Paleo Research Institute

MANUAL FOR POLLEN, PHYTOLITH, STARCH, FTIR, AMS RADIOCARBON, AND MACROFLORAL SAMPLING

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This guide is designed to provide advice on sampling techniques, to standardize field methods for collecting pollen, starch, phytolith, and macrofloral samples, and to provide a standard format for reporting field data to us.

When collecting pollen/starch, phytolith, and macrofloral samples it is important to remember that the purpose in sampling an archaeological site is to obtain specific information from various levels and/or features to address questions within the research design. To accomplish this, one must take care to sample only one specific level at a time. Do not mix levels or include surface material with soil from lower levels. The techniques outlined here are a guide to achieving thorough sampling with a minimum of contamination.

Pollen. Pollen may be transported by wind and form part of a record of local and regional vegetation. Some plants are pollinated by insects or other small animals and do not contribute much pollen to records of vegetation or human activity. Finally, pollen may be transported more selectively by humans in the course of working with plants. Pollen analysis can focus on interpretation of the past environment, for which stratigraphic samples are recommended. Pollen analysis also is a good tool for interpreting human exploitation of plants as foods, construction materials, or for a variety of utilitarian purposes. Pollen is surprisingly rugged and survives in sediments that many suppose would not be conducive to pollen preservation. We have developed methods for coaxing pollen from the sediments with which they are mixed. We find that making small changes in lab procedures improves recovery of pollen in geographic areas that have been difficult in the past.

Starch. Starch grains are white, tasteless, odorless, granular, solid complex carbohydrates (C6 H10 O5). They occur in starchy foods or foods high in carbohydrates, such as corn, grass seeds, cultivated and wild potatoes, biscuit root (*Lomatium*), acorns, etc. Any food that can be ground into a flour is a good candidate for yielding starches. Starch grains can be present in sediment samples from cultural contexts and stratigraphic samples, in vessel and in groundstone washes, and in dental calculus. Starch grains survive the normal processing of pollen and phytolith samples in this institute; therefore, any sample collected for pollen or phytolith analysis is a candidate for yielding starch grains.

Phytoliths. Phytoliths are silica bodies accumulated by plants when soluble silica in the ground water is absorbed by the plant roots and is carried up to the plant via the vascular system. Evaporation and metabolism of this water result in precipitation of the silica in and around the cell walls in plants that accumulate silica. The general term phytoliths, while strictly applied to opal phytoliths, can also be used to refer to calcium oxalate crystals produced by plants such as agave, prickly pear cactus, cattail, mesquite pods and other legumes, and in some other plants. Calcium oxalate crystals appear to be more susceptible to degradation and/or dissolution in sediments than opal phytoliths. Opal phytoliths, which are distinct and decay-resistant plant remains, are deposited in the soil as the plant or plant parts die and break down. They are, however, subject to mechanical breakage and erosion and deterioration in high pH soils. Phytoliths are usually introduced directly into the soils in which the plants decay. Transportation of phytoliths occurs primarily by animal consumption, man's gathering of plants, or by erosion or transportation of the soil by wind, water, or ice.

FTIR. Identification of the bonds between molecules that allows us to identify food residues (lipids, fats, proteins, carbohydrates) and other organics. Sediments, ceramics, fire-cracked or fire-affected rock, and other porous items such as charcoal all may absorb residues that are identifiable. Pack the samples either in aluminum foil or paper bags.

AMS RADIOCARBON. Charcoal, organic residues, dark soil – all may be dated. We can float your sample, recover and identify charcoal (or microcharcoal from dark soils that do not appear to have visible pieces of charcoal), or simply identify the piece(s) of charcoal that you have selected and submitted. It's best to collect your individual pieces of charcoal in aluminum foil. We can also remove charred residue from ceramics and date that. When in doubt, call and ask. Need your dates while you are still in the field? Call for our schedule (and no Rush fees).

Contamination

Contamination comes primarily from four sources at most sites: wind, plants, soil, and people. The wind is a constant source of pollen contamination at the site, carrying pollen from both nearby plants and vegetation farther away. It also moves dirt and remains contained in the local dirt, such as pollen and phytolith. It is preferable to sample for pollen on calm days to minimize contamination by modern pollen carried on the wind. If you must sample on windy days, try to shelter the area to be sampled during the sampling process, and conduct the sampling as quickly as possible. This might be accomplished by enlisting a crew member to serve as a windbreak for the period required to clean the surface and remove the pollen sample. Pollen samples must be collected immediately upon exposure of the feature or level to be sampled, since the surface will become thoroughly contaminated within 10-30 minutes (or less) of exposure. If immediate sampling is not possible, a small block of earth (1-2 cm thick) may be left in place over the area to be sampled. Note: this is not sufficient to protect the sample for long periods of time, or in case of rain. Plants near the site also are a source of modern pollen, which can contaminate samples. Surface samples (modern control) always should be collected at each site prior to removal of vegetation for excavation. As excavation proceeds, the soil that is removed becomes a potential source of contamination for both the modern surface and archaeological surfaces as they are exposed. The backdirt can introduce pollen from levels of occupation into the modern surface sediments. Smoking or chewing tobacco on site: Handling cigarettes, cigars, or chewing tobacco contaminates your hands. Your hands then contaminate the area in which you are working and any samples that you collect. You can introduce tobacco pollen into your samples, resulting in an interpretation of the presence and possible use of wild tobacco by occupants of the site. You can introduce pollen from weedy plants from the southeastern US, confusing the pollen record and interpretation. These weedy plants are found in many other parts of North America, as well. Dogs can contaminate a record. Their fur constantly accumulates pollen that is shed, along with hair, while dogs are at the site. Analysis of animal fibers recovered in samples will be hindered by the presence of dogs at the site, either permanently or as visitors. Notation of dogs at the site or back at camp should be made and a specimen of dog hair saved in an envelope for reference and submitted with the samples.
The presence of fields or piles of corn or other cultivated crops in the vicinity of the site also should be noted. If fresh (or leftover) vegetables, such as corn on the cob, are eaten at lunch or during the day at the site, please tell us.

Sampling Methods

Surface Samples

A surface sample should be collected at every site to be excavated before clearing or excavation begins. This will insure that the surface sample will not become contaminated by the activities of the archaeologists and by the soil from the occupation levels of the site. Every site that is tested, and might be a candidate for pollen sampling, should be sampled at the surface to provide data for comparison of the modern environment with that of the past. Soil samples from the surface should be collected by the modified pinch technique, i.e., a spoonful of sediment from various places within a diameter of approximately 100 feet (30 meters) around the site. Attention should be paid to local conditions such as leeward areas of rocks and plants and areas where the surface sediments are relatively finer, as these make excellent modern sampling loci. Surface samples collected in connection with a stratigraphic column, however, should be collected as the top 1 centimeter of the column. Sites that appear to be shallow (10 cm or less in depth) lithic concentrations do not need to be sampled.

Stratigraphic Columns

Sampling stratigraphic columns at archaeological sites accomplishes many purposes. A closely-sampled stratigraphic column provides information concerning vegetation change through time. The closer the sampling interval, the better the interpretation. Collecting samples at 2 cm vertical intervals is recommended from most sites. This yields an individual pollen sample each 2 vertical cm – they are contiguous.

If you are collecting stratigraphic samples at intervals greater than 2 cm, please do not lump more than 2 vertical cm of sediment into any one sample bag. This simply averages too much time in a single sample.

Stratigraphic samples provide an excellent control at archaeological sites for interpreting the pollen record of economic activities. Pollen recovered in samples from features may be compared with the pollen record from the stratigraphic column, particularly that from the cultural levels identified in the column, to identify anomalies that might represent cultural activity. In this way, stratigraphic sampling strengthens interpretations of cultural activity.

Types of Archaeological Samples:

Stratigraphic columns

Middens

Features

Living surfaces

Groundstone (manos, metates, mortars, pestles, etc.) surfaces

Ceramic vessels

Vessel fill: appropriate for recovery of macrofloral remains. For pollen, starches, and phytoliths this is a control sample.

Washes to recover pollen, starches, and/or phytoliths, FTIR

Residue: pollen, starches, and/or phytoliths, FTIR

The following is a general guide for collecting sediment samples for pollen, starch, and/or phytolith analysis.

All ceramic vessels and sherds considered for archaeobotanic analysis, and all sub-surface groundstone (manos and metates) should be bagged immediately in the field and sent to our lab for removal of extraneous sediment and pollen, starch, and/or phytolith washing.

Projectile points should be bagged and sent to the lab for protein residue analysis prior to rubbing or licking off the dirt. If you put them in small, clear zip-lock bags they can still be viewed and admired in the field.

Instructions for Collecting Pollen, Starch, and Phytolith Samples

1. Scrape trowel free of dirt, scrape area to be sampled to remove accumulation of modern pollen.

2. Clean trowel of dirt. Spray trowel with distilled water and wipe with paper towel.

3. Quickly remove pollen sample (approximately 100 cc or ½ cup) or combined pollen and phytolith sample (approximately 200 cc or 1 cup) and place into Whirl-pak or Zip-lock bag and secure. Sand does not contain as much pollen as silty or clay sediments, so you will want to increase the sample size by 50% in very sandy sediments. Remember that you are trying to recover a sample from the smallest time interval possible, use the trowel to scrape a vertically thin (less than 1 cm) sample. You will need to extend the sample as far as needed laterally within the stratigraphic unit to recover enough sediment to make roughly ½ cup (1 cup for combined samples).

4. Stratigraphic columns should be sampled so that the shape of the area sampled is rectangular and as thin vertically as possible. Extend the sample as far to the side as necessary to get an adequate volume of sediment without crossing stratigraphic boundaries. We prefer to work with a single sample to extract both pollen and phytoliths; and we will split the sample in the lab for our analyses. Collect stratigraphic samples every 2 cm from most deposits. Sample by natural levels, never collecting a sample that crosses level boundaries. When the natural levels are more than 4 cm in height, collect multiple samples from each stratum. For instance, recommended minimum vertical sampling distance is every 2 cm. More widely spaced sampling, such as the older standard of 10 cm intervals, misses significant differences in the vegetation in the site area. Ideally, samples should be taken at the closest interval possible to record fine scale changes in the biota. Samples from 2cm, or where, feasible 1cm intervals will return ecological information on a scale more closely approximating the time frame at which human decisions are made.

5. Place plastic sample bag into a second plastic bag or a paper bag and record sample data in pencil on an inventory card and place it between the two plastic bags or write using a Sharpie marker on the paper bag. Double bagging will help protect the sample bag from puncture and provide a convenient place to record sample information. Do not use paper bags as the only

means of containing your samples, even if they are taped closed seemingly securely. Relying on paper alone during shipping and handling is a certain way of cross contaminating your samples as the bags will fail during shipping. It's not worth the risk.

6. All whole vessels, sherds to be sampled, groundstone, manos, and metates should be bagged in the field prior to the removal of dirt, and sent to our lab. Garbage bags are good for bagging larger items. Projectile points should be bagged immediately if they are to be examined for fibers or protein residue.

7. Lab procedure for handling ceramics: Remove dirt from interior of vessel and use as control pollen, starch, and/or phytolith sample. This fill will ideally be used as a macrofloral sample. Bag this fill separately. This will not provide a pollen, starch, or phytolith record of the use of the vessel. Specific instructions for washing the interiors of the vessels to recover pollen, starch, and/or phytoliths follows separately at the end of this manual.

8.Lab procedure for groundstone: Collect a control sample from dirt in the vicinity of the groundstone. The dirt fill closest to the ground surface is not adequate for interpreting the plants ground. A careful wash of the use surface is better. The grinding surface may be washed per the instructions at the end of this manual.

Feature Types and Specific Pollen, Starch and Phytolith Sampling Instructions

Hearths and Roasting Pits. Sample fill for macrofloral analysis. Pollen and starch analysis is frequently not appropriate from portions of the fill. Pollen from the food processing area around hearths and roasting pits can enter the fill after the fire has cooled, thus preserving a record of economic activity, however direct sampling of areas adjacent to the hearth is more likely to contain a record of the economic use of plants. Open flames destroy pollen, but since the object is to cook, rather than char, foods, portions of hearths might not get hot enough to destroy pollen. In fact, we have recovered successive record of cooking foods in individual hearths, indicating that pollen can be preserved. In roasting pits, use of a buffering plant layer protects food, and the pollen and starch granules associated with it, from the intense heat of the coals. These remains can be preserved if left at the edges of the feature rather than removed by the prehistoric cooks. If the fill is stratified, take care to remove each stratum for pollen, starch, phytolith, and/or macrofloral sampling. Provenience of the sample for pollen, starch, and phytolith analysis is particularly important. FTIR – identification of fats, lipids, carbohydrates, proteins, and organic residues in general. Collect a small quantity of dirt (film cannister size) from fill that would have received cooking debris or been in place when fat dropped from meat being cooked.

Samples should be collected from the living surface adjacent to the hearth or roasting pit, if it can be defined. The work area around the hearth can be sampled as a single unit, by north/south or east/west halves, or in quarters.

Phytolith analysis should be conducted primarily when plants suspected to have been processed are known to produce phytoliths. Many foods do not produce phytoliths.

Storage Cists and Pits. The lower portion of the fill of these features should be sampled for macrofloral remains. Collect a scrape from the wall and bottom of these features for pollen, starch, phytolith, and/or FTIR analysis.

Burials. When possible, pollen, starch, phytolith, and/or macrofloral samples should be collected from burials in pits. Burials in trash middens are not good candidates for pollen or other sampling because trash middens contain such a wealth of remains unrelated to the burial. The best places for sampling include the stomach and pelvic areas where stomach and/or intestinal contents might have deteriorated. Other areas shown in Figure 4 are recommended for sampling only to recover information on potential ceremonial activity, if appropriate.

Metates. When groundstone is in situ a suite of samples is desirable. In addition to bagging the metate, pollen/starch and macrofloral samples should be collected in front of, behind, and to each side of the metate from the living surface. Each sample should be collected as a scrape of the living surface (Figure 3). If metates are recovered grinding side down, a sample should be collected from sediment in contact with the grinding surface. Manos or handstones should be bagged for pollen/starch and/or phytolith washes in the lab. Recovery of organics for FTIR analysis uses a solvent and is done in the lab.

Smashed Ceramics. The area containing smashed ceramics should be sampled for pollen/starch, phytolith, and/or macrofloral remains and for FTIR analysis. If the area burned during abandonment, charred macrofloral remains might be present. Ceramics displaying evidence of charred food residue are good candidates for phytolith analysis because phytoliths withstand the heat of charring and are trapped within the residue.

Maize cobs. Phytoliths recovered from maize cobs, maize cupules, and trapped within charred residue on ceramics can be isolated and measured. These measurements can then be compared with measurements of other maize phytoliths in an effort to identify race or variety of maize, as well as the growing conditions. Fragments of maize cobs or sherds bearing residue should be submitted for cleaning and recovery at Paleo Research Institute.

Structures, Living Surfaces, and Rooffall. Floors and the upper portion of rooffall that represents the roof surface during occupation represent living surfaces. Living surfaces contain a wealth of information concerning economic activity. Prepared or compacted floors should be sampled for pollen, starch, and phytoliths. Pollen/starch and/or phytolith samples should be collected as shallow scrapes of the living surface. Sampling in a grid pattern offers the best opportunity for interpretation. Quarter-meter grids are the best, but meter grids can be informative. Post holes represent areas within a living surface with little foot traffic and tend to collect pollen, the areas in close proximity to a recognized posthole are an excellent choice of a sampling location. We are often able to identify processing and storage areas within a living surface from changes in the pollen frequency of economic or ritual plants where samples are taken on a grid. Floor fill or unconsolidated floor surfaces should be sampled for macrofloral remains. If the budget is not sufficient to allow grid sampling, areas of the floor surrounding features should be sampled for pollen/starch to recover information concerning use of the features. Benches represent another surface used for processing and storing items and should be sampled methodically. Niches, bins, and cists all might have been used for storage of various vegetal items and should be considered for pollen sampling. If evidence of burning exists, macrofloral sampling also would be appropriate. Phytolith analysis can contribute substantially to interpretations of some types of economic activities. The best macrofloral records come from floor fill rather than compacted floors and are obtained when structures have burned. Floor fill also yields good pollen/starch and phytolith data. Floors and floor fill also are expected to carry organic debris, so sampling for FTIR analysis of the organics is a good idea.

Abandonment Mode

Abandonment mode is important in determining the extent of pollen, starch, and/or macrofloral sampling for an area. Catastrophic abandonment of a structure provides the best evidence for activities immediately prior to abandonment. Collapse of a structure that did not burn immediately might offer better pollen preservation. Burning chars macrofloral material, preserving it for recovery.

Rockshelters

Rockshelters with deep overhangs provide excellent preservation conditions for pollen, starch, phytoliths, and macrofloral remains. Preservation in areas with a minimal overhang will more closely resemble that in open sites. Sediments within rockshelters should be sampled stratigraphically for pollen for paleoenvironmental data. In addition, stratigraphic sampling of cultural sediments should assist in the interpretation of cultural activities and/or subsistence patterns. Features recovered in rockshelters should be sampled as described in previous sections. Better preservation conditions increase the possibility of retrieving data from various localities. Therefore, restrictions concerning sampling for pollen and macrofloral remains based on preservation considerations need not apply.

Ritual or Ceremonial Conditions

Sites or structures that exhibit evidence of use primarily as ritual or ceremonial centers often contain living or activity surfaces that can be identified. These surfaces include floors and the upper portion of rooffall that represents the roof surface during occupation. In addition, numerous features are expected in these situations. Floors should be sampled for pollen data concerning activity areas, if interpretations concerning activity patterns involving the utilization of plants or ceremonial pollen is desired. Features should be sampled for both pollen/starch and macrofloral remains and the floor surrounding features should be sampled for pollen/starch if interpretation of the significance of the features might rest on or be augmented by botanic data. Niches, bins, and cists all may have been used for storage of various vegetal items and should be considered for pollen sampling. If evidence of burning exists, macrofloral sampling also would be appropriate. Phytolith analysis can contribute substantially to interpretations of some types of economic activities. FTIR analysis has the potential to add to interpretation of ritual or ceremonial activities.

Ritual or ceremonial centers also must provide a certain amount of daily subsistence for the temporary occupation of the site during ceremonial activities or permanent occupation by caretaker(s). Interpretation of the archaeobotanic data in areas involving potential overlap of habitation and ceremonial function is more complex and relies even more heavily on the collection and transmission of excellent provenience data and information concerning the association of features and artifacts.

Ritual centers frequently were abandoned in a systematic way. If evidence of "ritual" or systematic abandonment is present, pollen and/or macrofloral sampling might be able to augment the interpretations of the preparations for abandonment.

Tooth Calculus

Phytoliths, starch granules, and to a limited extent pollen may be contained in human and animal tooth calculus. These remains are contained in the plaque build-up on the teeth and can be removed in the lab and studied. Calculus from human teeth often provides information concerning diet that might or might not be available from other data bases. Phytoliths are most common in calculus from teeth of grazing animals (deer, sheep, goats, etc.). Phytoliths recovered provide information concerning the grass composition in the areas that the animals have grazed. Phytoliths present in dental calculus can augment paleoenvironmental data. If animals have been allowed access to areas where cultivated crops in the grass family are grown or their debris, phytoliths accumulated on the teeth will register this fact. Teeth should be bagged and not washed until ready for analysis. The entire tooth or jaw should be submitted to Paleo Research for washing and analysis.

Historic Sampling

Gardens. Garden areas may be sampled for pollen, phytoliths, and macrofloral remains. Identifying the original garden level in the field can be difficult, but will provide the best surface to sample. If it is not possible to identify the original garden level, one should sample vertically to attempt to end up with samples from an original garden level. Combinations of the pollen, phytolith, and macrofloral records will provide evidence for garden location and content. Various plants will leave different evidence in the three records, making recommendation for utilization of a single data base difficult. Since most historic records in North America are relatively recent, it is reasonable to expect preservation of a portion of the macrofloral data base, even though it is not charred.

Collect samples of sufficient size to allow for both pollen and phytolith analysis – approximately 1 cup of sediment. A separate sample should be collected for macrofloral analysis. This sample should be at least 1 liter, preferably several liters in size. The quantity of organic matter in the sample and available funds for analysis will dictate the volume to be floated.

Collect samples from several locations within suspected garden areas. It is only through the identification of patterns of pollen or phytoliths that identification of garden elements can be made. There should be a sufficient quantity of pollen samples to be able to establish an expected pattern of background pollen representing wind-pollinated plants growing in the area. A good strategy to consider when sampling horizontally is to collect a single sample for pollen and phytolith analysis from every quarter-meter square (divide a one meter square into quarters). This will provide a rather intensive sampling of potential garden areas. Preliminary analysis might look at only one sample per meter, or one every several meters.

Pollen and phytolith samples should be collected from a limited vertical area. It is better to spread out a sample horizontally rather than vertically to obtain enough soil volume. This restricts the amount of time represented in each sample. Samples that span large vertical distances often represent several decades or several centuries of accumulation, which serves to dilute the specific historic data desired concerning reconstruction of plants from a limited occupation. A mixed record of vegetation through time results when samples are collected vertically.

Vegetable gardens may be separated from flower gardens, and at least some of the contents of each garden might be identified using pollen, phytolith, and macrofloral analyses. As an example, both pollen and phytoliths are expected from corn. Squash pollen is rarely recovered in garden settings, although phytoliths or silicified hairs might be present if fruit and leaves were

allowed to decompose in the garden. Bean pollen is virtually absent from pollen records, but depending on the area of the country and the local and cultivated vegetation, the presence of silicified hooked hairs might identify the presence of beans. Many plants produce pollen that can be identified from historic sediments, producing a record of ornamental flowers, food plants, weeds, and other vegetation. Seeds also might be preserved in the soils, representing a variety of plants.

Many flowers can be identified to family in the pollen record and others to genus or species. Pollen and phytoliths are grouped for interpretation by the type of plants they represent. We have recovered pollen and/or seeds from vegetables, weeds, flowers, and ornamental shrubs in historic gardens.

While few seeds live longer than a century, and most for a much shorter time period, it is possible to recover uncharred seeds from historic gardens. Seeds can frequently be identified to genus, and sometimes even species. Weed, flower, and vegetable seeds all might be recovered from gardens and trash pits. Trash pits, if present, provide the potential for recovery of charred remains, since trash frequently tended to be burned.

Trash Pits. Trash pit samples should be collected from the fill, paying attention to the presence or absence of natural stratigraphy. Trash pits exhibiting natural stratigraphy should be sampled within natural levels. If the natural levels are widely spaced, it is advisable to collect multiple samples from each stratum. For instance, ideal vertical sampling distance is every 5 cm. Therefore, for the pollen and phytolith records, it is advisable to collect samples every 5 cm vertically within a stratum. In a large feature horizontal sampling also should be considered to recover variety within the feature. Large features frequently accumulate through load dumping, which creates mounds of material from each episode. This type of dumping would not be well reflected in a simple vertical column placed in the center of the deposit. FTIR analysis of sediments is recommended, as trash pits and middens were used to discard organics. FTIR analysis identifies the bonds between molecules, which is used to identify organic residues.

PROTEIN RESIDUE COLLECTION MANUAL

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COLLECTION OF FIELD SPECIMENS

Flaked Lithics

All flaked lithic specimens should be placed directly into ziplock/whirl-pak bags with minimal handling. Please do not spit, lick, or rub on the artifacts as this may result in positive results for human proteins. Label the outside of the bag and if desired, place a second label inside the bag.

Control Samples

Since false positives may result from bacteria, animal feces, lippoproteins, and alkaline substances in the soil, it is necessary to test soil samples with the artifacts. Collect approximately 1 gram samples from soil surrounding the artifacts and place in suitable containers (film canisters work nicely). Other control samples:

Stratified sites:	Collect 1 gram samples from all cultural levels and from at least one, but no more than three, off-site areas.
Surface sites:	Collect 1 or 2 one gram samples from the site as well as 1 sample from off-site.

Control samples will be processed at our discretion and at no additional cost.

Groundstone and Pecked Stone

This category includes manos, mortars, pestles, etc. We prefer that groundstone and pecked stone artifacts be sent to our laboratory for extraction, especially if pollen and/or phytolith analyses are also requested. We will collect a protein residue sample from one side or area of the groundstone and pollen/starch and/or phytolith samples from a separate area of the ground surface. Starch granules represent starchy foods such as seeds and roots/tubers that often are ground in preparation for cooking.

Extracts for protein residue analysis from large metates or bedrock mortars should be collected by the archaeologist in the lab or field using the methods on page 4.

Ceramics

Ceramics can also be tested for protein residue, pollen, starch, and/or phytoliths. Whole pots or sherds can be sent for analysis and will be treated in a similar manner as groundstone. Protein residue analysis cannot be used on carbonized materials as the protein proteins are too severely denatured to yield results. Ceramics containing visible residue are excellent candidates for phytolith analysis using a new technique that can distinguish phytoliths of wild grasses from those of *Zea mays* and also can identify variety of maize. Ceramics should be handled in the same manner as flaked lithics.

Soils

Soils from suspected processing areas and/or kill sites frequently retain the blood of the animal(s) processed. These soils can also be tested. Small (1 gram) amounts of the soil(s) to be tested should be placed in a ziplock/whirl-pak bag or clean film canister and sent for analysis.

Curated Artifacts

Curated artifacts are also possible candidates for protein residue analysis. If the curated artifacts are stored in trays, they should be placed in separate, labeled ziplock/whirl-pak bags. If these artifacts are stored in other types of bags, such as brown paper, please send them in "as is."

ARTIFACT SELECTION/RESEARCH QUESTIONS

For very large collections of lithics, selection of artifacts sent for protein residue analysis should fit the research design. Some examples:

> Finished tools, projectile points with impact fractures, flakes with obvious use/wear.
>
> Specific classes of artifacts. For example, were projectile points of one class used on a single type of animal -- large points for large game, small points for small animals?

If artifacts were multi-purpose tools, it is sometimes possible to identify more than one type of protein residue, unless the animals are closely related such as sheep and goats, or deer and elk.

Different types of protein residue may be detected from the hafting area and from the tip of the same tool. Identification of proteins from the hafting area may identify the hafting medium. When sending artifacts for hafting area AND tip analysis, please

indicate that each area is to be tested separately. These areas are considered to be two separate samples and will be charged as two samples.

Protein residue analysis can be used in evaluating different aspects of prehistory. Examples in which it may be of benefit are:

1. As an objective test of tool function

2. As a test to determine whether animals represented in faunal remains were processed at the site.

3. To determine a possible range of animal resources exploited.

Reports

Our reports are interpretive, rather than providing just a list of positive reactions. We identify possible animals represented by positive results based on known distributions of the animals and known and tested reactions of the antisera.

Artifacts sent for analysis can be tested against the following antisera:

Bear	Elephant	Turkey
Bison	Goat	Agave (includes yucca)
Bovine	Guinea pig	Trout
Camel	Horse	American eel
Cat	Human	Gizzard shad
Catfish	Pig	Striped bass
Chicken	Rabbit	Atlantic croaker
Deer	Rat	Bay anchovy
Dog	Sheep	Weakfish

POLLEN/PHYTOLITH WASHES FROM GROUNDSTONE AND VESSELS

Equipment and Supplies:

- Bowl to collect liquid from wash
- Clean trowel
- Sonicating tooth brush (or you may use a stiff-bristled brush, such as a tooth brush or a paint brush with bristles cut to a length of 3/4 to 1 inch
- Jars with rubber gaskets to contain samples, or plastic bottles (lids must seal to prevent leakage in the mail or during transport), peanut butter jars or canning jars are good. Plastic bottles with plastic screw lids also are excellent sample containers.
- 2 plastic squirt bottles (1 for vinegar, and 1 for distilled water)
- "Canned air", pressurized air (Dust off, Tornado, etc.)
- Liquid household bleach
- Distilled water
- Vinegar (not flavored), distilled white vinegar is best
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Instructions:

All supplies must be "sterilized" prior to collecting the sample. Mix a bleach solution (1 part bleach to 3 parts water) and use to clean the bowl, brush, and jars. All supplies must be thoroughly rinsed with tap water to remove any bleach residue. Bleach oxidizes pollen, so any residue left on the sampling supplies will destroy the sample. Rinse copiously with tap water so there is no bleach smell left on the supplies. Then rinse lightly with distilled water to remove any particles introduced by the tap water. Fill 1 squirt bottle with vinegar and the other with distilled water.

Unwrap first piece of groundstone and remove any dirt clods adhering to the grinding surface (do not include these in the sample). Next, remove additional dirt using a clean trowel. Next, use "canned air" to blow any contaminants or loose sediment off the surface. You should have a fairly clean looking surface at this point. If not, loosen more dirt with the trowel and use the "canned air" again. If you cannot see the grinding surface of the tool, call Linda for further instructions, which might include lightly washing the surface with a gentle stream of water.

Wash non-grinding surfaces so that any liquid dripping down these surfaces does not collect additional sediment to contaminate the sample. Be certain not to wash the grinding surface during this process unless it is thoroughly covered with calcium carbonate (see next paragraph).

IF grinding surface is thoroughly covered with calcium carbonate rinse the entire artifact with water to remove all dirt, scrubbing with a brush to be certain it is clean. Otherwise, skip this step and proceed.

Hold groundstone so that liquid dripping from grinding surface will drip into bowl, but not run down the underside of the artifact. Squirt grinding surface with vinegar to wet. If surface bubbles, there is some calcium carbonate present, which must be dissolved (or the groundstone is made of limestone or other mineral soluble in acid). If the artifact is soluble in acid, only a limited quantity of vinegar should be used on the surface, as it will erode the surface. The wash should be continued with distilled water just as soon as any apparent carbonate deposits are removed. For non-soluble groundstone, use the vinegar until the bubbling stops, indicating that the carbonates have been dissolved. This will uncover the original grinding surface and allow the pollen accumulated on it to be removed. The grinding surface should be scrubbed with the brush (a sonicating tooth brush works wonders getting the surface clean) while the vinegar is being applied. This is easier with 2 people -- one to hold the rock and the other to squirt the vinegar and brush, or one to hold the rock and brush and the other to squirt the liquid. Do not brush so vigorously that you spray the vinegar or acid on the counter or on people. Remember both safety and that any pollen removed from the grinding surface is contained within the drops being sprayed around. When the surface no longer bubbles, indicating that the carbonates have been dissolved, continue washing with distilled water and brushing (in circles) with the brush. The object is to get the grinding surface clean enough to eat from. This insures that any dirt particles remaining in pores of the rock have been removed, and with them any pollen that had been ground into these pores. Brush only the grinding surface, not the non-grinding areas surrounding them. When the grinding surface is clean, rinse the brush into the collecting bowl with distilled water. Pour the sample into the jar (or jars) and seal. "Sterilize" all supplies with bleach before proceeding to the 2nd wash. Remember to rinse copiously again!

COLLECTING PROTEIN RESIDUE SAMPLES FROM GROUNDSTONE

Extraction of protein residues is usually performed with a 0.2 M Tris hydrochloride, 0.5 M sodium chloride, and 0.5 % Triton X-100 solution (most desirable), although a 5 % ammonium hydroxide solution (less desirable) also can be used. The components for these solutions can be purchased from Fisher Scientific and from Sigma Chemical Company. When washing groundstone, use as little solution as possible (1-5 mL). For an additional $12.00 charge, we can use a Centriprep-10 centrifugal concentrator with a 10,000 molecular weight cut-off membrane. This allows us to concentrate the proteins in a large volume of liquid down to a sample about 1-2 mL in size. If you opt to use the Centriprep device, a larger volume of solution may be used to wash the artifact (up to 50 mL).

Using a sterile (new) head on an ultrasonic toothbrush (preferred) or a new regular toothbrush, vigorously scrub the surface of the groundstone where a small amount of solution was applied. Decant the liquid into clean plastic container (do not use a glass container) using a pipette or syringe and repeat until a small area of the ground surface has been cleaned. Use additional solution from the pipette or syringe to "wash" the bristles of the toothbrush to get the residue out of it.

The decanted solution should be placed in a clean (preferably new) screw-capped plastic container with a tight seal. This container should be placed in a labelled ziplock/whirl-pak bag. If you have groundstone that was buried in the soil, be sure to scrape off the excess soil and include a soil control from the same area as the groundstone (preferably not what was scraped off the tool). Soils commonly contain compounds such as bacteria, lippoproteins, and animal feces that can cause false positive results for buried artifacts. A 1-2 gram soil control size is all that is required, and these soil controls are run at no extra charge.

DO THIS PROCEDURE FIRST, BEFORE WASHING THE REMAINDER OF THE SURFACE FOR POLLEN/PHYTOLITHS/STARCH.

DO NOT USE GLASS CONTAINERS FOR STORING PROTEIN RESIDUES, AS THE PROTEIN WILL ADHERE TO THE GLASS, MAKING RECOVERY DIFFICULT OR IMPOSSIBLE.

GUIDELINES FOR FIELD COLLECTION OF ARTIFACTS FOR PROTEIN RESIDUE ANALYSIS

In the Field:

1. Handle the artifact as little as possible.
   - Use trowel to pick up and place in plastic bag
   - Use plastic food service gloves (Some latex gloves have been shown to contain proteins)

2. Do not brush off or clean dirt adhering to artifact

3. Place artifact in clean plastic bag

4. In a separate plastic bag or clean film canister, add a small amount of adjacent dirt
   - Dirt will be used as a negative control
   - Dirt should be from under the artifact
   - Dirt should not have touched the artifact *in situ*