PILOT STUDY IN MICROBOTANICAL PLANT RESIDUE ANALYSIS, MARKET STREET CHINATOWN ARCHAEOLOGY PROJECT

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SUMMARY

Archaeobotany is the study of preserved plant materials from archaeological sites, including plant residues recovered from soils and artifacts in archaeological deposits. This report describes the rationale, methods, and analysis techniques used in a pilot study to evaluate the research potential of extracting botanical residues from sediment samples and ceramic vessels from the Market Street Chinatown archaeological collection. In fall 2011, researchers working on the Market Street Chinatown collection discovered that Archaeological Resource Services had collected sediment samples from the interior of some ceramic vessels, such as bowls and storage jars, during excavation in 1985-1986. This study evaluated the archaeobotanical research potential of the sediment samples and the vessels associated with them by analyzing 28 samples (16 residue samples and 12 sediment samples). This pilot study focuses on microscopic starch residues, a plant constituent that preserves relatively well in some archaeological contexts. The presence and absence of other residues such as phytoliths, pollen and fibers were also noted within this study. This is the first study to extract microbotanical materials from nineteenth century glazed Chinese and British ceramics within the context of an overseas Chinese community assemblage. This study allowed us to make the determination that sediments found within the vessels were related to depositional processes and not from domestic use. It also allowed us to make the determination that it is possible to use microbotanical analysis to gain more insights into daily lives of residents of the Market Street Chinatown.

TABLE OF CONTENTS

Section	Page #
Summary	i
Introduction	1
Residue Analysis Methodology	3
Sample Selection	3
Preparation: Reference Samples	3
Study Methods	4
Study Results	6
Vessel Residue Analysis	6
British Whiteware: 85-31/18-228 (V4)	6
Bamboo Porcelaneous Stoneware: 85-31/18-395 (V13) and 85-31/18-396 (V9)	7
Sediment Residue Analysis	8
Discussion	9
References Cited	11
Appendix A Residue Results	A-1
Appendix B Reference Starch Photographs	B-1
Appendix C Joint Starch and Phytolith Extraction from Sediment and Residues	C-1
Appendix D Residues	D-1

INTRODUCTION

The historical and archaeological analysis of the Market Street Chinatown collection has focused on expanding information about the first historic San Jose Chinatown, and gaining insights into the lives of Chinese immigrants to enrich Chinese American history, and Californian history more broadly. One way to expand the information that can be found through analyzing the Market Street Chinatown collection fully is through the use of archaeobotanical analysis. Archaeobotanical residues such as starch have been used in recent years to study environmental change, agricultural practices, and cooking practices (Torrence 2006). Specifically relating to this pilot study, starch and microfossils have been used to understand artifact uses in relation to decoration on stoneware pottery (Crowther 2005); in use-ware and residue analysis of museum artifacts (Fullagar 2006; Barton 2007); and to identify the usefulness of residue analysis on artifacts from survey collection (Hart 2011). While evaluating the potential of the collection for further analysis, this study can contribute to the broader discussion of whether residues can be found on glazed pottery, a subject which has been neglected to date in archaeological research.

Concurrent with this study, a second archaeobotanical pilot study was conducted at the PaleoResearch Institute to evaluate the research potential of bulk soil samples collected during the Market Street Chinatown excavations (see MSCAP Technical Report 3). The research reported here complements the PaleoResearch Institute study by assessing the potential for recovering starch and microfossil residues on ceramic vessels and the associated sediment samples taken from within the vessels. This study was conducted as an independent research course under Dr. Barbara Voss, Principal Investigator of the Market Street Chinatown Archaeology Project, during the completion of my graduate coursework at Stanford University. Megan Kane, collections manager for the Market Street Chinatown Archaeology Project, assisted in selecting the soil samples and ceramic vessels for analysis and coordinated access to the collections and project records. The work was conducted in Stanford University's Archaeobotanical Laboratory, where I was trained and supervised by Dr. Li Liu and Dr. Sheahan Bestel. Dr. Bestel and Dr. Voss also reviewed and edited draft versions of this report.

As a pilot study, the research questions guiding this project were: Are residue analysis methods useful in the analyses of the Market Street Chinatown collection and are there residues present on the ceramic vessels? Do the botanical residues present in sediment samples collected from inside the ceramic vessels relate to residues found on the vessel surfaces? And are these

results a record of cultural activity, or do they represent background environmental conditions?

RESIDUE ANALYSIS METHODOLOGY

Sample Selection

For this study, I selected twelve ceramic vessels with associated soil samples. Megan Kane assisted in vessel and soil sample selection. Three additional vessels were also selected for analysis because their fragmentary condition made sonication feasible. The criteria for the sample selection were: (1) to use vessels that were associated with recovered soil samples; (2) to select both vessels and soil samples from a variety of archaeological contexts; (3) to test a variety of vessel types; and (4) to select as many vessel and soil samples as possible from the same archaeological contexts as the bulk soil samples currently under analysis at the PaleoResearch Institute. The samples selected for analysis are listed in Appendix A, Tables A-1, A-2, and A-3 by catalog numbers, and by analysis numbers assigned to the various processed residue samples throughout the laboratory analysis.

Preparation: Reference Samples

In preparation for analyzing the starch residues from these sediments and vessels, I prepared modern reference samples of pickled ginger and cooked rice, as these were starches known from historic sources to be present in the diet of the historic residents of the Market Street Chinatown. Additionally, many of the vessels selected for this study are colloquially referred to as "rice bowls" and "ginger jars" because of the historic association. For each reference sample, over 100 starch grains were measured to create a visual and statistical range of comparison for preserved starch found on any of the Market Street Chinatown collections ceramic vessels. Modern pickled ginger (*Zingiber* sp.) starch exhibited granules with diagnostic shapes ranging between a bell and a fan shape, with a range of 4.8-10.74 µm ($\dot{x}$=7.1 µm; SD=1.10611). For the modern cooked rice (*Oryza* sp.) sample, I cooked Kokuho Rose brand rice in a rice cooker for approximately 35 minutes. Within the resulting cooked starch, some portions were gelatinized with damaged extinction crosses, while others were less damaged and so were measurable. The average length range of the non-gelatinized cooked rice starch was 2.4-7.9 µm ($\dot{x}$ =4.8 µm; SD=0.939631). Cooked rice starch has previously been described as follows: "grains are compound, subangular, faceted and small. Hilum is centric, closed. Crosses are radially symmetrical but usually quite faint. Individual grains range from 3 to 10 µm" (Henry 2009:917). Photographs of pickled ginger starch and cooked rice starch are presented in Appendix B. Modern reference samples aided residue identification along with the expert knowledge and extensive reference collection available in the Archaeobotanical Laboratory.

Study Methods

The methods used for analysis of the residue and sediment samples followed a lab protocol adapted from the Bestel-Liu Protocol (Appendix C). The artifacts listed in Appendix A Table A-2 were analyzed for residues. When possible selected pottery sherds (86-36/5-1535; 85-31/20-58 and 85-31/20-162) and fragmentary vessels (85-31/18-395, a fragment found in 85-31/18-396 and 85-31/28-3) were sonicated in an ultrasonic bath for three minutes. Piperno *et al*. (2009) have found that sonicating artifacts is useful for removing sediments held within tiny cracks. The liquids from the sonicated artifact sherds were then processed with the heavy liquids according to the Becks Protocol (Appendix C).

For ceramic vessels too large to be sonicated, residues were extracted manually. Visible residues on the inner or outer surface of the vessels were scraped onto slides, and then water was dropped on to the scraped area before residues were extracted with pipets. This technique has been shown to increase the number of starch granules recovered. Following the methodology outlined in Torrence (2006) and Piperno (2006) I removed residues from only small areas on the artifact surfaces, in contrast with methods which remove residues from the entire artifact (Hart 2011). Examples of the resulting residues are shown in Appendix D. By only sampling a small area of the vessel surface, residues were preserved on the vessels for future research, and none of the ceramic vessels were harmed during this process.

The sediment samples were processed by removing 800 mg (+/-20) or less (less than a quarter of a teaspoon of bulk samples) for analysis and were processed with the non-toxic heavy liquid sodium polytungstate (Na6-O39-W12), and cleaned with 10% Hydrochloric Acid (HCL) when sediment was too thick. While the extracted portions of the sample were effectively destroyed, 800mg is a very small percentage of each of the soil samples, and the remainder of the samples was unchanged. Sonicated sediment and wet samples were centrifuged and then put onto slides, which were analyzed using a Zeiss axioscope.A1 microscope with an attached AxioCam HRc digital camera and Zeiss Axiovision version 4.8 software. Due to the large number of slides to examine, a timed fifteen minute survey was used to identify the presence or absence of residues mounted on the glass slides. In cases where starch was recovered, slides were completely scanned. The presence/absence of microfossils and starch residues were noted using bright field and polarizing light filters as is common practice (see Fullagar and Barton 2006). DIC (differential interference contrast) or Nomarski filters were used to take images where appropriate. The presence and absence of residues other than starch within the vessel and sediment samples were also briefly noted but not thoroughly analyzed.

Those starch grains that were present are generally consistent with rice and other small grasses. One exception was a large, rounded grain, exhibiting what appears to be lamellae that are generally consistent with starch from the wheat tribe Triticeae. Many of the phytoliths were not diagnostic and were only identified as long cells and grass multicellular skeletons.

STUDY RESULTS

Vessel Residue Analysis

Out of fifteen vessels, ten (66.67%) had residues recovered through droplets, sonication and heavy liquid separation. Only three samples (20.00%) contained recognizable starch grains. In each case, the starch grains were recovered on unglazed, chipped, or broken areas of the ceramic vessels (85-31/18-228 and 85-31/18-395). Five (33.33%) of the samples yielded no discernible residues (from 85-31/18-6, 85-31/18-10, 85-31/20-75, 86-36/5-1583 and 86-36/5-1535). For two of these samples (from vessels 85-31/20-75 and 86-36/5-1583) the failure to produce results is likely related to slide mounting issues, and in the future glycerol will be used for slide mounting rather than water.

In terms of possible contaminants, six (40.00%) of the vessels sampled contained charcoal and burnt phytoliths at moderate levels, which is not surprising given the fact that all artifacts were recovered from lined and unlined trash pits that might have included incinerated trash; and because the 1887 arson fire that caused the Market Street Chinatown's demolition likely generated burned plant material. Only the sample from 85-31/2B-1 (06.67%) had bordered pits, which are residues that may be indicative of wood and in some cases can be diagnostic. Only two vessels (85-31/18-228 and 85-31/18-395; 13.33%) exhibited identifiable pollen grains, which is a bit surprising considering that pollen was likely prevalent in the environment and, as discussed below, was abundant in sediment samples. Eight vessel samples (53.33%) had fibers of some sort present within the residues, and as a subset of fibers only one vessel analyzed had a plant hair present. Finally, two of the vessel fragments tested contained phytoliths, although as with the starch grains these two fragments (pieces of 85-31/18-395) were found in association, and are likely parts of the same vessel. For more details about the vessel samples see Appendix A, Table A-3. It should be noted that in cases where residues were scratched from vessel surfaces and a wet sample was taken from the same location after removal of visible dry residue (85-31/18-6 [V2], 86-36/5-14 [V10], and 86-36/5-19 [V11]), the wet samples generally yielded more residues than dry ones. Sonicated samples did not yield more residues, but this is likely due to the cleanliness of the vessel sherds available for analysis rather than the methodology.

British Whiteware: 85-31/18-228 (V4)

Two morphologically different types of starch were present on Vessel 85-31/18-228 (V4). These residues were found on slide 4B only (see Appendix A, Table A-2). These starch grains are faceted compound starch with central

extinction crosses, and are similar to cooked starch from the Triticeae tribe as well as medium sized round starch grains. Images of these starch grains are presented in Appendix C. The faceted starch grains, although few, clearly resemble the modern cooked rice starch reference, and fit within the size range for rice starch granules.

Table 2: 85-31/18-288, Slide V4B: starch length average, range, standard deviation and total grain count.

85-31/18-288, Slide V4B Starch			
Faceted		Medium Round	
AVE	3.86 µm	AVE	11.38 µm
MIN	3.46 µm	MIN	8.61 µm
MAX	4.42 µm	MAX	14.42 µm
SD	0.4286	SD	2.39815
Total	4 µm	Total	4 µm

Bambo-Pattern Porcelaneous Stoneware: 85-31/18-395 (V13) and 85-31/18-396 (V9)

Three separate sample slides were processed for artifact 85-31/18-396 (V9); however, even with this intensified processing and three separate sample slides, only one type of starch granule was recovered. This cluster of granules exhibit faceted grains, with eccentric extinction crosses. This starch is morphologically similar to the cooked rice reference sample, and statistically it falls within the cooked rice size range.

Table 3: 85-31/18-396, V9: starch length average, range, standard deviation and total grain count.

85-31/18-396, V9 Starch	
Faceted	
AVE	5.125 µm
MIN	3.95 µm
MAX	6.93 µm
SD	0.766
Total	19 µm

Artifact 85-31/18-395 (V13) is a small fragment of the same vessel represented by 85-31/18-396. For 85-31/18-395 (V13), two morphologically different starches were recovered: faceted compound starch and a larger rounded starch grain. The faceted starch is a visual match for cooked rice starch and statistically falls within the appropriate size range for rice.

Table 4: 85-31/18-395 (V13) starch length average, range, standard deviation and total grain count.

85-31/18-395V13 Starch			
Faceted		Round	
AVE	5.118 µm	AVE	20.215 µm
MIN	3.91 µm	MIN	18.76 µm
MAX	6.36 µm	MAX	21.67 µm
SD	0.677	SD	2.058
Total	12 µm	Total	2 µm

Sediments Residue Analysis

All of the twelve sediment samples yielded some sort of residues, but none contained starch. Pollen was prevalent, and identified within nine samples (75%), which is an expected environmental background occurrence. All samples contained an abundance of charcoal and burnt phytoliths, which is consistent with the 1887 arson of the Market Street Chinatown as indicated by historical records. Two sediment samples (16.7%) contained bordered pits (86-36/5-16 and 86-36/5-20), , which are likely indicative of wood. As noted in the previous section, a single vessel sample from 85-31/2B-1 also contained residues with bordered pits. Ten samples (83.3%) contained fibers, four of which (33.3%) contained plant hairs, and two of which contained what appear to be feather barbs (85-31/20-9 and 85-31/18-396). All sediment samples contained some level of microfossils, predominantly grass long cell phytoliths, and multicellular grass phytoliths. In a few instances, possibly diagnostic sedge and rice husk phytoliths were noted (see Appendix A, Table A-3).

DISCUSSION

Small amounts of starch (N=41) were recovered from the non-glazed surfaces of porcelaneous stoneware and whiteware vessels. These starch grains were identified as small grained grass starch and Triticeae tribe grass starch. The category of small grained grasses includes thousands of family groups including rice, while the Triticeae grass tribe includes wheat and barley as well as other wild plants. It is difficult to distinguish between the small and facetted starch grains in grass family seeds, many of which are between 4-14 μm in size and are compound and facetted (Bestel 2012). Our hypothesis was that there would be rice starch present, and the findings in this case are consistent with rice starch being present as a residue on these vessels.

It is significant that starch grains were only recovered in residues taken from the vessels themselves, and not recovered from sediment samples that were associated the vessels. This, and the significant differences in residue type between sediment samples and residues from vessels, suggests that post-depositional contamination is unlikely to have affected the residues preserved on the ceramic vessels in the Market Street Chinatown collection. The high amounts of charcoal and other plant microfossils, including bordered pits and grass phytoliths, in the sediments contained within vessels, suggests that these sediments represent post-depositional infilling of the vessels with charred and burnt trash and midden deposits. This is also indicated by the contents of some of these sediment samples, which included metal nails and large fragments of charcoal as well as other inedible items. In short, these sediments do not represent vessels 'residues' or the vessel contents but rather a post-depositional infilling of the vessels that may have occurred within a midden or rubbish dump deposit. Considering the minimal overlap between the sediment and the vessel samples, it seems that there was not a high level of contamination and that the starch residues on the vessels are actually related to the vessels' use.

This initial study indicates that starch and phytolith residue analysis may be of potential use for further archaeobotanical study of the Market Street Chinatown collection. The benefit of starch research, however, may be limited compared to other archaeobotanical analyses. Starch residues were not found on the majority of vessels analyzed. Recognizable starches were identified on two out of fifteen vessels analyzed within this pilot study. In each case the recoverable residues were extracted from chipped or pitted breaks in each vessel's glaze. If I were to analyze more vessels within the Market Street Chinatown collection for plant residues, I would select only vessels with blemishes and glaze imperfections. For the best results in the future, more systematic scans of entire slides will yield the most complete starch counts. As was done in this study, it is beneficial to extract additional residues from

multiple sites on each vessel when possible, and to weigh each sample through each stage of the process to ascertain the representative significance of the observed starch residues within the remaining sediment and residue samples.

Plant microfossil and macrobotanical analyses would seem to be most fruitful for looking at the environmental background of the site. Given the large amounts of charcoal present in sediment samples, macrobotanical analyses may include an examination of possible wood types used to build the Market Street Chinatown buildings. Other future research would include a thorough scan of the sediment samples to identify phytoliths and give accurate phytolith counts, rather than a presence/absence measure as was done for this preliminary project. Phytolith references would need to be prepared to allow a thorough comparison with ancient samples, and minimum number of 300 phytoliths would need to be counted as is methodologically prudent (Piperno 2006:115). I might also be advisable to stain the slides for pollen analysis and identification.

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APPENDIX A
RESIDUE RESULTS

Table A-1: Correlation between ceramic vessels and associated soil samples

Soil Catalog #	Sample #	Vessel Type	Vessel Catalog #	Sample #
85-31/18-7	MS2	Stoneware Shouldered Jar	85-31/18-6	V2
85-31/18-11	MS3	Stoneware Spouted Jar	85-31/18-10	V3
85-31/18-231	MS4	British Whiteware Bowl	85-31/18-228	V4
85-31/18-396	MS9	Bamboo Bowl	85-31/18-395	V9, V13
85-31/20-9	MS5	Four Seasons Bowl	85-31/20-8	V5
85-31/20-74	MS6	Bamboo Bowl	85-31/20-62	V6
85-31/20-76	MS7	Celadon Bowl	85-31/20-75	V7
85-31/28-4	MS8	Stoneware Storage Jar	85-31/28-3	V8
85-31/2B-2	MS1	Stoneware Storage Jar	85-31/2B-1	V1
86-36/5-16	MS10	Stoneware Shouldered Jar	86-36/5-14	V10
86-36/5-20	MS11	Stoneware Shouldered Jar	86-36/5-19	V11
86-36/5-1582	MS12	Bamboo Bowl	86-36/5-1583	V12
X	X	Thin Storage Container	86-36/5-1535	V14

Soil Catalog #	Sample #	Vessel Type	Vessel Catalog #	Sample #
X	X	Storage Jar Lid	85-31/20-58	V15
X	X	Storage Jar Lid	85-31/20-162	V16

Table A-2: Vessel Sample Residue Results

Vessel Sample (Catalog Number)	Vessel Description	Residues Present	Starch	Pollen	Charcoal/ Burnt Phytolith	Bordered Pits	Fiber	Plant Hairs	Feather Barbs	Phytoliths	Comments
V1 (85-31/2B-1)	Stoneware Storage Jar	Y	ND	ND	ND	Y	Y	ND	ND	ND	Two kinds of pits
V2W (85-31/18-6)	Stoneware Shouldered Jar	ND	ND	ND	ND	ND	ND	ND	ND	ND	No diagnostic residue
V2D (85-31/18-6)	Stoneware Shouldered Jar	ND	ND	ND	ND	ND	ND	ND	ND	ND	No diagnostic residue
V3 (85-31/18-10)	Stoneware Shouldered Jar	ND	ND	ND	ND	ND	ND	ND	ND	ND	No diagnostic residue
V4A (85-31/18-228)	British Whiteware Bowl	Y	ND	ND	M	ND	Y	ND	ND	ND	
V4B (85-31/18-228)	British Whiteware Bowl	Y	Y	Y	M	ND	Y	ND	ND	ND	Compound starch consistent with rice
V5 (85-31/20-8)	Four Seasons Bowl	ND	ND	ND	ND	ND	ND	ND	ND	ND	No diagnostic residue
V6 (85-31/20-62)	Bamboo Bowl	Y	ND	ND	ND	ND	Y	ND	ND	ND	
V7 (85-31/20-75)	Celadon Bowl	ND	ND	ND	ND	ND	ND	ND	ND	ND	x
V8 (85-31/28-3)	Stoneware Storage Jar	Y	ND	ND	M	ND	Y	ND	ND	ND	
V9A (85-31/18-395)	Bamboo Bowl	Y	ND	ND	M	ND	ND	ND	ND	Y	No diagnostic residue outside the bowl
V9B (85-31/18-396)	Bamboo Bowl	Y	Y	ND	M	ND	Y	Y	ND	Y	Compound starch consistent with rice inside the bowl
V9C (85-31/18-396)	Bamboo Bowl	Y	ND	Y	M	ND	Y	ND	ND	ND	No diagnostic residue
V10W (86-36/5-14)	Stoneware Storage Jar	Y	ND	ND	M	ND	ND	ND	ND	ND	No diagnostic residue
V10D (86-36/5-14)	Stoneware Storage Jar	ND	ND	ND	ND	ND	ND	ND	ND	ND	No diagnostic residue

Vessel Sample (Catalog Number)	Vessel Description	Residues Present	Starch	Pollen	Charcoal/ Burnt Phytolith	Bordered Pits	Fiber	Plant Hairs	Feather Barbs	Phytoliths	Comments
V11W (86-36/5-19)	Stoneware Storage Jar	Y	ND	ND	M	ND	Y	ND	ND	ND	
V11D (86-36/5-19)	Stoneware Storage Jar	ND	ND	ND	ND	ND	ND	ND	ND	ND	No diagnostic residue
V12 (86-36/5-1583)	Bamboo Bowl	ND	ND	ND	ND	ND	ND	ND	ND	ND	x
V13 (85-31/18-395)	Bamboo Bowl Fragment from 85-31/18-396	Y	Y	ND	M	ND	Y	ND	ND	Y	Compound starch consistent with rice
V14 (86-36/5-1535)	Thin Storage Container	ND	ND	ND	ND	ND	ND	ND	ND	ND	No residues detected
V15 (85-31/20-58)	Storage Jar Lid	Y	ND	ND	ND	ND	Y	ND	ND	ND	No diagnostic residue
V16 (85-31/20-162)	Storage Jar Lid	Y	ND	ND	M	ND	Y	ND	ND	ND	No diagnostic residue

Key: Y=Present; ND= Not Detected; A=Abundant (≥100); M=Minimal (<100); x= an issue while processing. W=wet sample taken; D=Dry scratched residues. Vessel sample V4 and V9 had identifiable starch in the original quick scans, so multiple slides were made from the left over residue samples in the tubes. Corresponding sediment and vessel samples are associated with one another. V14, V15, and V16 are not associated with sediment samples and were very insubstantial because the vessels were so clean that it was not worthwhile to attempt residue extraction a second time.

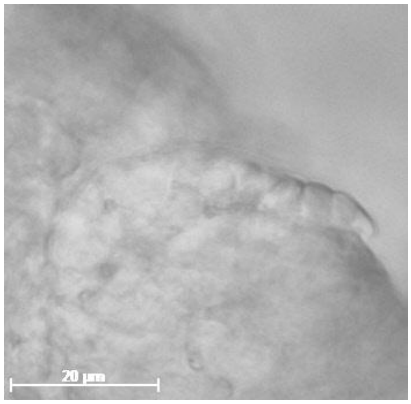
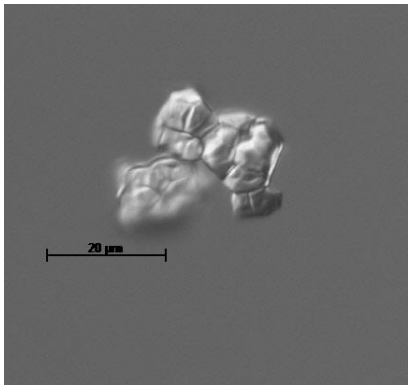
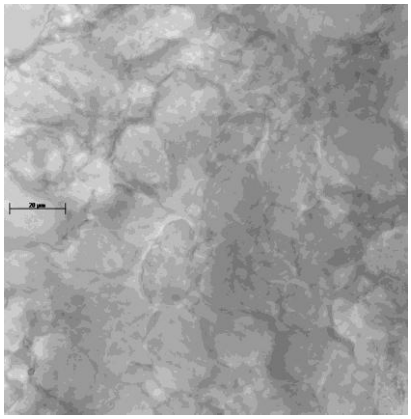
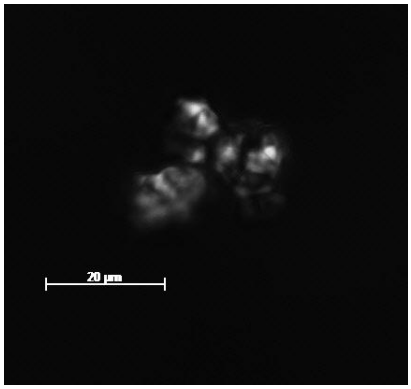
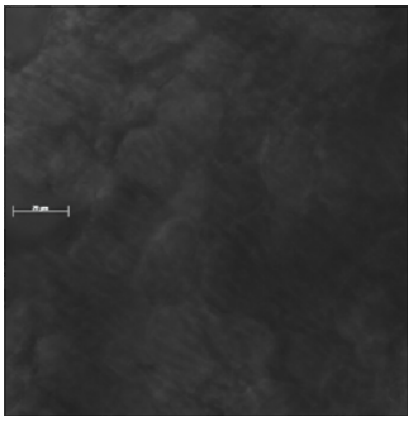
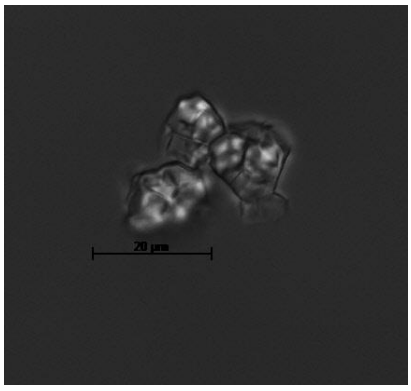
Table A-3: Sediment Sample Residue Results

Sediment Sample (Catalog Number)	Residues Present	Starch	Pollen	Charcoal/Burnt Phytoliths	Bordered Pits	Fiber	Plant Hair	Phytoliths	Feather Barbs	Comments
MS1 (85-31/2B-2)	Y	ND	Y	A	ND	Y	ND	Y	ND	
MS2 (85-31/18-7)	Y	ND	Y	A	ND	ND	ND	Y	ND	
MS3 (85-31/18-11)	Y	ND	ND	A	ND	Y	ND	Y	ND	
MS4 (85-31/18-231)	Y	ND	ND	A	ND	Y	ND	Y	ND	
MS5 (85-31/20-9)	Y	ND	ND	A	ND	Y	ND	Y	Y	
MS6 (85-31/20-74)	Y	ND	Y	A	ND	Y	Y	Y	ND	
MS7 (85-31/20-76)	Y	ND	Y	A	ND	Y	Y	Y	ND	

Sediment Sample (Catalog Number)	Residues Present	Starch	Pollen	Charcoal/Burnt Phytoliths	Bordered Pits	Fiber	Plant Hair	Phytoliths	Feather Barbs	Comments
MS8 (85-31/28-4)	Y	ND	Y	A	ND	Y	Y	Y	Y	Phytolith similar to rice husk but not diagnostic; long cell and multi-cellular grass phytolith
MS9 (85-31/18-396)	Y	ND	Y	A	ND	ND	Y	Y	ND	
MS10 (86-36/5-16)	Y	ND	Y	A	Y	Y	ND	Y	ND	
MS11 (86-36/5-20)	Y	ND	Y	A	Y	Y	ND	Y	ND	
MS12 (86-36/5-1582)	Y	ND	Y	A	ND	Y	ND	Y	ND	

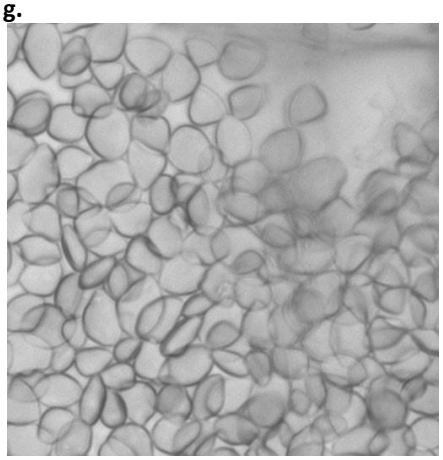
Key: Y=present; ND= not detected; A=Abundant (≥100). Grass long cells were present within most sediment samples, phytoliths were identified with lab reference collections and with guidance from Dr. Bestel. It is standard practice not to identify all microfossils to genus and species level, especially if they are not diagnostic.

APPENDIX B
REFERENCE STARCH PHOTOGRAPHS

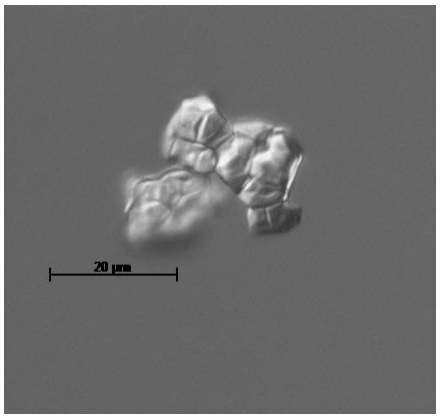
a-c: Modern reference sample of gelatinized rice starch under a bright filter (a), bright filter (b) and polarizing filter (c).	d-f: Modern reference sample of cooked rice under a bright filter (d), polarizing filter (e) and DIC filter (f).
a. 	**d.** 
b. 	**e.** 
c. 	**f.** 

g-i: Modern reference sample of pickled ginger starch under a bright filter (g), polarizing filter (h) and DIC filter (i).

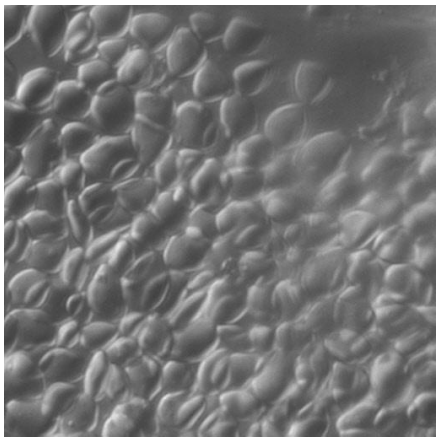
g.



h.



i.



APPENDIX C
JOINT STARCH AND PHYTOLITH EXTRACTION FROM SEDIMENT AND RESIDUES

The purpose of this process is to clean sediments samples, and to extract and condense starch and phytoliths from residues using heavy liquid separation.

Procedure:

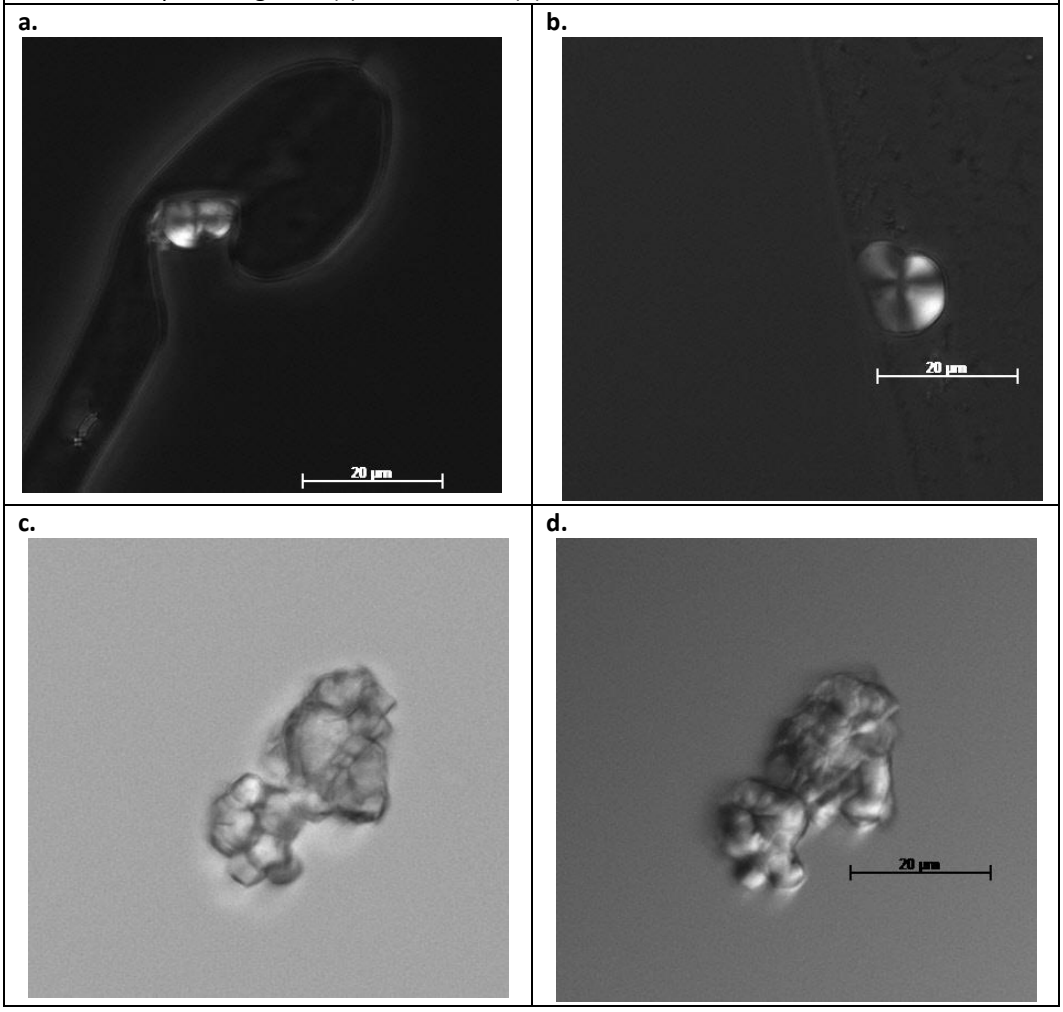
1. Weigh out 800 mg of sediment (.800g).
2. Place sediment into 1000ml beaker and add cold water to 800ml line. Stir well and let sit for 1 hour and 10 minutes.
3. Discard supernatant by tipping into disposable beaker.
4. Repeat steps 2 and 3 one to eight times. Two to three sedimentation processes are likely necessary.
5. Dry sediment overnight, and in an incubator at 40 degrees Celsius.
6. Weigh leftover sample in tube.
7. To clean out sediment samples (with gloves and pipette) add 2-4 ml HCL to dried sample and shake, or vortex well (3 min+) to let the chemical and sediments react. This step is to occur within biosafety cabinet, or fume hood. **If this step is not necessary skip to heavy liquid separation starting at step 12.**
8. Fill 15 ml tube the rest of the way with distilled water and centrifuge with at 1500 rpm for 5 minutes. Discard acidic contents in a hazardous material container and repeat at least once more.
9. Let the newly cleaned sample dry overnight in incubator at 40 degrees Celsius and weigh remaining sample in tube.
10. Note that for wet samples the sedimentation process (steps 2-5) may not be necessary, and heavy liquid separation may commence from step 6.Fill 15ml tubes with distilled water and centrifuge at 1500rpm for five minutes.
11. Using gloves and pipette at 3-4ml of non-toxic sodium polytungstate (SPT) mixed to 2.4 specific gravity to each tube containing sediment; vortex and then centrifuge at 1000 rpm for 15 minutes.
12. Using pipette, transfer upper 1-2mm of liquid at the top of centrifuge tube into new labeled clean tube—this is where the starch and phytoliths sample will be located. SPT from old tube may be recycled in the container.

13. Tube may be discarded or washed thoroughly, autoclaved and reused.
14. Fill new 15 ml centrifuge tube with distilled water and centrifuge at 1500 rpm for 5 minutes to rinse out STP. Repeat 2-3 times and discard water from tube into beaker.
15. Sample may be partially stored in glass vial or plastic tube until needed.
16. Mount samples on glass slide using non-toxic glycerol and seal with nail polish.

APPENDIX D
RESIDUES

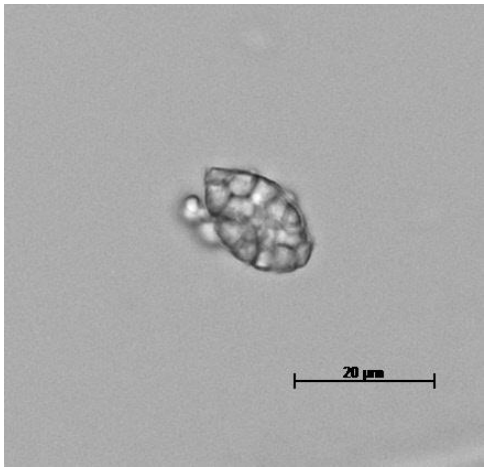
Figure D1: Images of starch samples residues from vessels

85-31/18-288 (V4) Larder round starch grains in polarizing light (a-b); gelatinized composite starch under polarizing filter (c) and DIC filter (d).

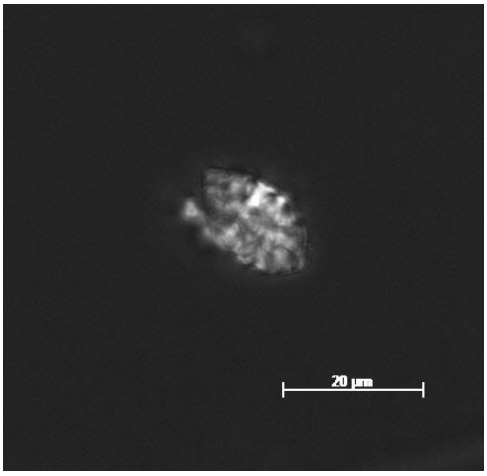


85-31/18-288 (V4) composite starch under bright filter (e) and polarizing filter (f).

e.

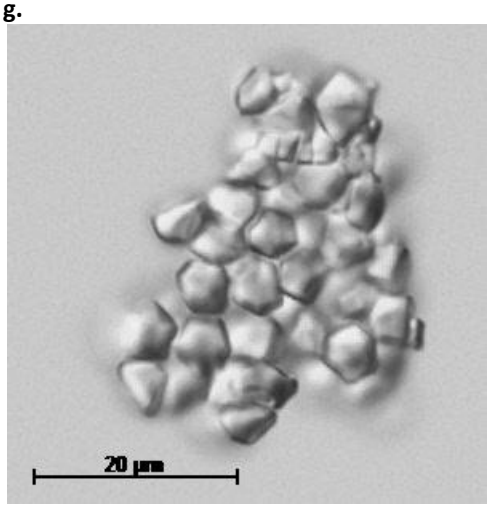


f.

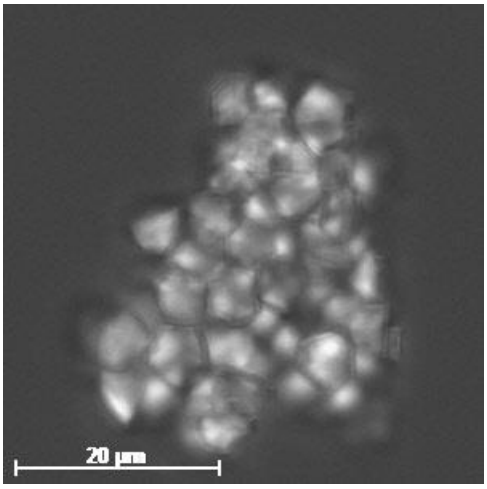


85-31/18-396 (V9) composite starch under bright filter (g), polarizing filter (h) and DIC filter (i).

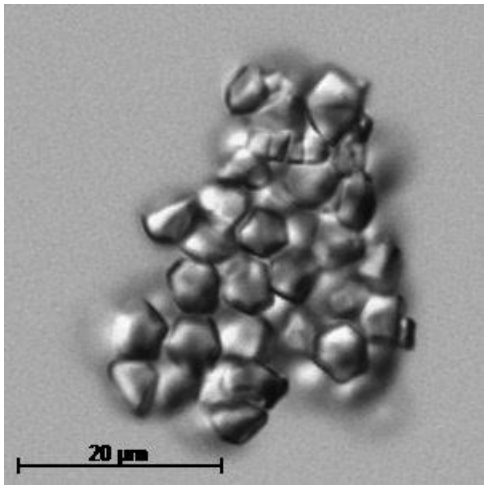
g.



h.

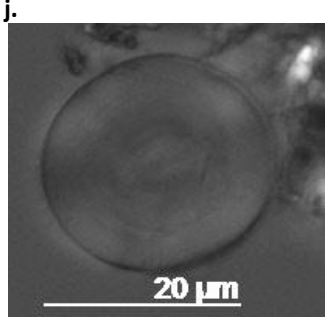


i.

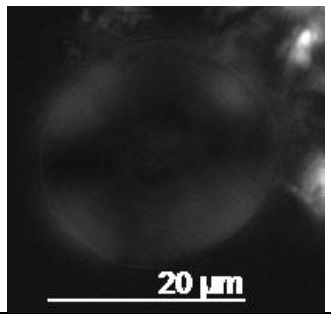


85-31/18-395 (V13) rounded starch under a bright filter (j); polarizing filter (k) and DIC filter (l).
85-31/18-395 (V13) damaged rounded starch in bright filter (m), polarizing filter (n) and DIC (o).

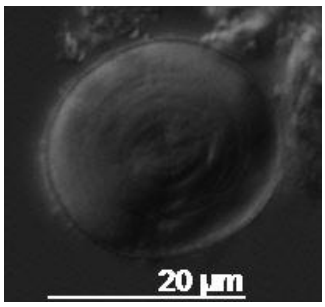
j.



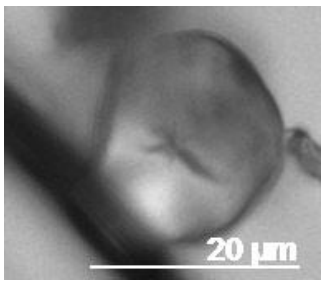
k.



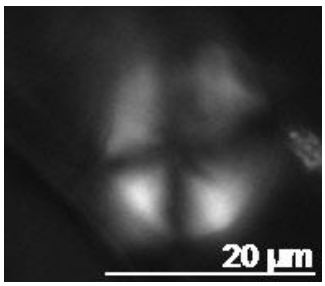
l.



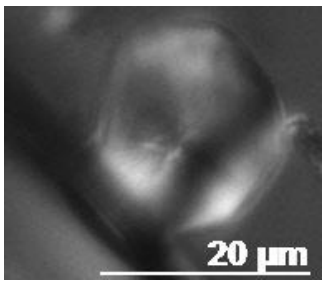
m.



n.

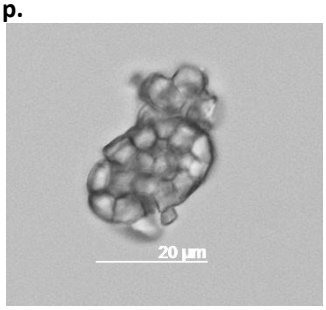


o.

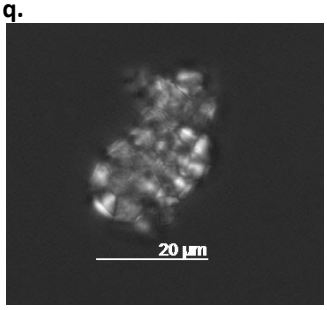


85-31/18-395 (V13) composite starch under a bright filter (p), polarizing filter (q) and DIC filter (r).

p.



q.



r.

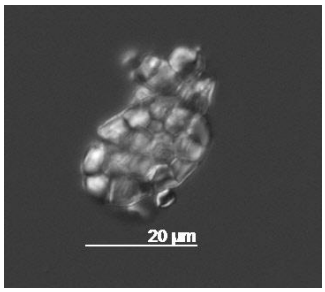
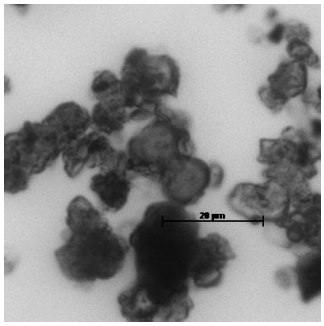


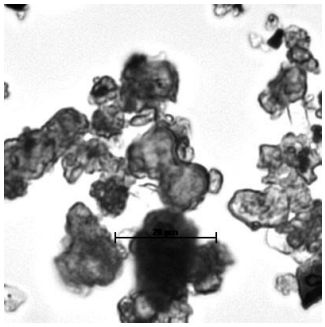
Figure D-2: Other Residue Examples

a-b: 85-31/18-7 (MS2) bilobe phytolith under a bright filter and (c) 85-31/20-9 (MS5) possible feather barb spines.

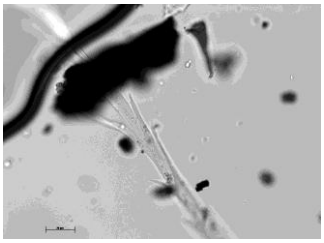
a.



b.

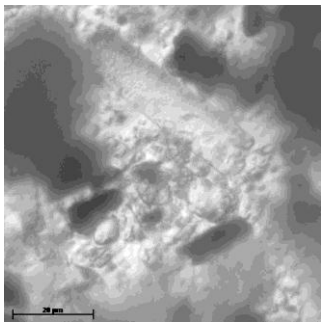


c.

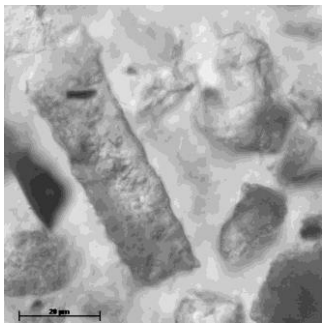


85-31/20-74 (MS6) long cell phytoliths (d-e); 85-31/28-4 (MS7) multi-cell phytolith (f).

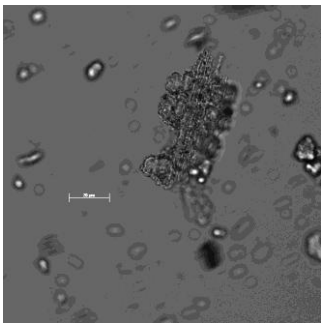
d.



e.

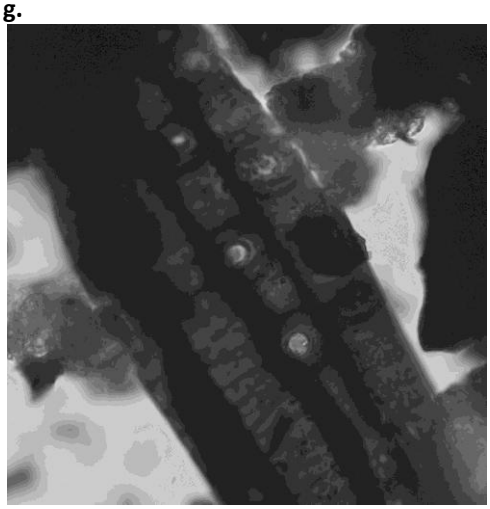


f.

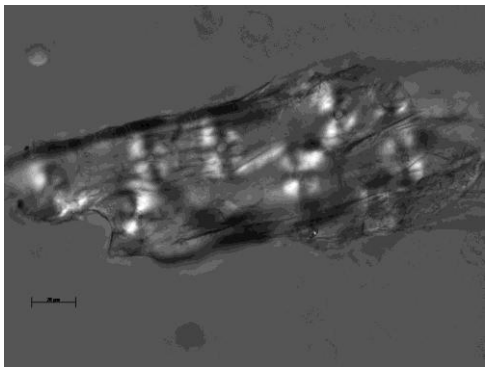


Bordered pits from 86-36/5-20 (MS10) under a bright filter (g), and vessel 1 bordered pit under a polarizing filter (h).

g.

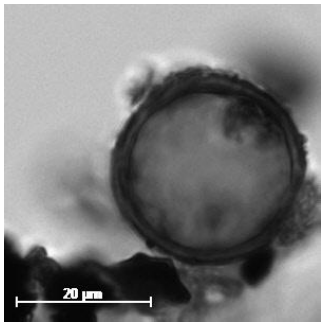


h.

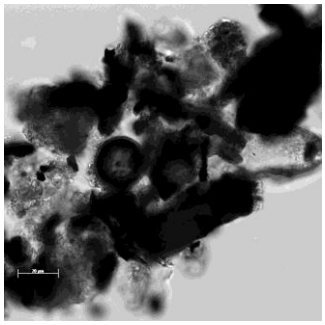


85-31/28-4 (MS8) pollen under a bright filter (m) and DIC filter (n) and 86-36/5-1582 (MS12) pollen under a polarizing filter (o).

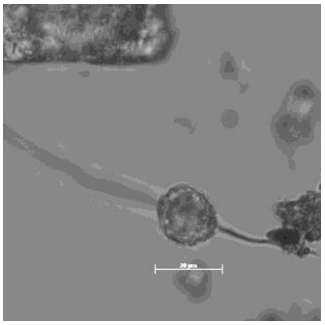
m.



n.

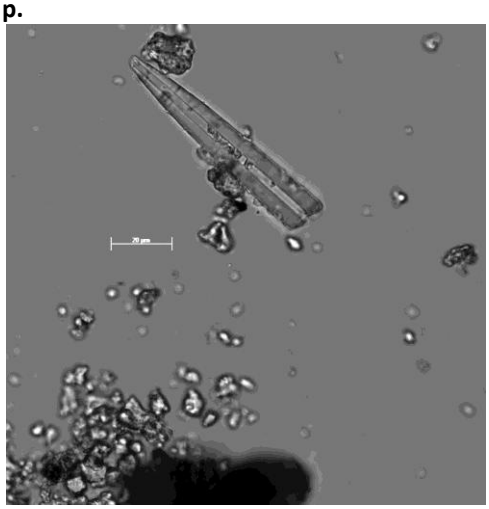


o.



85-31/20-76 (MS7) broken fragment of hair (p) and85-31/2B-1 (V1) fiber (q).

p.



q.