Protocol for Recovering Starch Grains from Lithic Tools

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Procedure for processing lithic tools to recover phytolith and/or starch grains from matrix sediments from lithic tool surface and those embedded in fine fissures/fractures/crevices. This protocol is exampled by processing four (4) lithic flakes that were potentially used in food processing at a maritime hunter/gatherer fish weir site. If possible, select artifacts that have not been washed after excavation recovery. The protocol covers steps beginning with documenting the lithic tools prior to laboratory processing, processing through three different levels of wash cycles (loose sediment, lithic surface sediment, crevice sediment), reducing the potential extracted materials to a compressed pellet, to the final stage of creating slides for analysis.

Equipment:
Glass beakers (250ml; one for each sample)
Glass pipettes
Disposable gloves
50ml centrifuge tubes (three for each sample)
15ml centrifuge tubes (six for each sample)
2.0ml Specimen Tubes (one for each sample)
New Toothbrushes (one for each sample)
Glass slides/slide covers
Corn Syrup
Toothpicks
Clear Nail Polish

The three wash levels are:

- **Wash A**: toothbrush with distilled water (removing loose sediment)
- **Wash B**: toothbrush or sonic toothbrush with distilled water (this will possibly provide phytolith/starch grains that are related to use).
- **Wash C**: sonicator (this will capture materials most likely related to use from the crevices in tool)

New disposable gloves should be used to handle each tool sample, and at every stage of processing whenever there is a possibility that the tool, or tool "wash" extracted materials may come into contact with your hands, or other surfaces, to ensure a minimum possibility of contamination.

In Preparation:

1. Create a laboratory log with columns for: date, sample number, photograph number, Wash #1, Wash #2, Wash #3.
2. Prior to phytolith/starch grain sediment processing, photograph each artifact with identifying sample number (if possible, include scale).

3. Wash 250ml glass beakers (one for each sample) with Contrex and tap water. Mark each beaker with a sticky note with Sample Number (e.g. 1A, 2A, 3A, 4A – the (1) stands for the lithic sample and the "A" stands for the wash cycle).
4. Label 50ml specimen tubes with artifact sample number and wash number. Each sample should have three labeled tubes (e.g. 1A, 1B, 1C; 2A, 2B, 2C; etc.) Also, mark each corresponding tube cap with the same information (to avoid mixing caps which can result in cross-contamination). Do this for the 15ml and 2ml centrifuge tubes as well.

Wash A (Tubes should be marked 1A, 2A, 3A, 4A)

Step 1: New gloves. Place lithic "1A" in 250ml glass beaker. Pour 100 ml of distilled water into the glass beaker. Using **new** toothbrush, wash lithic for two minutes or until visible loose sediment on surface has been removed. Pour sediment/water into appropriately labeled 50ml tube. Fill to the top with distilled water.

Do this for each specimen, using new gloves for each.

Place the 50ml specimen tubes in centrifuge and run @ 3000 rpm for two (2) minutes. After this first run, pour off excess water from 50ml tubes leaving a few milliliters of water in tube as the sediment which is compressed into a pellet at bottom of tube should not be disturbed. (Note: when pouring off centrifuged water from 50ml tube it must be accomplished in one pour or the sediment pellet will be disturbed and materials will pour out. If pellet is disturbed, must re-centrifuge to recompress the pellet before pouring off excess water.)

Repeat this process (pouring the extracted materials into the correct sample tube) for each specimen until the entire 100ml of extracted materials/wash water has been put through the centrifuge, resulting in one compressed pellet. It will probably take 3-4 cycles.

Step 2: Transferring extracted material pellet from 50ml into 15ml to further reduce liquid. With approx. 10ml of distilled water in 50ml tubes with the pellet, use vortex machine on the 50ml tube to homogenize the extracted materials within the water. Transfer mix into 15ml specimen tubes (by pouring and washing with an inverted squeeze bottle) and centrifuge @ 3000 rpm for two (2) minutes, following the same procedure as above to reduce and pour off excess water Use distilled water to squirt/flush all materials from 50ml into 15ml (takes practice). Repeat this process until all extracted materials/liquid is transferred from 50ml into 15ml tubes. After final transfer and centrifuge, pipette out the excess liquid without disturbing the pellet at bottom.

Step 3: Deflocculation. Fill each 15ml centrifuge tube with a 10% solution (i.e. saturated solution) of sodium hexametaphosphate or sodium bicarbonate. Place centrifuge tubes in shaker and allow them to shake for at least 12 hours. After shaking, centrifuge all tubes for 2 minutes at 3000 rpm and decant supernatant. Fill each tube with distilled water, vortex, centrifuge 2 minutes at 3000 rpm and decant supernatant. Use pipet to remove excess supernatant, leaving as little supernatant above pellet as possible without disturbing pellet.

Step 4: Heavy liquid flotation. To the label of each 15ml tube, add the suffix "HF" for heavy fraction. Label a second set of 15ml centrifuge tubes identical to the first but with the suffix "LF" for light fraction. Add 4ml of 2.1 g/ml sodium polytungstate to each 15ml centrifuge tubes, vortex, and centrifuge 2 minutes at 3000 rpm. Pour off supernatant into the corresponding "LF" 15ml centrifuge tube. Repeat the above flotation step once more. You now have the "HF" tube with a pellet containing particles that did not float (e.g. mineral particles, phytoliths, etc.) and sodium polytungstate supernatant. Fill each of these with distilled water, vortex, centrifuge for 2 minutes at 3000 rpm, and decant supernatant into a bottle to recycle sodium polytungstate. Each "LF" tube now contains floated particles (e.g. starches and organic material) and 8ml of sodium polytungstate solution. Fill each 15ml centrifuge tube with distilled water to dilute the sodium polytungstate solution so that starches will fall to the bottom of the tube. Vortex each tube and centrifuge 2 minutes at 3000 rpm. Decant supernatant into a container for recycling. Fill each 15ml tube with distilled water, vortex, centrifuge 2 minutes at 3000 rpm, and decant supernatant. Pipet off excess supernatant, leaving approx. 1ml of supernatant above pellet.

Note: If your second wash or sonicated extract is very small (ca. 2mg or less), the entire extract can be mounted on a slide without performing the heavy liquid flotation step. This saves time and avoids the possibility of losing starches in the process.

Step 5: Transferring "LF" pellet from 15ml into 2ml to further condense and in preparation for adding corn syrup to pellet as a medium to "float" the phytolith/starch grains for mounting on slide.

OPTION: For evaluation of density ratios for comparative analysis between proveniences, or to assess for contamination, this portion of Step 3 is a process to gain a "net weight" of extracted materials:

Prepare 2ml specimen tubes. Make sure each cap is still secured. Label each one. Tare the 2ml holder and record net weight of specimen tube.

To transfer extracted materials from 15ml into 2ml use a pipette. Because the extract in the 15ml was put through the centrifuge, the extract is in pellet form at bottom of tube with all excess water already pipetted off. Add (by drops) distilled H2O to the 15ml tube and pipette the extracted materials from 15ml to 2ml. You may need to vortex the 15ml to loosen the pellet. Do not "run the water" up the side of the tube because this will just cause the extract to stick to the upper end of the tube. Note: you can vortex the 15ml or you can "suck up" materials with pipette and push back into 15ml tube, and then re-suck it up, which will cause extract to mix/homogenize. Fill 2ml with extracted materials until almost full, centrifuge, pipette off excess liquid. Repeat until all materials from 15ml are transferred into 2ml tube. Pipette off all excess water without disturbing pellet. The final procedure is detailed following instructions for Wash B and Wash C.

REPEAT THE ABOVE PROCEDURES FOR WASH B.

REPEAT THE ABOVE PROCESS FOR WASH C, with the following amendments:

Wash C – sonication.

Fill sonicator with approximately three (3) inches of tap water. Place lithic in clean/relabeled 250ml glass beaker. Fill beaker with enough distilled water to completely cover artifact. Place the beaker with artifact in sonicator. If the beaker floats, remove cap from drain tube hose (hangs down into sink) and let water in sonicator run out until beaker stops floating.

The timer is broke on our machine, so simply plug in sonicator and let it run for 10 minutes. Remove lithic (wear new gloves). This liquid/sediment is now ready for processing, following same instructions as in Wash A.

FINAL STEPS FOR 2ml.

All extracted materials from washes (A,B,C) for each of the lithics should now be in 2ml tubes, with minimal amount of liquid. To remove all liquid, the 2ml tubes will be placed in oven to dry extracted materials thoroughly. OPTION: to speed up this process, fill 2ml tube with catalyzing drying agent such as acetone, ethanol or methanol (with pipette). Vortex tubes to mix thoroughly. Centrifuge at 3000 RPM for 5 minutes. Pipette off excess drying agent (down to 1ml above pellet). Leave caps open and place in oven at 40˚C for 1 – 1 ½ hours.

After dried: Tare 2ml holders and re-weigh the 2ml tubes with the extracted materials. Calculate net weight of extracted materials.

OPTION: At this point, can do "particle staining procedure" if desire, and follow the steps as set forth in that protocol.

MOUNTING PROCEDURES: Mark each slide with sample number/wash number.

To mount the extracted materials on slide, add corn syrup by drops (using a rounded toothpick) into the 2ml tubes. Wash A will require more drops, possibly need to fill the tube, because there will be a lot of sediment and there should be enough corn syrup to make extracted materials less dense for mounting and to identify individual phytoliths/starch grains. Wash B and C may require only 3-4 drops.

For each tube, once the corn syrup is added, stir vigorously for one full minute to homogenize the extracted materials with the corn syrup matrix. Now ready for mounting on slide.

With toothpick, place one drop of homogenized material on slide. Hold slide cover at 45 degree angle on the slide. Lower slide cover, gently pushing down to force bubbles to flow out as slide comes in contact with materials. Use nail polish to seal slide cover edges. Hint: do not put nail polish all the way to the corners, do inner edges first, make one last gentle effort to push any air bubbles out of the open corners. Then put nail polish on corners. Also,

apply nail polish to the edge that has materials closest to it to stop materials from squeezing out during this final mounting process.