# Lake Sediment Phytolith Extraction Method

*LacCore, National Lacustrine Core Facility* *July 2014*

## Contents

## Safety

All personnel are required to follow strict safety requirements. The chemicals, glassware, and equipment used in this procedure can be hazardous if mishandled. Lab staff must be specifically trained by experienced personnel.

Required protective gear: lab coat, splash goggles and gloves must be worn at all times. Staff must also wear closed toe shoes and long pants. Hair must be tied back.

All chemical reactions must be performed in a fume hood. Any sample with acid in it must be decanted in a waste bucket for neutralization before disposal. Once neutralized, these chemicals (along with the non-acids) may all be washed down the drain with tap water.

## Record Keeping

Notebook: Each preparer should have a lab notebook to log the process as it is done. The book should be a permanent bound volume, not just loose pieces of paper. We also keep a computer log. Make hardcopies of the printout, one going to each researcher that had samples processed in the batch.

All labeling is important. A perfectly prepared sample that is mislabeled is useless. Sample vial labels are permanent and part of the research archives. The label should include site name, location, core #, sample depth, sample volume, preparation date, preparer's initials, batch #, and spike quantity. Make sure to double check that the label matches what you have written in your lab notebook. Additionally it is critically important to label all tubes and beakers correctly to minimize errors related to the mixing up of samples.

There is no substitute for careful personal instruction in the subtleties of this procedure. Samples vary more than you might expect, and it is often necessary to give some of them individualized treatments. While learning, we encourage you to ask if you are unsure what to do. We want you to know that mistakes happen, even to those who have years of laboratory experience. The critical requirement is the *mistakes be noted and discussed* when they happen so corrections or adjustments can be made. It is generally best to start over.

## Quantitative procedure notes

This is a quantitative procedure**.** This means that in theory, there is no loss of phytoliths during the course of the treatment. Be aware of the causes of lost sample material as discussed below. Concentrate on what you are doing.

Phytolith data is only as good as the preparation. An analyst must be confident that the differences between samples are due to natural differences in the samples and not due to differences in the phytolith preparation procedures. The same lab technician should process all the samples in a project.

Contamination problems often come from poor housekeeping. Keep your area clean.

When transferring samples, make sure the entire sample is transferred. Be very careful in decanting samples as this is the greatest potential source of loss of materials in the sample short of dropping the sample.

## Reagents

- Reagents are dispensed from repipettes, squeeze bottles, or special anti-drip bottles if dangerous. To prevent contamination, do not touch the tip of the squeeze bottle to the side of a test tube. These chemicals are dangerous and should be treated as such. Please be familiar with the SDS (Safety Data Sheets) for each chemical before beginning.
- Hydrochloric acid (HCl), 36%, use as supplied.
- Nitric acid ($HNO_3$), 67%, use as supplied.
- Potassium hydroxide (KOH), 10%, 100 g potassium hydroxide pellets plus 900 mL DI water. Make sure the accuracy is to the nearest gram and measure DI water in a graduated cylinder. Put weighed pellets in container and SLOWLY add DI water.
- Sodium hexametaphosphate (SHMP), 5% = 950 mL DI water plus 50 g sodium pyrophosphate. Make sure the accuracy is to the nearest gram and measure water in a graduated cylinder. For sediments with a higher clay content, a 10% (900 mL DI water plus 100 g sodium pyrophosphate) SHMP solution may be necessary, as decided by the phytolith preparer and/or analyst.
- Soda ash-for neutralization.

## Equipment

- We currently use an Eppendorf 5810 centrifuge. Please see the Centrifuge SOP for proper use, care, and maintenance. You are responsible for following the information in the Centrifuge SOP. It is essential that the centrifuge buckets be balanced by weight.
- You need centrifuge vapor caps capable of capping both 15 mL and 50 mL centrifuge tubes. Since at some stages hazardous chemicals will be centrifuged, it is necessary to have a system that protects room air quality. The buckets are loaded and capped inside the fume hood to seal in fumes before being transferred to the centrifuge outside of the fume hood. We use the vapor caps supplied by Eppendorf for the 5810 centrifuge.
- Specific centrifuge tube adaptors are needed for each style of tube used in the centrifuge. We use adaptors supplied by Eppendorf with minor modifications.
    - 15 mL plastic conical tubes – 15 mL falcon adaptors, blue and the bottom layer of the adaptor is conically formed. Holds 9 tubes per bucket; maximum of 36 tubes in the centrifuge. (See Fig. 2 on p. 5 of Centrifuge SOP)
    - 50 mL plastic conical tubes – 50 mL falcon adaptors, orange with blue conical inserts that must be placed in the bottom of the adaptors before loading the tubes. The blue conical inserts must be used or the bottom of the tube may deform or split open. Holds 4 tubes per bucket; maximum of 16 tubes per centrifuge. (See Fig. 3 on p. 5 of Centrifuge SOP)

    - 1 dram vials – 3-15 mL adaptors, orange, includes rubber mat at base. Assemble adaptors but only use two orange layers on top of the base (with the rubber mat). Holds 12 vials per bucket; maximum of 48 tubes per centrifuge. (See Fig. 4 on p. 5 of Centrifuge SOP)
- A Griddle (to be used as large hotplate) or three small hotplates for heating beakers.
- Vortex mixer.
- Fume hood: all reactions must be done in a fume hood.
- Test tube racks. Need several for each test tube type.
- Conical centrifuge tubes: 15 mL polypropylene (Nalgene #3103-0015/Fisher #05-502-10A) and 50 mL polypropylene (Falcon/BD# 2098/Fisher #14-959-49A). *Do not use polycarbonate*. Carefully inspect all tubes (plastic and glass) before each use for any signs of crazing, cracking, or splitting. The multiple chemical assaults weaken them over time.
- Adjustable pipette and magnetic stir plate (if using LacCore Phytolith Spike suspension). We use an Eppendorf Research pipette that adjusts from 100-1,000 µL.
- Sieves system: We use "custom" designed 70 micron sieves. They are constructed out of screen, 1" plastic tubing, and plastic epoxy such as J-B Weld or Loctite epoxy plastic binder. Our current screen supplier is SEFAR 1-800-995-0531, their site is: www.labpak@sefaramerica.com. They sell by the yard, but the better deal is their LabPaks, which hold several 12" squares and are available in a wide range of nylon or polyester mesh sizes.
- Glassware: 400 or 500 mL tall form beakers, 100 mL graduated cylinders, microscope slides and cover slips for checking samples.
- Cleaning supplies: washbasin, sponges, paper towels, detergent, glassware brushes, gloves, etc.
- Funnels for holding sieves.
- Vacuum sipper set up. We use an Erlenmeyer flask with a side port connected to the in-house vacuum lines. A stopper and tube for sipping is attached to the top of the flask for sipping.
- Dispensing bottles (500 mL): Squeeze bottles and repipettes are appropriate for most reagents. For the screen steps, spray bottles are helpful in moving material through the screens.
- Benchkote for protecting the fume hood surface and countertops. This should be changed frequently to keep the area clean.

## Procedure

Follow the steps in sequence**.** The procedure is designed so that the steps do not interfere with each other. Examples: Potassium hydroxide breaks up the sample so it screens well. **Ask questions if you are not sure what to do next.**

This procedure takes roughly 3 days to prepare 12 sediment samples for phytolith analysis. Except where noted, it can be stopped at the end of each step. At the end of a work period, cover both the samples and the test tubes for holding the stir sticks. Label the test tube rack with the bill to/project code/user information, date, your name, and the current preparation stage. Leave the samples covered in the fume hood until you are able to continue the procedure.

There are 9 basic steps to the phytolith extraction procedure.

Step 1: Set-up
Step 2: Hydrochloric and nitric acid treatments
Step 3: Potassium hydroxide treatment
Step 4: Screening
Step 5: Gravity settling
Step 6: Freeze drying
Step 7: Heavy liquid separation
Step 8: Polystyrene spike
Step 9: Final storage medium/slide preparation
Step 10: Record completion
Step 11: Clean up

Steps can be conveniently divided up into 3 days. Day one usually consists of steps 1-3. Day two usually consists of steps 4-6. The final day is steps 7-10. Depending on initial sample volume and composition this may vary significantly.

## Step 1: Set up and spike

1. Before you begin, label the test tubes and racks you plan to use. You will need three test tubes for each sample: one 50 and two 15 mL (depending on sample size). Our current setup allows for 12 samples to be run simultaneously.
2. Transfer 1 cc of sediment into 400 or 500 mL tall form beakers.
3. Polystyrene spike will be added at the end of the procedure.

## Step 2: Hydrochloric and nitric acid treatments

1. Add 10mL 36% HCl, swirl sample and place on hotplate. Record the reactivity of the HCl with the carbonates (none, weak, moderate, strong).
    a. Add slowly, especially if carbonates are present.
    b. HCl will digest carbonates and oxidize some organics.
2. Add 50 mL 68% $HNO_3$ to beakers, swirl sample and place back on hotplate for 1 hour, or until oxidation has visibly stopped. Bring solution temperature up to 115C Cover beakers with a watch glass to prevent sediment from baking to the sides and swirl beakers every 15 minutes to prevent sediment from baking on (Figures 1-2).
    a. For sediments low in organics and/or charcoal, the acid solution will turn yellow when oxidation is complete. Otherwise, the solution may appear reddish-orange. It's possible that the boiling time may be > 1 hour.
    b. 10 mL HCl + 50 mL $HNO_3$ should be enough to completely immerse 1 cc of sediment. If more acid is required add it preserving a 1:5 HCl:$HNO_3$ ratio. The addition of HCl to $HNO_3$ lowers the boiling point of the solution and reduces the "knocking" of beakers on the hotplate.
    c. Nitric acid strongly oxidizes organics, and will work on carbonates as well. It will also remove iron (Fe) and aluminum (Al) oxide clays.
3. Once the reaction is complete, based on the color of the liquid and the gas trapped under the watch glass, remove the watch glass and boil-off enough liquid so that ~20 mL remains. Once 20 mL of

sample remains remove the sample from the hotplate and cool until the beaker can easily be handled (5-10 minutes).

4. Transfer the sample with high purity $dH_2O$ into 50 mL centrifuge tubes.
    a. Swirl the beaker, pour, and then rinse the beaker with a fine stream of high purity $dH_2O$ into a 50 mL centrifuge tube.
5. Rinse to neutral with high purity $dH_2O$.
    a. Rinses 1-2: centrifuge 3,000 rpm for 1min. Rinses 3-4: centrifuge 3,000 rpm for 3 minutes. Add 2-3 mL high purity $dH_2O$, vortex for 30 seconds in-between rinses, then top off to 40 mL with high purity $dH_2O$ using a squeeze bottle with enough force to mix the sample.
6. Transfer the sample with high purity $dH_2O$ into 15 mL centrifuge tubes. Vortex, and centrifuge 3,000 rpm for 3 minutes. Decant.

## Step 3: Potassium hydroxide treatment

1. Add 1 mL of room temp 10% KOH to each tube, vortex and add another 9 mL 10% KOH. Let stand for about 5 min. Vortex sample after 2.5 minutes if sample size is large.
    a. <u>Caution:</u> *Warm to hot 10% KOH can result in some phytolith dissolution after as little as 20 to 30 minutes exposure*.
    b. Potassium hydroxide is used to remove humates from the sample. KOH also acts to deflocculate the sample for sieving.
2. Top off with high purity $dH_2O$ and conduct another 5 reps of rinsing to neutral pH as described in step 8.
    a. This is a good time to stop in order to save screening and gravity settling for the next day. However, if sample size, clay-load, and diatom abundance require more than one day of gravity settling, Day 2 steps could be started at the end of Day 1.

## Step 4: Screening

1. Set up sieves. Use a small diameter sieve, placed in a funnel, which is then placed on top of a 100 ml graduated cylinder.
    a. 70 micron sieve will remove some of the diatom and sponge spicule fraction. Although gravity settling is a clay-separation technique, it also works to remove a portion of the diatom and sponge spicule fraction.
    b. Sieves are constructed of two pieces of 1 1/8" diameter PVS or ABS. A screen is glued into place between two cylinders with J-B Weld.
    c. Phytoliths typically range in size from 10 to 200 microns. Depending on the context and goals of the phytolith study, a larger sieve mesh (100, 150, 200, etc.) may be selected for use. Sieve size will be determined before the start of the extraction procedure. This procedure is optimized for the recovery of phytoliths diagnostic of wild rice (*Zizania palustris*), so the 70 micron sieve size was selected.
2. Using a squeeze or spray bottle filled with a 5% sodium hexametaphosphate (SHMP) solution, rinse the sample through the sieve (Figure 3).
    a. Use a spray bottle where you can adjust the water stream so that it is a wide forceful shower rather than a single stream.

b. Sieves can be placed upside-down in a sonic bath with 1% SHMP solution.

## Step 5: Gravity Settling

1. Once the sample is through the sieve, use a forceful stream of 5% SHMP solution from the squeeze bottle to thoroughly mix the sample as you fill the graduated cylinder to the 100 ml mark. Make sure the sample is well mixed and then allow it to settle for 1 hour.
    a. The sample can be more thoroughly mixed by covering the graduated cylinder and inverting it. Be sure to rinse the entire sample off of the cover and back into the graduated cylinder.
2. After 1 hour, sip the top 10 cm of supernatant in each graduated cylinder using a vacuum sipper and discard (Figure 4).
3. Next, use a squeeze bottle with 5% SHMP to deliver a forceful stream of solution to mix the sediment and to fill the cylinder back to the 100 ml mark (+/- 3 mL). Allow the sample to settle for 1 hour and then aspirate the top 10 cm as previously described.
4. Repeat steps 5.2-5.3 until the sample has reached a state where after 1 hour, the top 10 centimeters are completely clear (all clay-sized particles have been removed).
    a. When staring 1 cc of lake sediment, it typically takes 4 to 5 rounds of gravity settling. Starting more than 1 cc of sediment may require additional settling reps. It may be possible to conduct 1 or 2 rounds of settling after the acid/base steps on the 1st day of extraction.
    b. When it appears that only one more round of gravity settling will be necessary, only fill the graduated cylinders to the 75 ml level, and after 1 hour, aspirate 10 cm down from there. This should leave 20 ml of solution at the bottom of each cylinder.
    c. This method is based on Stokes law of settling, $= \frac{g(\rho_s - \rho_l)d^2}{18\eta}$, solved for time, $t = \frac{18\eta h}{v} = \frac{18\eta h}{g(\rho_s - \rho_l)d^2}$. Where $v$ =velocity of fall, $g$=acceleration due to gravity, $\rho_s$ =particle density, $\rho_l$=liquid density, $d$= particle diameter, $\eta$ =liquid viscosity, $t$ = time to sip, $h$ = depth to sip down to.
5. Transfer the samples from the graduated cylinder to 50 mL centrifuge tubes. Pick up the graduated cylinder with the first sample, swirl by hand to fully suspend the sediment and quickly pour into a 50 ml centrifuge tube. Keep cylinder inverted while using the $dH_2O$ water squeeze bottle to thoroughly rinse all sediments out of the cylinder and into the centrifuge tube.
6. Vortex, centrifuge (10 minutes at 3000 rpm), and carefully decant.
    a. Pellets at this stage can be fairly loose and easily disintegrate. Stop decanting when the meniscus reaches the pellet; however, enough water needs to be decanted to allow subsequent transfer into a 15 ml tube.
    b. If more than 4 ml of sediment remain after gravity settling, heavy liquid separation should be conducted in a 50 ml tube, otherwise continue by transferring the samples into 15 ml centrifuge tubes, spin for 10 min at 3000 rpm, decant all remaining water, and vortex to loosen pellet. Samples are now ready to dry for heavy liquid separation.

## Step 6: Freeze dry

1. (Optional) Freeze the samples, place in a vacuum chamber and dry overnight. Samples are typically dry when atmosphere in the chamber is less than 200 millitorr (mTorr). When dry, remove samples from the vacuum chamber, cap, and vortex for 30 to 45 seconds to loosen the sediment.
    a. Freeze drying helps to release clays and to separate particles that may otherwise adhere to each other. Drying samples will also ensure that the heavy liquid density (next step) is not reduced due to the presence of rinse water retained in the sample. This step may not be necessary and its inclusion or omission in the extraction procedure will be determined on a project-by-project basis.

## Step 7: Heavy liquid separation

1. Prepare a heavy liquid solution (LST – solution of lithium heteropolytungstates in water) with a density of 2.3 g/ml and add a 3 ml to each sample.
    a. The exact amount of heavy liquid that you add to each sample may vary. For 15 ml tubes, I like to have around 2 or 3 ml of separation between the sample pellet and the heavy liquid surface. For 50 ml tubes, I like to have at least 5 ml of separation.
    b. Caution – *sediments dried in a vacuum chamber are so dry that the addition of heavy liquid can result in a wisp of material out of the top of the tube. Slowly add the heavy liquid and use a fume hood to minimize cross-contamination between other samples and the heavy liquid container.*
2. Place caps on the centrifuge tubes and vortex thoroughly for at least 30 seconds (starting and stopping several times for through mixing), inverting once to check for complete mixing of each sample at the bottom of the tube.
3. Centrifuge samples for 10 minutes at around 1500 rpm (spinning at 3000 rpm can cause some phytoliths to sink that otherwise would not).
4. Decant the thin film of particles (phytoliths, diatoms, charcoal, etc.) and retain in either a 15 or 50 ml centrifuge tube.
5. Add another 2 to 3 ml of heavy liquid to each sample, mix, and spin as outlined above. Add this even thinner and possibly imperceptible film of residue to its respective tube from Step 24.
    a. The LST steps are repeated for thoroughness, to make sure that all phytoliths and exotic markers have been extracted from the sediment.
    b. The material that pelletized during the heavy liquid spins can be discarded.
    c. Please note – *the total amount of heavy liquid containing the phytolith extract should not exceed 6 ml in a 15 ml tube, and 20 ml in a 50 ml tube. This is because a $H_2O$ to heavy liquid ratio of 2.5 to 1 must be maintained in order to ensure that phytoliths sink and pelletize during the rinse steps outlined below*.
6. The tubes containing the retained heavy liquid and floated phytolith fraction need to be filled with $dH_2O$ water and very thoroughly mixed, often requiring inverted shaking to make sure that the less dense water fraction combines with the more dense heavy liquid fraction.
7. Centrifuge the samples for 10 minutes at 3000 rpm and decant, making sure that the meniscus does not disturb the phytolith-laden pellet while decanting.
    a. If recycling the LST, the decanted heavy liquid should be retained for recovery and reuse.

8. If the phytoliths that floated in heavy liquid were retained and rinsed in 50 ml tubes, they should now be transferred to 15 ml tubes for the remainder of the rinses.
9. The phytolith extract samples should be rinsed (mix with $dH_2O$, centrifuge, decant) at least 4 more times to make sure that all heavy liquid is removed.
    a. If it is not, the addition of alcohol (next step) with result in phytoliths sticking together during microscope slide prep. Rinse centrifuge times can probably be reduced to 5 min at 3000 rpm unless the phytolith extract pellets are too loose.

## Step 8: Polystyrene spike

1. After the last water rinse, add 1.0 mL of polystyrene spike, vortex, centrifuge, decant.
2. Rinse 3 times.

## Step 9: Final storage medium/slide preparation

1. After the last water rinse, add about 3 ml of 95 to 99 % alcohol to each sample, centrifuge (5 min 3000 rpm), and decant. Transfer using alcohol to 1 or 2 dram vials (dependent on sample size) for storage. Fill the vials with alcohol to prevent any drying out.
2. If diagnostic slides need to be made place 1 drop of sample onto a microscope slide, add 1 drop of immersion oil, mix, and let the alcohol evaporate (heating is fine). Place a cover slip over the sample and seal the edges of the cover slip to the microslide with nail polish. The nail polish may need to be added 2-3 times, with drying in between.

## Step 10: Record Completion

Make sure that everything is properly complete in your own lab book. Your book is sometimes checked for details on a procedure done to a specific sample, sometimes years later.
Transcribe your log into the FileMaker Pro log. Record any errors or discarded samples. Be certain the sample owner is aware of any problems; e-mail them to make them aware of any problems and the reasons for such problems. **Do not wait for the sample owner to get a copy of the log and ask about the problems, contact them first and notify a staff member.** Print a copy of the FileMaker Pro log entry and give it to the sample owner with their samples. Do not begin another batch until this is done. It is easier to find samples in this log if it is in chronological order.

Update the billing database with the number of samples processed for each project code.

## Step 11: Clean Up

Laboratory glassware: Wash every item after use. Wash with lab detergent and bleach, rinse 3 times with tap water followed by two $dH_2O$ rinses. Gloves and plastic apron are recommended when washing glassware. The glassware should have no spots or rings when dry. Put the dishes away when they are dry.

Countertops, shelves, hood surface: Clean regularly. Hood surface and countertops after each use and shelves at least once a month. Place benchkote on the surface of the fume hood and change it often.

Since the acids and bases used are inorganic, they may be disposed of down the drain after they have been neutralized (pH > 7) in the waste bucket.. All decanting involving hazardous chemicals was done into the waste bucket. Neutralize it with a scoop or two of soda ash as needed throughout the day. Foaming can be controlled by squirting the reaction with 95% EtOH (ethanol) or TBA (tertiary butyl alcohol). ALWAYS completely react wastes in the waste bucket by adding more soda ash until they test neutral or slightly basic using pH tape. DO NOT leave un-reacted wastes in the bucket overnight. Clean it for the next person.

## Document History

By Chad L. Yost (chadyost@email.arizona.edu) modified from Yost, Blinnikov, Julius (2013). Modified for use at LacCore by Jessica Heck (jheck@umn.edu, LacCore.org) and Jammi Ladwig (ladwi020@umn.edu).

## References

C.L. Yost, M.S. Blinnikov and M.L. Julius, 2013. Detecting ancient wild rice (*Zizania* spp. L.) using phytoliths: a taphonomic study of modern wild rice in Minnesota (USA) lake sediments. *Journal of Paleolimnology* 49(2), 221-236.

Aleman, J.C., et al., Estimating phytolith influx in lake sediments, Quaternary Research (2013), http://dx.doi.org/10.1016/j.yqres.2013.05.008

**Figures 1 -2. Hotplate and glass beaker setup for acid digestion (hydrochloric and nitric acid treatment)**

This is the suggested set up for Day 1, Steps 1 - 2 of the acid digestion (hydrochloric and nitric acid treatment) portion of the sample processing. Note the dark coloration of the gasses trapped beneath the watch glasses atop each beaker. This demonstrates that the reaction is not yet complete and additional time of sample heating is necessary. (photo credit: J. Ladwig)


**Figure 3. Small diameter sieve on graduated cylinder set up**

This is the suggested set up for Day 2, Step 5 sieving of sample into a graduated cylinder for subsequent gravity settling repetitions. (photo credit: C. Yost)


**Figure 4. Suggested set up for gravity settling steps**

Yellow slide markers are placed at the 10 cm level. Vacuum pump and Erlenmeyer flask with pipette on right used to aspirate the supernatant to the 10 cm level. (photo credit: C. Yost)

## Online Phytolith Resources

Online resources providing information pertinent to phytolith research/analysis are generally focused on the identification of different phytolith morphotypes via photomicrographs of phytoliths from different plant taxa, rather than discussing the laboratory techniques of phytolith extraction/preparation.

1) The Missouri Phytolith Database was created by Dr. Deborah Pearsall and her students at the University of Missouri's Paleoethnobotany Laboratory as a part of the Phytoliths in the Flora of Ecuador project. It provides a comprehensive view of phytoliths produced by the flora in this region of the New World.

- http://phytolith.missouri.edu/Welcome.html

2) The PhytCore Phytolith Database produced by the Research Group for Palaecological and Geoarchaeological Studies (GEPEG) is an actively-expanding searchable phytolith database representing flora from both the New and Old Worlds.

- http://gepeg.org/cercador.asp

3) The Colonial Williamsburg Foundation website includes a searchable phytolith database representing plant taxa from both the New and Old Worlds.

- http://research.history.org/archaeological_research/collections/collarchaeobot/phytolithSearch.cfm

4) The Institute of Archaeology (UCL) website includes a reference phytolith database created by Dr. Dorian Fuller that includes Old World plant taxa.

- http://www.homepages.ucl.ac.uk/~tcrndfu/phytoliths.html

5) Dr. Mikhail Blinnikov (Saint Cloud State University) created a phytolith gallery with both visible-light microscopy (VLM) and scanning electron microscopy (SEM) images of phytoliths produced by plants and recovered in soils from the Pacific Northwest of North America.

- http://web.stcloudstate.edu/msblinnikov/phd/phyt.html

6) TMI (Tool for Microscopic Identification) is designed to help both the novice and expert identify lacustrine and marine sedimentary components using microscopy. The tool emphasizes visual reference materials supported by written expert guidance on identification and interpretation. While not exclusive to phytolith/biogenic silica identification it serves as a helpful aid in the interpretation of other components that may be present on a given microscopic slide prepared from a lacustrine sample.

- https://tmi.laccore.umn.edu