Removing Starch and Phytoliths from Artifacts

This protocol is designed to remove potential starch and phytolith residues from artifacts and is composed of two parts, whole tool extraction and spot sampling. They take two days and a half hour, and are designed to take place in post excavation analyses and for the field, respectively.

FOR WHOLE ARTIFACT EXTRACTION:

Check the tool: if previously washed, proceed to sonication step. If previously unwashed, begin with washing step.

WASH ARTIFACT ~ 30 mins

1. Place artifact in large beaker.
2. Add DI water, wash artifact gently with toothbrush (boil toothbrush between uses).
3. As water gets dirty, pour off into separate beaker, add fresh water to beaker with tool, and continue washing until tool is relatively clean.
4. Collect all the water in one beaker.
5. Proceed to “Float Starches and Phytoliths” step.

SONICATE ARTIFACT ~ 6-20 mins + overnight

1. Place artifact in beaker large enough to have it lie flat. Cover tool with DI water.
2. Put beaker in sonicator (make sure water level in sonicator is high enough to cover tool as well).
3. Run sonicator for 5 mins. If artifact is dirty, dirt will “puff” off into the water. After 5 mins, remove beaker and inspect water.
4. If water is dirty, pour off into larger beaker, replace artifact in small beaker, add fresh water, sonicate again. Repeat if necessary.
5. If water is clear, lift artifact out of water with forceps, rinse with DI water (the wash bottles work well for this), and dry artifact.
6. Water in beakers should be allowed to settle overnight, and supernatant poured off, leaving sediment in a small amount of water. If necessary, water can be poured into 50 ml centrifuge tube, centrifuged at 1000 rpm for 5 mins, poured off, and more water added, repeatedly, until the beaker is empty.
7. Collect all water in one beaker.
8. A small amount of alcohol can be added to the sample to prevent algae or fungus growth, if the sample is not examined right away.
9. Proceed to “Float Starches and Phytoliths” step

FLOAT STARCHES AND PHYTOLITHS ~ 1 hr

This step is not always necessary, especially if the tool is already well washed. However, if the tool is dirty and thus the sonication gave many sediments, or if prewashing was necessary, the starch and phytoliths must be floated.

1. Pour artifact rinse liquid in batches into a test tube (14ml glass, 15 ml plastic, or 50 ml plastic depending on the amount of water and sediment), centrifuge, discard

supernatant. Repeat until all of the sample has been centrifuged to the bottom of one tube.
2. Fill tube approx. three-quarters full with K/CdI (2.3 specific gravity), mix well, cover with parafilm and invert, then immediately centrifuge 1000 rpm for 5 mins.
3. Pipette off top layer into fresh tube.
4. Repeat steps 2-3 once.
5. Add DI water to new tube to lower specific gravity, invert with parafilm, centrifuge 1500 rpm for 10 mins. Discard supernatant. Rinse off again with fresh water, spin, discard supernatant, leaving a small amount of water to suspend starches and phytoliths.
Be sure to rinse immediately, as K/CdI may damage starches

MOUNTING AND EXAMINING PHYTOLITHS AND STARCHES ~ 5 mins
1. Samples should still be in a small amount of water. If you are interested in representative samples (for counting), make sure that each samples has evaporated enough that you can mount all of the liquid on one slide. If there is still too much liquid, you can either place tube on low on hot plate, or centrifuge and pour off.
2. With Pasteur pipette, make sure all of pellet is suspended in water.
3. Transfer all of liquid to microscope slide, cover carefully with cover glass.
4. Keep the slide hydrated while examining it, using a small small squirt bottle of DI water (careful not to add too much water, otherwise, it will leak over edge of slide, and some of the sample will be lost).
5. Slides can be allowed to dry and stored flat indefinitely, though care must be taken so that cover glass does not fall off. Slide can be carefully rehydrated for re-examining.

<u>FOR SPOT SAMPLING</u>
This method is better when one wishes to establish whether the starches and phytoliths represent actual use of the artifact or contamination, either from surrounding sediments or post-excavation treatments. Extreme care is needed to prevent cross-contamination.
SPOT SAMPLE THE ARTIFACT ~ 10 mins
Choose at least two areas of the artifact to compare. These should, at the very least, be a “used” edge and an “unused” edge. More areas can be sampled, but these should be recorded on a drawing, tracing, or photograph of the tool so that their exact location can be noted.
1. Holding the surface of interest as level as possible, use a micropipette to place a drop of water on the area. If a larger area is to be combined, add more drops until the area is covered, but take care not to add so much that surface tension breaks and the water runs off the artifact.
2. Allow the water to sit for 30 sec.
3. Using micropipette, siphon water in and out in the area of interest, to facilitate removal of microfossils.
4. Finally, siphon water off completely, and place it into a small container (1 ml

microcentrifuge tubes are good for this).
5. Repeat at least once for the same area, combining the water from all samples.
Each area of interest is to be treated in the same manner, with new micropipette tips between each.

Revision History:
Created by Stephanie Simms, Boston University, 9/18/13
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