*. 3<sup>rd</sup> edition. Focal Press, Boston. xxxi + 359 pp.
- Bick, H. 1972. *Ciliated Protozoa: An Illustrated Guide to the Species Used As Biological Indicators in Freshwater Biology*. World Health Organization, Geneva. 198 pp. Available free of charge at <http://www.who.int/iris/handle/10665/40066>
- Foissner, W. 1976. Erfahrungen mit einer trockenen Silberimprägnationsmethode zur Darstellung argyrophiler Strukturen bei Protisten (Experiences with a dry silver impregnation method for revealing argyrophilic structures of Protista). *Verhandlungen der Zoologisch-Botanischen Gesellschaft im Wien*, 11: 68-79.
- Foissner, W. 2014. An update of ‘basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa.’ *International Journal of Systematic and Evolutionary Microbiology*, 64: 261-292.
- Génermont, J., C. Demar, G. Fryd-Versavel, H. Tuffrau, and M. Tuffrau. 1992. Polygenic control of an all-or-none morphological trait in *Euplotes* (Ciliata, Hypotricha). Evolutionary significance of naturally occurring morphological variation in Ciliates. *Genetics Selection Evolution*, 24: 89-105.
- Klein, B. M. 1958. The “dry” silver method and its proper use. *Journal of Protozoology*, 5(2): 99-103.
- Kodak. 1999. “Environmental Guidelines for Amateur Photographers.” Publication J-300. <http://www.kodak.com/global/en/corp/environment/kes/pubs/pdfs/j300.pdf>
- Kosinski, R.J. 2006. An introduction to phylogenetic analysis. Pages 57-106, in *Tested Studies for Laboratory Teaching*, Volume 27 (M.A. O'Donnell, Editor). *Proceedings of the 27th Workshop/Conference of the Association for Biology Laboratory Education (ABLE)*, 383 pages.
- Laybourn-Parry, J. 1984. *A Functional Biology of Free-Living Protozoa*. University of California Press, Berkeley and Los Angeles. 218 pp.
- Lynn, D. H. 2008. *The Ciliated Protozoa: Characterization, Classification, and Guide to the Literature*. Third edition. Springer, Dordrecht. xxxiii+605 pp.
- Montagnes, D., E. Roberts, J. Lukeš, and C. Lowe. 2012. The rise of model protozoa. *Trends in Microbiology*, 20(4): 184-191.
- Simon, M. and Plattner, H. 2014. Unicellular eukaryotes as models in cell and molecular biology: Critical appraisal of their past and future value. *International Review of Cell and Molecular Biology*, 309: 141-198.
- Williams, N. E., and O. H. Scherbaum. 1959. Morphogenetic events in normal and synchronously dividing *Tetrahymena*. *Journal of Embryology and Experimental Morphology*, 7: 241-256.





**Figure 1.** Sample images of stained organisms. A: *Euplotes*. B: *Euplotes*, closeup of oral membranelles and alveolar system. C: *Tetrahymena*. D: *Paramecium*, close-up of cytostome and oral ciliature.

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