Scanning Electron Microscopy
Preparations for Diatoms

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1. DIATOMS AND THE SCANNING ELECTRON MICROSCOPE

In studying, describing, and classifying diatoms, the scanning electron microscope (SEM) has become an indispensable tool for diatom taxonomists (1,2). The diatom surface, composed of organic materials and silica, provides excellent working material for use in scanning electron microscopy when properly preserved and fixed. The siliceous cell wall, also called a frustule, is often covered with organic material that can obscure key morphological details if not properly removed prior to viewing with the SEM (3). There are several procedures that will allow diatoms to be stripped of their outer organic coverings while keeping the frustule intact. Here, we describe the most common techniques used for SEM analysis of diatoms. Whenever possible, we have included slight derivations suggested in the current literature to these classical techniques.
2. ACID TREATMENT METHOD

Hasle and Fryxell (1) developed the classical method of cleaning diatoms by acid treatment in the late 1960s. This technique is probably the most commonly used for both light and scanning electron microscopy analysis and can be found published in many current taxonomy and morphology papers such as Hasle (4) and Villac and Fryxell (5). Examples of cells prepared using the acid treatment method are pictured in Fig. 1. The method published by Hasle and Fryxell (1) involves the following steps, which should be carried out under a chemical fume hood:

1. Pour equal amounts of concentrated sulfuric acid (H₂SO₄) and the diatom sample into a 150-mL beaker and agitate gently.
2. Add freshly made potassium permanganate (KMnO₄) dropwise until the sample turns from brown to purple. Agitate the solution gently between each addition of KMnO₄ to oxidize the organic matter. (Note: Round et al. (3) recommend a method of KMnO₄ oxidation that includes the addition of concentrated HCl until the solution becomes clear instead of using step 3).
3. Add freshly made oxalic acid (COOH₂) dropwise until the solution becomes clear. Agitate the solution gently between each addition of (COOH₂). The solution will bubble significantly.
4. Transfer the solution to a centrifuge tube and centrifuge down to a loose 5 mL pellet. We recommend a medium speed for 10 min. Decant off excess liquid. (Note: Miller and Scholin (6) developed a system using a filter tube and filtration manifold that reduces sample processing time and causes less cell damage than centrifuging.)
5. The sample is washed by adding distilled water, agitating the sample, centrifuging as described, and removing the supernatant several times.
6. Check the sample to see if any organic material remains on the cells by placing a drop under a light microscope. If organic material remains, repeat the procedure starting at step 2.

If no organic material remains, the sample is ready to be analyzed under the SEM.

A final step that we have found useful, and recommended in Postek et al. (7), involves rinsing the sample with distilled water at least 6 times to separate the cells before mounting the specimens.

3. HYDROGEN PEROXIDE METHOD

Removal of the outer organic layer can be achieved quickly and effectively through the introduction of hydrogen peroxide (H₂O₂) (8). Preparation of diatom samples for SEM analysis using H₂O₂ is described in the following protocol, the results of which can be seen in Fig. 2.