

Some techniques for preparing, manipulating and mounting dinoflagellates

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ABSTRACT—Twentytwo techniques from among those in use in the palynological laboratory at Stanford University are outlined. Many of them are adaptable to different scales of production and to different purposes of research or industrial service work. Sophisticated equipment beyond that conventionally present in any moderately well equipped palynological laboratory is not necessary. Techniques applicable to fossil dinoflagellates are emphasised but a few procedures for obtaining and preparing modern dinoflagellates are included.

INTRODUCTION

Techniques for the recovery, preparation and study of palynomorphs – like the kitchen techniques of a good cook – are generally simple and unexciting in themselves, however fundamental they are to the success of the undertaking. The superior effectiveness of modern palynological techniques compared with those of, say, 25 years ago, is immediately obvious when representative microscope slides or published illustrations from “then” and “now” are compared. Although currently new major improvements in palynological techniques are announced infrequently, they tend to be communicated more effectively than many individually minor improvements whose collective benefit, nevertheless, may be substantial. The innovations among the techniques discussed here are definitely minor ones, but they have been well tested by my students and myself at Stanford and have been incorporated to great advantage into our routine procedures for working with dinoflagellates. They will not appear to be highly sophisticated procedures to those at home in a modern biological laboratory or familiar with some of the practices now common for preparing palaeontological materials for study under the scanning electron microscope. However, they can be carried out in a relatively simple laboratory with more or less readily available materials and without the aid of specialised equipment (e.g., a micromanipulator). Some were developed at Stanford, others were adapted from other laboratories, but, as with the analogous kitchen recipes, it is difficult to appropriately allocate credit for the original ideas.

Just as no culinary recipe will appeal to all cooks or to all appetites, or be suitable for all occasions, these techniques fit different scales and sizes of operations with different objectives. If some of them seem to be directed towards details too trivial to be of concern to the industrial palynologist, remember how details of foraminiferal aperture structures were once regarded as important only for the academic specialist. Dinoflagellate specialists

are just beginning to pay close attention to certain details of morphology that, although difficult to examine, seem likely soon to join the list of criteria essential for identifying genera and species.

Most of the techniques reviewed here will require modification to meet individual needs and facilities. Therefore, dimensions given are not limiting, but represent items used in the laboratory at Stanford. Similarly, amounts and concentrations of reagents may require or permit adjustment to suit specific circumstances. To avoid confusion, “glycerol” is used in the following remarks to refer to reagent glycerine, whereas “glycerine jelly” is used for the glycerine-water-gelatine combination used as a mounting medium.

SPECIFIC PROCEDURES

The following steps constitute a routine processing procedure that is satisfactory for extracting cysts from most fossil and Recent sediment samples:

1. Percussion, if necessary to finely divide sample.
2. Hydrochloric acid to remove carbonates.
3. Hydrofluoric acid to remove silica and some silicates.
4. Boiling in concentrated hydrochloric acid to remove fluorosilicates.
5. Heating to near boiling in nitric acid to remove pyrite and some unwanted organic debris.
6. Sodium hypochlorite (about 5%) to oxidise and remove some unwanted organic materials.
7. Short centrifugation with detergent to remove clay-sized residue.
8. Centrifugation in zinc bromide solution (sp. gr. 2.0) to separate organic and inorganic residues.
9. Acetolysis to colour cysts, with side effect of removing additional unwanted organic residue from some samples. Alternatively, biological stains (e.g. safranin, basic fuchsin, Bismark brown) may be used for colour.
10. Sieving to separate a convenient number of size

fractions.

11. Final preparations: a) dispersing and sealing a sample in strew mounts, b) orientating individual specimens for inspection or photography, or c) drying specimens for mounting for the S.E.M.

Abbreviating this sequence of treatments to include just steps 2–3 and 9–11 provides a suitable method for concentrating cysts (but not thecae, which are disrupted by sodium hypochlorite and destroyed by acetolysis) from a modern plankton sample.

ADDITIONAL COMMENTS

1. Note regarding Step 6 (treatment with sodium hypochlorite). This treatment substitutes for the widely used sequence of treatment with Schulze's solution followed by alkaline rinse. It is highly effective for many samples, and prolonged or vigorous treatment will not damage most cysts. Procedure: Add to the residue (which should have been centrifuged but need not be rinsed, following treatment with hot hydrochloric acid) about 25 ml of approximately 5% aqueous sodium hypochlorite (household bleach). With a glass beaker conveniently close to receive overflow, stir to thoroughly mix with residue, pouring into beaker as appropriate. When ebullition subsides, add small amounts of concentrated hydrochloric acid and more sodium hypochlorite alternately. The reaction, which may follow a delay of one to five minutes and vary in intensity with the sample from nearly imperceptible to surprisingly – on occasion, startlingly! – vigorous, is usually accompanied by at least a definite change in colour from blackish to brownish and some generation of heat. Terminate the treatment with an excess of hypochlorite; that is, with the solution alkaline.

2. Note regarding Step 11a (final mounting of specimens). Two properties of glycerine jelly make it a superior mounting medium for dinoflagellates. First, its low index of refraction assures a highly desirable optical contrast, which increases the visibility of thin and delicate features of surface ornament and wall structure. Consequently, details which may be barely discernible in media such as polyvinyl alcohol or carboxymethyl-cellulose are rendered much more readily visible (and photographable). Secondly, the ease and speed with which glycerine jelly can be remelted and restiffened makes selective orientation of individual specimens a feasible possibility. Selective orientation, in turn, facilitates extraction of maximum information from a minimum number of specimens.

There are also some disadvantages to glycerine jelly. Slide preparation is somewhat slower, owing to the necessity of cleaning and sealing the slides after mounting. Also, ultimate permanence of slides is assured only if the seal remains unbroken, which is clearly impossible to guarantee over the long term if slides are likely to be used by many different persons without effective

curatorial care. Finally, the slides must be protected from more than brief exposures to temperatures high enough to melt the glycerine jelly.

For large scale slide production, as in many oil company palynological laboratories, this technique may seem impractical for routine use. However, occasional preparation of glycerine jelly slides, especially in conjunction with techniques that permit more thorough investigation of a single specimen than is possible in most strew mounts as normally made, may well be worth the extra time and effort, in the interest of more reliable identifications. Whereas familiar species whose morphology is well understood can often be identified confidently from fragmentary or poorly preserved specimens, this is not true of unfamiliar species. Use of a mounting medium that provides optimal conditions for observation and also permits manipulation of specimens, as well as techniques for such manipulation, are likely to become increasingly important as specific identification comes to depend on increasingly fine points of morphology.

SOME SPECIAL PROCEDURES AND TECHNIQUES

The following comments are arranged approximately in the order in which procedures would be applied to a sample or specimens, except that the comments about the handling of modern dinoflagellate thecae follow those about dinoflagellate cysts.

1. **To make and use a "Bostick" tube for heavy liquid separation.** (This extremely convenient device was developed by Neely H. Bostick.) The directions are for a tube to be used with a 50 ml centrifuge tube. Cut a piece of tygon* tubing (5/8" inner diameter × 1/16" wall thickness) 10 cm long and insert through a circular metal ring having 5/8" inside diameter. Insert into the end of the tubing the small end of a rubber stopper of such size that the metal ring, when slid down tightly over the end of the stopper, will seat firmly against the taper. Pour into the tube about 5 ml of residue which has been mixed thoroughly with 2.0 specific gravity aqueous zinc bromide solution; fill to no less than 1.5 cm from the rim with additional zinc bromide; cover the end with a protected finger (rubber "finger cots" serve excellently) and mix thoroughly by inverting several times. Add one drop of concentrated hydrochloric acid to the top surface; do not mix. Place Bostick tube in 50 ml plastic centrifuge tube and fill space between tubes with water. Centrifuge 20 minutes (countertop clinical model at nearly top speed, or equivalent). Remove Bostick tube and pinch it tightly with crook-necked pliers a short distance below the level where the organic material is

* Editorial note: Tygon tubing is flexible transparent tubing which is pvc based.

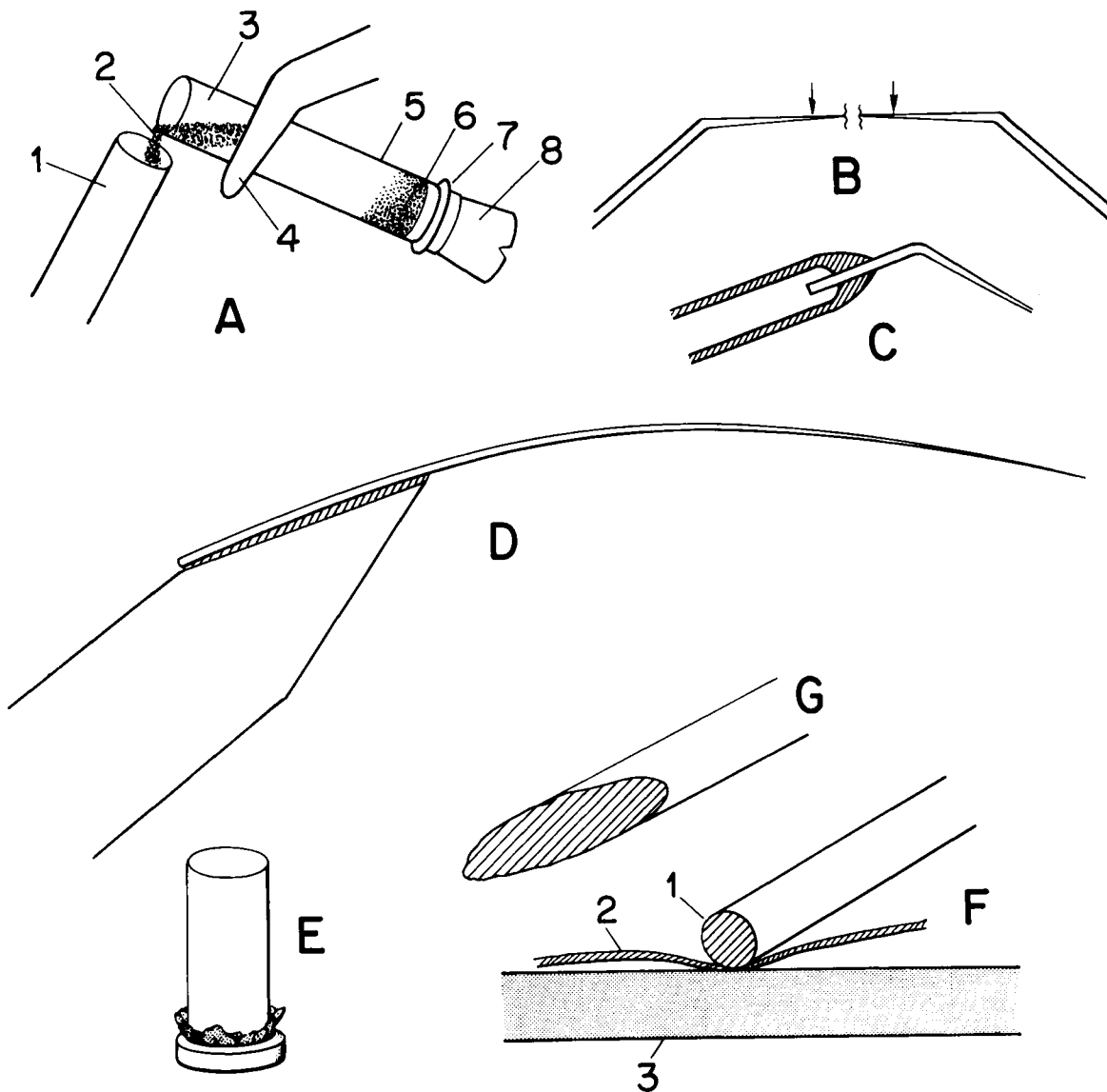


Fig. 1. Some Aids to Sample Processing and Sample Manipulation.

- A. Decanting floated organic residue from Bostick tube after centrifugation in sp. gr. 2.0 zinc bromide: 1, 15 ml glass centrifuge tube; 2, organic residue being decanted; 3, tygon tube; 4, tip of crook-necked pliers pinching tube below floating organic residue; 5, zinc bromide solution; 6, insoluble mineral matter; 7, metal ring around tygon tube and rubber stopper; 8, grooved rubber stopper closing end of plastic tube.
- B. Glass blood capillary, finely drawn in microburner flame at an angle to its length. Arrows show points at which to break after scratching with diamond scribe.
- C. End of micropipette. Detail of junction between plastic tube and capillary (unseen end of plastic tube is attached to medical syringe).
- D. Eyelash affixed to tapered end of small-diameter wooden dowel.
- E. Assembled sieving unit consisting of 2.5 cm square piece of nylon monofilament bolting cloth stretched over one end of a short section of 15 ml centrifuge tube (glass or rigid plastic) and held in place by a tightly fitting rubber ring.
- F. Transversely broken end of finely drawn glass stirring rod (1) being used to cut through a dinoflagellate cyst wall (2) by pressing it against a glass microslide (3) with a "rocking" motion (arrows).
- G. Portion of a microknife for the same purpose, made from a randomly broken oblique section of a similar rod.

concentrated at the top of the contents (Fig. 1A). Tip the open end of the plastic tube into a 15 ml glass centrifuge tube (short conical bottom), aiding the transfer of the organic material with a jet of water from a wash bottle. By this means, any desirable residue that may be left in the Bostick tube can be flushed out into the glass tube. Fill the centrifuge tube with water, close the opening and mix thoroughly by inverting the tube several times to avoid density stratification of the water and the zinc bromide suspension. Centrifuge and decant; repeat as needed to remove all bromide solution.

Disassemble "Bostick" tube; pour out remaining heavy liquid (which can be reclaimed and sieved for re-use) and flush out residue; clean all parts thoroughly with test tube brush and detergent. Dry for reassembly and re-use.

Note: To prevent suction from holding the rubber stopper to the bottom of the 50 ml tube after centrifugation, incise an "X" across the large end of the stopper with the angular edge of a small file.

2. To make temporary strew of residue in glycerol. For a temporary preparation from which to pick individual specimens, place one or two drops of residue in water suspension on a standard glass microslide and add one drop of glycerol. Mix well with small glass stirring rod and spread out over a conveniently sized area of the slide. Allow the water to evaporate, speeding the process on a warming table if desired.

Examine the strew without a coverglass under a dissecting microscope or with the low power objective of a compound microscope. The high viscosity of the glycerol will retard the movement of specimens and facilitate picking them up with a micropipette; the glycerol will also insure against both desiccation and electrostatic flocculation.

3. To make a micropipette. Soften a glass blood capillary tube (available from medical supply firm) at its mid-length in a gas microburner. Remove the tube from the flame and, holding the straight sections at about 90° to one another, pull ends apart to produce two tapered tubes. Scratch the surface of each finely drawn portion with a diamond scribe at a point where the diameter is appropriate to the size of specimens to be handled (Fig. 1B); flex sharply between the fingers to break the tube. Check the broken ends under the microscope and reject tubes with oblique or irregular breaks. Soften one end of a 50 cm piece of tygon chemical tubing (4 mm inside diameter, 1.5 mm thick wall) over the microburner flame. Insert the untapered end of the capillary and, squeezing with the fingers cautiously to avoid burns, form the soft plastic tightly around the capillary (Fig. 1C). Allow to cool. The capillary can be removed and reinserted or replaced by another as desired. Attach

the opposite end of the plastic tubing to a medical syringe.

4. To use and care for micropipette. The pipette can be used under either a stereoscopic or compound microscope for picking up and manipulating specimens in a variety of fluids and for transferring them from one fluid or container to another. Use the plunger of the medical syringe for gross action only. For more delicate action in sucking up, discharging, moving, and cleaning specimens, simply squeeze the plastic tubing between the fingers of one hand while directing the point of the pipette with the other hand. A half day of practice will make a budding expert.

A number of specimens can be collected in the pipette before discharging any of them as follows: Insert the tip of the pipette into the fluid (i.e., glycerol or water, whatever the strewn material is in), either on the slide under study or in a separate small container, and squeeze the tube between the fingers to force out a few bubbles of air. Then, release the fingers and, as soon as the fluid has begun to flow into the pipette (i.e., well before the vacuum has been satisfied), withdraw its tip from the fluid. The surface tension at the tip of the pipette will prevent an inflow of air, thus maintaining the lower pressure within the tube. A series of specimens can then be sucked up individually without squeezing the tube further by quickly inserting the tip of the pipette next to each specimen and withdrawing it again from the liquid as soon as the specimen has been sucked into the orifice. Until considerable skill has been developed, it is easier to pick specimens from glycerol than from water, owing to the greater viscosity of the former, which slows the movements of all particles.

This technique also enables one to "blow" debris away from the vicinity of a specimen, or to move a specimen to another area of the slide. Alternatively, picked specimens can be separated from associated debris by successive transfers from one drop of glycerol to another, either on the surface of a clean, flat microslide or in cavities of a cavity-slide. Once placed in a drop of glycerol, picked specimens are not only protected from accidental desiccation, but, with moderate care in a dust-free container, are relatively secure indefinitely. Also, specimens being picked from a strew can be sorted according to species by discharging them from the pipette into (minute!) drops of glycerol arrayed in columns and rows on a clean microslide.

The glass pipette must be kept meticulously clean to prevent specimens from sticking to its inner surface (still in sight, perhaps, but frustratingly out of reach!). If the orifice of the pipette is contaminated with even the smallest amount of glycerine jelly, care should be taken to clean it thoroughly before further use by inserting the tip into warm water (e.g. a drop on the warming table) and flushing water in and out. At all times avoid sucking

fluid or specimens beyond the large end of the capillary tube as they will be very difficult to recover from the inside of the plastic tube. A clean pipette can be assured by removing it from the plastic tube when not in use and storing it, point down, in a 10ml beaker containing about 2 ml of chemists' glass-cleaning solution (concentrated sulphuric acid saturated with potassium chromate). Before re-using the pipette, rinse it well with flushes of fresh water.

5. To make and use an eyelash manipulator. Affix the base of a single eyelash to the tapered end of a short (c. 10cm) piece of fine (eighth-inch or 3mm) wooden dowel with a non-water-soluble cement, such as fingernail lacquer (Fig. 1D). Use the eyelash as a probe to move and adjust specimens.

An eyelash tapers gradually to a very fine point. It is ideal for moving or orientating specimens in individual mounts, for isolating specimens from debris prior to picking them up in a micropipette, and for dislodging debris that may be adhering to them. Even particles of debris inside cysts often can be removed successfully by inserting the eyelash tip through the archeopyle and agitating it (a rare benefit of an unsteady hand!). A dry, clean eyelash can also be used for transferring and arranging dry cysts preparatory to mounting for the S.E.M.; electrostatic attraction will suffice to hold specimens to the eyelash, yet permit easy release.

An eyelash can be used freely in water, glycerol, or glycerine jelly, but sodium hypochlorite solution will quickly soften and distort it by attacking the animal protein.

6. To make graded sieves. First, cut sections about 3.5cm long from a piece of glass or rigid plastic tubing having an inside diameter of about 1.5cm. Then obtain rubber or plastic rings (e.g. rubber garden hose washers) which fit snugly around the tubing. These parts will make the sieve-holder and are for re-use after washing. Next, cut 2.5cm squares of several grades of nylon bolting cloth (e.g. 100, 65, 35, and 20 μ m mesh sizes). The bolting can be handled most conveniently if placed between sheets of paper and cut with a sharp paper cutter.

To assemble the sieve, place the elastic ring on a firm, clean surface, lay a square of nylon on top. Then press the tube into the opening of the ring washer, which will stretch the nylon across the end of the tube (Fig. 1E). To clean, simply disassemble the sieve and discard the nylon; wash other parts with detergent and test tube brush of suitable diameter.

7. To sieve a sample of residue. Pour the water-suspended residue through a sieve assembled from the

coarsest mesh to be used, catching the through-flow in a small beaker. Flush the last fines from the coarser fraction caught on the sieve with a jet of water from a wash bottle. Then invert the sieve over a watchglass and retroflush the coarser fraction into the watchglass with a gentle jet of water. This fraction is now ready for examination, specimen selection, or strew mounting.

Proceed similarly with finer sieves. If the volume of through-flow water with its suspended fine residue becomes excessive, let the suspension settle (or centrifuge if in a hurry); then decant to concentrate, and continue sieving. Although the finer sieves will clog quickly with residue, this problem can be minimised by adding a drop of non-sudsing detergent and vigorously "pumping" the fluid in and out of a medicine dropper whose tip is held close to the sieve surface. Add more unsieved residue and detergent as appropriate. Alternatively, an ultrasonic probe inserted in the suspension in the sieve tube may relieve the clogging (but beware of vibrations that might tear the dinoflagellates).

Note: Sieving a sample unalterably biases it for quantitative analysis, such as determination of relative abundance of species or of specimens per unit weight or volume of sediment.

8. To make a permanent glycerine jelly strew mount. Place the water-suspended residue in a watch glass, allow the particles to settle briefly, then concentrate them in the center by a gentle swirling. Withdraw a fraction of the concentrate with a medicine dropper and place it on a clean coverslip. Add a drop of warm glycerine jelly and mix thoroughly with a small glass rod, spreading the mixture evenly over the coverslip except for a narrow strip along all edges. Place the coverslip on a warming table and allow the strew to dry thoroughly, testing its dryness by holding a cool glass microslide close to the surface and checking for condensation. Remove the coverslip from the warming table when dry, and allow it to cool thoroughly.

On a clean glass microslide which has been warmed slightly, place one or two drops of warm glycerine jelly. Invert the cool coverslip onto this and gently press from above, forcing the fluid jelly to flow quickly to the edges of the coverslip before particles of the residue are remobilised. Allow any excess jelly to extrude beyond the coverslip margins. Put this mount aside to cool, and handle it with special care until cleaned and sealed.

After one to three days, run a razor blade along all edges of the coverslip, then peel away such excess of glycerine jelly as can be removed this way and clean away all the rest by wiping along the coverslip edges gently but thoroughly with a piece of soft tissue moistened with warm water. When the surface of the slide has dried completely, seal around the coverslip edges with a generous application of fingernail lacquer, using the brush provided or a finely drawn medicine dropper or

medical pipette. Special slide-ringing compounds offer a more secure seal and should be used if they are available.

Note: Do not use Canada Balsam, "Permout", "Elvacite", or similar mountants that are dissolved in xylene or toluene, as the final mountant for a strew in glycerine jelly. Highly undesirable fogging on the inner surface of the slide will surely result.

9. To acetolyse a single specimen. Distribute a very fine line of silicone fluid around the edges of two cavities in a glass cavity-slide. This will prevent reagent fluids from creeping over the glass surface. Place a drop of glacial acetic acid in each cavity. Under the stereoscopic microscope, transfer a specimen into one drop of acid with as little water or glycerol as possible. Evacuate the water from the micropipette, then agitate the specimen with a jet of acid created by squeezing the plastic tube, to ensure mixing of the fluids. Now transfer the specimen to the drop of acid in the second cavity and repeat the agitation. With an ordinary pipette, replace the acid in the first cavity with acetic anhydride. Introduce the specimen into this with as little acid as possible, reserving the acetic acid in the second cavity for use later as a rinse. Add to the acetic anhydride about one-eighth its own volume of concentrated sulphuric acid, and mix thoroughly with a jet from the micropipette. Place the slide on a small hotplate stabilised at about 150°C for 5 to 10 minutes, being careful to add more acetic anhydride as needed to compensate for evaporation. By the end of this time, the mixture will have turned from colourless to amber or brown (if not, increase the temperature). Remove the slide from the heat and allow it to cool. Add enough glacial acetic acid to the sulphuric acid to nearly fill the cavity and, under the stereoscopic microscope, withdraw the specimen in a micropipette, transferring it to the reserved acetic acid in the second cavity. Agitate the specimen in this fluid and then rinse it further by transferring it to water in another cavity. Repeat the water rinse.

For maximum security and control, carry out the entire process under the stereoscopic microscope. This will require a suitably small hotplate, and adequate lighting, as well as a shield against the fumes that rise from the slide.

Acetolysis of modern specimens can be used to destroy either the theca surrounding a cyst or the protoplast within (usually also opening the archeopyle in the process); it will also impart a yellowish brown colour to most specimens. The reaction appears to be approximately equivalent to fossilisation with regard to its affect on organic materials. Thus, except for deformation that may result from sedimentary compaction, and corrosion from extended oxidation, modern cysts treated by acetolysis are visually equivalent to fossil cysts.

10. To stain specimens individually or in small numbers. Transfer specimens into a small quantity of water in a glass cavity-slide. Add a drop of stain with the micropipette and stir with the eyelash. If the process is carried out under the stereoscopic microscope, the staining can be controlled closely by withdrawing the specimen(s) into the pipette when the stain has reached the desired intensity. It is much easier to stain slowly and with several additions of relatively dilute stain than to remove stain from overstained material. If necessary, an overstained specimen can be bleached by immersing it in water to which a little 5% sodium hypochlorite has been added. This treatment will also bleach any colour that has been imparted by acetolysis. Retreatment with acetolysis or staining is possible after very thorough washing to eliminate all traces of hypochlorite, which otherwise might cause eventual colour loss after mounting.

11. To orientate a single cyst in a selected position. Place a minute spot of glycerine jelly on a clean glass microscope slide. Melt the jelly on a warming table or small hotplate and spread it out with the aid of the eyelash manipulator so that the surface convexity of the jelly mass is low. Then, allow the jelly to cool and solidify. Using the micropipette, introduce a cyst onto the surface of the jelly mass from either water or glycerol, leaving the specimen with as little fluid as possible. Warm the slide to melt the glycerine jelly and return the slide at once to the microscope stage, immediately adjusting the cyst to the desired orientation with the aid of the eyelash. Again, allow the jelly to cool. Without adding a coverslip, check the orientation of the specimen by examining it under the microscope. It may be necessary to use the compound microscope if extra magnification is required to see desired detail. Dry objectives up to 63-power can be used without difficulty if the glycerine jelly mass is sufficiently thin. Warm the slide and readjust the orientation for fine control of position if necessary. Specimens mounted this way (i.e. in glycerine jelly without a coverslip) can be examined without difficulty under an oil immersion objective if the thickness of jelly covering the specimen is sufficiently small. After examination, the oil should be removed completely from the upper surface of the glycerine jelly by tilting the slide and applying successive drops of xylene directly onto the jelly mound. When all xylene has evaporated from the surface of the jelly, the specimen can be mobilised again with heat, and reorientated for viewing in different directions, or a minute drop of water can be added to the warm glycerine jelly and the specimen can be removed with the micropipette. Alternatively, the specimen and its surrounding medium can be incorporated in a permanent mount under a coverslip.

13. To rough-cut a section through the wall of a specimen.

Draw out a small glass rod (3 mm × 6 cm) after softening it at midlength in a microburner flame. Scratch the surface and break the drawn rod to get a clean transverse break at a point where the diameter is appropriate. Apply the sharp edge of the break to the specimen in a drop of glycerol on a glass slide under the stereoscopic microscope and, with a rolling action, cut through the specimen to the surface of glass slide (Fig. 1F). Alternatively, break the glass rod without scratching and use the obliquely broken edge as a knife, pressing it through the specimen against the surface of the glass slide (Fig. 1G).

14. To dry a specimen from a liquid for mounting for S.E.M. Transfer specimen(s) to be mounted into tertiary butyl alcohol which has been placed in a thoroughly cleaned glass cavity-slide. If the specimen is being introduced from glycerol or water, agitate it with the eyelash if the turbulence of the mixing fluids is not sufficient to do so, and transfer the specimen into a second amount of alcohol to further dilute any remaining water or glycerol. Now let the alcohol evaporate completely from the cavity-slide, leaving the dry specimen(s), or speed the process by transferring the specimen(s) with a minimal amount of alcohol onto the surface of a clean glass microslide.

The low surface tension of tertiary butyl alcohol will permit cysts with all but the thinnest walls to dry without collapse, leaving them easily moveable with a dry, clean eyelash. Drying from water or ethyl alcohol is also possible, and is satisfactory for specimens with relatively stiff walls, but many of the more delicate cysts will collapse under this treatment as the liquid evaporates and the full force of the surface tension is exerted against the specimen.

Freeze drying or critical point drying may be necessary for specimens too delicate to withstand collapse when dried from liquid tertiary butyl alcohol.

15. To eliminate air from a dry cyst preparatory to remounting. Air contained within hollow cysts that have been dried previously (e.g., for S.E.M. study) and are to be placed again in water, glycerol, or a mounting medium can be removed quickly and surely by placing the cysts in a small amount of n-butyl alcohol in a cavity-slide. After a few minutes, the air will have been absorbed completely by the alcohol, which can then be replaced by water or glycerol before further study or subsequent steps in remounting.

16. To obtain modern thecae from fresh or marine plankton. Use a small plankton net (approximate dimensions of a suitable net, obtainable from a biological supply firm: 10 cm diameter ring-supported opening, 30 cm overall length; 35 μ m or larger mesh size as

desired) equipped with an adapter ring for a glass collecting bottle. Attach the net by a line to a conveniently long pole and add a 500 gm fishing weight to keep it submerged during towing. Tow the net through the surface water from a pier, boat or pondside, occasionally washing the catch into the bottle by removing the net from the water and moving the bottle vigorously in a circular path while the net is held stationary.

Much of the catch will stay alive for hours or a few days if the natural ambient temperature is maintained and the concentration of organic matter is not too great. To preserve the sample, first add a little FAA (mixture of equal parts of formalin, glacial acetic acid, and ethyl alcohol), or ethyl alcohol alone, to the bottle; then centrifuge the mixture gently or allow to stand until the catch settles. Finally, decant as much fluid as possible and fill the bottle with more FAA or alcohol, close tightly and store. Several rinses with alcohol will remove the chlorophyll from a rich green freshwater sample.

17. To concentrate modern thecae in diatom-rich plankton sample. Centrifuge a convenient amount of sample in a plastic centrifuge tube; decant. Add hydrofluoric acid (c. 50%) and stir. Allow to stand a few minutes; wash and centrifuge repeatedly until all acid is eliminated. Treatment with hydrofluoric acid will not ordinarily cause thecal plates to separate. If remains of diatom cell contents persist, agitate with a small amount of liquid detergent and wash/centrifuge until the detergent is eliminated.

18. To dissect a theca. Thecae concentrated in a drop of residue that has been placed already on a clear glass microslide can be dissociated into their constituent plates on a gross scale by adding a drop of about 1% aqueous sodium hypochlorite solution, then a coverslip. After a few moments, the plates will dissociate spontaneously or they can be jarred loose by tapping the coverslip with the tip of the eyelash dowel or a glass stirring rod. Alternatively, place one theca or several selected for dissection in a very small drop of water on a microslide; add a coverslip and relocate the specimen(s) under the microscope. Remove any excess water from the margin of the coverslip with an absorbent tissue, and add a very small amount of about 1% sodium hypochlorite solution at the edge of the coverslip. While keeping one specimen in sight, touch a tissue to the opposite edge of the coverslip, and allow the hypochlorite solution to be drawn past the specimen, or let diffusion bring the hypochlorite to the specimen. Tap the coverslip to speed dissociation of plates if desired.

For precise dissection, rinse a single theca briefly in hypochlorite solution, using the micropipette technique for handling individual specimens already described. Then place the specimen in an uncovered droplet of glycerol on a clean microslide and, under the microscope,

tease the theca with the eyelash tip until the plates fall apart. Individual plates can then be manipulated, observed in different orientations, or photographed as desired.

High optical contrast between the cellulose of the thecal plates and the water medium makes for excellent photography in bright field, phase contrast, or interference contrast illumination. If small plates are jostled about objectionably by Brownian motion or if the convexity of larger plates prevents sharp focus over a substantial area, the plates can be immobilised or flattened by withdrawing water from the slide with absorbant tissue at the edge of the coverslip until the plates are pressed firmly between the opposing glass surfaces. Problems resulting from relatively rapid evaporation of the water medium can be avoided by using glycerol instead, but some of the favourable optical contrast inevitably will be lost.