COPAS Cytometric Sorting of Pinaceae Pollen for Subsequent AMS Radiocarbon Dating

Objective

The objective of this experiment was to test if the COPAS flow cytometer could be used as a means of concentrating Pinaceae pollen from lake sediments. This QTN has been adapted from and full details of these experiments may be found in the paper "Cytometric Sorting of Pinaceae Pollen and its Implications for Radiocarbon Dating and Stable Isotope Analyses" presented as a poster at the 20th Annual PACLIM Meeting, Asilomar, California, April 6-9, 2003. In the paper, first use of flow cytometry as a means of concentrating Pinaceae pollen from lake sediments is reported.

Introduction

The COPAS SELECT instrument is able to analyze and sort large objects (40-300 microns) at a high rate (up to 50 objects per second) on the basis of the physical characteristics of size, density, and fluorescence signals. A red diode laser (670 nm) is used to measure both the size and optical density of the objects, and a multi-line (488, 514 nm) argon laser is used to excite the autofluorescence.

Use of flow cytometry will make it much easier to concentrate fossil pollen for Accelerator Mass Spectroscopy (AMS) radiocarbon dating and will also make it easier to use fossil pollen for stable isotope analyses of carbon, hydrogen, and oxygen. In this experiment, researchers focused on Pinaceae pollen, or more specifically pine (*Pinus*) and fir (*Abies*) pollen, because these pollen types are typically large, i.e., more than 50 micrometers in long dimension, and therefore relatively easy to concentrate in quantities needed for mass spectrometry. Pinaceae pollen also auto-fluoresces (Figure 1), making it a candidate for sorting by use of fluorescence.

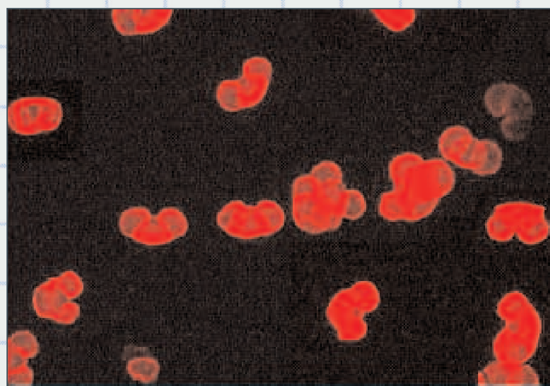

Figure 1. Pine pollen showing autofluorescence in red wavelengths (610 nanometers).The excitation wavelength is green (488 nanometers).

How much pollen is needed for a Mass Spectroscopy?

The preferred mass of pollen required for mass spectroscopy depends upon the sensitivity of the spectrometer and the isotopes being analyzed. In this study, researchers aimed to concentrate samples with a dry weight of at least 400 micrograms. According to Zetsche and Vicari (1931), the composition of sporopollenin is 65.3 percent carbon, 8.6 percent hydrogen, and 26.1 percent oxygen. Assuming these proportions are correct, a 400 microgram pollen sample represents 260 micrograms of carbon.

Reference:

Cytometric Sorting of Pinaceae Pollen and its Implications for Radiocarbon Dating and Stable Isotope Analyses
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In order to determine how many pine pollen grains were needed for a 400 microgram sample, researchers added a known quantity of *Lycopodium* spores to known amounts of dry pine pollen. The results indicate that if we take the conservative case and assume that a sample is made up entirely of *Pinus contorta* pollen, the number of pollen grains needed for carbon isotope analysis would be ca., 20,000 grains.

Results

The autofluorescent signals emitted by pollen were analyzed at the 488 nm and 514 nm excitation wavelengths on the COPAS SELECT. Using 540 nm or 610 nm emission filters, researchers were able to accurately separate pollen from non-pollen material such as charcoal and diatoms. Figure 2 shows a characteristic profile of pollen and non-pollen contaminants at 610 nm emission under 488 nm excitation. Each dot represents a particle recognized by the instrument. The researchers were able to sort 30,000-60,000 pollen grains in an hour from sieved samples. The sorting rate depends largely on the purity and concentration of sample analyzed.

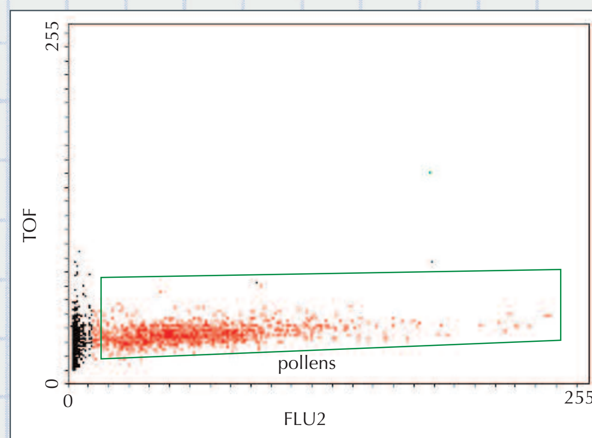

Figure 2. A profile of pollen and non-pollen components in a mixed sample. The X-axis indicates the intensity of red fluorescence whereas Y-axis indicates the size of particles. The particles in the population appear largely homogenous in size, consistent with the fact the samples have been sifted through a 64 micron filter before sorting. Note that non-pollen materials represented by black dots are non-fluorescent. The rectangular box defines the sorting criteria that was set for sorting pollen from contaminants. The intensity of autofluorescence of pollen varies considerably, possibly due to the variable amount of fluorescent materials left in the pollen grains.

Cytometrically Sorted Pinaceae Pollen and AMS Radiocarbon Dating

The direct dating of fossil pollen concentrates by Accelerator Mass Spectroscopy was first reported on by Brown et al., (1989). Since then it has been widely used especially for dating sediments especially in situations where reservoir effects preclude the use bulk sediment samples. The use of flow cytometry to concentrate pure pollen samples makes it much easier to prepare samples for AMS dating. The preparation of pollen samples for AMS dating has traditionally involved sieving and chemical extractions. However, prepared samples often contain resistant material that is the same size as pollen, such as charcoal, insect chitin, fungal spores and colonial algae. Such material may or may not have the same radiocarbon activity as pollen, so it is necessary to purify the residue by visually sorting the pollen from the non-pollen fraction. Visual sorting is necessarily time consuming because a minimum of 10,000 large pollen grains, such as pine pollen, are needed for a reliable age determination (Mensing and Southon 1999).

CONCLUSION

The results of this study clearly establish that flow cytometry can be used to concentrate pure pollen residues from sediments. This new technology will be useful in preparing pollen samples for AMS radiocarbon analysis and will also make possible the direct use of fossil pollen in studies of stable isotope variation through time.

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