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# Effectiveness of Cleaning Methods for Investigating Diatoms

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Diatoms are routinely chemically cleaned of all organic matter in order to study the silicious wall (frustule) ultrastructure and to make accurate taxonomic determinations. Since the 1800‘s many methods have been developed for cleaning organic matter from diatoms [2, 6, 7]. Currently most techniques use concentrated acids, hydrogen peroxide or bleach. Recent developments in electron microscopy facilities include instruments for cleaning such as the plasma cleaner, UV (Zone) cleaner and sputtering by focused ion beam (FIB).

Although chemical cleaning methods remain useful, they are harsh, not often highly selective, nor well controlled. Diatoms have complex wall structures, with as many as 50 components impregnated with 10-72% amorphous silica [6] and a complex organic coating lines the inside and outside of the wall [6, 2]. Chemical cleaning can destroy some features of diatoms useful for taxonomy or other studies, especially those diatoms constructed of thin or delicate silicious-organic frustules, processes or with chitin strands. Researchers wanting to study 3D frustule structure [4], the organic casing [6], energy dispersive x-ray spectroscopy (EDS) and structures of mineral-organics [4], polysaccharide and chitin secretions, structural and connective relationships with epiphytic, symbiotic or parasitic organisms, such as bacteria or diatoms [6], may require other techniques for gentle and controlled cleaning. Thus we researched different chemical methods and plasma, UV and FIB instruments to determine their effectiveness in a wide range of applications that require gentle, staged and controlled removal of organics or cutting and cross-sectioning of diatom cell structures.

Most current cleaning techniques use concentrated acids (nitric and sulphuric), 30% hydrogen peroxide ($H_2O_2$) or bleach (5% sodium hypochlorite), often in combination with oxidizers (most commonly: potassium permanganate and potassium dichromate) [1, 2, 5, 6]. We first reviewed the effectiveness of seven commonly used cleaning techniques for marine diatoms, via light microscopy and SEM examination: 1) concentrated and diluted nitric and sulphuric acids (in combination and singly), 2) 5% sodium hypochlorite (household bleach), 3) 30% hydrogen peroxide, 4) 30% hydrogen peroxide and hydrochloric acid, 5) 30% hydrogen peroxide and nitric acid, 6) above chemical methods with acetone, 7) Hydrogen peroxide with potassium permanganate and potassium dichromate. Today, many researchers favor using 30% $H_2O_2$ and a hot-water bath because it does not require concentrated acids, strong oxidizers (with potential violent reactions and Cr toxicity) a fume-hood and careful safety precautions. The chemical cleaning of diatoms is partly a technical art, with each genus requiring modifications of the technique. We report on a generally excellent, quick and relatively safe chemical method that also cleared organics and salts: hydrogen peroxide, nitric acid, hexane or acetone, followed by washes with distilled water. This method can be varied somewhat to control the removal of organics.

We demonstrate, with marine diatoms, that plasma and UV cleaning can be highly effective, especially for the gentle and controlled removal of layers of organics (sometimes only 10 nm thick), keeping delicate frustules intact (see figs. 1 and 2) and studying environmental samples. Their limitations and considerations of use, especially when combined with FIB are discussed. And we

show that these EM cleaning and cutting/ablation techniques provide novel opportunities for a wide range of research avenues previously mentioned.

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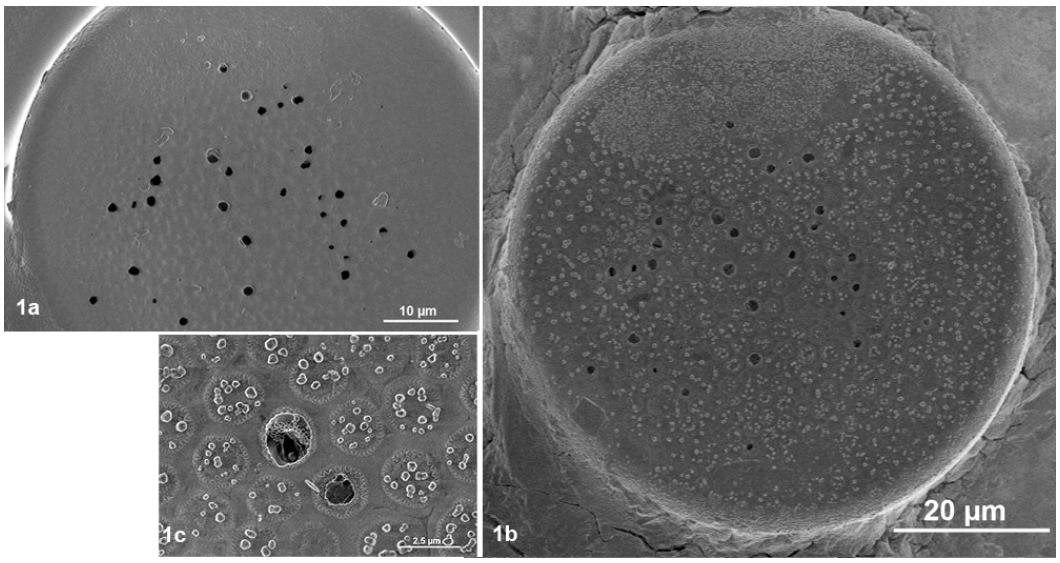


FIG 1. UV Zone cleaning. 1a: 0 minutes; 1b: 20 minutes; 1c: higher power to show details of 1b.

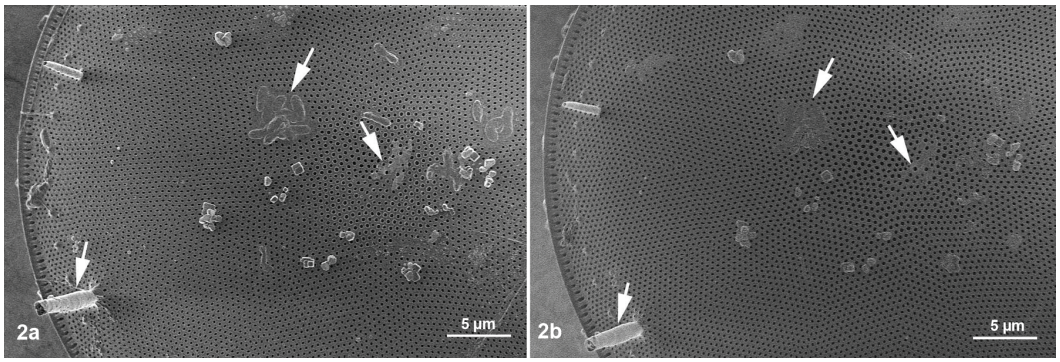


FIG. 2. EtOH/acetone first then Plasma cleaning. 2a 0 minutes plasma cleaning, 2b after. 20 minutes plasma cleaning. Shows how the cleaning is gentle and even (arrows). Further cleaning removes most of the organics including the bacteria.

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