

Microscope Photography, Measurement and Image Stacking

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Laboratory Report #91

10/27/23

Olympus DP74 Camera Photography Instructions Using cellSens

Instructions for Users Opening CellScens for the First Time

- 1) Check the box to **accept the license agreement**, then press **Continue**.
- 2) Assign yourself **Power User** status when prompted.
- 3) Select desired toolbar layout, recommended Acquisition and Measure (**NOT simple**). Layout can be changed by selecting **Layout** in the top right corner of the screen, then **Manage Layouts**.
- 4) Verify that camera is connected by selecting **Acquire** from the top toolbar, then **Devices**, then **Device List**. Verify that settings match the example below, press **OK**, then verify that the settings match the next example and press **OK**.

Camera Preparation (Adapted from Lab Report #68)

- 1) Remove the plastic cover from the Olympus SZ61 Microscope, and check if the camera is attached on top (Olympus DP74, a round black object). If the camera is not attached, or attached to the Olympus BX51:
- 2) Remove the cable protruding from the back of the camera by squeezing the two buttons on the side and then pulling rearward.
- 3) Unscrew the camera by turning it to the left.
- 4) Remove black cap from SZ61 and place on top.
- 5) **Gently** screw the camera on by turning it to the right.
- 6) Verify that the microscope is in camera mode. **The black pin on the right side of the microscope should be pulled all the way to the right.**

Sample Preparation (Adapted from Lab Report #87)

- 1) Place white paper underneath petrie dish in the microscope viewshed.
- 2) Make sure to retain the label near sample, outside of viewshed, but within association.
- 3) Place sample and physical scale in petrie dish. Adjust so that the scale is within viewshed but can be cropped out of the image later.
- 4) Turn on attached overhead light using dial.

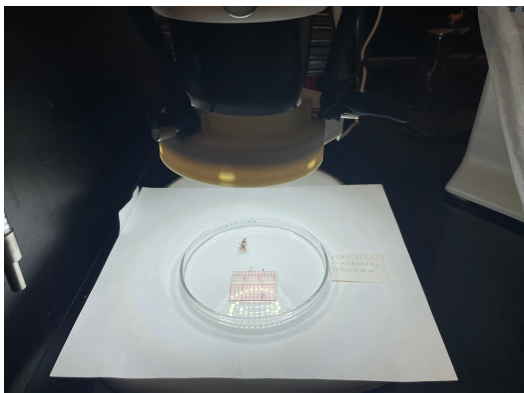
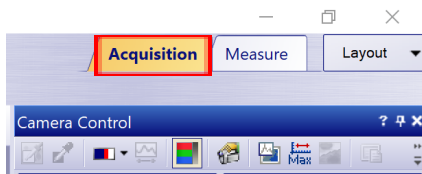
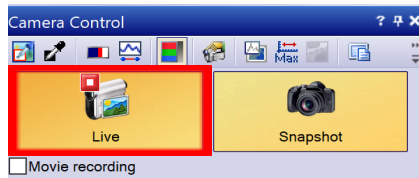


Image Preparation (Adapted from Lab Report #87)

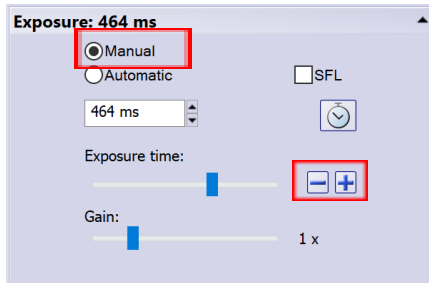
- 1) In your account, open the program cellSens from the desktop
- 2) Select the **Acquisition** tab, at the top right of the program screen



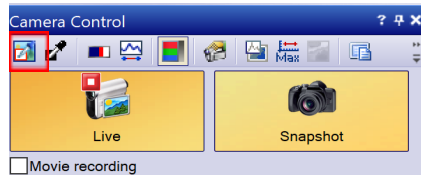
- 3) Press the **Live** button at the top of the **Camera Control** window.



- 4) Adjust **Exposure** within **Camera Control** by selecting **Manual**, then adjusting using the + and - until a clear image appears. **Automatic**, if selected may select correct exposure, but verify and adjust as necessary. If taking multiple images for CombineZP, set the exposure to **Automatic** to adjust and then switch to manual to lock in the same consistent exposure for each of the images you will take.

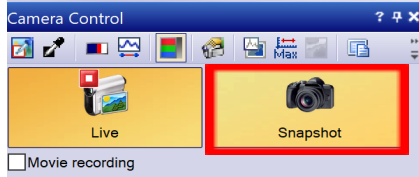


- 5) Arrange the physical lighting for your sample using the exposure tool and the physical overhead light, watching your image on the screen. High quality photos will have few to no shadows.
- 6) Select the **White Balance** box in the right side toolbar. Next, left click and drag a box on the live image screen that contains mostly white (background) with a small section of the sample. Click again to complete your box. This allows the program to automatically correct the white balance in your image. OR, In the 'Simple' tab, navigate to the white balance button and perform the same steps.

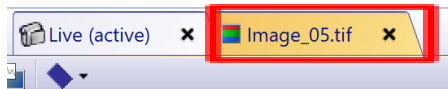


Imaging (Adapted from Lab Report #87)

- 1) Focus first on the uppermost point of your sample. The manual focus is the largest black knob on the back side of the microscope.
- 2) To image, click **Snapshot** in **Camera Acquisition**, which will create a new tab with the image in the upper left. Alternatively, you can use the **F8** command to take snapshots.



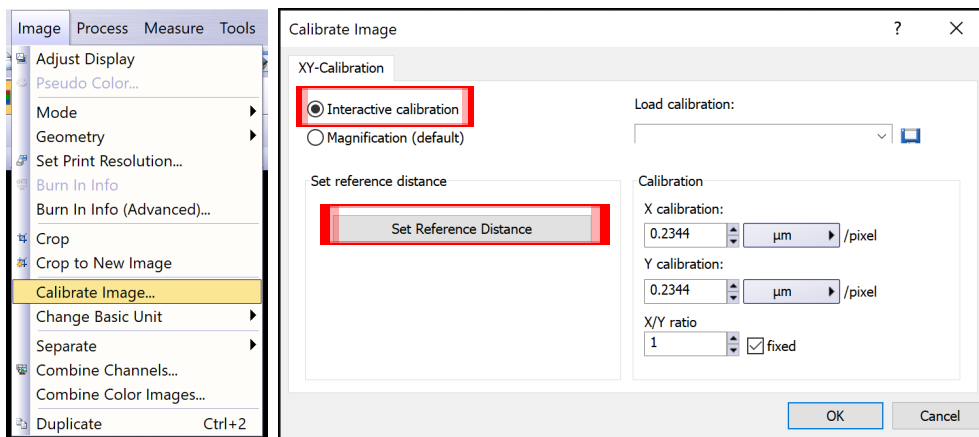
- 3) If taking multiple photos for CombineZP, take multiple photos by focusing on progressively lower areas of the sample. Adjust the knob a couple of degrees each time. Make sure there's some overlap between each refocused image so you can combine photos later in CombineZP. If the object has irregular topography (like a maize cupule) you will have to take more photos to get all of the uneven surfaces into focus.
- 4) Click on the image tab in the upper left (which will highlight it in yellow)



- 5) In that image, in the top toolbar, select **File**, **Save as**, and save your image as a .tif file. You should save your images to your own desktop or flash drive, with unique file names that indicate your samples' identifying information. (or use **Control + Shift + S** to complete the same operation)

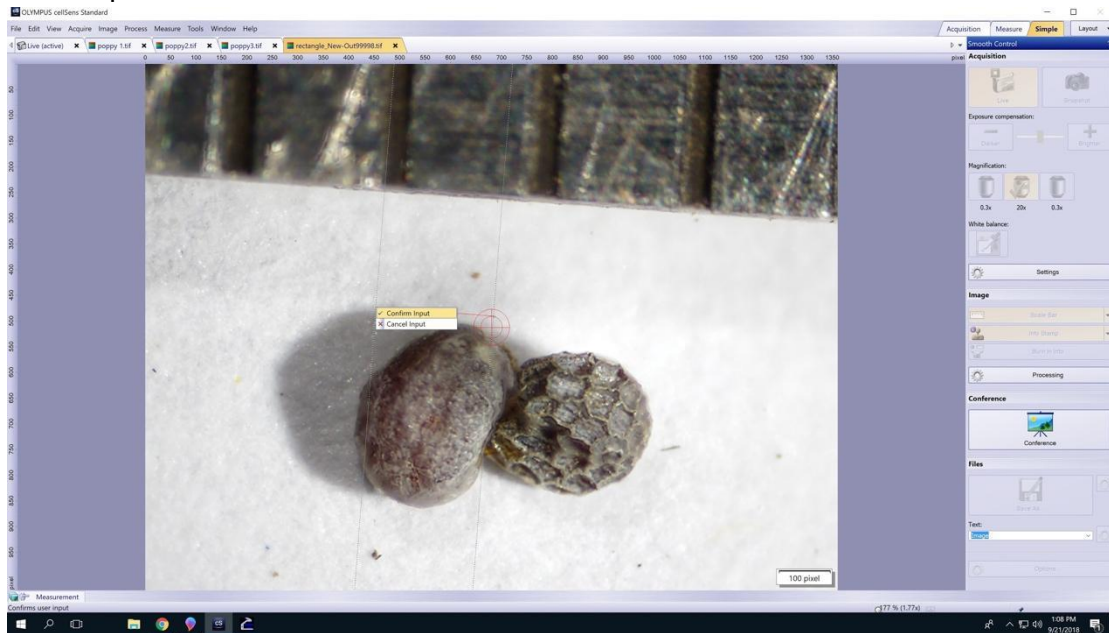
Add a Permanent Scale Bar (Adapted from Lab Report #87)

- 1) In CellSens, open your saved image.
- 2) Select **Image** in the toolbar, then **Calibrate Image**. Select **Interactive Calibration** then select **Set Reference Distance**. This creates a malleable "ticked" rectangle which will be aligned with the physical scale bar in your image.

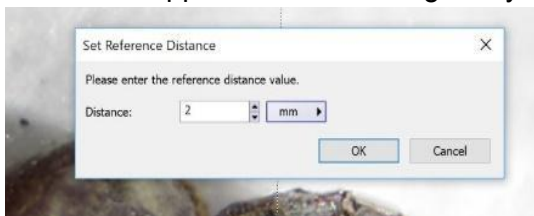


Setting Reference Distance (Adapted from Lab Report #87)

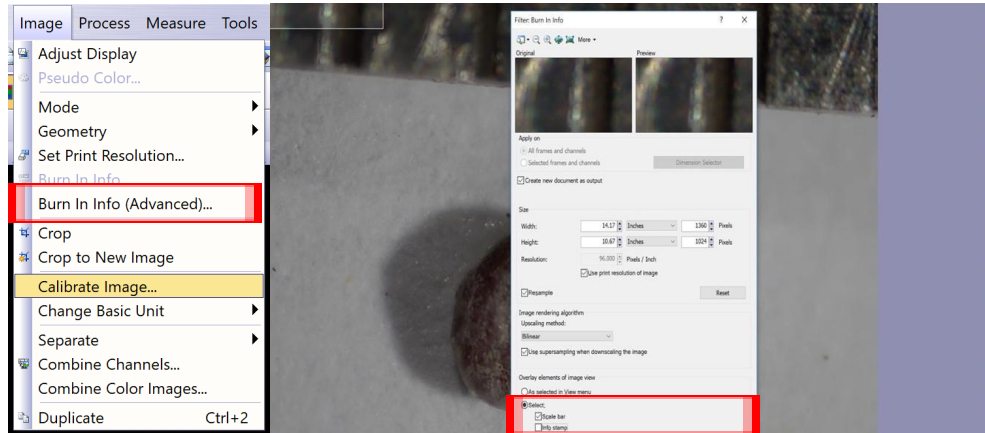
- 1) To select the length of your scale bar, first left click over the physical scale bar at the left side of the distance you want to create for your scale bar, then drag over to the other end of your distance and left click again. Always try to create an equal mm length by clicking on the same point on your physical scale for the first and second point, i.e. if you begin with the left side of the black line then use the left side of the black line for the second point.



- 2) **Right click** and select **Confirm Input**
- 3) A dialogue box will open and ask you what distance reference you just calibrated (i.e. 2mm, 3mm). Write what you chose in the box and press **OK**. Press **OK** again. Your scale bar should appear in the lower right of your screen.



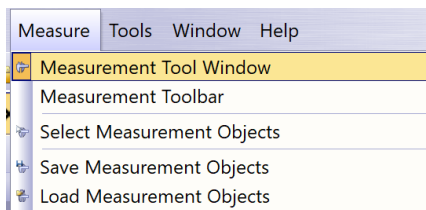
- 4) Select **Image** in the toolbar, then **Burn In Info**. At the bottom of the dialogue box that will appear, select **Select** and make sure the **scale bar box is checked** to burn the scale bar into the image. Press **OK**.



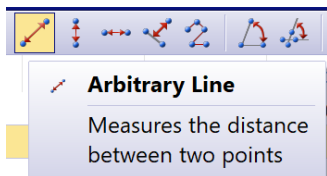
- 5) **File**, then **Save** as your image as a .tif file. You should open it back up to make sure you have burned in the scale bar.
- 6) Finally, open photoshop and crop out physical scale bar, any unwanted dust, etc. If you only need to perform very simple cropping, you can alternately open up Paint on the computer, use the select rectangle to select the portion you want, and select crop.

Measuring Tools

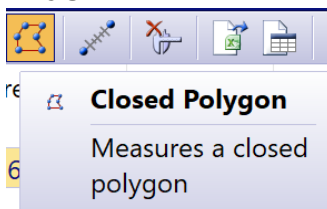
- 1) After calibrating image, select **Measure** window, then **Measurement Tool Window**.



- 2) A toolbar will appear that allows you to select various measurement devices. Measurement is interactive, and measurements are displayed below on toolbar.
- 3) **Arbitrary Line** measures the distance between two points.



- 4) **Polygon** provides area and perimeter measurements. Right click to close polygon.



Optional “Quality of Life” Features

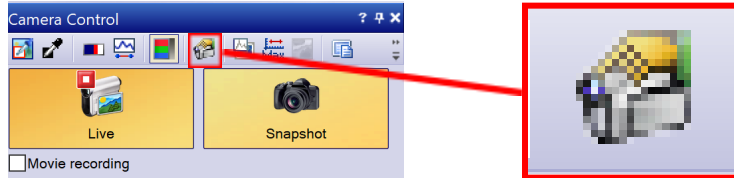
Automatic Image Saving

Feature Description

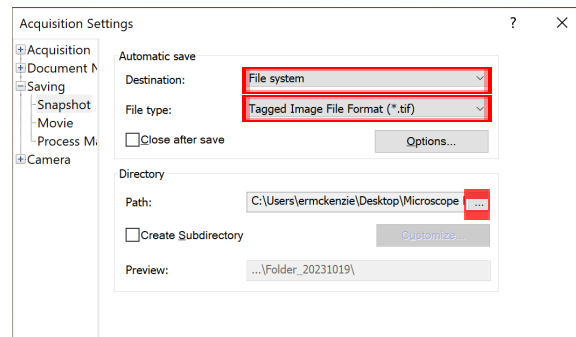
Rather than saving each capture individually, a user can establish a process for automatically saving an image in a designated file location upon capture.

Process

- 1) Select the **Acquisition Settings** button, located at the top of the **Camera Control** window



- 2) In the **Acquisition Settings** window, select **Saving**, then **Snapshot**
- 3) Under the **Automatic save** heading, select **File system** from the drop-down menu



- 4) Change **File type** under the next drop-down menu, if desired
- 5) Under the **Directory** heading, select the ... button, then choose or create a folder
- 6) Select **Apply to All**, then **OK**

Notes

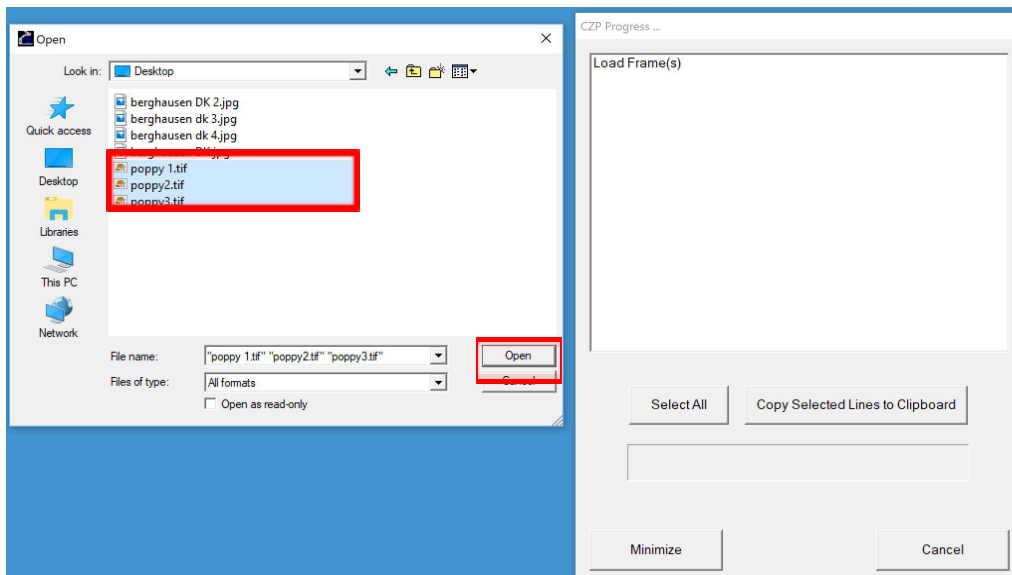
All images will now automatically save to the designated folder upon capture. Images will save with an automatically generated file name (i.e Image_01.tif). Upon capture, the image will briefly show an asterisk next to its file name, which will then disappear to confirm that the image has been saved. If the asterisk does not disappear, or if an error message displays, the file must be saved manually. In this case, make sure to manually save the image to the designated folder, as to not misplace the image. As a suggestion, create a “Microscope Photos” folder on the desktop as your designated folder. This folder will then act as a workspace as stacked images are created using CombineZP. Final images can then be renamed and moved to other folders, and the cache of images created by CombineZP deleted from the “Microscope Photos” folder.

Stacking Images Using CombineZP (Adapted from Lab Report #87)

- 1) Open CombineZP software from your desktop.
- 2) To begin creating a stacked image: Click **New** in the top left of your screen.



- 3) This folder will pop up. **Select/highlight all the photos you want to combine**, then select **Open**. You may need to navigate to where you have saved your images.



- 4) From the drop down menu at the top of your screen, select **Align and Balance Used Frames (Quick)** in the drop down menu, then **Go**.



- 5) From the drop down menu, select **Do Stack**, then **Go** which will prompt a progress bar. Your new, combined image, will appear on the screen.
- 6) You can either crop now or do all work in photoshop. If you want to crop now: Adjust the frame by clicking the "shrink and expand active rectangle". This will cut out the excess/out of focus background into a more usable angle.
- 7) Press **Save** and select **100% quality**. Name your new image file.