

Using the SZ61 Microscope to Take Images with Optional ZStack in CombineZP:

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Laboratory Report #87

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Camera Preparation (from Alan Farahani's Lab Report #68):

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:

1. Remove the cable protruding from the back of the camera by squeezing the two buttons on the side and then pulling rearward.
2. Unscrew the camera by turning it to the left.
3. Place the camera on top of the SZ61 (there should be a black cap that should be removed).
4. Screw the camera on by turning it to the right. Be gentle!

Sample Preparation:

1. Place white paper under your petrie dish in the microscope viewshed.
2. Place sample and physical scale in petrie dish. Adjust so that the scale is within viewshed but can be cropped out of your image later.

Taking Images:

Opening:

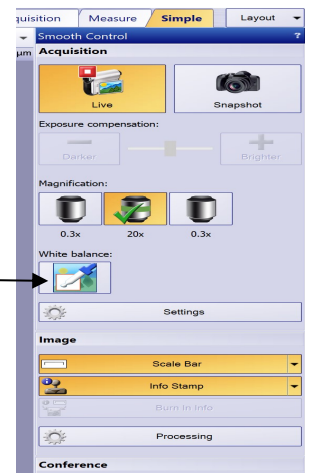
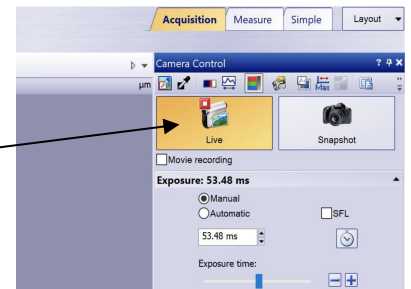
1. In your account, open the program cellSens from the desktop (see troubleshooting section for access issues).

Preparing:

2. Click "live" in the top right corner to see the camera view in CellSens:
3. Arrange the physical lighting for your sample, watching your image on the screen. High quality photos will have few to no shadows. Physically move the lights until you achieve your desired result.
4. On the right side of the screen, navigate to the 'Acquisition' tab (other options: Simple and Measure). *Alternatively, proceed to step 6 to use the 'Simple' tab.*

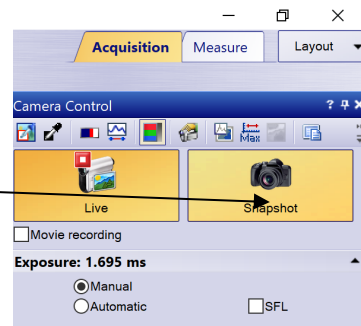
Balancing White:

5. 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.
6. **OR,** In the 'Simple' tab, navigate to the white balance button and perform the same steps.



Imaging:

7. Make sure you're in the Acquisition tab.
8. Set your exposure—if you are taking a single image of your sample, select automatic. Unless you want to change the exposure in the manual setting, you are ready to take the photograph.
9. *If you are taking multiple images for ZStacking*, 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. You can also use this manual setting to adjust to a preferred exposure at any time.
10. Focus first on the uppermost point of your sample. The manual focus is the largest black knob on back side of microscope.
11. Once you have it in focus, take your image: click “snapshot” on the right toolbar (under acquisition **OR** simple), which will create a new tab with the image in the upper left. Alternately, you can use the F8 command to take snapshots.
12. If you are taking a single image, continue following steps 13-22 to calibrate a scale bar and save your image. *If you are taking images for ZStack*, proceed to step 23 to follow that sequence.



Save your image:

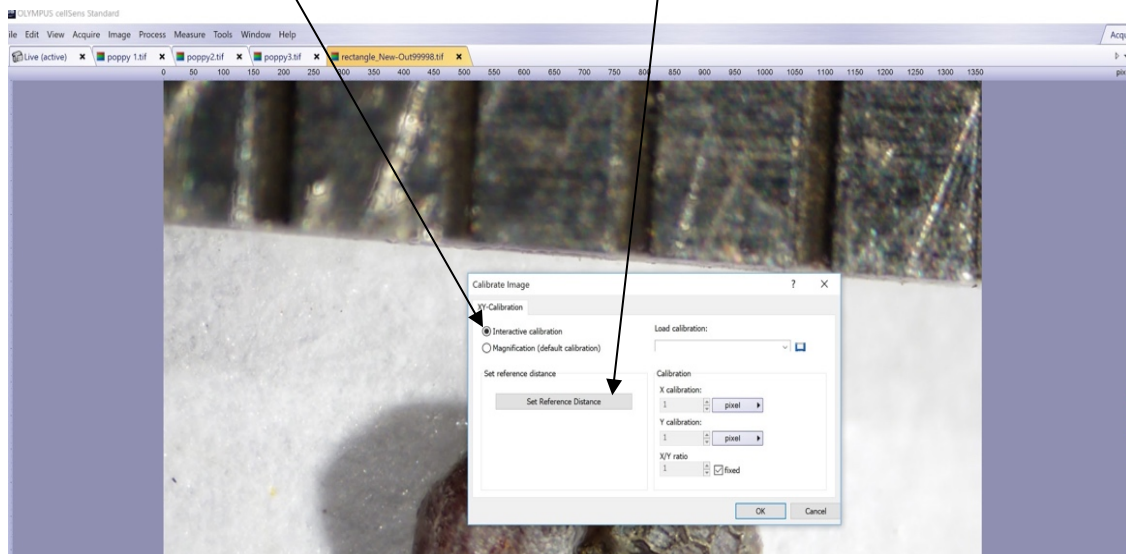
13. Click on the image tab in the upper left (which will highlight it in yellow):



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)

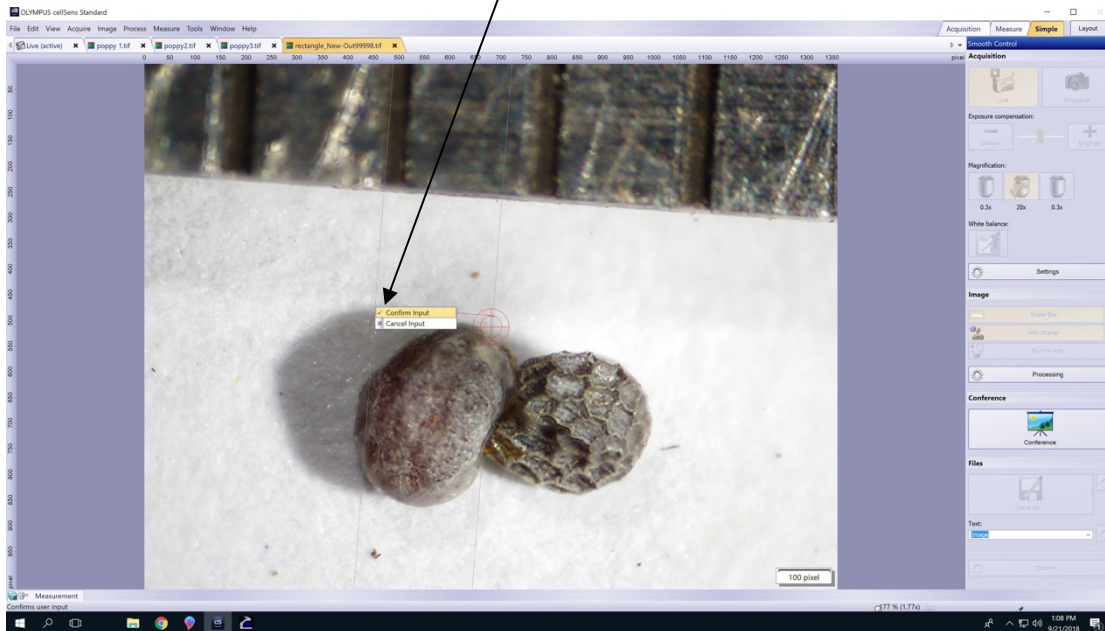
Adding a Permanent Scale Bar:

14. In CellSens, open your saved image.
15. Select Image in the tool bar→ in drop down select calibrate image→ check the Interactive Calibration bubble→click set reference distance box. This allows you to create a malleable “ticked” rectangle which you will align with the physical scale bar in your image (step 16).

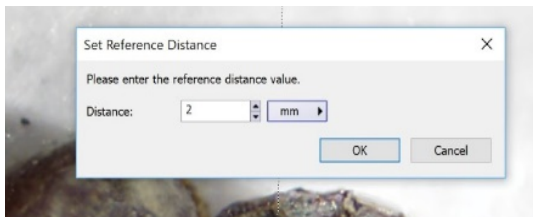


Setting Reference Distance:

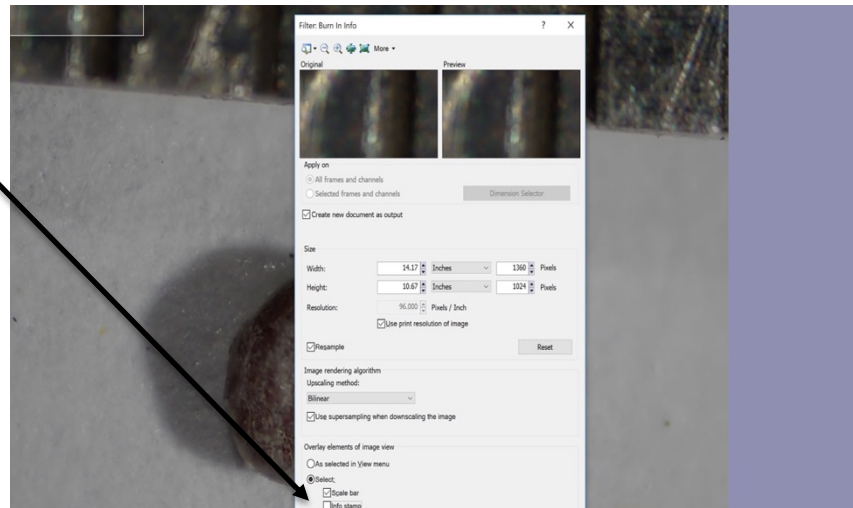
- To select the length of your scale bar, first left click over physical scale bar at the left side of the distance you want to create for your scale bar, then drag over to the other end of your distance and left click again. Always try to create an equal mm length by clicking on the same point on your 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.
- Right click and select “confirm input”



- A dialogue box will open and ask you what distance reference you just calibrated. Write what you chose in the box and press ok. Press okay again to exit dialogue box. Your scale bar should appear in the lower right of your screen.



- Navigate to ‘Image’ in the dropdown toolbar → click “Burn in info”. At the bottom of the dialogue box that will appear, select the ‘select’ bubble and make sure the scale bar box is checked to burn the scale bar into the image.



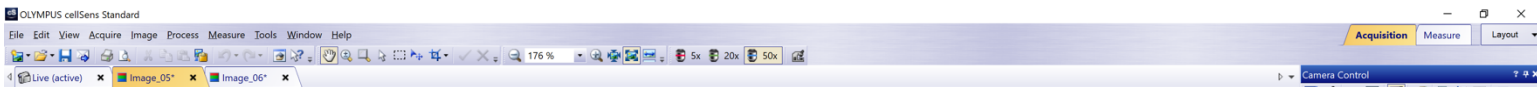
20. Hit ok
21. File→Save as your image as a .tif file. You should open it back up to make sure you have burned in the scale bar.
22. Finally, open photoshop and crop out physical scale bar, any unwanted dust, etc. *If you only need to perform very simple cropping, you can alternately open up Paint on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

Taking Multiple Images for Zstack:

23. *If you are taking multiple photos for combining into an in-focus image:* take multiple photos by focusing on progressively lower areas of the sample. You only need to twist the knob a couple of degrees each time (it's a tense dial so you'll need to practice). 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. You will return to CellCens to burn in your scale bar after completing the ZStack procedure.

Saving:

24. Once you have taken all of the images that you need (they will be in tabs from left to right across the top of your screen): click on the image tab in the upper left (which will highlight it in yellow). In that image, in the top toolbar, select file→save as, and save your image as a .tif file. You



should save each of your images to your own desktop or flash drive, with unique file names that indicate your samples identifying information. Save your images in sequence so that you can easily access them for stacking.

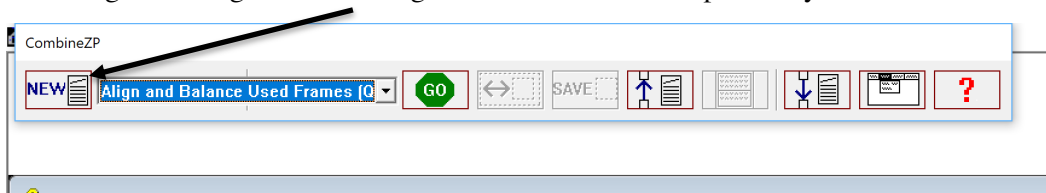
Tip: You can also save your images as you take them (this is a matter of preference). Don't rename the picture until you have saved it permanently so that you know the order you took it in. When saving the image, put context number (or some other identifying feature) in the name so you can look back on it later and know where it was from. There will be an asterisk next to file names in the tabs of photos that are currently unsaved.

25. When all of your images are saved (to your file on the desktop, or to your flash drive), move on to the CombineZP process.

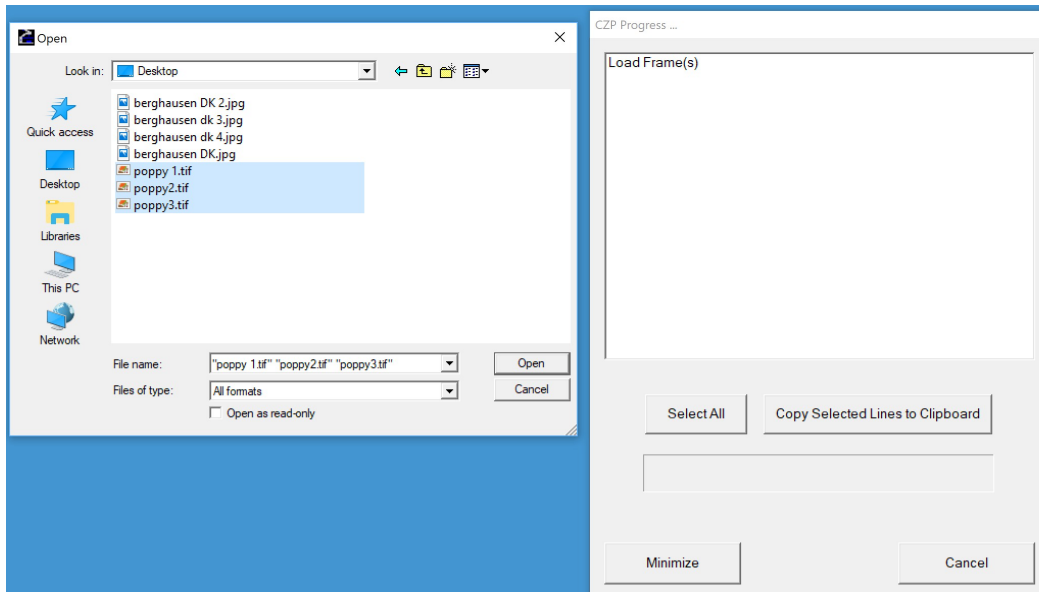
Using CombineZP Zstack Software to Combine Images:

Opening:

26. Open CombineZP software from your desktop.
Tip: If you have not stacked images on your account yet, you may need to download CombineZP at <https://combinezp.software.informer.com> in order to use the software.
27. To begin creating a stacked image: Click “new” in the top left of your screen.

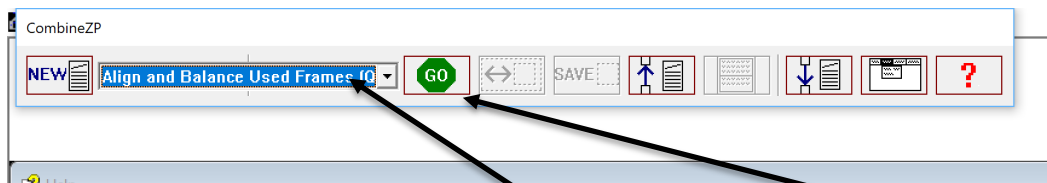


28. This folder will pop up. Select/highlight all the photos you want to combine → click 'open'.

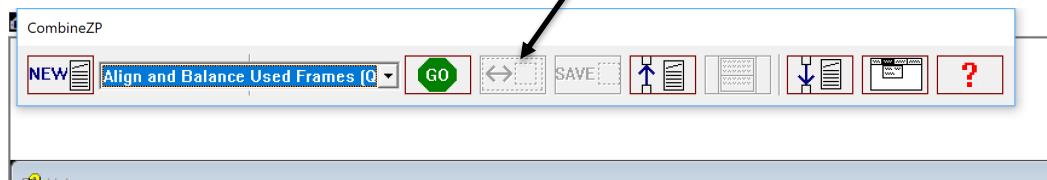


Stacking Images:

29. From the drop-down menu at the top of your screen:



- Select Align and Balance used Frames in the drop down menu → hit Go. When prompted, select 'thorough' or 'quick' (thorough takes longer, and is typically unnecessary).
 - From the drop down menu, select "Do Stack," → hit Go which will prompt a progress bar.
 - Your new, combined image, will appear on the screen.
30. 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. *If there are still things you don't want in the photo, you can use photoshop later.*



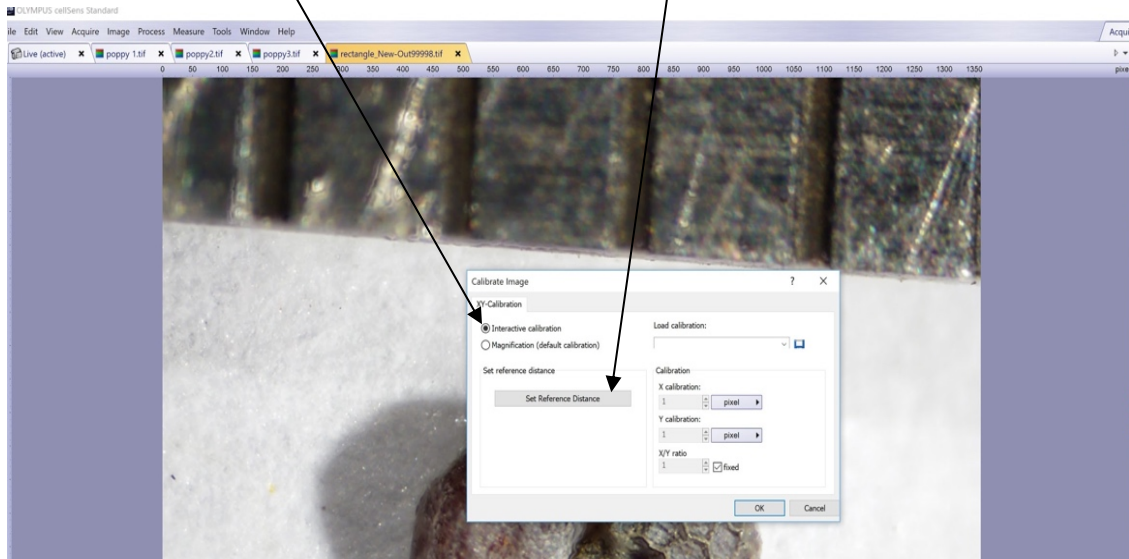
31. **To save** this new combined image as a .tif file with a new name. (go up to file → save as) and name your new image file.

Tip: If you are having trouble opening the saved image: The computer sometimes processes the save incorrectly, so make sure that when your image is saved it is several mb. If it is 1 kb or less the file has been saved incorrectly. If this occurs, you must return to the beginning of step 31 to resave the image as a jpeg. Next, open it in cellSens and re-

save it as a tif file. As usual, save your file in a way that indicates the unique sample that you are imaging.

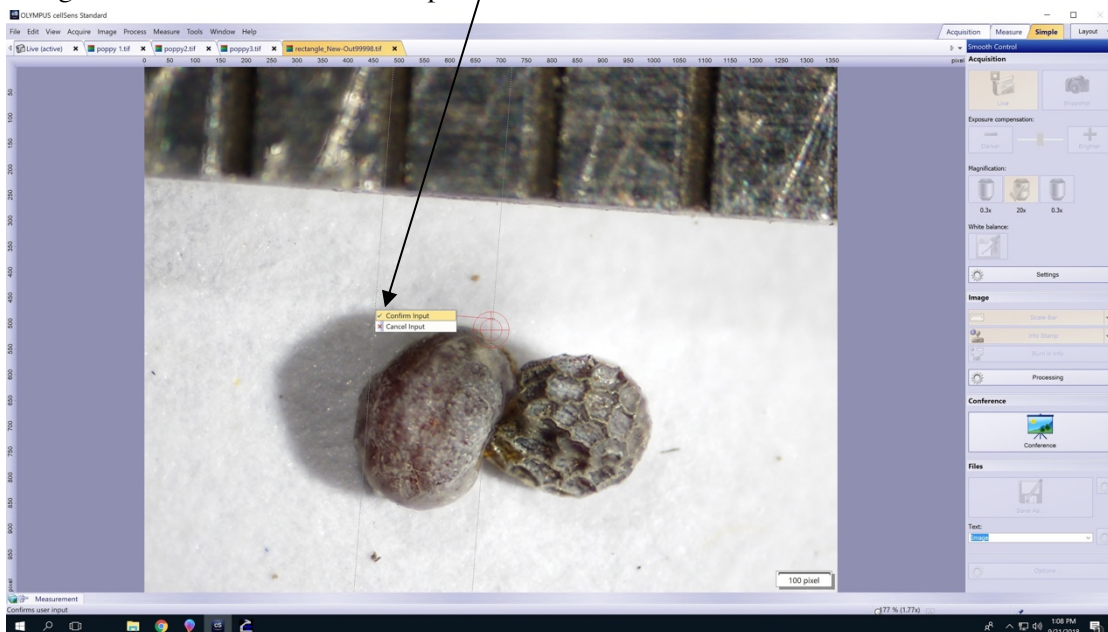
Adding a Permanent Scale Bar:

32. Close CombineZP, reopen CellSens from your desktop, and go to file→open your stacked image (you have named this previously in step 31).
33. Select Image in the tool bar→ in drop down select calibrate image→ check the Interactive Calibration bubble→ click set reference distance box. This allows you to create a malleable “ticked” rectangle which you will align with the physical scale bar in your image (step 34).

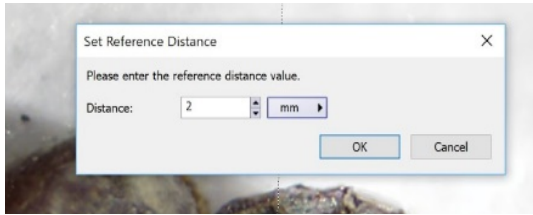


Setting Reference Distance:

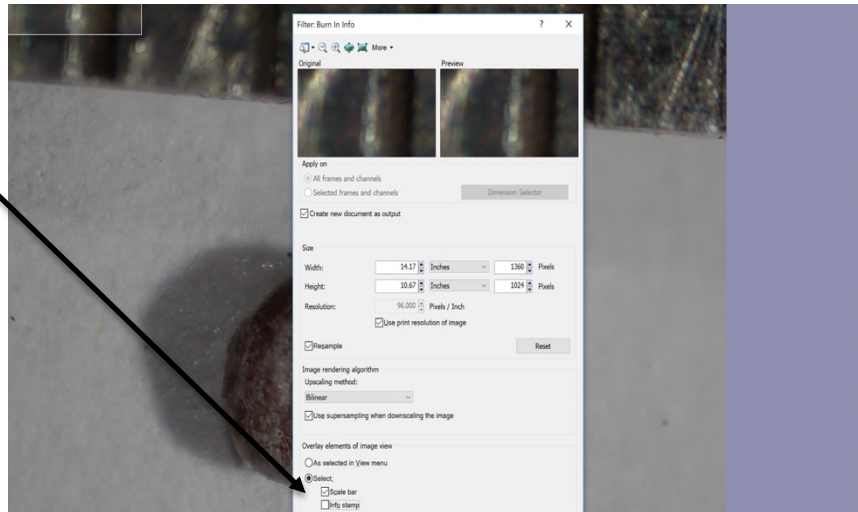
34. To select the length of your scale bar, first left click over physical scale bar at the left side of the distance you want to create for your scale bar, then drag over to the other end of your distance and left click again. Always try to create an equal mm length by clicking on the same point on 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.
35. Right click and select “confirm input”



36. A dialogue box will open and ask you what distance reference you just calibrated. Write what you chose in the box and press ok. Press okay again to exit dialogue box. Your scale bar should appear in the lower right of your screen.



37. Navigate to 'Image' in the dropdown toolbar → click "Burn in info". At the bottom of the dialogue box that will appear, select the 'select' bubble and make sure the scale bar box is checked to burn the scale bar into the image.

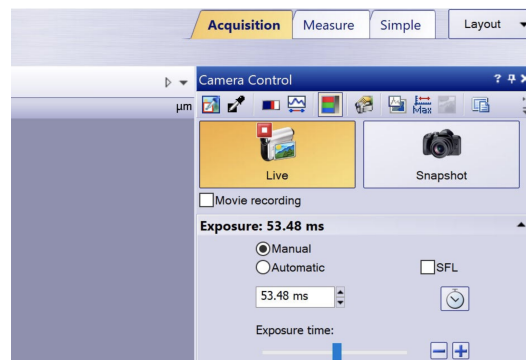


38. Hit ok
 39. File→Save as your image as a .tif file. You should open it back up to make sure you have burned in the scale bar.
 40. Finally, open photoshop and crop out physical scale bar, any unwanted dust, etc. *If you only need to perform very simple cropping, you can alternately open up Paint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

Troubleshooting:

If you sign in on your account and open the CellSens successfully, but the actual image is all black, check the exposure!

On the right hand toolbox, select the Acquisition tab and choose the option "exposure" and change it to automatic (if it is on manual). From here, return to manual and lock in your exposure for your set of images.



Account access issues:

- a) This appears as an error with the cable connection upon opening CellSens. Go into a folder called "Programs -> Olympus -> Cellsens -> Program Data Olympus". All folders need full access under folder permissions.
- b) The lab account does not seem to be working (as of 09/21/18) for CellSens. Use your own account.
- c) If Cellsens refuses to run in your account (still) you have to right click and run as administrator (picture of blue and yellow shield next to it) from the desktop.

Admin account password is in top right drawer on cd case. Username: ./labadmin