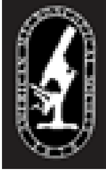

Diatoms: Cleaning and Mounting for Light and Electron Microscopy
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DIATOMS: CLEANING AND MOUNTING FOR LIGHT AND ELECTRON MICROSCOPY[1]

GRETHE R. HASLE[2] and GRETA A. FRYXELL[3]

HASLE, GRETHE R., & FRYXELL, GRETA A. 1970. Diatoms: cleaning and mounting for light and electron microscopy. Trans. Amer. Microsc. Soc. 89: 469–474. Morphology of the siliceous valves of diatoms has been and continues to be important in identification and classification. Organic material and cell contents obscure the valve structure. A simplified method of cleaning diatom frustules of organic matter is presented, utilizing sulphuric acid, potassium permanganate, and oxalic acid. No boiling is required. For light microscopy, a cleaned sample is first mounted on a cover slip, rather than directly on a microscope slide, to decrease waste. The cover slip is then mounted permanently on a microscope slide with a medium of high refractive index. Cleaned samples may also be mounted on coated grids for transmission electron microscopy or stubs for scanning electron microscopy.

INDEX DESCRIPTORS: Diatoms, Bacillariophyceae; Methods; light microscopy; scanning electron microscopy; transmission electron microscopy; cleaning; mounting; Technic.

For many years diatom structures of increasing fineness have been used to test the resolving power of light microscope objectives. They were first used for the same purpose in transmission electron microscopes in the 1930's. Diatomists realized this new tool could aid their study with its greater resolving power, magnification, depth of focus, and contrast, thus making much more information on the structure of the diatom cell wall available.

DIATOM MORPHOLOGY

Various morphological characters have been used for the classification of diatoms, such as the shape and number of chromatophores and the shape and type of colony formation. Chromatophores lose their shape after a period in storage, and colonies break up. Because most taxonomic studies of diatoms are performed on preserved material, these characters do not suffice for proper identifi-

[1] The authors wish to express their thanks to Dr. Sayed Z. El-Sayed who collected the diatoms pictured during the International Weddell Sea Oceanographic Expedition, 1968. This investigation was carried out, in part, under National Science Foundation Grant (GA 13836). A Leitz Laborlux microscope was used for Figures 1 and 2. A K Square Ultrascan scanning microscope was used for Figure 3; thanks are expressed to Dr. Heinz P. Studer, E & P Research Center, Shell Development Company, Houston, Texas.

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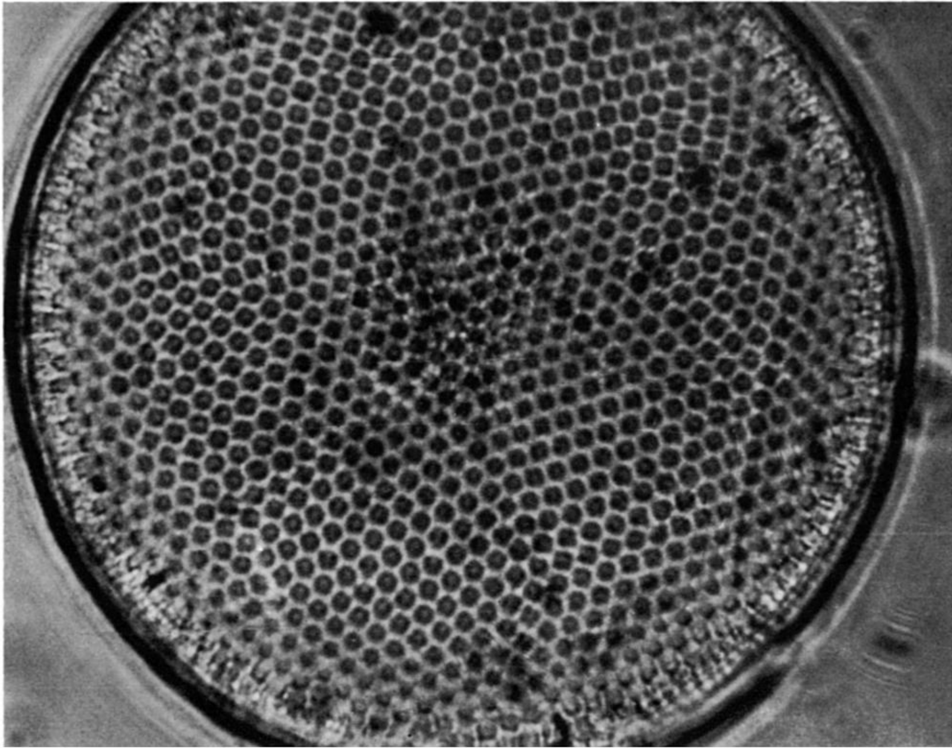

FIG. 1. Uncleaned cell of *Thalassiosira tumida* (Janisch) Hasle in a water mount in valve view, photographed in phase contrast. Note that central pores are obscured although areolae pattern is clear. × 2000.

cation. Diatomists realized that they must base relationships upon the silica frustule. Subsequent investigations have done little to change this opinion.

There are patterns over the greater part of the diatom frustule which originate in differences of silicification resulting in perforations, weakly silicifed areas, and heavily silicified areas called ribs. Complicated silicified structures and pores (slit-shaped, circular, or oval) contribute to the ornamentation of the frustule. Morphological characters used for classification include: valve outline, the presence or absence of a raphe or pseudoraphe and its location, setae, horns, apiculi, mucous pores, and arrangement of the various processes and of valve areolae and ribs.

In some genera (e.g. *Dactyliosolen, Grammatophora, Rhabdonema, Striatella*) the girdle with its numerous intercalary bands is the taxonomically important part of the frustule. In most genera (e.g. *Coscinodiscus, Thalassiosira, Navicula, Nitzschia*) valve processes and the valve structure (the pattern formed by the gradation of silicification) are more important than the girdle bands.

The raphe, pseudoraphe, areolae, ribs, and valve processes cannot be observed with sufficient accuracy in entire frustules in water mounts. The refractive index of the silica wall is similar to that of water; there is too little contrast between the diatom and its surrounding medium for perception of details of structure. Moreover, the cell contents complicate the image of the valve and its processes (Figure 1).

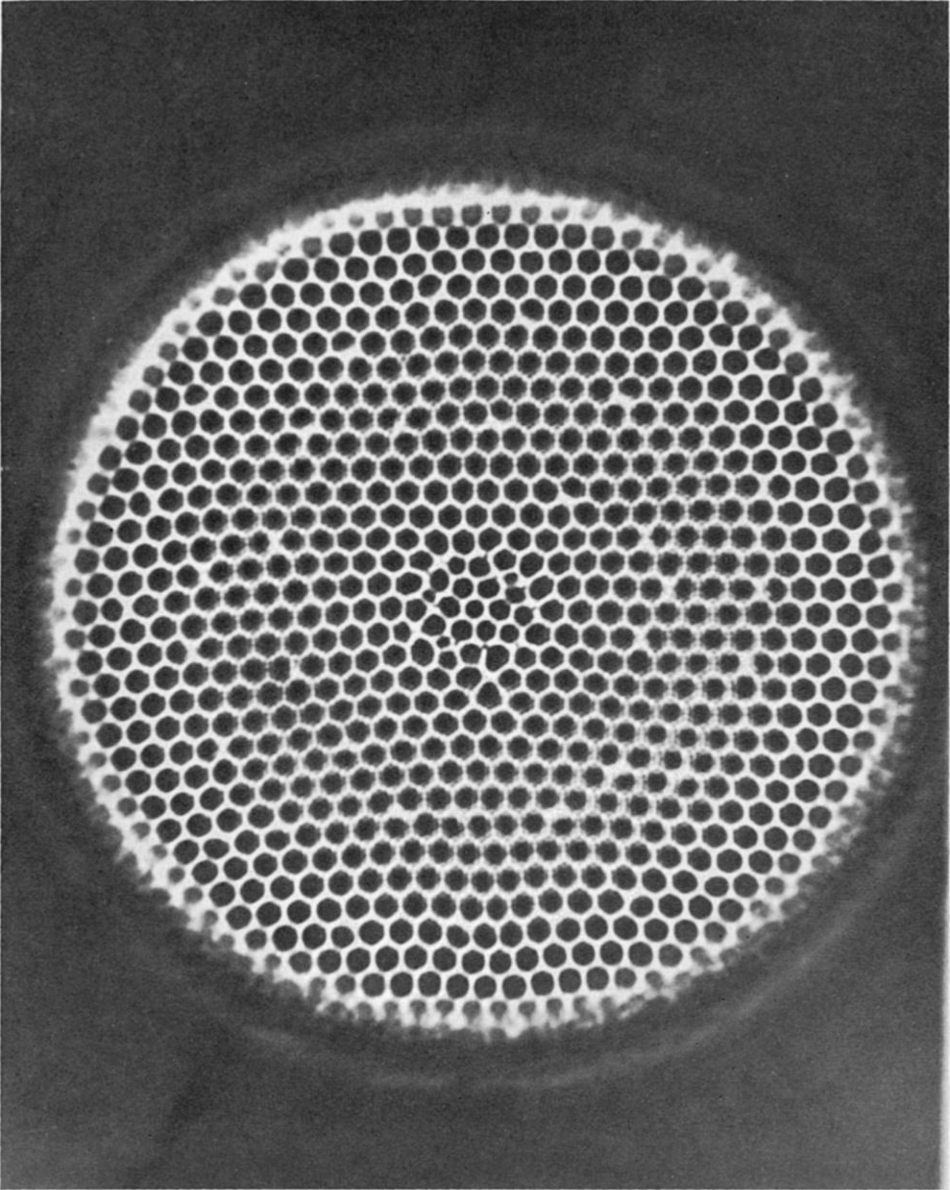

FIG. 2. Cleaned valve of *Thalassiosira tumida* mounted in Coumarone, photographed in phase contrast. Note the central pores and the focus only on the central and marginal areas which indicates the inflated shape of the frustule. × 2000.

PREPARATION FOR THE LIGHT MICROSCOPE

In order to improve conditions for observation of these morphological elements in the light microscope, samples may be: (1) oxidized to remove the cell contents and the organic material in and around the external shell; and (2) mounted in a resin of high refractive index (Figure 2). Diatom frustules may be cleaned in

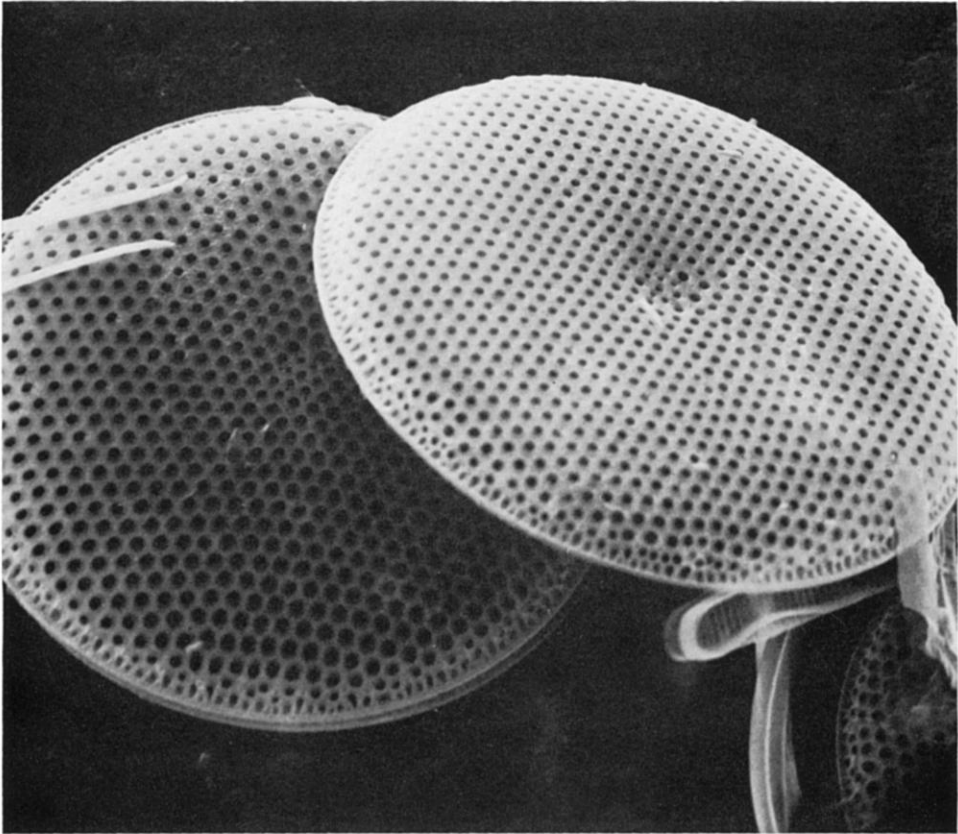

FIG. 3. Scanning electron microscope view of *Thalassiosira tumida* showing cleaned valves. The central pores, areolae patterns, and the shapes of the valves are evident. × 1350.

many ways; one way is given below and several others are included in the literature list. The method described below is convenient since it does not require boiling in acid in contrast to many other methods. Several resins have been used for mounting diatoms, such as Hyrax (refractive index of 1.7), Coumarone, and Aroclor (both with a refractive index of about 1.6).

PREPARATION FOR THE TRANSMISSION ELECTRON MICROSCOPE

For examination in the transmission electron microscope (TEM) also, the diatom frustules must be free of organic material, and the same methods may be used as for light microscope studies. For examination in the TEM, the cleaned diatom sample is transferred to a small grid covered with a supporting film—usually Forvar film.

PREPARATION FOR THE SCANNING ELECTRON MICROSCOPE

When the scanning electron microscope (SEM) was introduced to diatomists in the 1960's, promising possibilities were offered for still more detailed knowledge of the diatom frustule. The SEM gives a three dimensional picture (Figure 3) which formerly had been attained only by means of pairs of stereopictures

from the TEM. For examining in the SEM, the cleaned diatom sample is mounted as for examination in the TEM or on a small piece of cover slip. Then the grid or cover slip is cemented to a metal stub by colloidal silver. The cleaned diatom sample may also be mounted directly on the metal stub and dried of the distilled water or alcohol in which it was stored. The mounted sample is then coated with gold-palladium to obtain maximal resolution.

Cleaning Diatom Frustules

1. Pour a well mixed sample into a new beaker (about 150 ml size). Twenty ml of sample are enough if the water is cloudy with diatoms. Add about an equal quantity of concentrated sulphuric acid. Agitate gently. Work under a hood or near an open window.
2. Add potassium permanganate (saturated $KMnO_4$ in distilled water, freshly made) a little at a time. The sample bubbles up and turns brown at first. Continue adding $KMnO_4$ until the liquid has a purple tint. Agitate gently after each addition. This is an important step in oxidizing the organic matter inside the cells; if there is a great deal of organic matter, this step will take more time.
3. Add oxalic acid (saturated $(COOH)_2$ in distilled water, freshly made) a little at a time, agitating gently after each addition, until the solution becomes clear. It bubbles vigorously.

$$2KMnO_4 + 3H_2SO_4 + 5(COOH)_2 \rightarrow K_2SO_4 + 2MnSO_4 + 10CO_2 + 8H_2O$$

4. Centrifuge the sample, concentrating it to about 5 ml. Decant excess liquid.
5. Dilute the sample with distilled water, mixing thoroughly. Then centrifuge to concentrate and decant rinse water several times. Observe a drop under the microscope to see whether the organic matter is completely oxidized or not. If there are still remainders of chromatophores, the procedure must start again at point 2.
6. More than six rinses may be needed, especially if the cells tend to clump together and rinse poorly (e.g. *Chaetoceros* or *Corethron*). The sample needs further rinsing if cells do not dry thoroughly over low heat on cover slips or if the diatoms get rings of crystals around them on the slides.

Preparing Slides of Cleaned Diatoms

1. Clean No. 1½ cover slips (0.17 ± 0.02 mm) with alcohol to remove all oils so that the slides can be wetted by the samples.
2. Lay cleaned cover slips on a labelled tray.
3. With a new disposable pipette, place one to four drops of cleaned sample on each cover slip, depending upon the density of the sample. It is desirable to have frustules distributed evenly so that one can be viewed or photographed by itself. If it is a dense sample, use one small drop with three drops of distilled water to spread it. Use the pipette to spread the water evenly on the cover slip, and use that pipette for one sample alone to avoid mixing.
4. Dry over gentle heat or overnight. Protect from dust.
5. Add two to four drops (depending on viscosity) of resin with a high refractive index to give contrast to the silica in the frustules.
6. Dry over gentle heat or overnight. Protect from dust.

7. Adjust a hot plate to moderate heat (calculated at about 200° C) and clean and mark microscope slides. Heat slides, leaving one end off the plate for easy handling.
8. Place one slide face down on a prepared cover slip. (A pattern under glass helps to center the cover slip.) Turn it over quickly when the resin melts enough to stick, and replace the slide on the hot plate.
9. Heat until resin has spread under the entire cover slip. Do not boil. Use a wood stick to tap the cover slip gently to remove bubbles, and slant the the slide so that gravity helps in removing the bubbles. Be patient. Work with a few slides at a time at first. The bubbles will come out faster with some resins than others.
10. Cool the slide. Trim excess resin with a knife or use a solvent. Seal with nail polish. Affix permanent label.

* * *

Other techniques of microscopy and articles of special interest are listed in the literature section. The method above of cleaning not only avoids the hazards of extended periods of boiling in acids, but minimizes the transfer of the sample from one vessel to another. Thus a valuable sample is conserved, as it is by mounting the sample on the cover slip instead of strewing it on the microscope slide and wasting part of the material by extrusion. This method results in a minimum of detritus deposited in the center of the mount for study and photography.

Literature

HART, T. J. 1957. Notes on practical methods for the study of marine diatoms. J. Mar. Biol. Ass. U. K. 36: 593–597.

HAY, W. W. & P. A. SANDBERG. 1967. The scanning electron microscope, a major breakthrough for micropaleontology. Micropaleontology 13(4): 407–418.

HENDEY, N. I. 1964. An introductory account of the smaller algae of British coastal waters. Part V: Bacillariophyceae (Diatoms). Fishery Invest., Lond., Ser. 4: 1–317.

NEEDHAM, G. H. 1958. High refractive index media. In: *The Practical Use of the Microscope*. Charles C Thomas, publisher, Springfield, Illinois, pp. 384–387.

SWATMAN, C. C. 1941. A simple method of cleaning fresh diatom gatherings. J. Quekett Micr. Club, Ser. 4, 1(4): 191.

SWIFT, ELIJAH 5th. 1967. Cleaning diatom frustules with ultraviolet radiation and peroxide. Phycologia 6 (2 & 3): 161–163.

VAN DER WERF, A. 1955. A new method of concentrating and cleaning diatoms and other organisms. International Association of Theoretical and Applied Limnology. Proceedings Verh. Int. Ver. Limnol. 12: 276–277.