UC BERKELEY MCCOWN ARCHAEOBOTANY LABORATORY REPORT #77
***CAPSICUM* SPP. PROJECT PROCEDURE FOR SEED PHOTOGRAPHY**
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This document serves as a brief summary of the procedure utilized by K. Chiou, C.A. Hastorf, and members of the University of California, Berkley Undergraduate Research Apprentice Program (URAP) to photograph and measure *Capsicum* spp. seeds, both archaeological and modern, using the Olympus SZ-61 microscope, the Olympus DP-72 camera, the Olympus MicroSuite program, and the CombineZP program located at the microscope photography computer station located within the McCown Archaeobotany Laboratory (65 Kroeber), University of California, Berkeley. The instructions below detail the 3 photographs that will be generated and the measurements/qualitative assessments that are associated with them. Figures that illustrate attributes are located within Lab Report #74 accessible at the website archaeobotany.berkeley.edu (Chiou and Hastorf 2012). For a general explanation of SZ-61 procedures with screenshots and images, please refer to Lab Report #68 (Farahani 2011).

(1) **Station Set-Up**: Remove the cover on the SZ-61 microscope. Before use, confirm that the DP-72 camera is located atop the SZ-61 stereomicroscope and not the BX-51 slide microscope. If it's not, please ask K. Chiou or one of the other graduate students in the lab to move the camera for you. This one camera is shared between the two microscopes, so may or may not be on the appropriate one when you begin.

(2) **Program Set-Up**: Open up MicroSuite located on the computer desktop. Make sure all the measurements are taken in mm (at times, those using the BX-51 slide microscope will measure in microns so the settings need to be checked and altered, if necessary) by opening up the calibration window (Image→Calibrate) and selecting "Unit." The basic unit should be "m" and the scale should also be "m." Then click on the measurement tab (the one with the calipers) and click on "Select Measurements." Then click on "File" and load "Katie Caps Measurements." Click "OK." Click on the rightmost button in the Measurement tab "Measurement Properties" and select the "Measure" tab. Make sure the scale dropdown menu has "m" selected. Click "OK." Open the scale bar menu (Image→ Scale Bar→ Properties) and click on the "Format" tab. Make sure the Unit is "mm." Click "OK."

(3) **Opening Video Camera**: Make sure the pin (located next to the eye and camera icon) on the microscope is pulled out—this allows for the video feed. Click on the video camera button to begin getting a video feed. Adjustments to the video feed can be made by clicking on the button located to the right of the camera button called "Camera Control" (e.g., exposure, contrast, white balance, etc.).

(4) **Photo 1: Flat Lying Seed:** Place the seed, lying flat, above a scale with the beak facing the right on the screen on a petri dish (place a white piece of paper beneath for the background). Use the

zoom adjustment knob to frame the seed. Then use the focus knob to take several photos of the seed with different areas in focus. Typically, this is done by twisting the knob until everything is out of focus and slowing turning it back until the top of the seed comes in focus. Click on the camera button to take a photo. Click on the video camera button to return to the video feed. Slowly continue turning the knob until another part comes in focus. Take a photo. Repeat. Normally, photos of the seed lying flat require 3-5 photos, depending on topography (the less flat the seed is, the more photos it will require). Select all the photos on the left sidebar and save using the dropdown menu or CTRL + S. Save the images in the folder of your choice.

a. *Creating a Z-stack in CombineZP*: Open up CombineZP located on the computer desktop. "NEW" and an "Open" window will appear. Select all the images you just saved. Click "Open." Select "Align and Balance Used Frames" (first option) from the drop down menu. Click "GO." Once this process has finished, select "Do Stack" from the dropdown menu. This will generate the Z-stack (stitched together) image. Click on the dotted box with a double-headed arrow button to select the area. Then click "Save" and name the image, making sure to include the seed ID number. Close the window after saving. If the image looks sufficiently clear, delete the unstitched photos (e.g., TV1, TV2) in your folder.
b. *Measurements in MicroSuite*:
    i. Calibration: Open up the image in MicroSuite. Select Image→ Calibrate. Then click on the "Calibrate" button and select 3 mm along the scale bar. Click "OK" to set the calibration.
    ii. Scale Bar: Select Image→Scale Bar→ Draw Into Overlay. The scale bar should say 2 mm. If the scale bar looks okay, use the cursor tool to drag the scale bar underneath the image. Click the "Burn Overlay" button to permanently burn the overlay into the image. Then save the image (keep the same name and overwrite the old file).
    iii. Binarizing the Image (from Color to Black and White—): Select Process→ Set Color Thresholds. Adjust the settings so that the seed is one color or the background outlines the seed. Click "OK". Then select Process→ Binarize Color Image. This will generate a black and white image. Should this procedure fail, use the closed polygon tool to draw around the seed (right click to close the polygon).
    iv. Measurements: Click on the measurements tab. Select the magic wand tool from the toolbar and hover within the seed boundaries. Right click and several measurements should instantly appear. Then click on the vertical length measurement tool and measure the seed along the midpoint of the body from top to bottom. Then click on the horizontal length measurement tool and do the same from left to right. These are your relational length and relational width measurements. Click on the arbitrary distance measurement tool and select the midpoint of the beak (center of the beak, usually at the tip_, measuring to the other side. Then measure the width perpendicular to that length measurement, nearly intersecting the relational length and width measurements. These are

your maximum length and perpendicular length measurements. Record all the relevant measurements in the appropriate spreadsheet for that seed.

v. Other Observations: Record your observations for seed shape, testa texture, and beak prominence. Then, using a protractor (the actual tool not found in the program) and measure the beak angle. Record these in the spreadsheet.

vi. End: Save the black and white image (the default name is fine) and close out of all the files in the sidebar by selecting them and clicking the delete key on your keyboard. Click on the video camera button to begin Photo 2.

(5) **Photo 2 Attachment Scar**: The photo of the attachment scar is taken by placing the seed in putty so that the attachment scar is facing upright. In the photo, the attachment scar should be oriented so that it will be in the left of the photo with the scale bar underneath. Similar to the procedure above, frame the picture using the zoom adjustment knob and then use the focus knob to slowly zoom in, taking several photos as you go. Make sure to get a clear picture of the attachment scar—there is no need to see the bottom of the seed, as that would result in a large number of photos which would cause the CombineZP program to have difficulty stitching the images together. After the set of photos is completed, save them and open them up in the CombineZP program. Align the photos, and then do the stack. Select the area then save and name the image. After you have an acceptable clear image, close out of the program.

a. *Calibration*: Calibrate the image (3 mm) in the same manner as above. Do not bother with a scale bar.

b. *Measurements:* Pull up the measurements tab. Using the closed polygon tool, draw around the attachment scar (right click to finish). Measurements will immediately appear. Then use the arbitrary distance measurement tool to measure length and width. Record the area of the attachment scar, the length, the width, and the sphericity in the spreadsheet.

c. *End:* Save the photo, making sure to label it with its ID number and click on the video camera tool to resume video feed.

(6) Photo 3 Transverse Cross-Section: Take a razor blade and lay the seed flat on a hard surface like a glass petri dish. Cut the seed along the medial line (cut exactly where you took the relational length measurement when seed was oriented flat). Take one half of the seed and place it in the putty so that the outline of the testa (seed coat) is visible to the camera. Following the procedures above, take several photographs (again, like with the attachment scar photo, only the top of the seed is important). Save the stacked, crisp photographs and open them up in the CombineZP program. Align, do stack, select the area, then save and name the file. Close out of the program.

a. *Calibration*: Calibrate the image (3 mm) in the same manner as above. Do not bother with a scale bar.

b. *Measurements:* Pull up the measurements tab. Using the arbitrary distance measurement tool, measure three well-spaced measurements along the curved part of

the seed margin and 3 measurements along the long portion of the testa where the seed lies flat. Enter these into the spreadsheet. Save the image.

(7) Finish and Store: You are now finished with the 3 images associated with each seed. You have also gathered measurements that we can use to compare to other seeds. Place the seed back into its capsule and begin with the next seed, repeating the steps explained above, remembering to carefully document the seed ID number on all images.