Automated Diatom Analysis Applied to Traditional Light Microscopy: A Proof-of-Concept Study

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1. Introduction

Diatoms are microscopic algae with siliceous cell walls (**Figure 1**). Globally, diatoms are responsible for ~20% of net primary productivity (Young and Morel, 2015). They are important proxies for marine and freshwater biotic conditions, including ecosystem health and they are subject to environmental change (Armbrust, 2009).

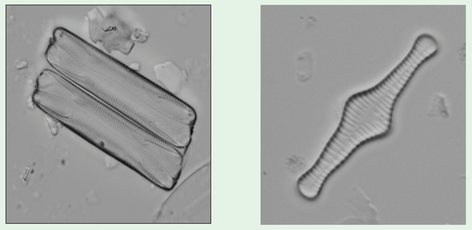

Figure 1: Silicon (SiO_2) cell walls of diatoms.

Light microscopy is used to identify and enumerate the silicon structures of diatom cell walls. This method; however, is time-consuming, labor-intensive, and requires expertise from a very limited number of diatom scientists (Álvarez et al., 2013), thus motivating the need for automated analysis. Automated particle analysis is widely applied, using a flow cell and aqueous medium, but this approach is less successful for diatoms since they are small (<100 μm).

Incorporating automated particle analysis software, VisualSpreadsheet, with high-resolution light microscopy would aid scientists working in academic institutions or federal agencies to conduct more intensive diatom research in a timelier manner with less demanding effort, thus allowing for more environmental health research.

Problem:

Automated particle analysis of diatoms has not yet been applied to high-resolution light microscopy. In this study, trials under different operating conditions were evaluated to determine the potential for automated diatom analysis.

2. Methods

1. 183 images of a broad field of view were captured using an Olympus BX53 light microscope, with 100x oil immersion (1.4 NA) objective. A set of 33 calibration images was also captured.

2. Images files were named in a systematic-fashion, (image_000.tif, image_001.tif, image_002.tif and so on) using InfranView64. Calibration files appeared first in the sequence, followed by test images.

3. The images were imported into VisualSpreadsheet, an image analysis software.

4. Parameter configurations were specified in each trial using the "Context" menu (**Table 1**).

5. Each trial was evaluated for a number of parameters and the diatom particle images were stored.

6. The results of each trial were compared against visual assessment of the diatom particle images.

Table 1. Initial operating conditions for each trial.

Parameter	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Distance to Nearest Neighbor(µm)	1	2	5	10	20
Close Holes (iterations)	5	5	5	5	5
Particle Segmentation: Dark Threshold (0-255)	31	31	31	31	31
Particle Segmentation: Light Threshold (0-255)	70	70	70	70	70
Basic Size Filter: Minimum Diameter (ESD)(µm)	8	8	8	8	8
Basic Size Filter: Maximum Diameter (ESD)(µm)	10000	10000	10000	10000	10000

3.1 Results: Parameter Evaluation

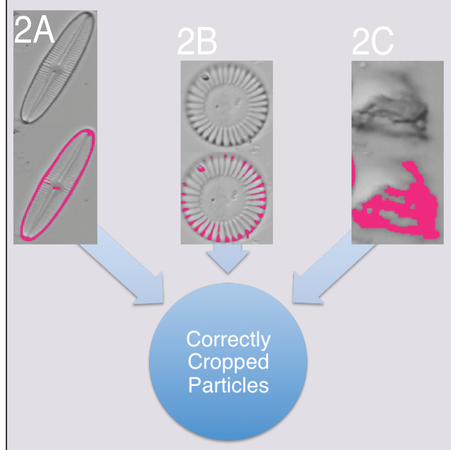

Figure 2: Diatom images and binary image overlays (in pink), showing correctly cropped particles.

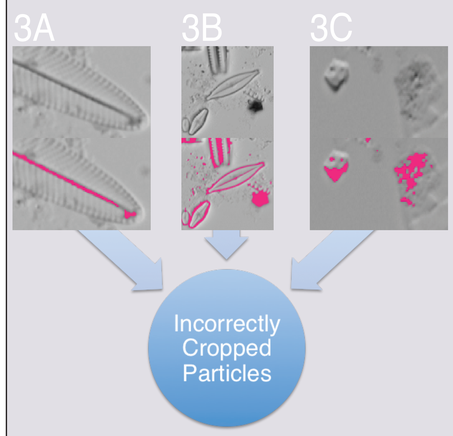

Figure 3: Diatom images and binary image overlays of incorrectly fragmented particles.

Table 2: Results.

Measurement	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Sampling Time (seconds):	68	80	84	104	149
Total Particle Count:	276	321	251	103	6
Diatom Particle Count:	216	211	137	57	4
Diatom Particles Correctly Cropped:	170	168	88	16	4
Diatom Particles Incorrectly Cropped:	46	43	49	41	0
Inorganic Matter Particle Count:	60	110	114	46	2
Inorganic Matter Particles Correctly Cropped:	50	71	56	4	0
Inorganic Matter Particles Incorrectly Cropped:	10	39	58	42	2
Number of Particles Correctly Cropped:	180	239	144	20	2
Number of Particles Incorrectly Cropped:	96	82	107	83	4

Using 150 light microscope diatom images, 255 diatom particles (complete valves and valve fragments) were manually counted in 400 seconds. In comparison, 85% or 216 total diatoms (out of a possible 255) were sensed and processed by the software in 68 seconds. Of the 216 diatom particles, 79% or 170 were successfully cropped by the software.

"Distance to Nearest Neighbor (μm)" expressed significant effect on the quality of diatom and inorganic matter image crops depending on the selected values from the range: 0-1000.

"Distance to Nearest Neighbor (μm)" is a parameter that defines the minimum pixel distance (μm) that two particles can be from one another without being incorrectly processed and cropped as one individual particle.

Lower values yielded more high quality analysis and cropping ability of individual diatoms and inorganic matter particles (**Figure 2A, 2B, 2C**). Trials 1 and 2 had the largest number of properly cropped diatoms, yielding 170 and 168 particles, respectively.

Higher values yielded lower quality image crops with fragments of individual diatoms (**Figure 3A**) or multiple particles per cropped image (**Figure 3B and Figure 3C**). Trials 4 and 5 had the least number of properly cropped diatoms, yielding 16 and 4 particles respectively.

3.2 Results: Software Sensitivity

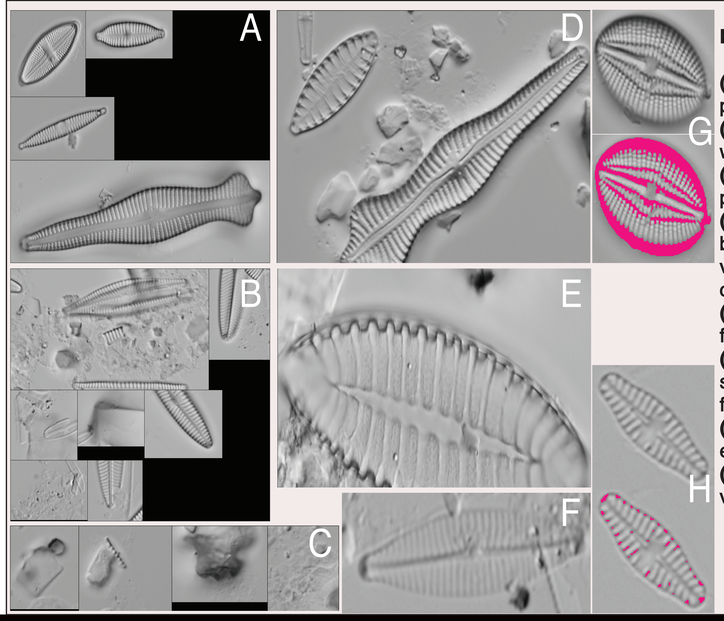

Figure 3: Various particle image cropping results.

(A) Heavily silicified diatoms that expressed dark color pixels with well-defined outlines yielded the best crops.
(B) Lightly silicified diatoms were light in pixel color and were often fragmented into multiple cropped images.
(C) Not only did the software pick up on diatoms, but it also picked up on numerous inorganic matter particles.
(D) When the pixel distance between multiple particles is below the defined "Distance to Nearest Neighbor (μm)" value then issues with two particles being processed as one individual particle occurred.
(E) Curved ends on diatoms often resulted into multiple fragmented image crops.
(F) Very lightly silicified valves were not picked up by the software due to pixel color values being lower than the defined segmentation threshold value.
(G) Example of a diatom with well-defined edges with an enclosed binary image overlay (in pink).
(H) Example of a diatom that is very lightly silicified and has very little binary image overlay.

4.1 Discussion: Segment Thresholding

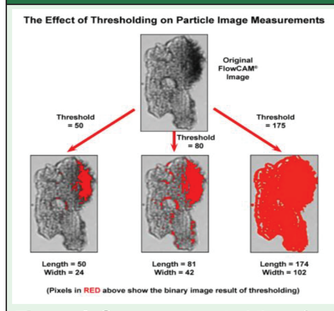

Figure 4: Segment thresholding effects with different defined values (Fluid Imaging Technologies, 2014).

Thresholding is used to distinguish the foreground (objects) from the background of a grey-scale image (Sezgin et al., 2004). If pixels are darker or greater than a defined threshold pixel color value, then those pixels are processed as a "particle."

Issue: Diatoms express similar pixel color values to the background due to the transparent valves and sometimes were not sensed by the software.
Issue: Diatom valve outline pixel colors were typically darker than the threshold value, whereas the interior valve pixels color values were lighter.

4.2 Discussion: Binary Image

Figure 5: Binary image produced by VisualSpreadsheet.

White pixels indicate "particle" and black pixels indicate "non-particle" (Sezgin et al., 2004).

Issue: The software was unable to produce binary image overlays (BIO) that captured all the interior and exterior valve structures.
Issue: Statistical library filters may not work properly since the software does is unable to account for all the measurement data of the entire diatom.

4.3 Discussion: Imaging Conditions

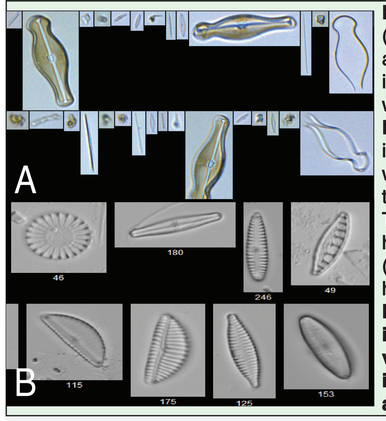

Figure 6: (A) FlowCam (Bishop et al., 2017) images and **(B)** light microscope images captured by the software are compared.
Benefit: (B) Microscope images imported into the software express higher resolution than FlowCam images **(A)**. The light microscope images have a resolving power of 0.25 (μm) whereas FlowCam images have a value of ~ 3-4 (μm).
Higher resolution facilitates in-depth analysis of diatom valves and structures, which is critical in diatom taxonomy and enumeration.

5. Conclusions and Future Work

- The software is able to quickly and efficiently analyze diatom particles.
- It has potential, but improvements in the binary image overlay must be made to allow for development of statistical filters and automated species identification.
- Currently, the software could be used for diatom analysis, but should be used in conjunction with traditional light microscopy techniques.

- Develop library and classification filters to make automated identifications of various taxa.
- Improve imaging conditions by preparing valves that do not touch in the microscope image and adjusting contrast values without losing high resolution.
- Improve the binary image overlay abilities of the software.

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