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Automated SEM Diatom Surveys and Correlative Light Microscopy

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Diatom surveys are commonly used to evaluate lake sediments as an indicator of changes in water quality and regional climate change [1]. Typically, after removal of organic material with acid and peroxide, the remaining silica cell walls of the diatoms ("valves") are dried on a coverslip and mounted on a slide under a high-refractive-index mounting medium, such as Naphrax (Brunel Microscopes). Diatom valves are then identified and tallied with bright-field light microscopy using an oil-immersion 100X lens. Common problems are: clumping of diatoms in the sample, difficulty identifying overlapping diatoms in thick samples and microscope limitations to the number of people that can count samples. We have overcome these problems by attaching samples to poly-D-lysine-coated coverslips, reversibly mounting them on SEM stubs and creating automated panoramas of SEM images. The result is a uniformly-dispersed sample and a stitched SEM panorama of the sample which can be electronically distributed for identification, counting and archiving. If desired, the SEM-imaged coverslip can be re-mounted for light microscopy.

A drop of processed diatom suspension from a core sediment sample from Michi Lake, Michipicoten Island, Lake Superior, was added to a Corning BioCoat 12 mm circular, poly-D-lysine-coated coverslip and air-dried on a warming plate. The negatively-charged silica valves bind strongly to the amine-derivatized coverslip. The dried suspension forms a circular spot ca. 5 mm dia., with some clumping of suspension at the circumference of the spot, but uniformly distributed internally. The coverslip is reversibly mounted to an aluminum SEM stub on a circle of a 3M PostIt note attached to the stub with a Pella carbon-adhesive dot. It is then sputter-coated with a thin layer of Au/Pd and imaged at 1000X in secondary-electron mode with an Hitachi S-3400 NII SEM at 3 kV acceleration. A single 2 mm x 100 um X-axis scan of each sample begins from well inside the clumped circumference toward the center of the spot and recorded as a series of 19 overlapped 5 MP micrographs, using the automated zig-zag function of the motorized stage. The images are stitched together using panorama stitching software, dragged to a large-format PowerPoint slide and converted to PDF format**.** This results in a 5MB PDF panorama of the 2mm x 100 um strip that can be distributed electronically. (See Figure 1A.) The image can be easily enlarged on any computer monitor and panned for identification and counting. To compare the SEM panorama with light microscopy imaging, we unmounted the coverslip, placed it in a coverslip holder and imaged the same scan area under 100X objective without oil. An LM panorama of the SEM scan area in Figure 1B was created for comparison (Figure 1C). The coverslip was then permanently mounted in a drop of Naphrax high-refractive-index mounting medium on a slide for conventional LM identification and counting. From coverslip preparation to SEM panorama took ca. 90 min for one sample, correspondingly less time with multiple samples.

Identifications and counts of SEM vs. LM are shown below in Table 1. Tally results are comparable among methods. Counting, however, was notably easier and more rapid for the more concentrated, more uniform PDL coverslip samples. It was also found that diatoms on the PDL coverslip imaged with SEM kept their orientation when mounted in Naphrax (not shown). The SEM panorama adds the advantages of semi-automation, portability, electronic archiving and easier identification of diatoms, especially in thicker areas of the sample. With the superior depth of focus of SEM, overlapping diatoms

remain in-focus, whereas LM of the same areas requires changing focus continuously. (Compare Figures 1B and 1C.) For this reason, using panoramas of light microscope images for on-screen identification, counting and archiving would be impractical.

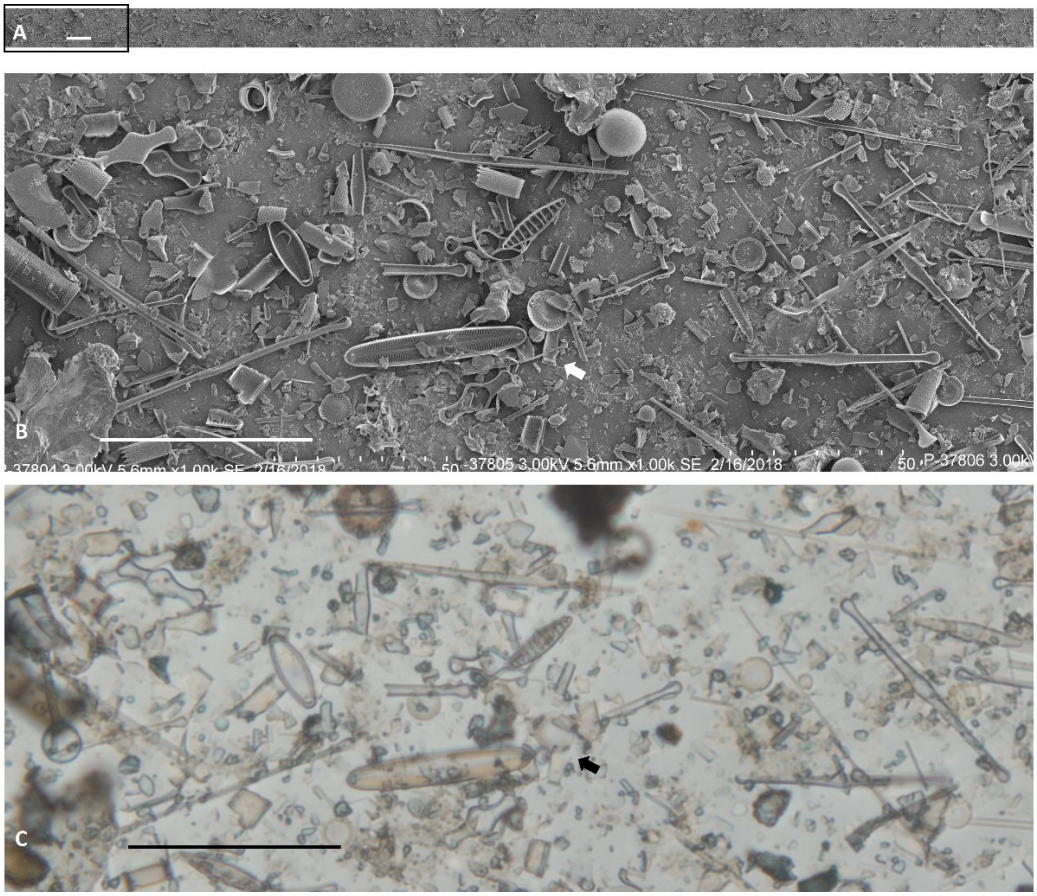
References:

[1] Slemmons KE, ML Rogers and JR Stone, Hydrobiologia **800** (2017), pp. 129.

Table 1. Comparison of LM and SEM for identification and tally of Michi Lake core sample no. 25 (depth, 12.0-12.5 cm) using five most abundant species. SEM panorama coverslip became the Naphrax coverslip. Results are expressed as percent of whole diatom assemblage counted in each sample.

	Discostella stelligera	*Aulacoseira ambigua*	*Tabellaria flocculosa*	*Lindavia rossi*	*Staurosirella pinnata*
Original slide (LM)	28.4%	18.0%	15.9%	10.7%	3.1%
Naphrax coverslip (LM)	22.4%	14.2%	19.8%	11.0%	6.5%
SEM panorama	23.2%	12.8%	17.0%	13.5%	4.9%

Figure 1. **A.** Stitched, 19-image SEM panorama of a 2 mm x ca. 100 um strip of Michi Lake sample no. 25. **B.** Enlargement of outlined section of SEM panorama. (A monitor image can be magnified ca. 5X higher.) Note the high definition and the ease of distinguishing overlapping diatoms (white arrow). Original magnification: 1000X. **C.** LM bright-field image of area in 1B. Note absence of several diatoms seen at arrow in 1B at this level of focus (black arrow). Calibration bars: 50 um.



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