May 09, 2024

Slide Preparation and Transect Counting of Paleoecological Microfossils - SPaTCoPM

DOI
dx.doi.org/10.17504/protocols.io.eq2ly7ormlx9/v1

Megan C. O'Toole[1], Richa Patel[2], Jacopo Niccolo Cerasoni[2]

[1]Department of Chemistry, Loyola University Chicago; [2]Department of Biology, Loyola University Chicago

Archaeo-Evolutionary Cre…

Jacopo Niccolo Cerasoni
Department of Biology, Loyola University Chicago

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.eq2ly7ormlx9/v1

Protocol Citation: Megan C. O'Toole, Richa Patel, Jacopo Niccolo Cerasoni 2024. Slide Preparation and Transect Counting of Paleoecological Microfossils - SPaTCoPM. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2ly7ormlx9/v1

Protocol status: Working
We use this protocol and it's working

Created: May 22, 2023

Last Modified: May 09, 2024

Protocol Integer ID: 82271

Funders Acknowledgement:
NSF
Grant ID: CAREER (EAR 2145830, awarded to Prof. Y.E. Stuart)

Abstract

Paleoecology attempts to reconstruct history through geochemical isotopic studies, trace fossil analyses, examination of microbe communities, and the presence or lack of microfossils. Ancient lakes are useful for reconstructing paleoecology because they accumulate sediment through time. Paleobotanical microfossils, including diatoms and phytoliths, allow inference of vegetation, pH, salinity, water chemistry, and environmental temperature, among others. Here, we propose a protocol split into two sections to prepare slides and count diatoms and phytoliths along transects. Researchers can develop an ecological history by analyzing microfossils from different locations and times. As an example, we apply this method to study the palaeobotanical microfossils of an 820 cm deep stratigraphy, composed of 10.3my Miocene diatomite, from a high-elevation desert paleolake in Northern Nevada (Fernley District). The preliminary results hint at a complex climatic and environmental variance. Another application of this method includes the development of environmental interpretations and hypotheses regarding the future of modern vegetation.

Guidelines

Cross-contamination needs to be prevented at every stage. Ensure you use KIM Wipes to clean slides and coverslips before starting each sample. Disposable glassware should be used when available, and equipment should be cleaned with acetone between samples. Also, ensure that you are wiping the table/environment you are working in between each sample.

Materials

	Reagents/Chemicals	Glassware	Other
	Distilled Water (dH2O)	Clear glass slides and coverslips	Metal spatula
	Acetone	Falcon tubes (1.5 mL)	Needle tool
	Immersion Oil (non-drying)		KimWips
			Nail Polish
			Aluminum foil

Protocol materials

Immersion Oil (non-drying TYPE B) **Cargille Catalog #**CODE 1248 Step 5

Safety warnings

Wear proper personal protective equipment (lab coat, safety goggles, gloves), follow normal lab procedure, and become familiar with the safety and first aid supplied. When using chemicals turn on the intake fan in the fume hood.

Read the safety data sheet (SDS) and adhere to each chemical's storage specification.

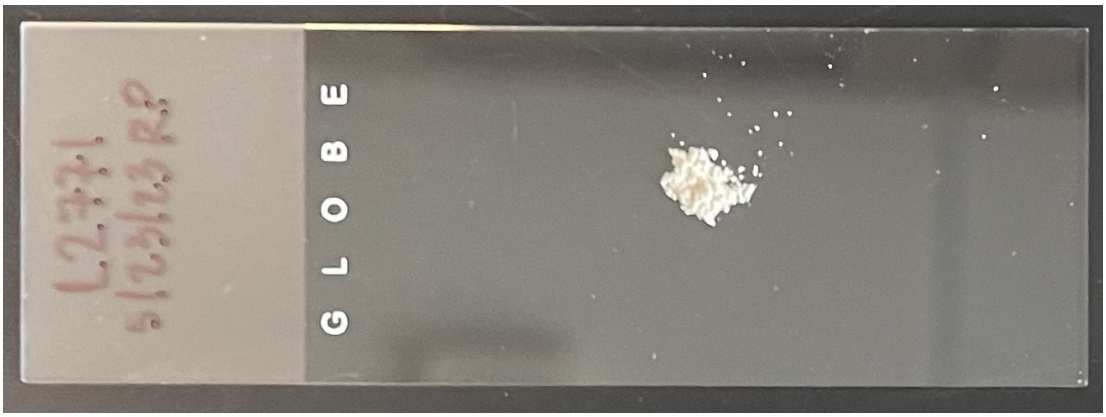
Before start

Make a database of all samples and an orderly sample inventory. Include important information such as country, site, feature, level, etc., to help identify the samples. Give each sample a unique label code (using SITE, YEAR, or ID NUMBER). Digitally create and print an appropriate form for logging observations made while samples are prepared in the lab.

Slide Preparation

1 Using a needle tool, grind the sample inside the falcon tube to create a fine powder.

2 Using the needle tool, put about 6 mg of the sample onto the slide.

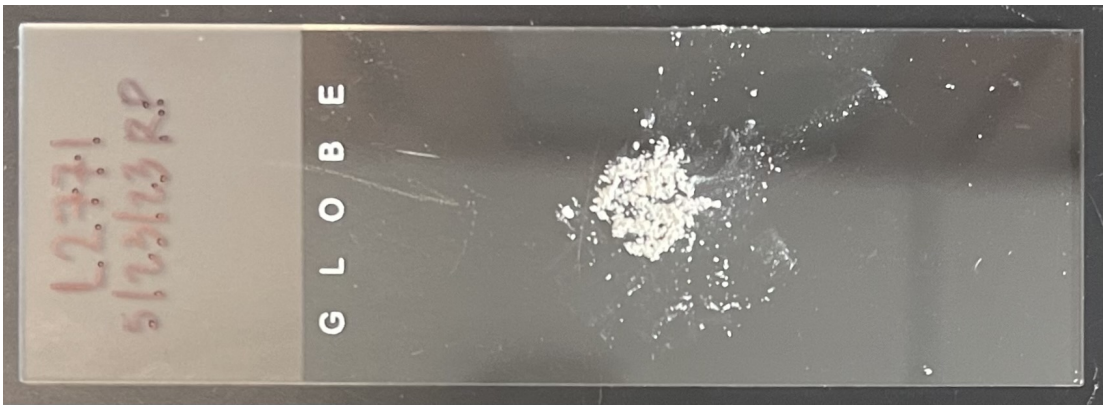


Note

We tried different amounts of sample to create different densities of microfossils for the slides. To create a light density, we used 3 mg; to create a medium density, we used 6 mg; and to create a high density, we used 10 mg. For one drop of immersion oil, we decided that 6 mg was the perfect amount for us.

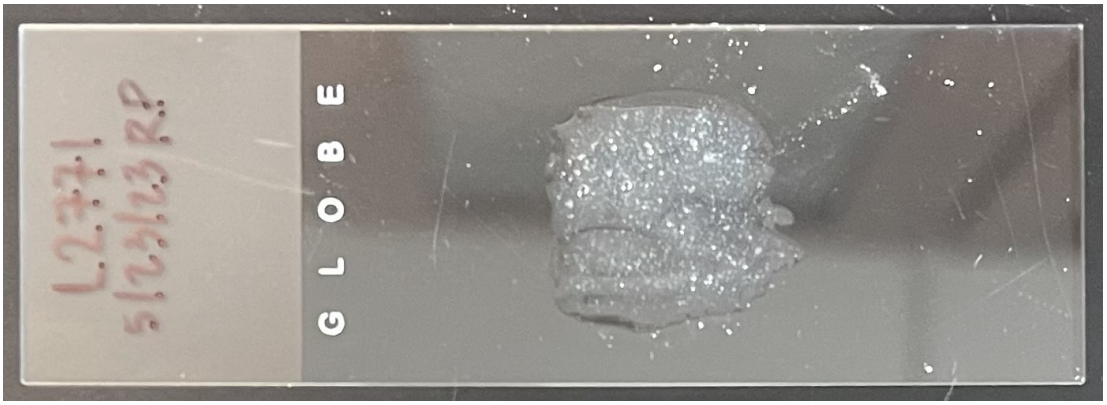
3 Using a metal spatula, grind up the sediment onto the slide. Make sure that you are not pressing too hard as possible phytoliths in the sample could scratch the glass.

4 Using a needle tool, break up any clumps created by the metal spatula. Use it also to gather the sample towards the middle of the slide.

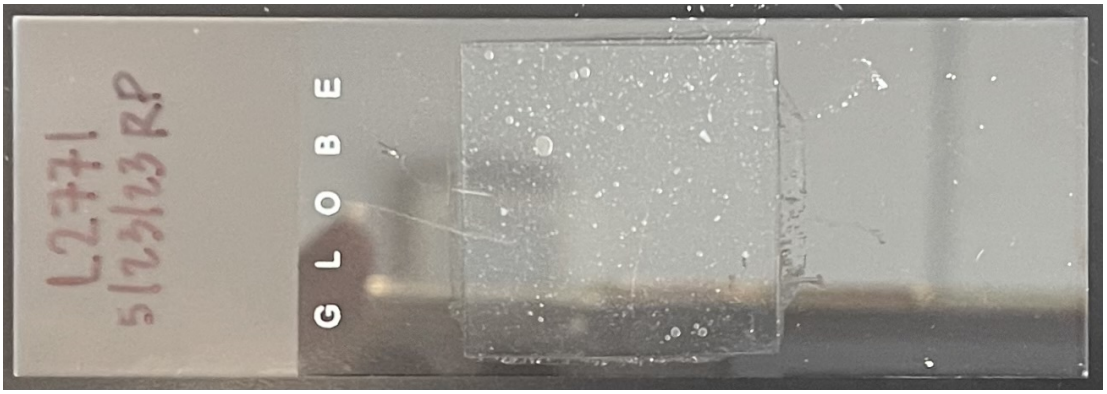


5 Use Immersion Oil (non-drying TYPE B) **Cargille Catalog #**CODE 1248 , and place ONE DROP on top of the sample.

6 Use a needle tool to help mix the immersion oil and sample together. Make sure to break apart any clumps and spread the sample around to create an even layer across.



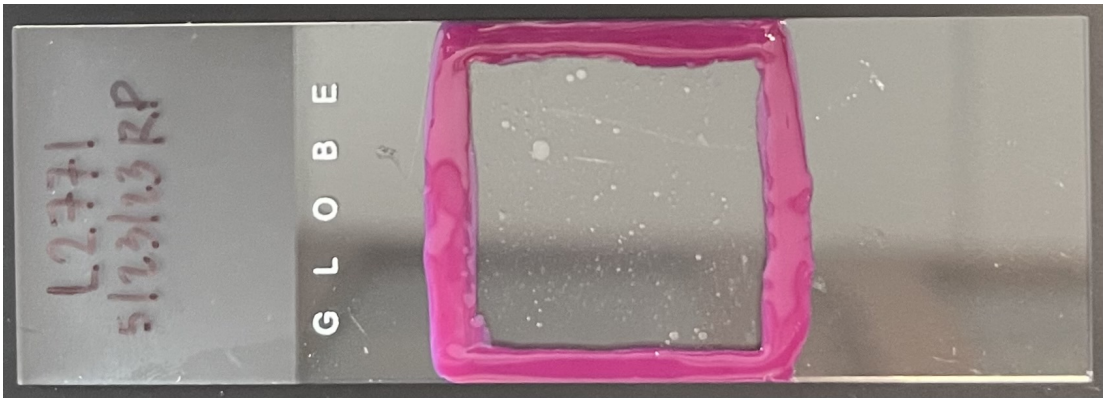
7 Place a COVER SLIP onto the slide and let it sit for about 30 seconds.



Note

Letting the slide sit helps to get rid of any air pockets. We want to ensure that the entire coverslip is touching the oil. If there are any parts that aren't, gently press on the coverslip to help move the oil. If you are still unable to get it covered, make a new slide.

8 Seal the outside edge of the coverslip using Sally Hansen Xtreme Wear Nail Color. Use three coats of polish to ensure that everything is properly sealed, letting each coat dry in between.



9 Store slides according to needs.

Microscopy (Brightfield) and Transect Counting

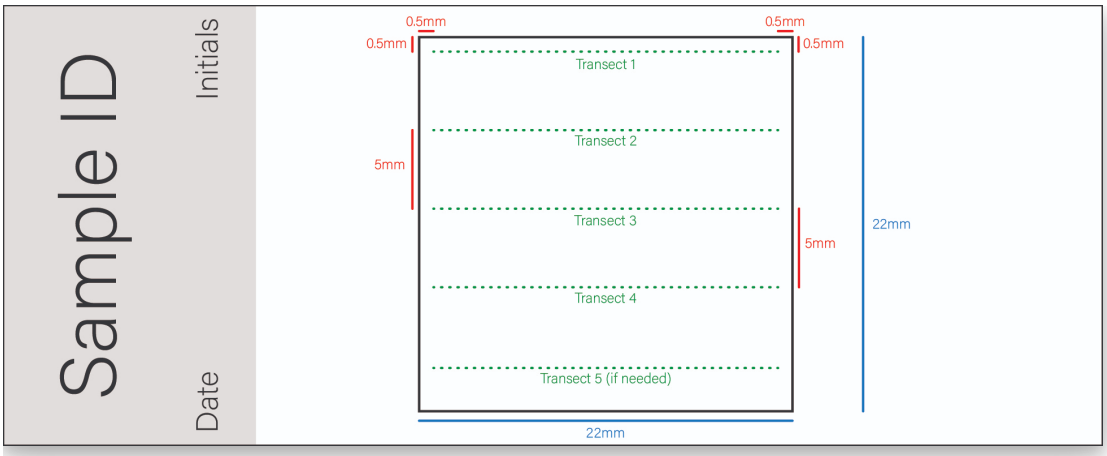
10 Use a compound microscope, binocular or trinocular, for brightfield viewing (light placed below the sample, viewing from above).

11 A polarizing lens will help discern shapes and features in phytoliths and diatoms.

12 A magnification of 40x is good for the initial observation. For individual identification of microfossils and microscope photography, a magnification of 100x will be required.

13 Up to five transects are recommended. The total number of counted transects depends on microfossil density of the specific sample.

The first transect is placed at 0.5mm distance from the upper and lateral sides of the cover slide. All other transects are placed 5mm below the previous transect.



Visual representation of transect placement within cover slide.

13.1 Start the first transect at 0.5 mm down and 0.5 mm to the right of the top left corner of the slide.

13.2 Go across the slide, horizontally, until reaching the end of the slide. Count diatom and phytolith morphotypes present in the field of view.

Note

Morphotypes were easily counted using a clicker.

13.3 Start the second transect 5 mm below the first. This would be at 5.5 mm down the slide, and still 0.5 mm to the right of the top left corner. Continue counting microfossils across the transect using the clicker.

13.4 Start the third transect 5 mm below the second, now at 10.5 mm down from the top of the slide, and still 0.5 mm to the right. Continue counting microfossils across the transect using the clicker.

13.5 Start the fourth transect 5 mm below the third, now at 15.5 mm down from the top of the slide, and still 0.5 mm to the right. Continue counting microfossils across the transect using the clicker.

13.6 If necessary, start the fifth transect 5 mm below the fourth, now at 20.5 mm down from the top of the slide, and still 0.5 mm to the right. Count the microfossils across the transect using the clicker.

Note

A fifth transect was used if there were less than 100 microfossils counted in the four previous transects.

14 Once all microfossils are counted along each transect, ensure no novel morphotypes exist in the rest of the slide. Scan the entire slide for microfossils that have yet to be counted and are of a different morphotype than what has been counted thus far. Identify the new morphotype rather than count it.

15 Note the number of each morphotype in each sample in an Excel database.

Final Results

16 A complete spreadsheet will be produced from the application of this method, with microfossil counts represented by type and/or morphotype as separate transects per each sample. Below is an example of a completed count for a single sample.

Microfossil_Type	Morphotype	Genus_Type	Species	Variety	Sample_ID	Transect	Count
Diatom	A	1	a	x	ID_0001	1	7
Diatom	B	2	b	x	ID_0002	1	8
Diatom	C	3	c	x	ID_0003	1	2
Phytolith	D	4	d	x	ID_0004	1	43
Phytolith	E	5	e	x	ID_0005	1	12
Diatom	A	1	a	x	ID_0001	2	3
Diatom	B	2	b	x	ID_0002	2	90
Diatom	C	3	c	x	ID_0003	2	35
Phytolith	D	4	d	x	ID_0004	2	14
Phytolith	E	5	e	x	ID_0005	2	76
Diatom	A	1	a	x	ID_0001	3	35
Diatom	B	2	b	x	ID_0002	3	76
Diatom	C	3	c	x	ID_0003	3	21
Phytolith	D	4	d	x	ID_0004	3	1
Phytolith	E	5	e	x	ID_0005	3	56
Diatom	A	1	a	x	ID_0001	4	1
Diatom	B	2	b	x	ID_0002	4	0
Diatom	C	3	c	x	ID_0003	4	57

	Phytolith	D	4	d	x	ID_0004	4	23
	Phytolith	E	5	e	x	ID_0005	4	1

Example table of final results. The table structure was based on Wickham (2014).
Reference: Wickham, H. (2014). Tidy data. *Journal of statistical software*, *59*, 1-23.