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Chapter 19

A Toolbox for Working With Living Invertebrates

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Charlie Drewes received a BA in biology from Augustana College (SD) and an MS and PhD in zoology from Michigan State University. Currently, he is a professor in Ecology, Evolution and Organismal Biology at Iowa State University. His research focus is on rapid escape reflexes and locomotion, especially in oligochaete worms. Charlie teaches courses in invertebrate biology, neurobiology and bioethics. During summers, he leads hands-on, residential workshops for high school biology teachers at Iowa Lakeside Lab. In 1998, he received the Distinguished Science Teaching Award from the Iowa Academy of Science and, in 2002, he received the Four-year College Biology Teaching Award from the National Association of Biology Teachers.

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Introduction

Over the past 10 years, I have been involved in educational outreach activities, such as hands-on workshops, which serve college-level and pre-college biology educators. One goal of these activities has been to develop and share new methods for using invertebrates in biology classroom investigations. One result has been development of numerous, innovative, 'low-tech' tools and gadgets that enhance abilities of teachers and students to collect, handle, view, and experiment with small, living invertebrates. I gratefully acknowledge support and inspiration of many teacher colleagues, who regularly give credence to the well-known saying, *"Necessity is the mother of invention"* (credited to Plato).

Students in my invertebrate biology course routinely and enthusiastically use these tools for collection, handling, observation, and investigative study of small aquatic and terrestrial invertebrates. I expect these tools to be useful in general biology lab teaching at all levels. [For more tools and related resources, see: *http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/*]

Note for Instructors

Since tool assembly is time-consuming and involves careful use of sharp instruments, I suggest making tools before rather during class. It is helpful to provide each student group with a small container for protective storage and convenient access to these tools and materials.

I. FleX-Acto Invertebrate Detachment Tool

Purpose of tool:

This spatula-like tool enables rapid detachment and minimally destructive removal of small, living organisms that adhere to irregular or smooth surfaces in both field and lab contexts. The tool is especially well suited for removal of snails, snail egg masses, planaria, leech cocoons, caddisfly larval tubes, and other small, delicate organisms that adhere to rock, wood, or aquarium surfaces. Additional desirable features of this tool are that it (a) has a very thin, flexible blade, (b) has an adjustable "blade" length, (c) floats, (d) is inexpensive, (e) is made from recycled materials, (f) is safe to use, and (g) may be re-configured in a variety of blade shapes and sizes.

Required materials

- craft sticks (popsicle-type sticks; available at most craft stores in several lengths and colors)
- new, single-edge razor blade
- very flat, smooth work surface for cutting (such as smooth countertop, metal or glass plate)
- flexible "snap-top" lids from margarine, sour cream, yogurt, or other re-sealable container [***Note***: Light colored lids, such as white or yellow) are preferred because they improve viewing contrast while dislodging dark-colored organisms.]
- thin rubber bands (e.g., Universal® or Plymouth® brand #8 rubber bands; 7/8"x1/16"x1/32")
- heavy scissors

Assembly and use of this tool:

1. Use scissors to cut the flexible plastic lid into long, rectangular strips. Each strip should be about 4-7 mm in width and about 5-6 cm in length.
2. Lay one of these flexible strips on a very smooth (non-scratchable) surface or counter top and tightly grip the distant end of the strip with your thumb and forefinger. The free end of the strip should lie flat on the counter surface pointing toward you.
3. Using a brand new single-edge razor blade held in your other hand, place the edge of the razor blade in contact with the free end of the strip, several mm from the end (Fig. 1A). The cutting edge of the razor blade should be positioned as follows: (a) tilted at a steep angle (Fig. 1B), (b) parallel with the cutting surface, (c) directed toward you, but (d) positioned well away from any part of your body.

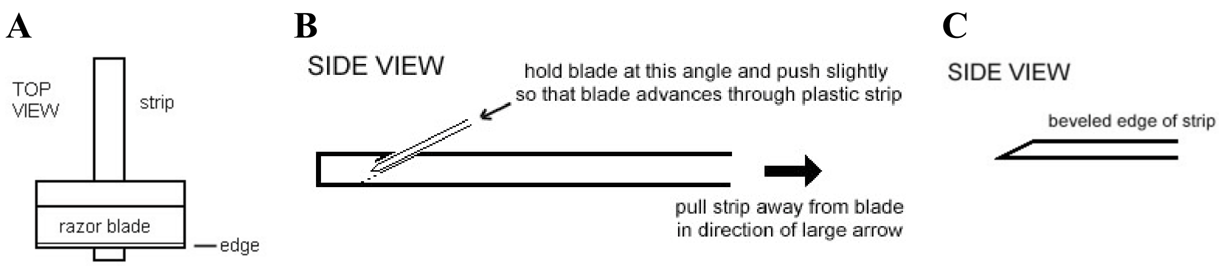


Figure 1. Making beveled blade for *FleX-Acto* tool.

4. With the razor blade tilted at this steep angle, pull the blade slightly toward you and downward while using your other hand to firmly and slowly pull the strip away from you. The blade should smoothly slice through the strip creating a beveled edge at the very end of the strip (Fig. 1C). Make a similar beveled edge at the other end of the strip.
5. Now, place the finished, beveled strip in between two craft sticks with one beveled end extending about 1 cm beyond the ends of the sticks. Make a second beveled strip with a different shape or color and sandwich it between the sticks at the other end (Fig. 2A).
6. Finally, wrap each end of the stick tightly with a thin rubber band (Figs. 2A & 3A). Assembly time per tool is about 5 min. Estimated cost per tool is less than 5¢.]

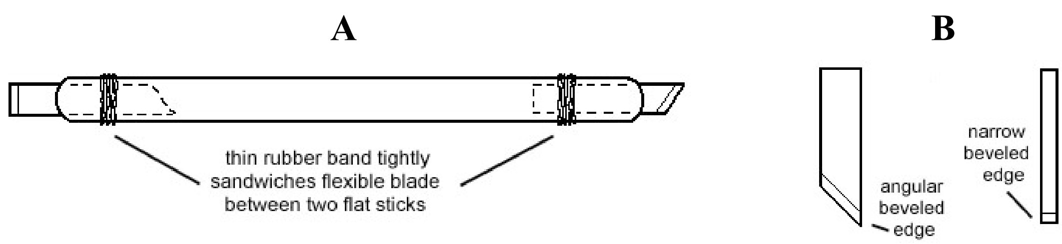


Figure 2. Assembled FleX-Acto tool and accessory blades.

Suggestions for using this tool:

1. To remove aquatic organisms from the underside of rocks or submerged objects, lift the rock out of the water and invert it (Fig. 3B). Then, gently slide the beveled end of the strip under the organism, making sure that the plastic edge remains in contact with the substrate beneath the organism that you are attempting to dislodge. Use the tool to transfer the organism to a storage container with water. Replace the rock, as found.

A B

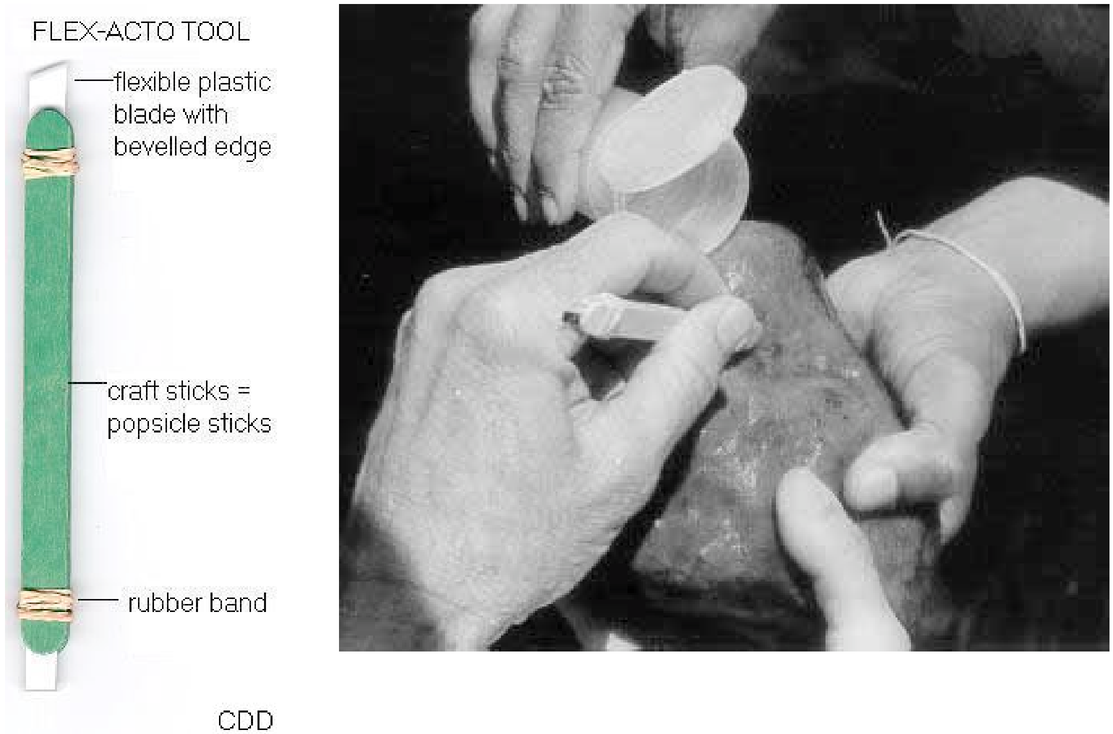


Figure 3. Photos of assembled FleX-Acto tool (A) and its use in the field (B).

2. If the organism is attached to the side of an aquarium, then you may also need a disposable, large-bore plastic pipet to quickly suck up the dislodged organism before it reattaches. Then, transfer the organism to another container for viewing or storing.

3. You can change the flexibility of the beveled strip by changing the distance that the strip extends beyond the end of the stick. Longer, stiffer, narrower, or bent strips may be useful for detaching animals from hard-to-reach areas. With practice, beveled ends with other shapes and angles can be made (Fig. 2B).

II. Pour- Person's Plankton Net

Purpose of tool:

This tool provides a quick, inexpensive, and simplistic alternative to a plankton net. It is most useful in situations when there is insufficient space or distance to use a plankton net. The tool effectively collects and concentrates zooplankton. To use this net in the field, water is collected from a dock or shoreline in a pail-sized container. The net is held level by one person while another pours the water sample(s) through the net. Sieved material is then back-flushed into a collection container. Alternatively, the net may be held vertically and guided through pond water (or aquarium water in the lab) using figure-eight motions. Then, the net is quickly leveled and lifted out of water. Concentrated plankton are back-flushed into a storage container.

Required materials:

- Scissors
- Netting material (Recommend: heavy chiffon fabric or light-weight nylon. Both are available at most fabric stores or in the craft section of large discount department stores for less than $2/square yard.)
- Super glue
- Wooden embroidery hoops with screw tightening mechanism (Suggested size: 6' diameter hoops. Cost is about 50 cents per hoop set.)

Assembly and use of this tool:

1. Cut out a square of material. The size of the square should be about 2-3 inches greater than the diameter of the hoop (Fig. 4).
2. Separate inner and outer hoops; clamp the square of material loosely in the center.
3. Using circular motions of your fingers, press down very gently in the center of the clamped material (Fig. 5). The idea is to create a 1-2 inch-deep pocket in the material, while keeping the material loosely clamped around the entire circumference of the hoop.

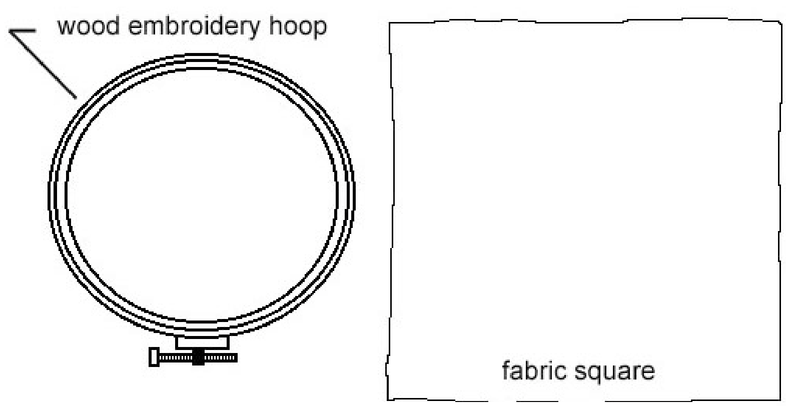


Figure 4. Top view of embroidery hoop and fabric square.

4. Next, tighten the screw clamp and use scissors to trim edges of the material (Fig. 5).
5. Apply a small amount of super glue all around the hoop circumference where the inner and hoops meet. This will permanently secure the netting to both hoops (Figs. 5 & 6).

[Total cost of one assembled net is less than $1. Estimated assembly time is about 15 min/unit.]

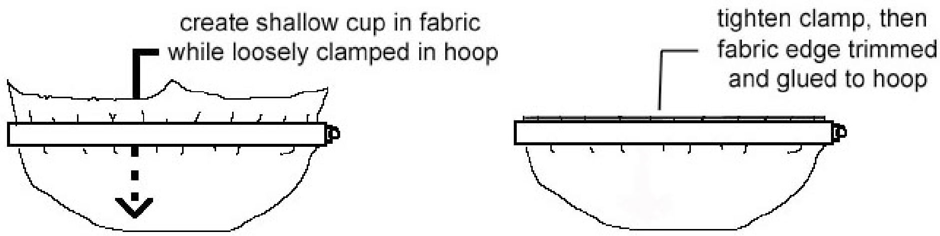


Figure 5. Clamped fabric with shallow pocket and trimmed edges.

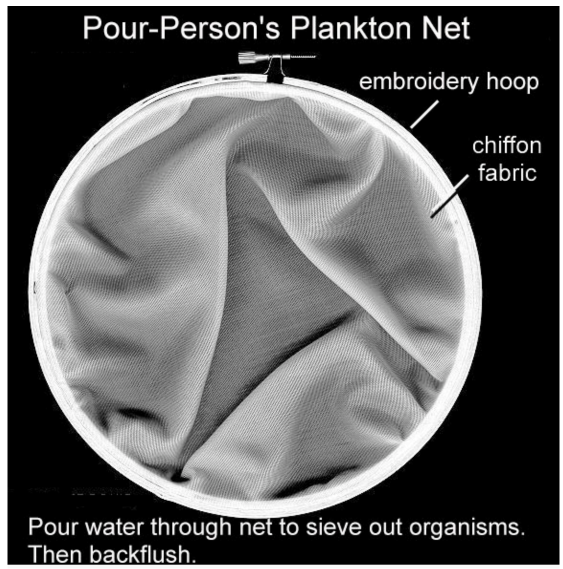


Figure 6. Photograph of completed pour-person's plankton net

Collection Procedure

The following are helpful for field use of the Pour-Person's Plankton net: (a) small to medium-sized pail, (b) pour-person's plankton net, and (c) wide mouth, unbreakable collection jars with tight lid (about 500-1000 ml capacity). A two-person team is also suggested.

1. Use a small bucket to gather a water sample from the edge of a lake, pond, or stream. Collecting samples from a dock may also work if the water surface is within arm's reach. Avoid collecting from areas where the water samples have a significant amounts of suspended algae or macrophytes. These will clog the net. Avoid falling in the water!
2. While one person holds the hoop level over the water, a second person slowly pours the water through the net. The net acts to filter the zooplankton out of the water sample. Make sure the water does not overflow the edges of the net, or zooplankton may be lost.
3. Now, obtain a few cups of clear water. Turn the net over. While centering the net over an empty wide-mouth collection jar, slowly and carefully pour the small volume of water through the net and into the jar. This "back-flushing" procedure will dislodge plankton which collected on the net and these now will be concentrated in the collection jar. If the plankton yield is small, obtain another pail-full of water and repeat the filtration and back-flushing sequence.
4. Do not allow the plankton sample to heat up and do not keep the plankton sealed in the collection container for more than an hour (preferably less).
5. In the lab, place the sample in a large, transparent, labeled container where zooplankton may be easily viewed and withdrawn with a pipet. Dilute the sample with spring water, if needed, to prevent overcrowding. Gentle aeration of samples is strongly recommended to avoid mass die-off. Use spring water to replace water lost by evaporation. Feed plankton tiny amounts of powdered *Spirulina* algae (Recommend: Algae Feast Aquatic Eco-Systems, Inc., Catalog #SP1; 1.2 lbs/$24; www.aquaticeco.com). Do not overfeed.

III. Stretch Pipets

Purpose of tool:

This simple, reliable, and easy-to-use tool is extremely useful for very finely controlled transfer of small, slow-moving microorganisms from one place to another using a very small water volume. Stretch pipets are preferable to glass transfer pipets because stretch pipets are unbreakable, more maneuverable, and can be used with much finer control of suction and water level than standard glass pipets with rubber bulbs. Microorganisms that are easily transferred with stretch pipets include: large protozoans, rotifers, ostracods, tardigrades, nematodes, and small aquatic oligochaetes. Stretch pipets are also very useful for making tiny adjustments of liquid within well slide chambers (see Figs. 9 & 12). Lastly, stretch pipets are ideal for precisely delivering tiny body fragments of *Lumbriculus variegatus* (blackworms) to small predators, such as hydra and planaria, for observing predatory attack and feeding behaviors.

Required materials:

- Disposable polyethylene pipets (smooth, uncalibrated)[e.g. Fisher Cat.#13-711-7]
- Pliers
- New, single-edge razor blade

Creation and use of this tool:

Place the plastic pipet with its tip extending over the edge of the counter. Grip the bulb firmly with one hand and rest this hand on the counter. Use the other hand to firmly grip about 5-6 mm of the tip with the pliers. Then, very slowly, firmly, and steadily pull the tip straight out and away from the pipet until it is stretched about 3-4 cm (Fig. 7). Stretching beyond this point or stretching too rapidly will cause the tip to break away, ruining the effect you are trying to create. When properly done, the stretched portion of the pipet will appear clear with a uniform diameter of about 1-2 mm. Use a new, single-edge razor blade to squarely cut off the flattened tip at a point shown in Fig. 7.

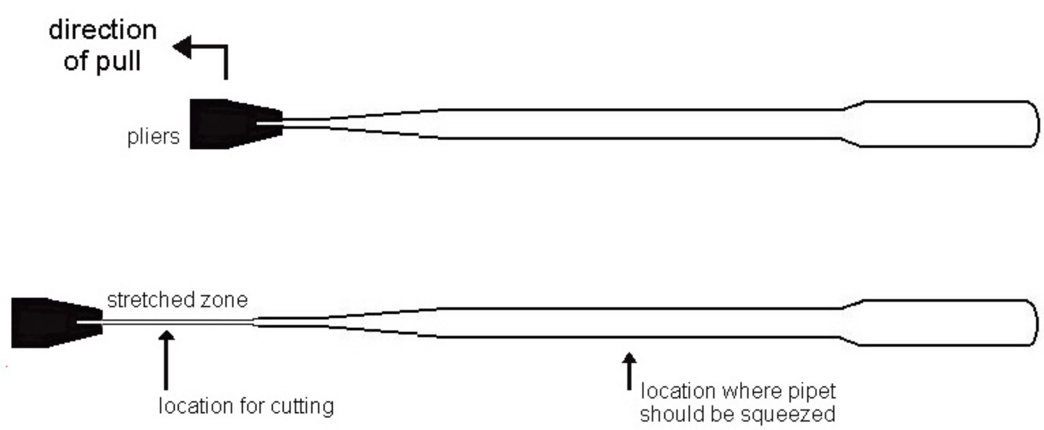


Figure 7. Procedure for stretching plastic pipets.

SPECIAL NOTE: When using stretch pipets, it is highly preferable to grip pipets by the barrel region, just as you would hold a pencil. Then, fluid levels in the pipet are very precisely controlled by gently squeezing or releasing the barrel (Fig. 8), rather than by squeezing the bulb.

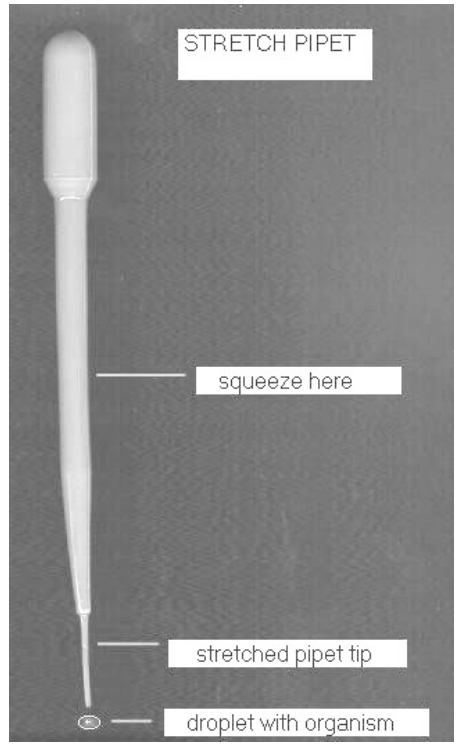


Figure 8. Final configuration of stretch pipet. Note the fine tip. By gripping and squeezing the pipet along the barrel, like a pencil, it is possible to draw up and precisely transfer very small volumes of fluids containing one or more microorganisms.

IV. Foam-well slides

Purpose of tool:

These versatile well slides are made from *Foamies*®, an inexpensive craft product that is non-toxic, non-absorptive, easy to cut, and quick to assemble. When exposed to water, the adhesive backing on the foam sheet will not loosen from the plastic or glass surface to which it is attached. These clear-bottom well slides may be used with either dissecting or compound microscopes, with or without the benefit of unbreakable coverslips over the wells. Wells may be made in all shapes, sizes, and depths. The well slides are particularly useful for viewing small crustaceans (such as daphnids, amphipods, isopods, brine shrimp), small mollusks (snails), small insect larvae, and many other small organisms. Slides are unbreakable and easily cleaned.

Required materials:

- Clear, heavy acetate transparency sheets for floor of well slides [Alternate floor materials: clear plastic lamination sheet, plexiglass, Lexan® clear polycarbonate sheet, or glass slide.]
- Foam sheets made of EVA (ethylene vinyl acetate) that are available in many hobby/craft stores, or craft sections of discount stores. [Option #1: *Darice Foamies*® white, sticky-back sheet. An on-line source is "ArtCity.com". Cat. # DRC-114413 specifies white *Foamie*s® 9"x12"x2 mm, 10 pack for $3.97. Sheets 6 mm thick are also available.] [Option #2: KIDS Funky Foam Sticky Back EVA Sheet. Item #10506 at Hobby Lobby stores.]
- Hand-held paper punch for making circular or oblong holes [Alternative: X-Acto® knife with new blade for making custom-shaped wells.]
- Marking pen
- Scissors
- Soft cloth or facial tissue

Assembly and use of this tool:

1. Use the pen to mark off 1" by 3" rectangles on the paper backing on a foam sheet. Cut out the rectangles with a scissors.
2. Mark the locations and size of the desired wells on the paper backing.

3. With paper backing still attached to the foam sheet, use the paper punch to punch two or three round holes in the sheet (Fig. 9). [Alternatively, use an X-Acto® blade to cut rectangular wells by making repeated vertical slicing motions of the blade that extend completely through the foam and into styrofoam backing or cardboard backing. Avoid cutting movements that shred the foam.]
4. Now, remove the paper backing and carefully lay the foam rectangle on the clear transparency sheet, aligning it along the straight edge of the sheet (Fig. 9). Repeat steps 1-3 until several rows of foam rectangles are attached to the transparency sheet.
5. Then, turn the transparency sheet over so that the clear side faces up. Use a soft cloth or tissue to press down firmly on all surfaces of the transparent sheet so that it thoroughly adheres to the foam strip and all air bubbles under the sheet are forced out.
6. Use a standard plastic pipet (or stretch pipet, as shown in Figs. 7 and 8) to place the specimen into the well and to adjust fluid level in the well. If no cover slip is used, the water level should be about even with the top of the well.
7. If a cover slip is desired, then add enough fluid so that the well is slightly over-filled, creating a convex meniscus. Then, cover the fluid-filled well with a small rectangular strip of clear transparency sheet. Press very gently on the cover slip and tilt the slide sideways to drain off excess water drops. The specimen is now ready for viewing.
8. CAUTION: Make sure that the microscope light source does not overheat organisms within the well.
9. After making observations, slide the cover slip off the well and use a pipet to flush organisms back into their original container.
10. Wells should be rinsed thoroughly in distilled water and air-dried.

[Total cost is less than 10 cents per well slide. Estimated assembly time is less than 5 min/unit.]

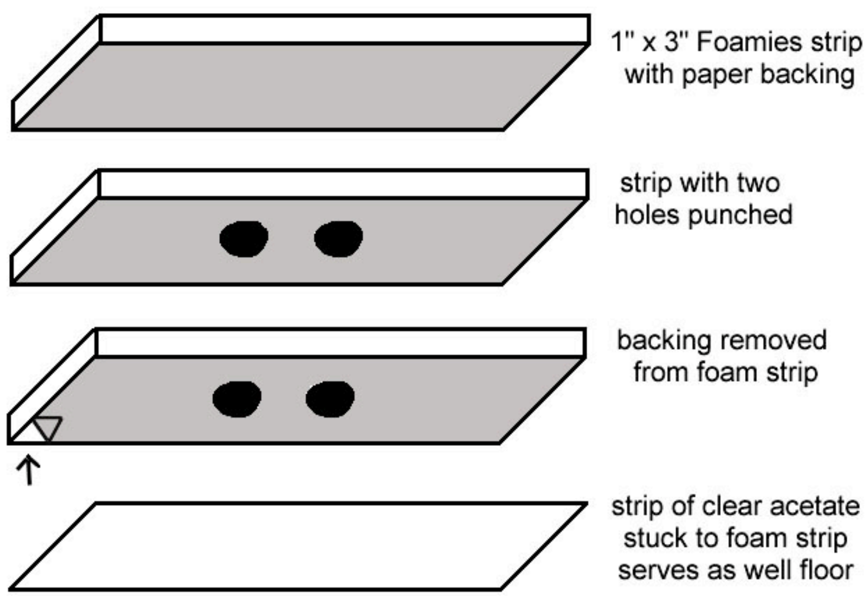


Figure 9. Assembly steps for foam well slides.

V. Tape well slides

Purpose of tool:

Tape well slides are well suited for non-destructive observation of small organisms while they are encapsulated in a relatively thin and uniform plane of focus. The slides are especially useful for viewing living, whole specimens of aquatic oligochaetes, such as *Lumbriculus variegatus* (Drewes and Lesiuk, 1999). These worms are thigmotaxic and prefer refuge in the narrow space between the floor of the well slide and cover slip. The well slides are also excellent for viewing water samples containing microorganisms, such as protozoa, rotifers, nematodes, etc.

Required materials:

- Clear plastic tape [Brands: (1) Frost King® clear plastic weatherseal tape; 2” x 100’ ft roll; sold at home improvement stores, or on-line at: http://www.castlewholesalers.com/
  (2) Scotch® Colored Plastic Tape - Clear, Cat. #190; 0.75” x 125”
- Metal straight edge ruler
- Sharp-tipped forceps
- Marking pen
- New, single-edge razor blade
- Heavy scissors
- Heavy-duty, flexible clear plastic [Suggested materials: Lexan® clear polycarbonate sheet (30 mil thickness), or extra-heavy clear plastic lamination sheet, or thin plexiglass] [Alternate: glass microscope slides]

Assembly and use of this tool:

1. Use a marking pen and straight edge ruler to mark off the desired rectangles that will become the floors of the tape well slides (Fig. 10).
2. Carefully align a long strip of clear tape over a series of the rectangles, as shown in Fig. 10. If wide (2”) tape is used, it is important to minimize air bubble formation under the tape. To minimize such bubbles, use one finger to press down and smooth the tape, beginning at the center line of the tape and working outward toward the outer edges.
3. Add multiple layers of tape, as desired.

   SPECIAL NOTE: Each layer of Frost King® weatherseal tape adds approximately 75 microns (0.075 mm) of depth to the well. Each layer of Scotch® Clear Plastic Tape adds approximately 60 microns (0.060 mm) of depth to the well. Three layers of weatherseal tape nicely accommodate average-sized blackworms (*Lumbriculus*) in the wells.
4. Using a metal ruler and new single-edge razor blade, make vertical cuts through the tape layers, as shown in Fig. 11. These cuts define the well size. Make sure cuts are overlapping at the corners. This insures easy removal of the tape that covers the well.
5. Now, insert the tips of a sharp-tipped forceps under the corner of tape covering one well. Lift the corner of tape to remove all tape layers covering the well (Fig. 11). Next, use a heavy scissors to cut out the rectangles from the sheet. Use a single-edge razor blade to trim away any tape that extends over the edges of the cut rectangles. Cut two narrow strips of tape and attach them to the underside of the well slide, as shown in Fig. 12. These tape strips act as spacers or ‘feet’ that

slightly elevate the well slide above the microscope viewing stage, thus helping to reduce scratching or marring of the floor of the well slide.

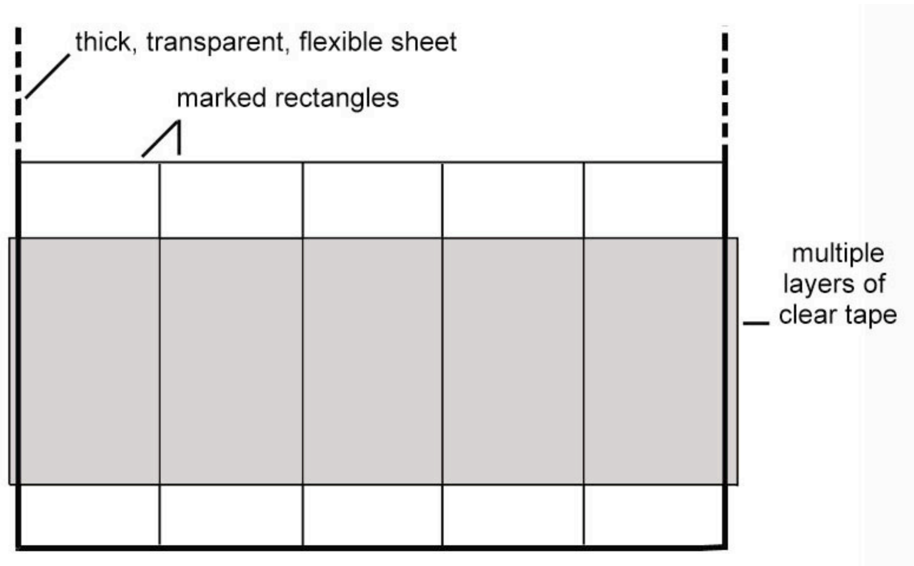


Figure 10. Rectangles on transparent sheet covered by multiple layers of clear tape.

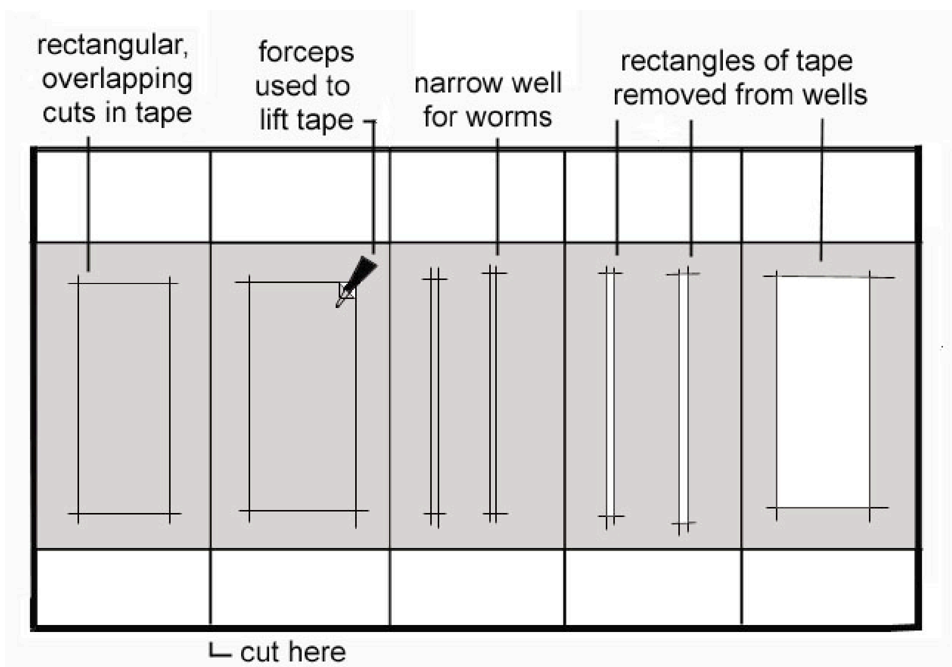
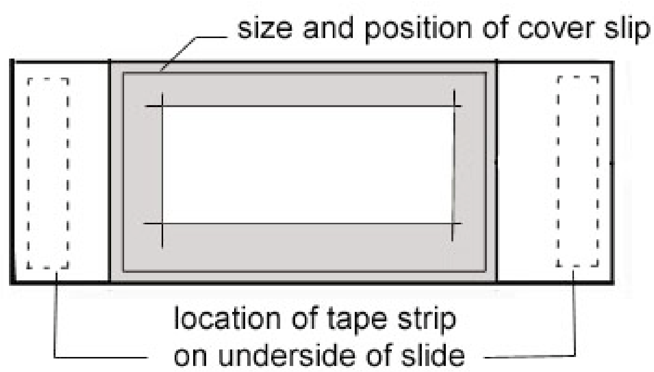


Figure 11. Suggested position and size of cuts for tape well slides. Note that forceps tips are used to pry up the tape at one corner of the well.

6. On a clear transparent sheet, mark off a series of rectangles that define the cover slips to be used over the tape wells. The size of the cover slips should be larger that the well but slightly less that the length and width of the tape that it will rest on (see Fig. 12).
7. Use a plastic pipet (or stretch pipet, as shown in Figs. 7 and 8) to place a living specimen in the well, along with a few drops of water.
8. Cover the fluid-filled well with one of the cover slips made in step 8. Press slightly on the cover slip and tilt the slide sideways to drain off excess water drops. Now, the specimen is ready for viewing
9. CAUTION: Be sure the microscope light does not overheat organisms in the well slides.
10. After making observations, carefully slide the cover slip off the well and use a pipet to flush the organism back into its original container.
11. Wells should be rinsed thoroughly in distilled water and air-dried.

    [Cost is less than 10 cents per well slide. Assembly time is less than 10 min/unit.]

Figure 12. Completed tape well slide showing size and position of cover slip. Note the tape 'feet' on the underside of the well slide (dotted lines).



VI. Widgets

Purpose of tool:

This tool allows students to gently touch, nudge, or push small aquatic or terrestrial organisms, such as mollusks, annelids, crustaceans, insects, etc. Widgets are particularly useful for testing tactile sensitivity of specific parts of small, soft-bodied organisms, such as the end of a worm or the tentacle of a snail. These probes deliver touch stimuli of consistent strength, while minimizing injury or insult to organisms.

Required Materials:

- Long, slender rubber bands for widget tips [*For thick widget tips*: Universal® or Plymouth® brand #19 rubber bands; 3-1/2"x1/16"x1/32"] [*For thin widget tips*, two options exist: (1) Stretch-Rite® Metallic Elastic Cord. This product is sold at many fabric stores and contains eight slender white bands within the metallic braid; (2) "Rubber legs" colored elastic strands are sold at fly fishing supply stores. Rubber legs have exceptional elasticity properties.]
- Wooden applicator sticks
- Heavy scissors or side cutters
- Small disposable pipet tips (e.g., 10 microliter size)
- Small ruler
- Super glue (quick-drying cyanoacrylate glue)

Assembly and use of this tool:

1. Firmly insert one end of an applicator stick into a small, disposable pipet tip (Fig. 13).
2. Use heavy scissors to cut off the pipet tip just beyond the inserted end of the stick.
3. Obtain multiple lengths of elastic band, each about 3-4 cm in length. [Note: If thin rubber strands are obtained from braided metallic elastic bands, then it will be necessary to remove the metallic braid by teasing out and pulling apart the individual strands of rubber band that compose the core of the band.]
4. Insert one end of an elastic band into the trimmed pipet tip, with about 1 cm of band extending beyond the narrow end of the pipet tip, as shown in Fig 13.
5. Insert one end of an applicator stick into the large end of the pipet tip. Firmly wedge the stick against the elastic band and the pipet tip, making sure that at least 1 cm of elastic band still extends beyond the pipet tip.
6. Apply a small drop of super glue into the large end of the pipet tip. The glue should contact the rubber band and wooden stick within the pipet tip. Allow the assembly to dry.
7. Use a scissors to trim off the rubber band, as needed.
8. NOTE: Many other widget configurations are possible. Some are shown in Fig. 14. Thin-tipped widgets may be made from very slender rubber band material, or even human hair. Looped widget tips are very useful for stimulating tail or head segments of blackworms (*Lumbriculus*). Such stimulation reliably initiates helical swimming and body reversal behaviors in these worms, respectively (Drewes and Cain, 1999)

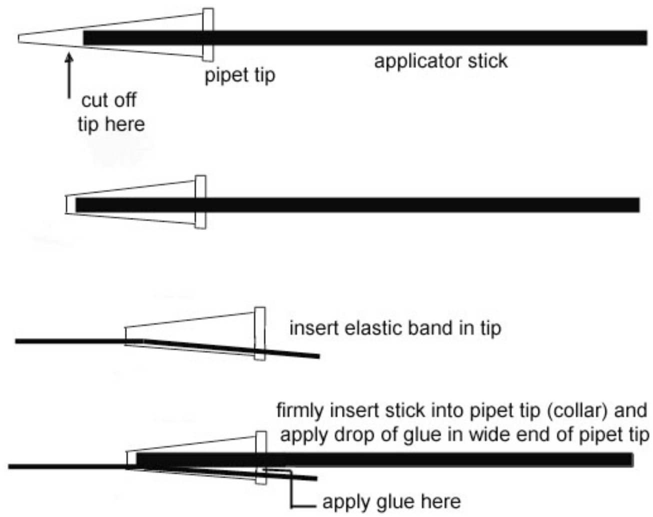


Figure 13. Assembly steps for making widgets.

[Total cost per widget is less than 5 cents. Assembly time is less than 5 min/unit.]

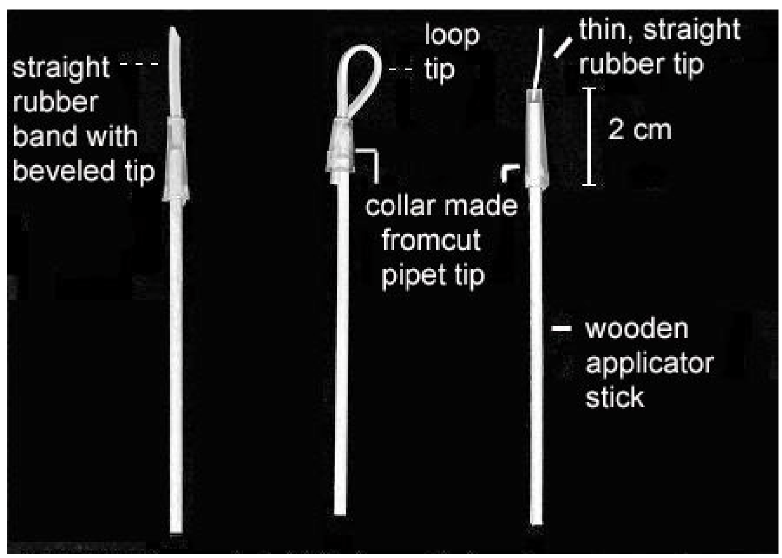


Figure 14. Examples of three widgets. The loop widget in the center is particularly useful for initiating helical swimming and body reversal in blackworms (*Lumbriculus*).

Literature Cited

Drewes, C. D. and K. Cain. 1999. As the worm turns, *American Biology Teacher*, 61:438-442.

Drewes, C. D. and N. Lesiuk. 1999. Blackworms, blood vessel pulsations and drug effects, *American Biology Teacher*, 61:48-53.

Related Web Images and Resources

http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/

http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/flexacto2.jpg

http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/flexacto.jpg

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http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/micFOSS-pipetFIN.JPG

http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/foamslide1.jpg

http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/widgets.jpg

http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/micFOSS-widgFIN.jpg