# TECHNICAL NOTE

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# Scanning Electron Microscopy: Application in the Identification of Diatoms in Cases of Drowning

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**ABSTRACT:** A simple and rapid method is described for processing organ and water samples for the identification of diatoms so that they can be studied and recorded for taxonomic and diagnostic purposes. Samples are treated with concentrated nitric acid; the fluid obtained is centrifuged, and its sediment is dried, coated, and examined under the scanning electron microscope. The method does not alter the morphology of diatoms and allows the study of freshwater and seawater species present in the organs of bodies found in the water, making possible the diagnosis of drowning under specific conditions.

**KEYWORDS:** pathology and biology, drowning, diatoms, microscopy, scanning electron microscopy

The diagnosis of drowning is one of the most difficult tasks in forensic pathology. As a result, a number of ancillary tests have been proposed to confirm this diagnosis such as the detection of chemical changes in blood [*1–6*], vitreous humour [*1,7,8*], and in cerebrospinal fluid [*9*]. Moreover, histological changes have also been reported [*10–14*], although most of these changes are considered inconstant and could occur irrespective of drowning [*15*].

The diatom test is the only one still considered reliable by some authors [*15–18,19*]. According to them the test provides supportive evidence of drowning especially in putrefied bodies where no other tests are possible, and it also gives evidence of the probable site of drowning. However, the use of this method as an indicator of drowning is very controversial, and its reliability has long been disputed [*20,21*].

The validity of the diatom test, and therefore its reliability, depend upon the exclusion of contamination and the correct interpretation of the results, which involves a complete taxonomic analysis of the diatoms recovered from water samples and from the organs of

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the deceased [*18,19*]. Scanning electron microscopy (SEM) is probably the most effective technique for identification and classification of diatoms [*22*]. Therefore, the study of water and organ samples under SEM will facilitate their identification, photographic record, and taxonomic analysis. We describe an easy and quick method of processing the samples for such purposes.

## Method

Samples of water from the site where the body was found or where the immersion probably occurred, are collected in clean containers and isolated from extraneous detritus by overnight sedimentation. The gross volume is carefully decanted, the remainder is centrifuged, and the deposit examined under dark-field microscopy to determine if diatoms are present. If the sample contains diatoms the material collected at the postmortem examination is processed. Blocks of approximately 100 g of the organs to be studied are cut, avoiding contamination, dropped into new Kjeldahl flasks, and boiled in twice the volume of concentrated analytic nitric acid (specific gravidity 1.42) under a fume hood. The fluid obtained is allowed to cool at room temperature; it is centrifuged in a standard centrifuge at 2.500 rpm. for 25 min, and the supernatant acid is poured off and replaced with free-diatom distilled water. The process is repeated three times until the fluid is transparent with a final spin at 3.000 rpm to produce a small button of deposit. After removing the supernatant, the sediment is air dried on a metallic stub for SEM, then it is coated with a conductive layer and examined under the microscope.

Fatty sediments from bone marrow, brain and kidney can be transferred into the SEM stubs for air drying on a hot plate at 60°C. Sediment of the water sample is also air dried, coated, and examined in the same manner.

## Results and Discussion

For taxonomic purposes, and regardless of the method used, the desired end product for SEM is a dried sample without artifacts. Diatoms possess a solid exoskeleton (frustule), do not require fixation, and are sufficiently strong to tolerate air drying [*23*]. It has been suggested that the use of concentrated nitric acid could lead to partial or complete dissociation of the exoskeleton [*22*]. Nevertheless, with the method described, complete freshwater diatoms recovered from the kidney (Fig. 1) and seawater diatoms recovered from the lung (Fig. 2) were observed. In fact, details of the frustule, very important for the identification and classification (Fig. 3) and small diatoms, extremely difficult to find with the light microscope, could also be recorded (Fig. 4). A similar method using sulfuric acid and membrane filtering has been outlined [*24,25*].

The presence of the same species of diatoms in the water sample and in organs other than the lungs is considered indicative of drowning [*15–19*]. However, qualitative diatom analysis is more an exercise for the expert diatomologist rather than a routine laboratory test [*18*]. For this purpose, the present method may provide a helpful, simple, and relatively rapid alternative with a permanent photographic record suitable for taxonomic analysis.

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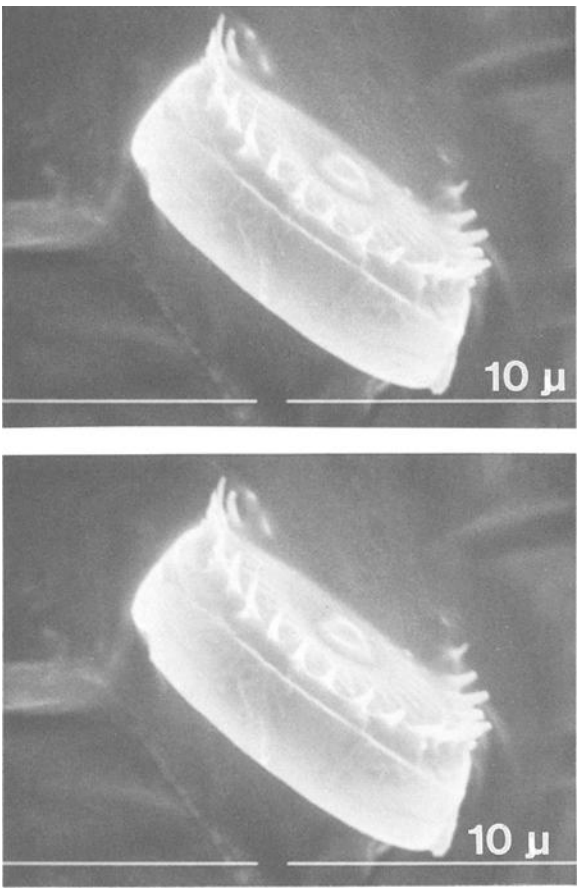


FIG. 1—*Complete fresh water diatom "frustule" SEM X7000.*

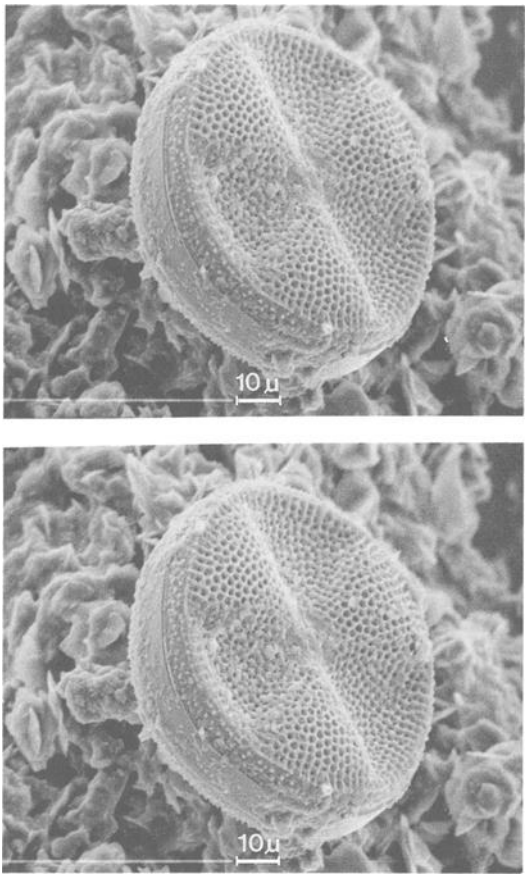


FIG. 2—*Complete seawater diatom (Actinocyclus Senarius Ehrenberg)* [26] *SEM X1000.*

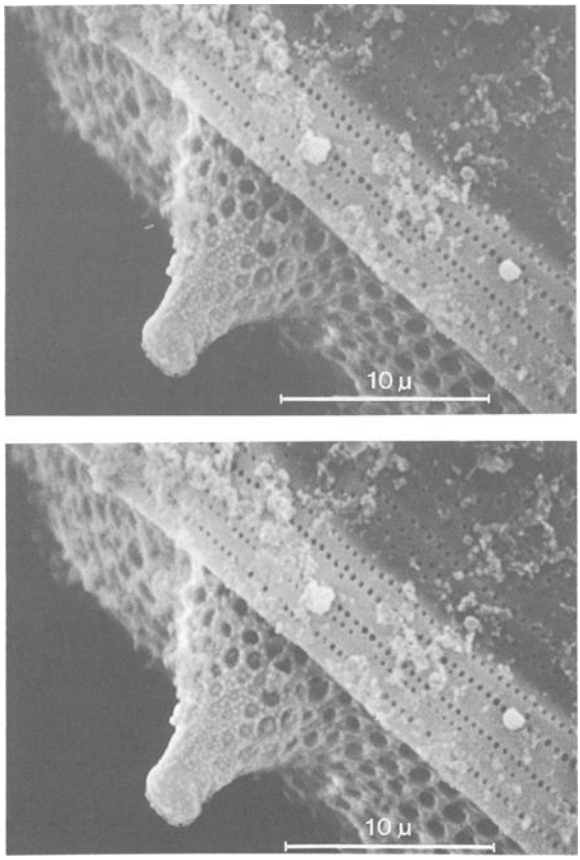


FIG. 3—*Details of exoskeleton (Ceratulus Smithii Ralfs in Pritchard)* [26] *SEM X5000.*

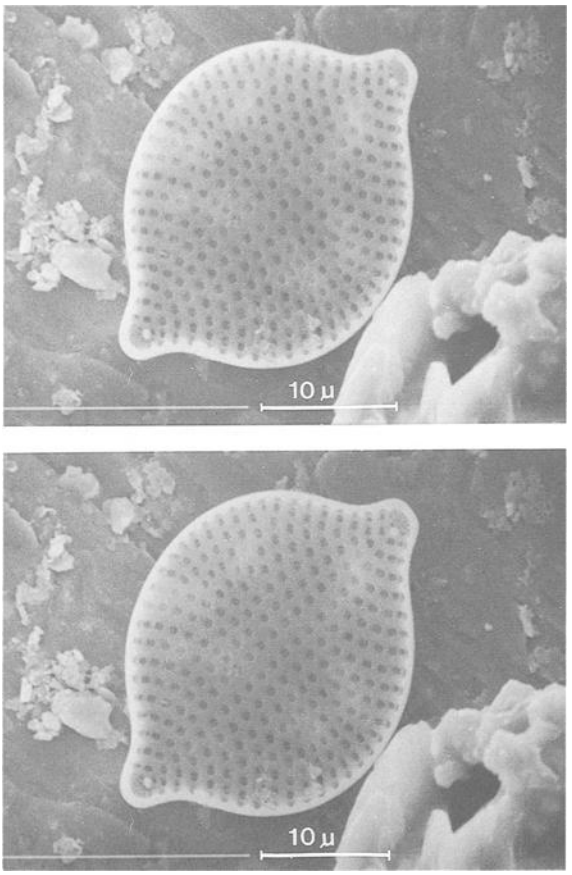


FIG. 4—*Small diatom (Rhaphoneis Amphiceros Ehrenberg)* [26] *SEM X3000.*

## References

[1] Plueckhahn, V. D., "Alcohol and Accidental Submersion from Watercraft and Surrounds," *Medicine Science and the Law*, Vol. 17, No. 2, 1977, pp. 246–250.

[2] Lorente, J. A., Villanueva, E., Hernandez Cueto, C., and Luna, J. D., "Plasmatic Levels of Atrial Natriuretic Peptide in Drowning: A Pilot Study," *Forensic Science International*, Vol. 44, No. 1, 1990, pp. 69–75.

[3] Yu-Chuan, Ch., Zhao-ke, D., and Jia-Zhen, Z., "The Significance of Detecting Serum Fluorine Level in the Diagnosis of Drowning," *Forensic Science International*, Vol. 46, No. 3, 1990, pp. 289–294.

[4] Abdallah, A. H., Hassan, S. A., Kabil, M. A., and Ghanim, A. E., "Serum Strontium Estimation as a Diagnostic Criterion of the Type of Drowning: Water," *Forensic Science International*, Vol. 28, No. 1, 1985, pp. 47–52.

[5] Piette, M., Timperman, J., and Parisis, N., "Serum Strontium Estimation as a Medico-Legal Indicator of Drowning," *Medicine Science and the Law*, Vol. 29, No. 2, 1989, pp. 162–171.

[6] Karkola, K. and Neittaanmaki, H., "Diagnosis of Drowning by Investigation of Left Heart Blood," *Forensic Science International*, Vol. 18, No. 2, 1981, pp. 149–153.

[7] Adjutantis, G. and Coutselinis, A., "Changes in Magnesium Concentration of the Vitreous Humour of Exenterated Human Eye Balls Immersed in Sea Water," *Forensic Science*, Vol. 4, No. 1, 1974, pp. 63–65.

[8] Farmer, J. G., Benomram, F., Watson, A. A., and Harland, W. A., "Magnesium Potassium, Sodium and Calcium in Post-Mortem Vitreous Humour from Humans," *Forensic Science International*, Vol. 27, No. 1, 1985, pp. 1–13.

[9] Coutselinis, A. and Boukis, D., "The Estimation of Mg Concentration in Cerebrospinal Fluid (C.F.S.) as a Method of Drowning Diagnosis in Sea Water," *Forensic Science*, Vol. 7, No. 2, 1976, pp. 109–111.

[10] Kelemen, G., "Temporal Bone Findings in Cases of Salt Water Drowning," *Annals of OtoRhinoLaringology*, Vol. 92, No. 2, 1983, pp. 134–136.

[11] Babin, R. W., Graves, N. N., and Rose, E. F., "Temporal Bone Pathology in Drowning," *American Journal of OtoLaryngology*, Vol. 3, No. 3, 1982, pp. 168–173.

[12] Torre, C. and Varreto, L., "Scanning Electron Microscopy Study of the Lung in Drowning," *Journal of Forensic Sciences*, Vol. 30, No. 2, 1985, pp. 456–461.

[13] Lunt, R. W. and Rose, A. G., "Pathology of the Human Heart in Drowning," *Archives of Pathology and Laboratory Medicine*, Vol. 111, No. 10, 1987, pp. 939–942.

[14] Karch, S. B., "Pathology of the Heart in Drowning," *Archives of Pathology and Laboratory Medicine*, Vol. 109, No. 2, 1985, pp. 176–178.

[15] Knight, B., "Immersion Deaths," in *Forensic Pathology*, Edward Arnold Publishers, London, 1991, pp. 360–373.

[16] Cameron, J. M., "Drowning," in *Gradwohl's Legal Medicine*, 3rd ed., F. E. Camps, A. E. Robinson, and R. G. B. Lucas, Eds., Wright and Sons Ltd., Bristol, England, 1976, p. 353.

[17] Camps, F. E. and Cameron, J. M., "Immersion in Fluids," in *Practical Forensic Medicine*, 2nd ed., F. E. Camps and J. M. Cameron, Eds., Hutchinson Medical Publications, London, 1979, pp. 319–320.

[18] Hendey, N. I., "Letter to the Editor: Diatoms and Drowning. A Review," *Medicine Science and the Law*, Vol. 20, No. 4, 1980, p. 289.

[19] Auer, A., "Qualitative Diatom Analysis as a Tool to Diagnose Drowning," *The American Journal of Forensic Medicine and Pathology*, Vol. 12, No. 3, 1991, pp. 213–218.

[20] Peabody, A. J., "Diatoms and Drowning. A Review," *Medicine, Science and the Law*, Vol. 20, No. 4, 1980, pp. 254–261.

[21] Foged, N., "Diatoms in Drowning. Once More," *Forensic Science International*, Vol. 21, No. 2, 1983, pp. 153–159.

[22] Round, F. E., Cranford, R. M., and Mann, D. G., *The Diatom Biology and Morphology of the Genera*, Cambridge University Press, Cambridge, England, 1990, pp. 7–20.

[23] Gabriel, B. L., "Chemical Fixation," in *Biological Scanning Electron Microscopy*, Van Nostrand Reinhold Company, New York, 1982, pp. 51, 118.

[24] Peabody, A. J., "Diatoms in Forensic Science," *Journal of the Forensic Science Society*, Vol. 17, Nos. 2 and 3, 1977, pp. 81–87.

[25] Torre, C., Varetto, L., and Gili, A., "Identifying Diatoms from Water Courses and from Post Mortem Samples using Scanning Electron Microscopy," *Minerva Medico-Legal Archives Antropology and Criminology*, Vol. 104, No. 4, 1984, pp. 295–297.

[26] Hendey, N. I., *An Introductory Account of the Smaller Algae of British Coastal Waters*, Fishery Investigations Series IV, Part V Bacillariophyceae (Diatoms), Mildner and Sons, London, 1964, pp. 95, 106, 154.

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