MONTANA STATE UNIVERSITY

Center for Biofilm Engineering

Developing a Model for the Flow Cytometric Analysis of Ice Cores

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INTRODUCTION

Glacial ice contains a wealth of information about past climate, geologic events, and biological activity. Much of this information can be obtained through the analysis of the particles and gasses trapped in this ice. The goal of this project is to develop a rapid method to characterize both biotic and abiotic particulates in samples from glaciers.

This system will allow us to run a large volume of glacial samples in a short amount of time. This type of standard analysis procedure will process large amounts of data into parameters that can be used to quantitatively describe particle distributions.

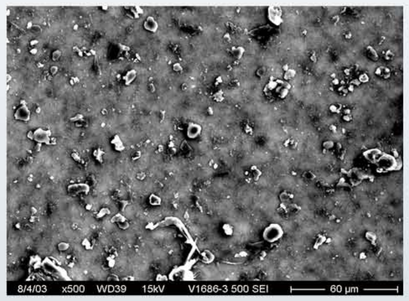

Fig. 1 Scanning electron microscope image of particles from a sample of ice from glacial Lake Vostok in Antarctica. This image shows both sediment particles and microbial particles, although it s very difficult to distinguish between the two using this microscope technique.

DATA COLLECTION

The ice samples are analyzed using a flow cytometer, a laser particle counter. This device obtains data describing particle sizes and concentrations. The counter can be used in conjunction with a fluorescent stain to analyze biological aspects of the particles. Nucleic Acid stains have been used to distinguish between biotic and abiotic particulates. The resulting output consists of two different particle size distributions, a biotic distribution and an abiotic distribution.

In order to analyze the samples, they must be melted and treated with SYTO 60, a red fluorescent stain. The solution is placed in a one milliliter tube, and analyzed in the machine.

Figure #2. The Microcyte Flow Cytometer uses a laser and an infrared detector to measure particle size and concentration. It can be used to detect both sediments and microbes.

METHOD TESTING

The flow cytometer was tested using a variety of methods. Polystyrene calibration bead standards were used, as well as stained samples of cultures from bacteria isolated from glaciers. Comparisons were made between the results of the flow cytometer and the results of epi-fluorescent microscopy.

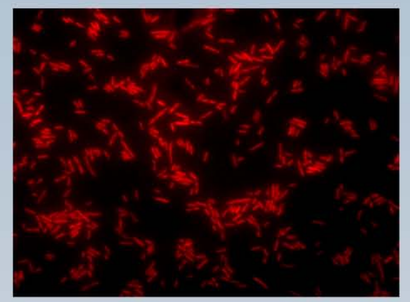

Figure 3. A sample of glacial bacteria, CG2 (Cotton Glacier 2), that was stained using SYTO 60 and analyzed with fluorescent microscopy. The cells in this sample were counted by hand. This is a time consuming process that is prone to operator error. The flow cytometer is intended to eliminate the problems associated with microscope counting.

Three samples from Lake Vostok were analyzed using the flow cytometer. The samples were at depths of: 1686m, 2334m, and 3612m. These samples were previously studied using scanning electron microscope and fluorescent microscope techniques. The distributions obtained from the flow cytometer were compared to the results of the other two analysis techniques.

The flow cytometer total particle counts were found to be within an order of magnitude of the scanning electron microscope total particle counts. The biotic particle counts were found to be an order of magnitude greater. This difference will be further investigated. This either indicates greater sensitivity of the flow cytometer, or that the fluorescent stain may be binding to sediment particles.

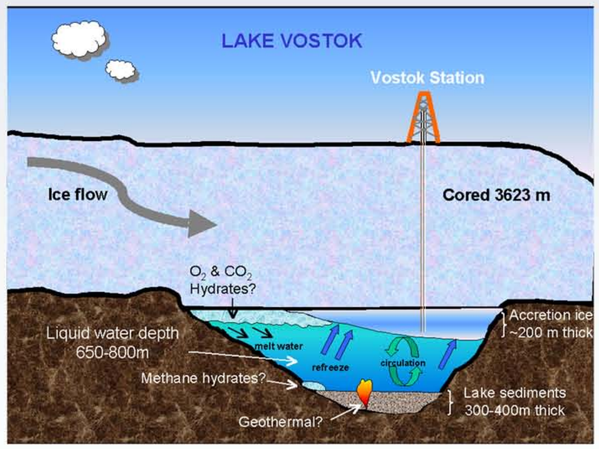

Figure 4. Lake Vostok, a glacial lake deep beneath the Antarctic ice sheet, was sampled to a depth of 3623 m. Some of these samples were used to test the flow cytometer. This system is designed to be able to analyze long sections of ice core samples at regular depth intervals, such as this series of samples from Lake Vostok.

DATA ANALYSIS

Through research and literature review, it was found that these size distributions could reasonably be fit to distribution curves. Weibull distributions tended to fit the data best. A curve fitting procedure was developed to analyze and describe the raw data that was output by the flow cytometer.

The data acquired from the Lake Vostok samples were studied using this curve fitting technique. Numerical descriptions of the distributions were obtained.

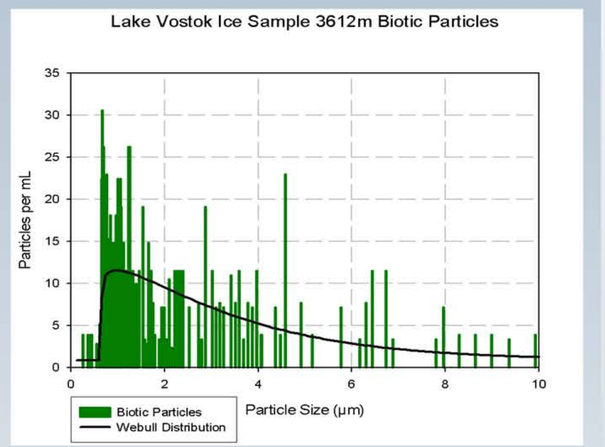

Figure 5. A particle size distribution from a ice sample at a depth of 3612 meters. The distribution was fit with a Weibull distribution. This curve has an R-squared value of: 0.8843. This graph shows that the biotic particles tend to be around one-half to one micron in size, this is the typical size of bacteria.

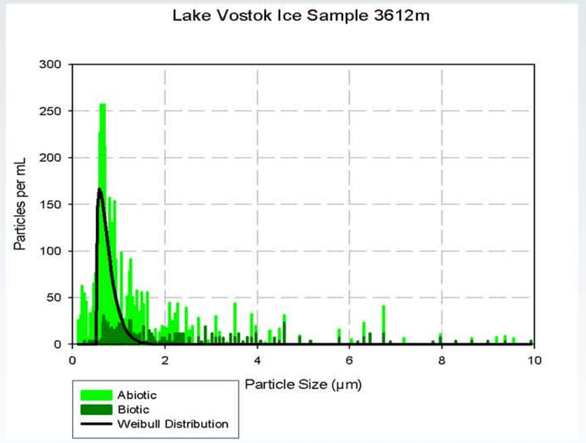

Figure 6. A sample from a depth of 3612m showing the distinction between biotic and abiotic particles with a curve fit to the abiotic distribution. The curve had a R-squared value of: 0.8564

DISCUSSION

The functions that are fit to the distributions can be used to quantify the data. These continuous functions can then be described using statistical parameters such as mean, median, and mode. These parameters can be used to show the characteristics of the particle distributions.

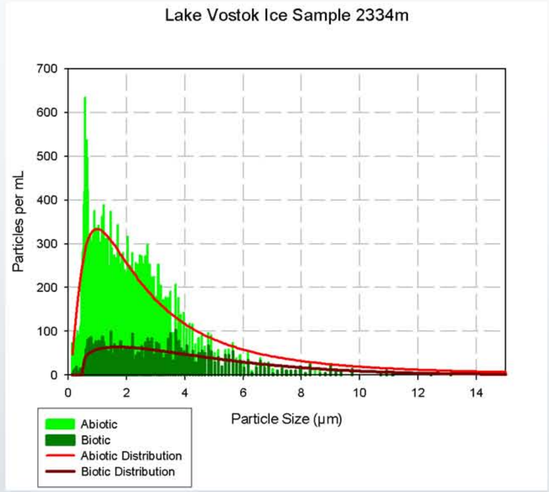

Figure #7. Two distributions, one describing biotic particles and one describing abiotic particles. The differences between the two can be quantified by using the curve fits. The median particle sizes are: 2.4 µm for the biotic curve, and 1.9 µm for the abiotic curve. This indicates that the microbial particles tend to be larger than the sediment particles. Overall, there is one order of magnitude more sediment particles than microbe particles.

FUTURE WORK

This preliminary work is intended to allow for the future use of this system in the analysis of ice samples from Arctic and Antarctic regions. We hope to be able to be couple this system to a chemical analysis system that is currently is use at the Desert Research Institute in Reno, NV. This automated system analyzes ice samples continuously and very rapidly.

When our system is ready, we will attempt to couple it to this chemical analysis system. This has the potential to offer valuable information about the ecosystems contained in glacial ice, and climate changes throughout geologic history.

ACKNOWLEDGEMENTS

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