UC Berkeley
McCown Archaeobotany Laboratory Reports

Title
Fall 2011 Microscopy and Measurement Protocol for the SZ61 Microscope

Permalink
https://escholarship.org/uc/item/8bt411mt

Journal
UC Berkeley McCown Archaeobotany Laboratory Reports, 68

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Publication Date
2011-09-01

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Fall 2011 Microscopy and Measurement Protocol for the SZ61 microscope
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McCown Archaeobotany Laboratory / Laboratory Report #68
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Before you begin, read the microscope and camera manuals to learn how to operate the equipment properly. These are in the drawer to the right of the station (i.e. directly beneath the computer). Additionally you should read a digital photography manual to gain knowledge on how to interpret and utilize different camera options (e.g. aperture, ISO, etc.).

CAMERA PREPARATION

1. Remove the plastic cover from the Olympus SZ61 Microscope, and check if the camera is attached on top (Olympus DP72 -- a round black object). If the camera is not attached, or attached to the Olympus BX51:
    a. Remove the cable protruding from the back of the camera by squeezing the two buttons on the side and then pulling rearward.
    b. Unscrew the camera by turning it to the left.
    c. Place the camera on top of the SZ61 (there should be a black cap that should be removed).
    d. Screw the camera on by turning it to the right. **Be gentle!**

SAMPLE PREPARATION

1. Take a piece of either white or colored paper, and place it beneath the microscope view shed.
2. Place a petri dish *upside-down* on top of this paper (this reduces glare from the overhead fluorescent lighting), and add a scale as well. BONUS: Shut off the middle light of the lab for extra glare-reduction.

OPTIONAL 1: At this point, you do not know how much light is necessary in order to procure a light-balanced image. If it is determined that more light is necessary, position the arms of a fiberoptic lightbox as needed in relation to the petri-dish, and adjust light to desired intensity by turning the black knob on the lightbox SLOWLY clockwise. **NB:** If the light is too bright, it will wash out the image you are trying to take.

OPTIONAL 2: To stabilize the samples, use mounting putty (pliable, clay-looking substance), and smooth out a thin piece between your fingers. Place this on the petri-dish. Another option is to place mesh-sorted salt crystals (found in the wet lab) in a petri dish and place samples on top of the salt crystal bed.

3. Place samples on the petri dish (or mounting putty).
4. Look through microscope binoculars to set up the sample(s).
    a. The pin behind the zoom knob (the zoom knob has white numbers printed on it, and the pin has a graphic in front of it of an eye and a camera) shifts the light from the microscope to the camera.

   b.   Push the pin in to use the microscope, pull it out to use the camera.
5.   To set up the image, make sure that the pin directly behind the zoom knob is pushed in all of the way, and adjust them zoom / coarse adjustment knob (large black knobs in the back) until the samples are in view. Once in view, pull the pin back out.

TAKING PICTURES

1.   Double click on Microsuite on the desktop (if the computer asks you if you want to run this program, click yes)

2.   On the top of the screen, under the word "Special" click on the button that looks like a camcorder.
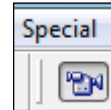
3.   You should now see an image (if you do not see an image do not panic, read inset below). Adjust the intensity of the light, the zoom, and course adjustment knob until image looks satisfactory.

   I SEE NO IMAGE! The exposure time is probably too high. Click on the "Camera Control" button directly under the "Window" option at the top of the screen.
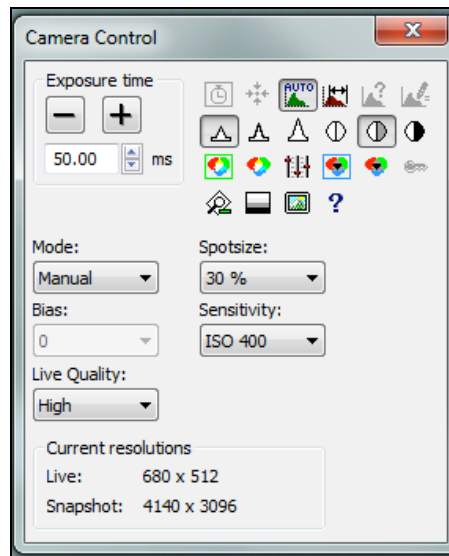
   This panel adjusts exposure time, sharpness, contrast, and white / black balance.
   The Mode should usually be "Manual"
   To modify the brightness of the image, do not adjust lighting or white balance, increase the exposure to make the image brighter (the big plus button), and decrease it to make it darker (the big minus button).

4.   Now click on the button that looks like a regular camera to the right of the image that looks like a camcorder -- this takes a picture.
5.   The picture will now be saved in the panel to the left, as "TV1".
6.   Repeat steps 2-5 as necessary.

BUT WAIT! MY SAMPLE HAS DEPTH AND I WANT TO REMOVE THE DEPTH OF FIELD EFFECT! If the edges of your image are blurry, then these images need to be "stacked" in order to remove the depth of field effect. In order to do this, multiple images of the sample(s) must be taken with different areas of the sample(s) in focus. The best strategy is to use the course adjustment knob while the image is visible in microsuite, start with one "edge" in focus and the rest blurry, take a picture, turn the course adjustment knob slightly in the direction required, take another picture, and repeat as many times as desired.

7. Once done taking all necessary images, click on the first image in the image bar to the left of the screen, and then hold SHIFT and click on the last image.
8. Go to File > Save As > Users > YOUR FOLDER HERE

If further image processing (e.g. "stacking") is necessary, see **Processing Images** below. If not, see **SETTING UP A SCALE**.
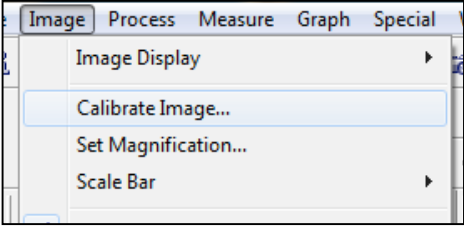
PROCESSING IMAGES (Using CombineZP)

Use this procedure if you have more than one image of the same sample(s) in which you'd like to remove the depth of field effect.

1. Double click on CombineZP.
2. The pulldown bar should read "Align and Balance USED Frames (Quick Method).
3. Click New.
4. Navigate to your folder and select all of the images by Shift or CTRL clicking them.
5. Click OPEN.
6. Click GO.
7. Wait.
8. Once complete, click on the pulldown bar and choose DO STACK.
9. Click GO.
10. Wait.
11. Click on the "Shrink / Expand Active Rectangle Button" immediately to the right of GO. If unsure about the correct selection, hover over the box and it will supply its name.
12. Click SAVE (immediately to the right of the above)
13. Rename the file to required specification
14. Exit
    a. If prompted "do you want to save anything?" do not despair, your image is actually saved, so press "NO".
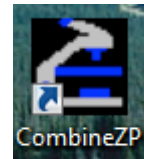
SETTING UP A SCALE

This section assumes that you are already in the Microsuite program. Not sure where or what that is? See **TAKING PICTURES** above.

1. Click on an empty image slot in the image bar to the left. If the image is already open, click on that image, and skip to step 3.
2. Click open, navigate to the proper folder, select the image, and click open.
3. Click Image > Calibrate Image (at the top of the screen).
4. Click Unit and change Basic Unit to 'm', Scale to 'm'.
5. Change calibration length to the maximum distance displayed. This means that if the scale has a maximum of 10mm in view, set the calibration length to 10mm.
6. Click calibrate, and now left click on the scale to set the first calibration point (visualized as a straight green line) and then click on the scale again to set the second calibration point that equals the distance desired. This usually means, if using a ruler, clicking on the inside of a band representing 1mm to the outside of a band in the distance desired.
7. Press ok. The image is now calibrated.
9. To check if the image is calibrated correctly, see **GATHERING MEASUREMENTS.**

MANIPULATION OF THE SCALE BAR

First ensure that a scale bar is present. Often after **SETTING UP A SCALE**, it is not. To do so, click Image > Scale Bar > Show in Viewport. Also ensure that Overlay Bar is selected as well.
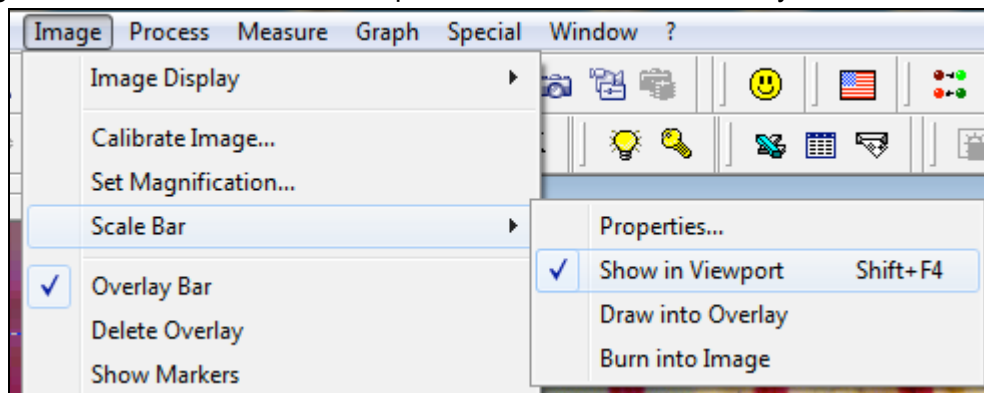
1. Click Image > Scale Bar > Properties. Select the attributes desired for Display, Format, and Size. The font size (under the "Size" Tab) recommendation is 48 as Microsuite likes multiples of 12 (Note: you will not be able to "see" your changes in the view screen, but

the attributes will show up once the image is burned). Adjust both the "Clipboard / Burn
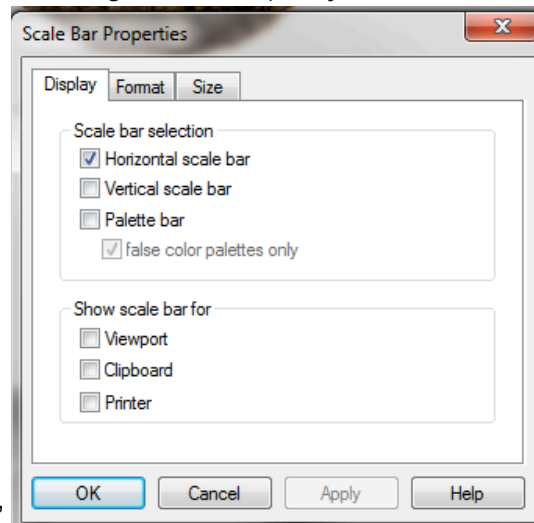
image" and the "Overlay / Print".

2. In order to see this newly adjusted scale, click Image > Scale Bar > Draw into Overlay. If it appears that there are too many scales, click Image > Scale Bar > Show in Viewport. The latter step removes the scale that cannot be adjusted / moved.
3. Click on the Arrow Icon on the overlay bar on the bottom of the screen.

4. Click on the Scale Bar, move to the desired location, and right click to finalize.
5. To burn this new scale bar into the image so that it remains with the image, in the Overlay Bar click on the picture of the Sun with a down-arrow next to it. When prompted, click "OK". Or *** To add the scale bar: Go to image scale bar and then burn into image
6.
7. The scale bar is now burned in the image. Save the image as you would regularly.

GATHERING MEASUREMENTS
1. Click on the Tab on the bottom left of the screen that looks like a pair of calipers.

2. Click any of the measurement options on the vertical bar of icons.
3. To take a measurement, click once for the first measurement point, again for the second. If the software does not seem to take the measurement right away, right click to process the image.
4. The measurements and accompanying vector-types will be displayed in the measurement screen to the left.
5. This is a useful way to **assess how well an image is calibrated**. After calibration, use the measurement tool to assess how closely the measurement as displayed matches the actual length of a physical scale.
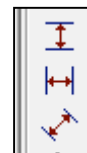
Repeat Steps 1-3 as many times as needed.