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Article *in* Silicon · May 2012

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Silicon (2013) 5:13–17
DOI 10.1007/s12633-012-9115-1

ORIGINAL PAPER

A Micro-manipulation Technique for the Purification of Diatoms for Isotope and Geochemical Analysis

Andrea Marie Snelling · George E. A. Swann · Melanie J. Leng · Jennifer Pike

Received: 29 February 2012 / Accepted: 17 April 2012 / Published online: 22 May 2012

Abstract A method is described for the purification and extraction of diatom samples for isotope and geochemical analysis. The technique involves a micro-manipulator attached to an inverted microscope with a cellular micro-injector system used to remove contaminants or separate assemblages into single-species samples. Whilst time consuming, the advances associated with this technique, eliminating issues related to contamination and the impact of species-dependent fractionation factor/isotopic effect, allows ultra-clean as well as species and/or size specific diatom samples to be analysed in palaeoenvironmental research down to seasonal timescale resolution. By further altering the inner dimensions of the commercially available capillary tubes, (3.5–150 μm) this technique can be extended for use with other microfossils including radiolaria (30 μm to 1 mm), pollen (6–100 μm) and phytolith samples (1–200 μm).

Keywords Diatom · Purification · Single-species · Isotope · Micro-manipulator

1 Introduction

Increasing research is being devoted towards obtaining isotope and geochemical records from diatoms, unicellular siliceous algae, for palaeoenvironmental investigations [1–5]. Such research is believed to be essential in high latitude regions in both lakes and oceans where carbonates and other biological proxies suitable for isotope or geochemical-based research are either absent or not preserved in sufficient quantities [5, 6]. An essential pre-requisite for such work is the necessity of ensuring that samples are clean of all contaminants (>98% purity). Whilst a variety of chemical and physical techniques exist for this (e.g. [7–9]), small amounts of contaminants can often remain behind leading to "spikes" in the data [10–12]. Even in instances where pure material is extracted, samples are often analysed as bulk-species samples containing large numbers of taxa due to the small size of individual frustules (typically 5–40 μm) and the need for comparatively large sample sizes (e.g., 1–8 mg for $\delta^{18}O$ and $\delta^{30}Si$ [13], 20–90 mg for $\delta^{13}C$). The use of bulk-species, however, can be compromised by inter-species differences in: 1) seasonality, depth habitat and life strategies [e.g. 14, 15]; and 2) disequilibrium effects including size and species differences [e.g., 16, 17]. Whilst the overall impact of these processes remains unknown, such issues have the potential to dominate any temporal change in

Electronic supplementary material The online version of this article (doi:10.1007/s12633-012-9115-1) contains supplementary material, which is available to authorized users.

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the isotope/geochemical record and lead to potentially erroneous palaeoenvironmental reconstructions [9].

A number of techniques are currently available to assist with separating diatom species and so generate the data required for high precision palaeoenvironmental reconstructions. These include sieving at different size fractions to take advantage of the different size range of individual taxa [9, 18] and gravitational split-flow thin fractionation (SPLITT) in which individual species can be separated within the laminar flow if differences exist in the densities of individual taxa [7, 19]. Neither of these techniques, however, are capable of routinely producing mono-specific samples.

An alternative approach is to physically extract individual frustules from the sediment assemblage. Whilst time consuming, this approach has the advantage of allowing the analyst to individually select and check each frustule, ensuring both that the analysed sample is comprised of a single taxa and allowing frustules showing signs of dissolution or other diagenetic features to be avoided. Early workers used a pig's eyelash to individually pick diatom frustules by hand [20, 21]. More recently, Stoll et al. [22] demonstrated the use of a tungsten needle attached to a micro-manipulator to extract coccoliths. Whilst a significant improvement, this method remains unsuitable for extracting the large numbers of diatoms required for isotope/geochemical analysis with 1 mg approximately equating to c. 0.4 million × 25 μm diameter centric marine diatoms. Here we demonstrate the application of a glass capillary micro-manipulator system for both removing residual contaminant after existing cleaning techniques have been applied and for extracting mono-specific diatom samples. This method is both quicker and more efficient than existing techniques for isolating large numbers of frustules for isotope/geochemical analyses and has potential for enabling more robust diatom-based isotope and geochemical research.

2 Material and Methods

2.1 Equipment

The micro-manipulator system in use at the NERC Isotope Geosciences Laboratory (NIGL) consists of: 1) a Zeiss Axiovert 40 C inverted microscope with ×10 magnification eyepieces in addition to ×10 and ×40 long distance lens; 2) a CellTram vario hydraulic micro-injector which has a coarse and fine drive (transmission ratio = 10:1) and uses oil for pressure transmission to enable controlled and constant suction; 3) fitted glass capillary tubes (Eppendorf) for the extraction of diatom frustules/contaminants; and 4) a Tranferman NK2 micro-manipulator (Eppendorf) for positioning of the capillary tubes through the use of a joystick which can execute movement in three dimensions (X, Y and Z axis), and can be set at centimetre to sub-micron increments enabling precise movement of the capillary tubes (Fig. 1).

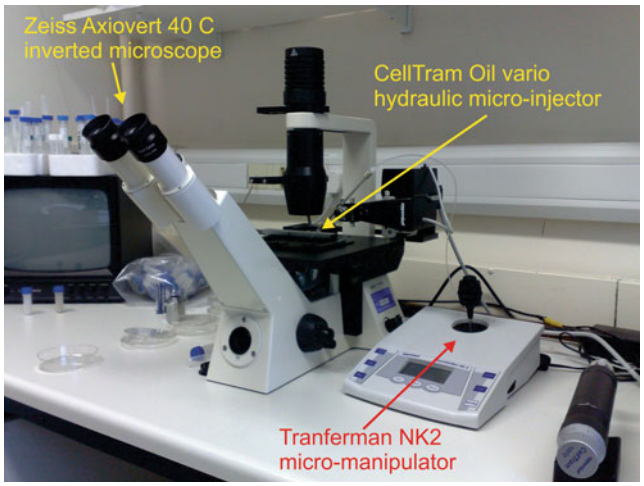

Fig. 1 Set-up of the micro-manipulation system at NIGL showing the inverted microscope, and the hydraulic micro-injector attached to the micro-manipulator

Glass capillary tube dimensions can be customised for use with the large size range of diatom frustules that may be present in different diatom assemblages with, for example, the inner diameter of the tubes able to range from 3.5–150 μm. During the development of this method it was found that smaller dimension tubes quickly become blocked when extracting large numbers of frustules. As such, it is recommended that the inner dimensions of the capillary tubes be considerably larger than the frustules being extracted. For the c. 20 to 40 μm sized frustules/contaminants extracted in this study, capillary dimensions of c. 60 μm were found to be necessary to avoid blockages. For larger diatoms capillary tubes with an internal diameter of 100 μm may be more useful.

2.2 Procedures

The following technique applies to both the removal of the residual contaminant matter and extraction of mono-specific diatom samples and is summarized in Fig. 2, along with the pre-manipulation cleaning technique. Prior to using the manipulator samples should be cleaned and purified of contaminants as far as possible using one of the previously established chemical/physical techniques [e.g. 9]. It is considered, based

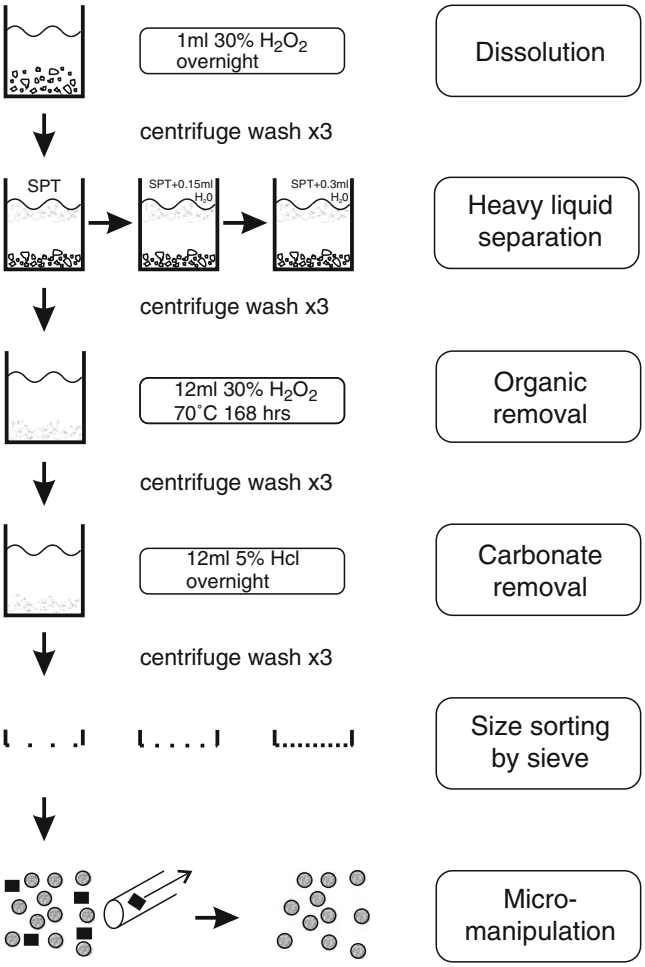

Fig. 2 Flow diagram showing the stages in the cleaning method for obtaining pure diatom samples for oxygen isotope analysis (adapted from [9])

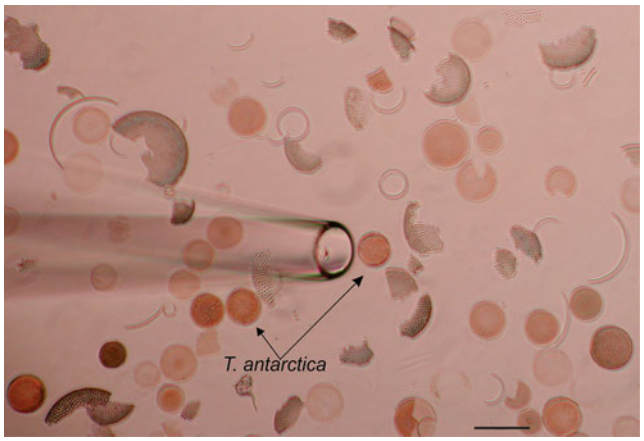

Fig. 3 Light microscope image showing the extraction of *Thalassiosira antarctica* frustules (approx. 25 μm diameter) through a glass capillary tube (capillary tube here has a 40 μm internal diameter). Scale bar represents 50 μm

on experiments in this study that samples should have approximately less than 30% contamination remaining prior to beginning micro-manipulation for the technique to be a viable option otherwise the amount of time required to clean the sample becomes unworkable. Following standard cleaning techniques, samples are placed in a plastic Petri dish in c. 1–2 cm diameter drops with deionised water such that the density of diatom material is low enough so that individual frustules can be seen and isolated. We recommend a density similar to that shown in Fig. 3 (this equates to approx. 20,000 particles of pre-cleaned fossil Antarctic diatom material per 1 ml of water). The glass capillary tube is fitted to the manipulator arm and, with the objective in the ×10 position, positioned above the sample using the joystick control and then lowered so that it just breaks the water. To prevent the capillary tube from bending/breaking on the Petri dish it is recommended that the joystick control used in a fine-resolution setting. The capillary tube is moved into the field of view under the ×40 objective and lowered until both it and the diatoms are in focus (Fig. 3). The most effective position was found to be c. 1–5 μm from the bottom of the Petri dish. The CellTram micro-injector is controlled by a simple turning mechanism to either suck or expel material by generating differences in pressure, the intensity of which is controlled by the movable piston. Using the coarse drive, a complete revolution of the dial corresponds to a change in volume of c. 9.8 μl and 0.96 μl for the fine drive. The extent to which the dial is turned governs the speed with which the material is aspirated or dispensed. If the material is moving into the capillary tube too quickly then simply turning the dial in the opposite direction will reduce the speed/capillary pressure. The capillary tube can remain stationary as the microscope stage is moved, making it easy to move around the sample and collect material (Online resource 1). With a capacity of <2 ml the capillary tube will require periodic emptying, which is easily and quickly achieved by raising the system to its default "home" position and ejecting material into a separate vial.

3 Assessment and Discussion

Two types of sample have been tested: 1) an almost single-species sample (*Thalassiosira antarctica* resting spores) from Palmer Deep, west Antarctica (ODP Site 1098A), with residual mineral contamination; and 2) a sample from Lake El'gygytgyn, Siberia, with two diatom species: *Cyclotella ocellata* and *Pliocaenicus costatus var. sibiricus*, a centric and pennate species

respectively in proportions of c. 4:1. Both samples were pre-cleaned following standard methods [9, 23]. These samples were chosen because they represented samples which following standard cleaning and preparation techniques, still contained a small amount of contamination or a mix of species to a level that was felt could be easily removed and is often frustratingly encountered in diatom isotope preparation. When using the micro-manipulator, the most effective method for the Antarctic material was to remove the contaminant (average size 10–20 μm), using a 60 μm diameter capillary tube leaving behind a cleaned diatom sample. For the sample from Lake El'gygytgyn it was more effective to extract the most dominant *C. ocellata* taxa, again using a 60 μm diameter capillary tube. Both of these methods were successful in producing clean diatom single-species samples that could be used for isotope analysis (Fig. 4c,d). The choice of whether to remove the contaminant or the required diatom species needs a pre-assessment of the individual sample. When extracting diatoms, it was found that better results were achieved in removing centric diatoms due to the tendency of pennate diatoms to rotate sideways when trying to extract them increasing the risk of a blockage in the capillary tube.

In terms of sample preparation time, to micro-manipulate a 1 ml drop of water diluted material (approx 20,000 × 25 μm diameter marine diatoms) can take c. 5 h but several drops may be required to get enough material for isotope analysis depending on the method used and the amount of sample needed for a single analysis. Analyses that require only 0.8 mg of material may take only 7–8 h to manipulate whereas an 8 mg sample would take around 70–80 h (based on calculations using a sample containing single-species 25 μm diameter marine diatoms). In terms of practicality, this method is best suited where only a small sample size is required and where the unwanted material in the sample constitutes less than 30%. However, the method enables contaminated samples previously rejected for analysis to be cleaned-up and run following a relatively small investment of time. In addition, the extraction of single-species fractions allows issues of inter- and intra- species disequilibrium effects as well as seasonality/habitat to be assessed on a finite number of samples, allowing an appropriate sampling strategy to then be devised for other samples within a core. Where individual taxa can be related to specific seasons, the micro-manipulator can be used to create a seasonal, as opposed to annually-resolved, isotope/geochemical record. Such work will significantly improve attempts to constrain the response of lacustrine and marine systems to palaeoenvironmental events, particularly as the research agenda becomes increasingly focused towards investigating the nature, timing and response of rapid climate changes.

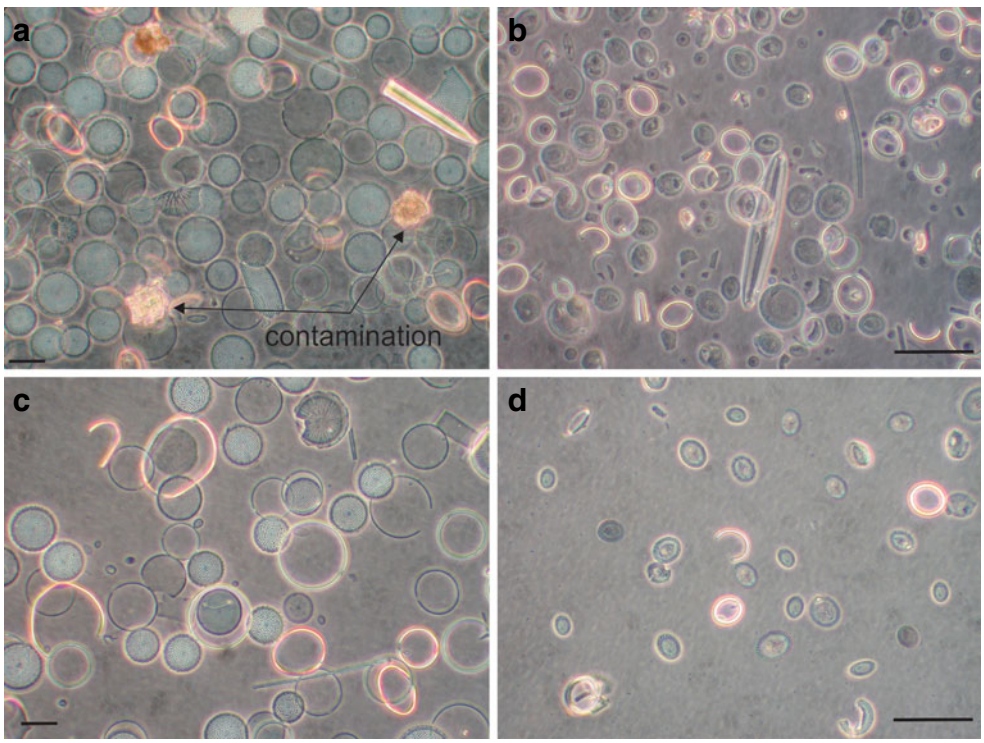

Fig. 4 Images of **a**) single-species (*Thalassiosira antarctica*) sample with mineral contamination from the west Antarctica Peninsula (ODP Site 1098) prior to micro-manipulation; **c**) same Antarctic sample following extraction of contamination; **b**) Mixed species sample from Lake El'gygytgyn showing *Cyclotella ocellata* and *Pliocaenicus costatus var. sibiricus* prior to manipulation and **d**) extracted *C. ocellata* frustules from the same sample. All scale bars 20 μm

4 Comments and Recommendations

The micro-manipulation of diatom samples to remove residual contamination and/or to extract species/size-specific diatom samples in the quantities necessary for isotope analysis represents a significant advance in attempts to use diatom isotope/geochemical records in environmental reconstructions. Whilst it may not be suitable for all sample types, it brings closer the possibility of routinely analysing single-species samples, therefore minimising offsets as a result of size, species or seasonality differences. Such approaches will bring diatom isotope/geochemical work in line with that currently being carried out on foraminifera and ostracods which routinely analyse single-species samples. Although not investigated here, this approach is readily applicable for use with other microfossils such as radiolaria, pollen and phytolith samples and authigenic minerals, particularly where only a small sample size is required.

Acknowledgements We are grateful to the reviewers for their useful and constructive comments. This research was supported by Natural Environment Research Council (NERC) grants NE/G004137/1 to M.J.L. and G.E.A.S. and NE/G004811/1 to J.P.

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