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Title
Imaging Protocols for the UCSB Invertebrate Zoology Collection

Permalink
https://escholarship.org/uc/item/2rv3m3q2

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Publication Date
2019-07-01

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IMAGING PROTOCOLS FOR THE UCSB INVERTEBRATE ZOOLOGY COLLECTION

By Rachel Behm, Chelsea Chung, Lina Solomon & Katja Seltmann
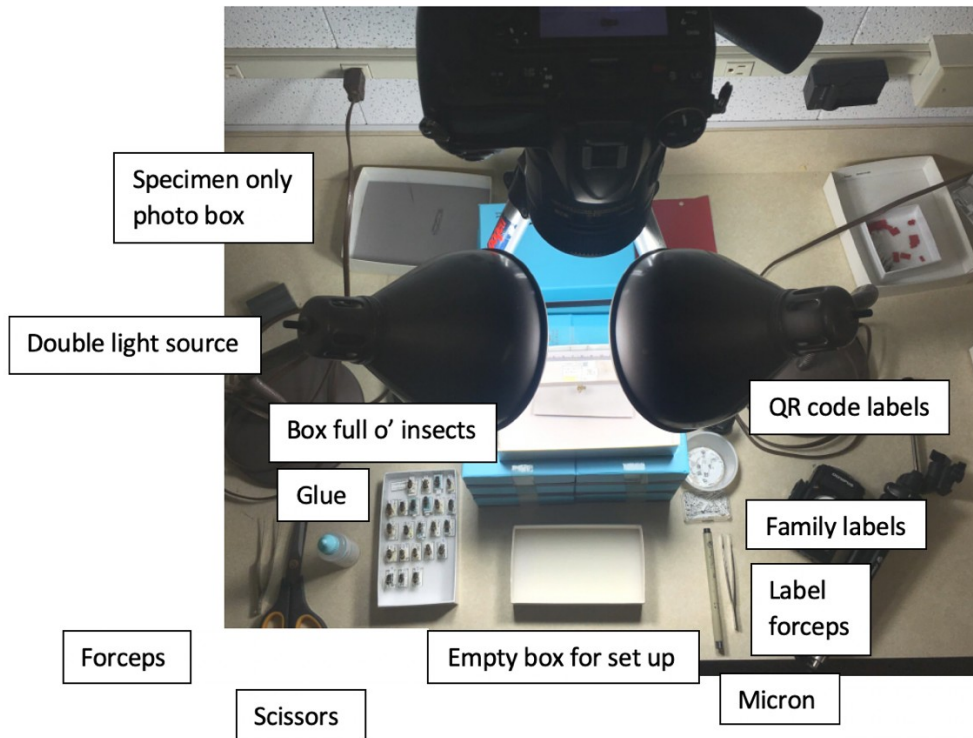
CHEADLE CENTER FOR BIODIVERSITY AND ECOLOGICAL RESTORATION UNIVERSITY OF CALIFORNIA, SANTA BARBARA
HARDER SOUTH, BUILDING 578
JULY, 2018

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UCSB Invertebrate Zoology Imaging Protocols

INTRODUCTION

This guide was developed at UC Santa Barbara as a general guide for how to image specimens and labels of pinned invertebrate zoology specimens for use as exemplar specimens and as vouchers for databasing. The guide was created by students to help new collection curation students learn and understand the imaging protocols specific for our collection.

The UCSB Invertebrate Zoology Collection is a small collection with taxonomic strengths in Hymenoptera and Diptera. Originally used as a teaching collection for a general entomology course taught from 1961 to 1993, this collection's diversity (9,000 insects in 21 orders and 246 families) makes it a valuable historical record of insects in endangered coastal California habitats. This report was developed as part of the Institute of Museum Library Services Grant (award #MA-30-16-0387-16) to curate and digitize the Adrian Wenner Historic Insect Collection at University of California, Santa Barbara.

LABEL IMAGING PROCEDURES

This section of the UCSB Invertebrate Zoology Collection imaging guide was developed by Chelsea Chung

## CAMERA SETUP 1: NIKON D300S & LENS
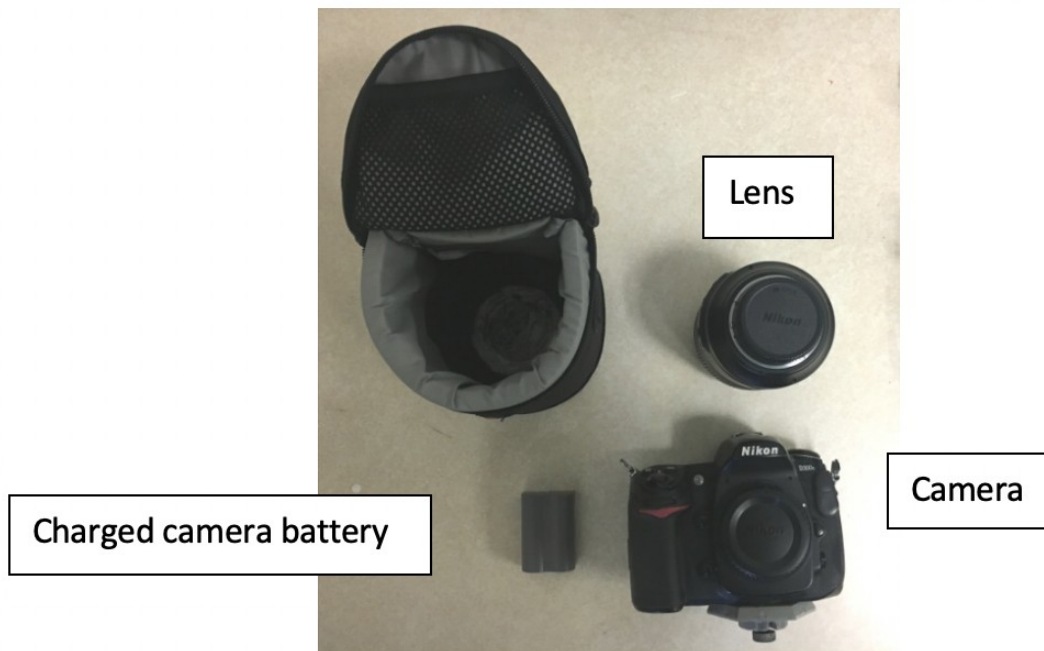
1. Check out the lens from the monitoring cabinet in the main office
2. Put in the camera battery into the battery slot

3. Putting on the camera lens: Line up the white circle on the non-focusing side of the lens with the white circle on the camera. Rotate the lens counter-clockwise into place.
Removing the lens: Press the lens release button and rotate the lens clockwise.
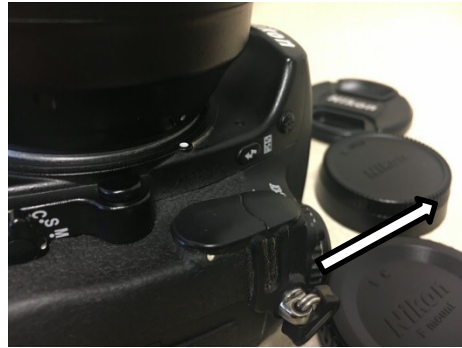
Button to release

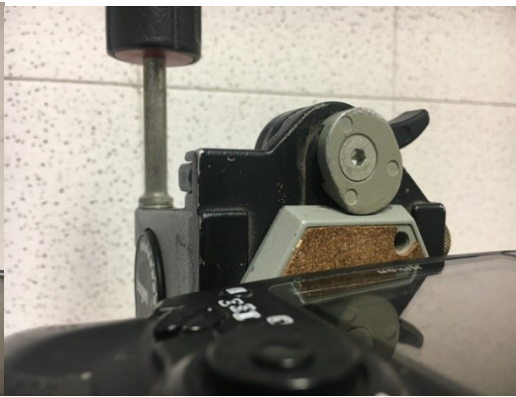White aligning

4. Lock the camera into the tripod. Place the metal section of the camera against the tripod opening, snap into place using the left and central clasps of the tripod (Central clasp should snap in automatically) Both must be flipped upwards to release the camera.
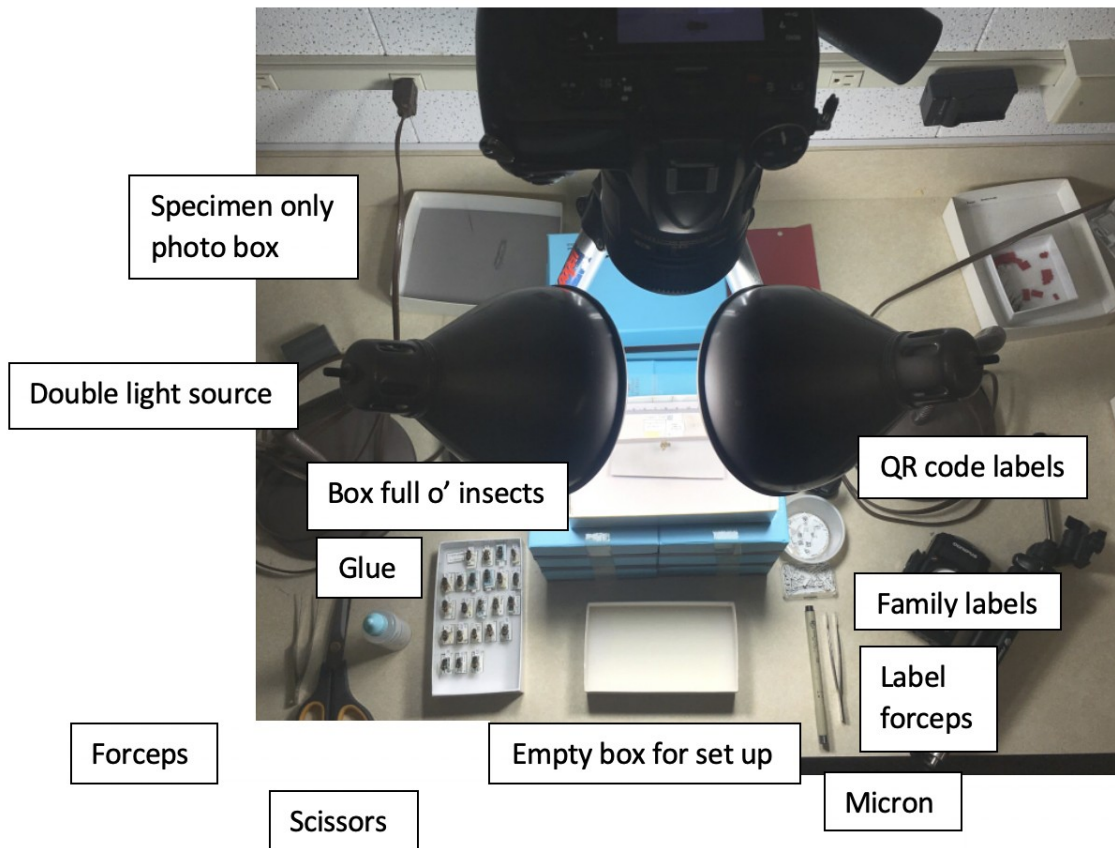
Open   Closed

The tripod camera height can be adjusted with this knob.

GENERAL SETUP

Labels on image:
- Specimen only photo box
- Double light source
- Box full o' insects
- Glue
- Forceps
- Scissors
- Empty box for set up
- QR code labels
- Family labels
- Label forceps
- Micron

CAMERA SETUP 2: OLYMPUS TG-4

The photography setup is much the same, only the camera and tripod are different. A lens is not required.

1. For insects already in focus, the camera mode should be on Microscope mode.
2. For insects not in focus, the camera should be on Focus Stacking Mode, signified by a ladybug. Shift the mode dial to the microscope icon, then move the joystick to the side to set the mode to Focus Stacking. Taking a photo in this mode
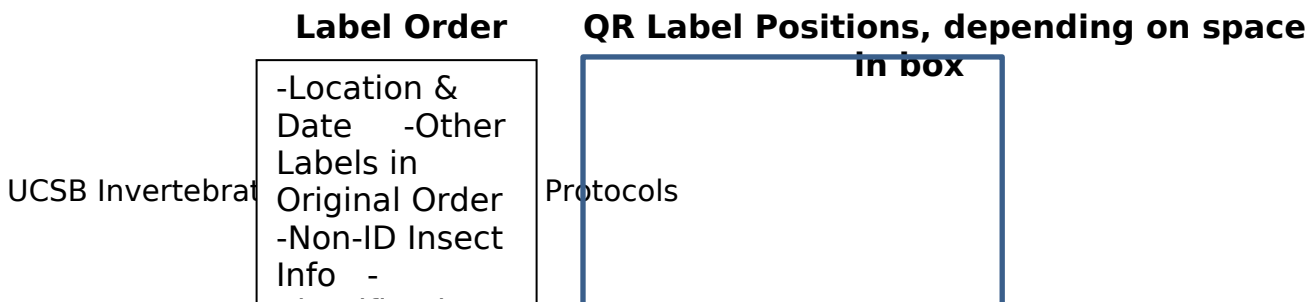

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*To take a photo with the Nikon, press the Live View button first to see the image in view.

IMAGING PROCEDURE

1. Set up labels on the higher stage in box.
    a. Include the proper family label at the top, handwritten on archival paper by yourself.
    b. If there is information on both sides of the label, make a label with info of one of the sides, and place within the photo along with the original label.
2. Set insect in place on the lower level in the pin hole
    a. Shift the paper around as needed to have the insect fit.
3. Focus camera, take picture.
4. Take out insect & put on labels, each slightly separated, **neatly** aligned.
5. Put pin with insects and labels **neatly** back into the box, insect head facing up, QR code at least partly visible. Position insects compactly with enough space for fingers to pick up pins.

Label Order   **QR Label Positions, depending on space in box**

UCSB Invertebrat

-Location & Date   -Other Labels in Original Order
-Non-ID Insect Info   -

Protocols

QR

QR

Quick explanation of insect collection boxes

The large blue labels usually denote the families of the insects after the box it is in
The red-orange labels denote a needed update in the database, imaging, or both
Most boxes have the lowest found identification level in the upper left

*Some of the older labels i.e. <2000 QR Code number have had their photos lost – best to check the first and last specimens in the box on Symbiota to check.
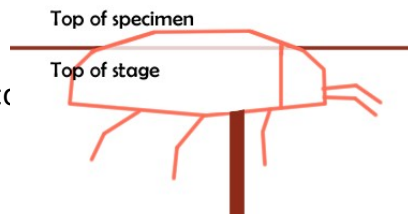
PHOTOGRAPH SETUP

The main goal is to make a well-organized photograph with visible, in-focus, orderly labels. The insect is secondary to the labels.

(Top view) Labels and top of ruler are in focus and visible, insect is right

(Front view) The top of the insect is just over the

Top of specimen

Top of stage

Labels
- QR code is entirely visible, in the upper right corner of the image
- Family identification and lower level identification labels are visible
- Labels are clear and compacted
- All are in focus
-

Insect
- Insect is level with the stage (see fig. 2)
- Insect is in the center of the image, below the labels
- Head of specimen faces the right, exceptions:
    o **If specimen is pinned on poin**t point faces the right

        *If the insect is too large or more visible when facing the right, you can photograph normally

    o **If 2/3 or more of the specimen is cut off from the photo (**even with tripod adjustments) you can point the head upwards. Half of the specimen being visible is fine.

    o **If the specimen is too wide to fit (>2 inches wide) or a Lepidopteran** (For most cases, large size applies to grasshoppers, dragonflies, beetles, crane flies, some leps) Take photos of the labels

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and the insect separately. QR code and ruler should be visible in both photographs. Pin QR code to a pin to keep level with insect.

UPLOADING & BACKING UP PHOTOS

1. Upload photos from camera to Desktop into a new folder titled with the date of upload ("MonthDayYear")
2. Make a copy ASAP as a backup while editing. If applicable, first separate photos that need to be focus stacked from those that do not
3. Back up folder to Box and to the LocalBackups folder on the Desktop
4. Put folder with photos into insect-image-processing. Photos that *only* contain labels should go into label > toBeFlipped. Photos that need to be focus stacked should go into habitus > toBeStacked. All other photos should go into habitus > toBeFlipped.

   o   NOTE: This should technically be the other way around (photos with only the insect into *habitus,* every photo with visible labels in *label*), I just did not set up folders that way.

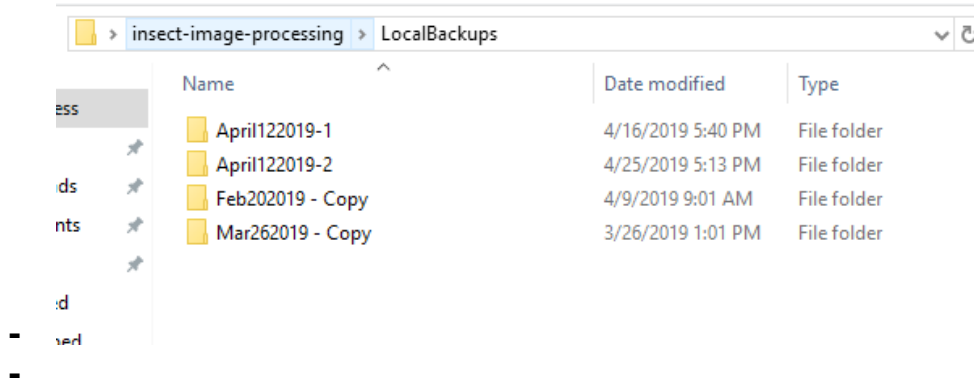UCSB Invertebrate Zoology Imaging Protocols

- bad-images: Photographs that are blurry, crop off information, or otherwise unclear. These should be retaken.
- finalFlipped: Photographs that have been edited.
- toBeFlipped: Photographs to be edited.
- stackedToBeFlipped: Stacked photographs to be edited
- toBeStacked: Photographs to be focus stacked in Helicon Focus Pro

Box Backup
- Back up new photo uploads as a folder named as the date of upload ("MonthDayYear") through Box into the Insect Collection images folder that should be shared with you

Local Backup
It's a good idea to back up folders locally (onto the Computer) for editing. (Desktop > insect-image-processing -> LocalBackups) They can be deleted after the original files are edited, or remain there for further use.

|	insect-image-processing > LocalBackups				
	Name	^	Date modified	Type	
ess					
	April122019-1		4/16/2019 5:40 PM	File folder	
ds	April122019-2		4/25/2019 5:13 PM	File folder	
nts	Feb202019 - Copy		4/9/2019 9:01 AM	File folder	
	Mar262019 - Copy		3/26/2019 1:01 PM	File folder	

EDITING PROCESS

1. Focus stack photos if applicable (see next section)
2. Edit stacked photos in Gimp (.jpg extension) if applicable
3. Edit normal photos in Gimp (.JPG extension)

FOCUS STACKING

If the insect is too tall to be in focus/non-blurry with one photograph, it might need to be focus stacked. This is when you take multiple pictures (2-4) to bring different body parts in focus, then merge them with focus stacking software. At the time of this writing, we use Helicon Focus Pro.
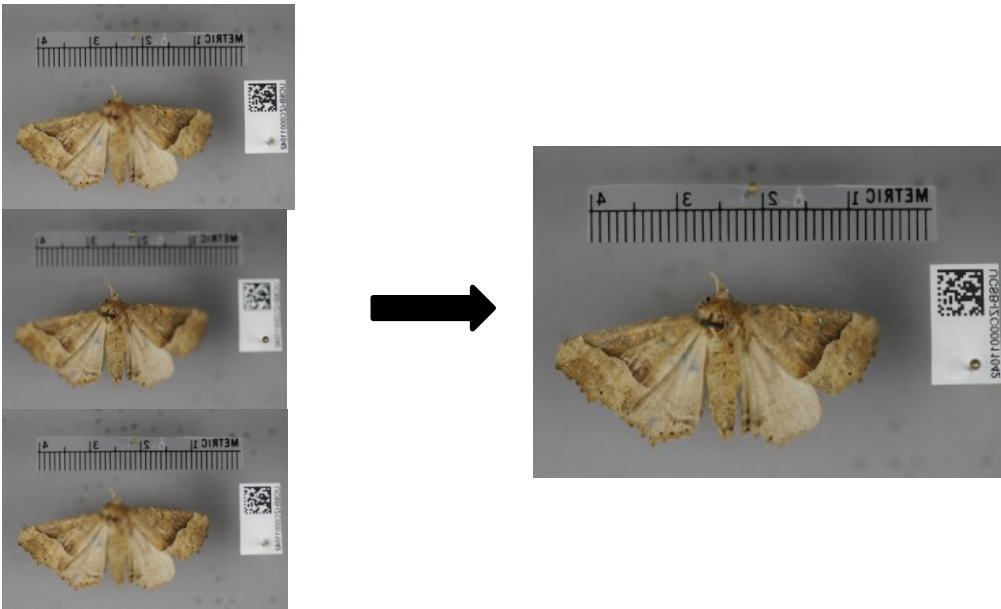
USING HELICON FOCUS PRO
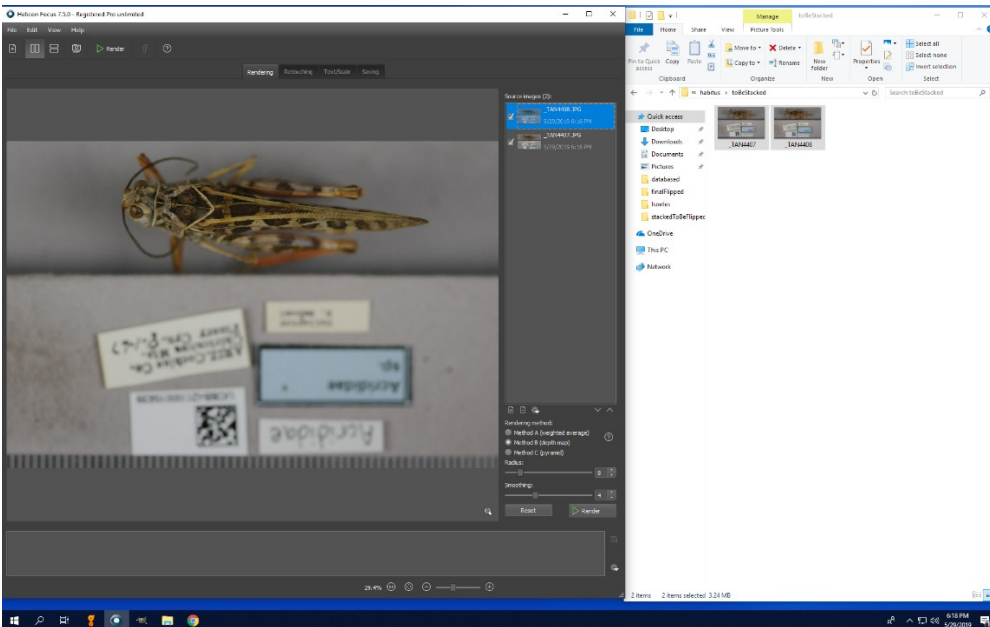
1. Drag photos to be stacked into the Source Images window
2. Choose a Rendering Method. There's no clear-cut best way – I usually go with Method A or C.
3. Press Render. Repeat 2 and 3 as many times as needed to get a well-done photo. (It makes a misaligned photo sometimes for no reason, just render again).
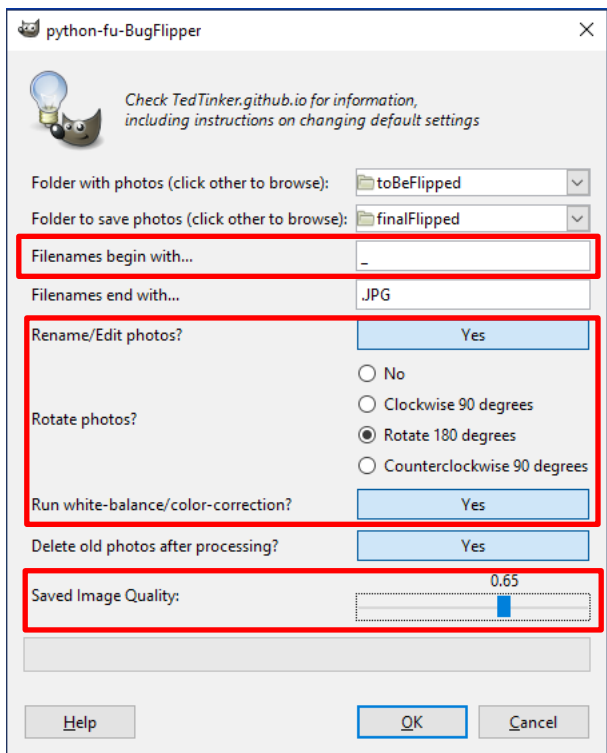
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4. Finished photos go into the output window at the bottom. Right click and save output to insect-image-processing > habitus > stackedToBeFlipped. Rename as: "_TAN[sourcePhotoNumbersCombined]"
5. The photo now ready to edit in Gimp.
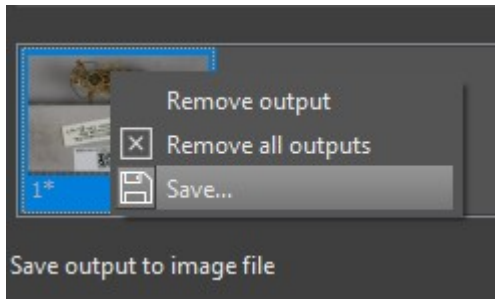

FLIPPING & EDITING IN GIMP
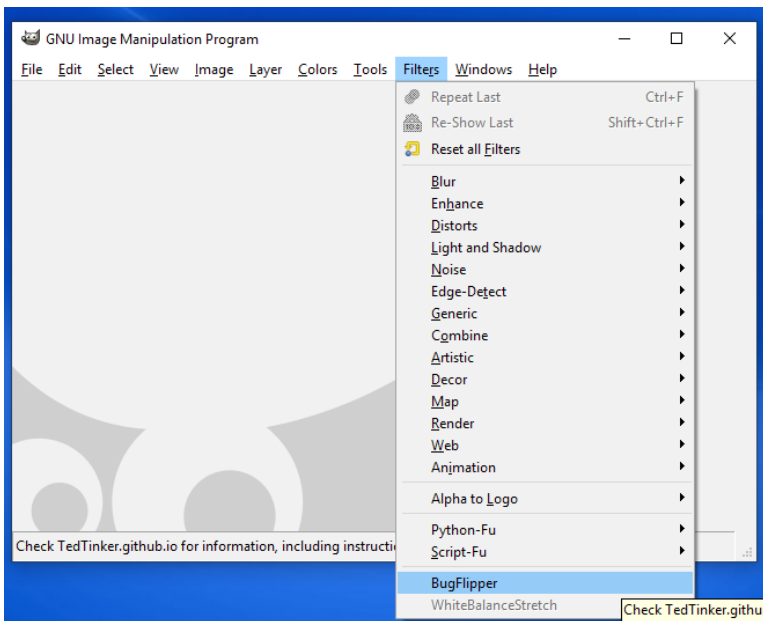
1. Open up Gimp (The shortcut is on the taskbar) Select the BugFlipper tool under Filters.
2. Here are the settings. Select the folder with photos to edit from and the folder to save to. Change the extension of the photos to be edited as needed. Move this settings window to the side & press OK.(The window stays in place during the editing process) Fields en-squared do not have to be changed.

python-fu-BugFlipper ✕

Check TedTinker.github.io for information,
including instructions on changing default settings

Folder with photos (click other to browse):	📁 toBeFlipped
Folder to save photos (click other to browse):	📁 finalFlipped
Filenames begin with...	_
Filenames end with...	.JPG
Rename/Edit photos?	Yes

Rotate photos?
- ○ No
- ○ Clockwise 90 degrees
- ● Rotate 180 degrees
- ○ Counterclockwise 90 degrees

| Run white-balance/color-correction? | Yes |
| Delete old photos after processing? | Yes |

Saved Image Quality: 0.65

| Help | OK | Cancel |

VERY IMPORTANT:
- **Do NOT save to the same folder.** This will delete all the photos in that folder. There are different "toBe…" and "final…" folders for normal photos, focus stacked photos, and photos with only the label.
- **ALL the photos in the "Folder with photos" option MUST have the filename extension you choose in "Filenames end with…"** Not doing so will delete the photos in the folder that do not have that extension. **Photos**

*You can automatically delete old photos if you want, or you can manually delete them after editing
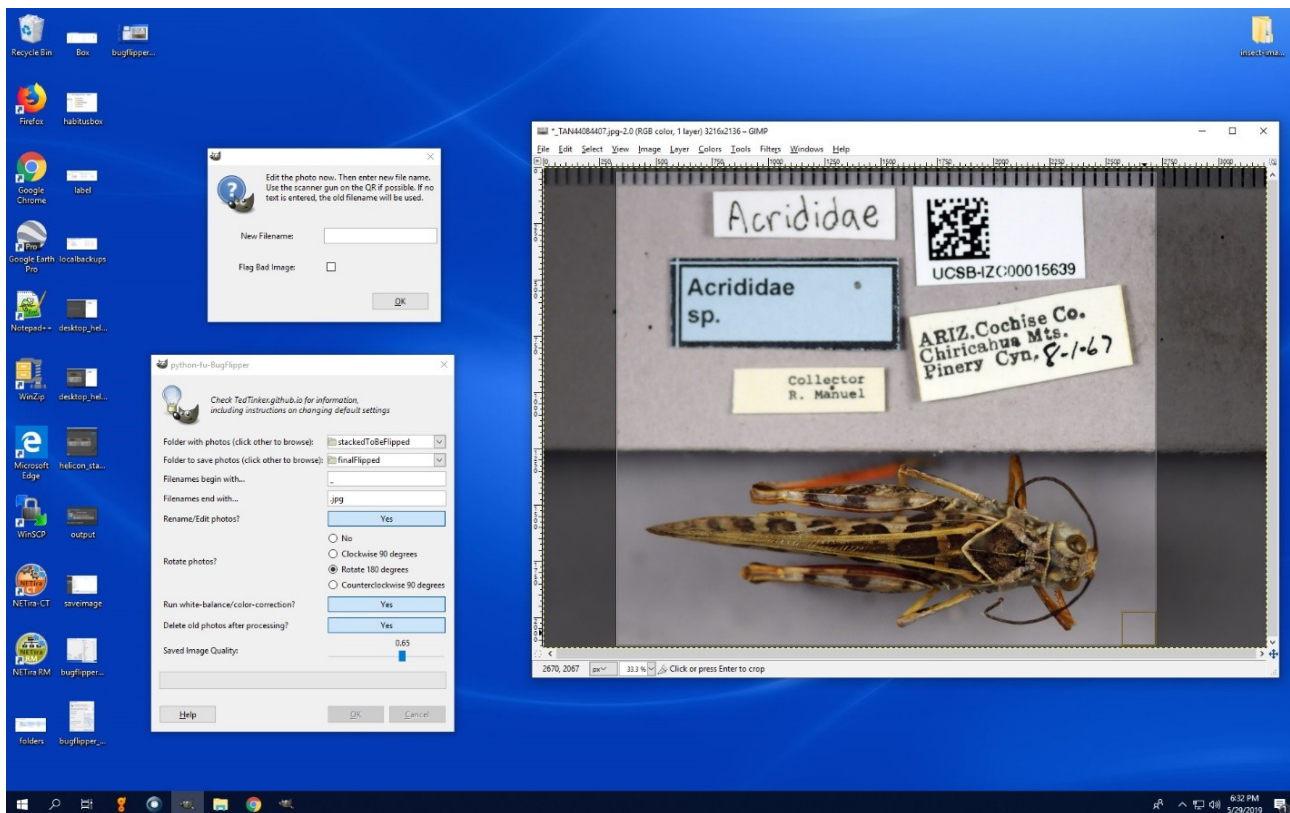
2. Crop the photo in Gimp's main window with the crop tool (Shift-C). Enter cropping changes by pressing enter or clicking the square in the corner.

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3. Click into the "New Filename" field and scan the QR code with the QR scanner. Gimp will automatically go to the next photo until the folder is empty. **VERY IMPORTANT** This will overwrite any other photo with the same QR code. If you find a duplicate, make sure you don't use the same filename by entering it in manually ie "QRCODE_2"

You are then finished with the imaging work! Good job. The finished photos are now ready to be uploaded to our Symbiota database by someone with access.

DORSAL/LATERAL WHOLE SPECIMEN IMAGING

This section of the UCSB Invertebrate Zoology Collection imaging guide was developed by Rachel Behm and Lina Solomon

PROGRAMS NEEDED:

1. Helicon Focus 7 (for stacking)
CCBER has a license for it

2. Camera Control Pro 2 (for remote imaging on the Nikon)
$179.95 or 30-day free trial download via the website

SET-UP

1. Acquire Nikon camera lens from the cabinet of room 1004 (key 9 in green key box)

2. Sign out lens with name and date on sheet in the red folder
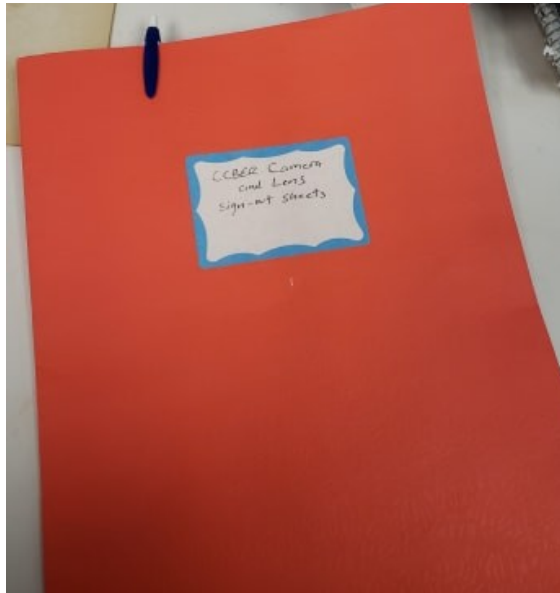
Lock up the cabinet and return the key once you take the lens

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3. Bring lens to imaging station in room 1043 (last door in speech and hearing area across from the insect work room)

4. Remove camera from tripod, by holding camera securely with one hand and lifting the large lever on the right with the other (make sure the latch on the left side is in the upwards position)

5. Attach lens to camera (GENTLY line up the white pegs and rotate), **making sure it securely clicks into place** (will make loud click noise)

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6. Remove battery from charger and put into camera (the slot is in the back)

Add another battery to the charger (if one needs to be)

7. Secure camera to the tripod
Lift large lever back up and put camera into place

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Pull large lever back down but <u>DO NOT</u> let go of the camera until the small latch is in the <u>downwards position)</u>
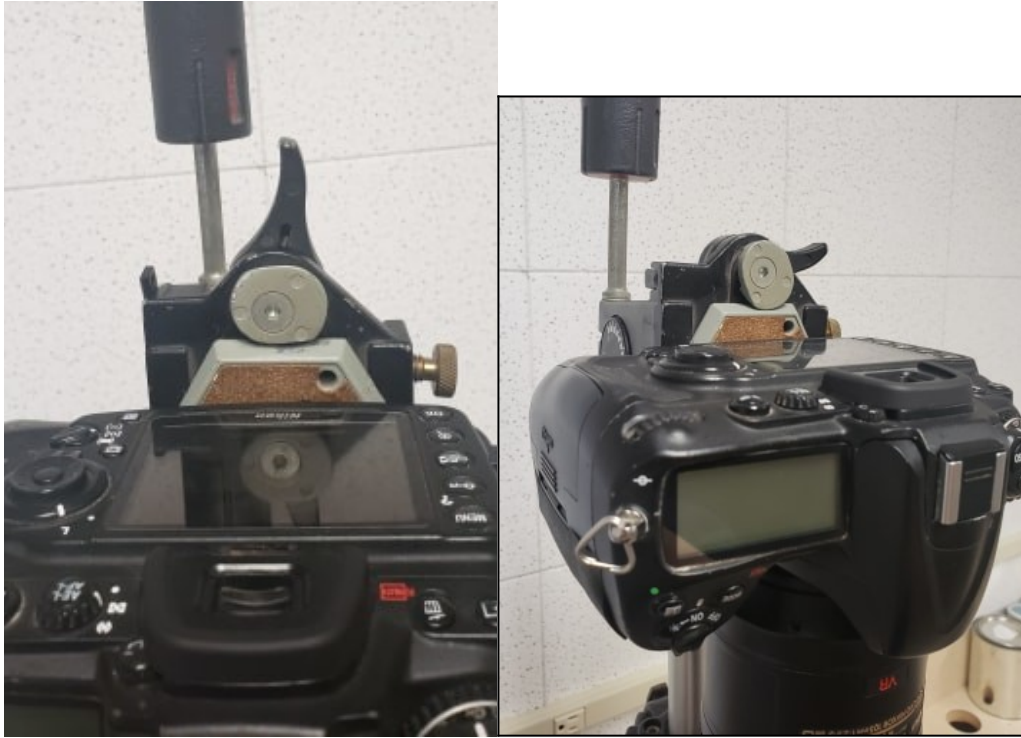
8. Turn on camera via switch below shoot button
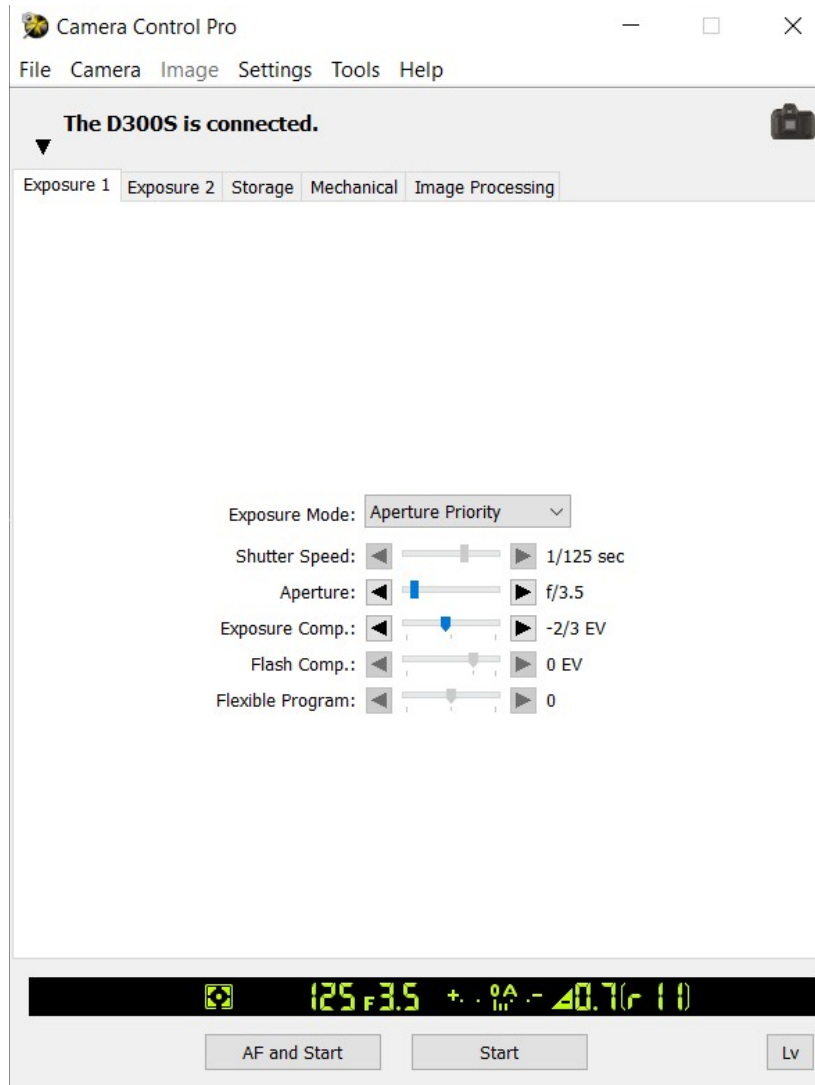

9. Connect camera to computer via USB cable

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10. Open Camera Control Pro 2 on your computer
If you get a window that looks like this...
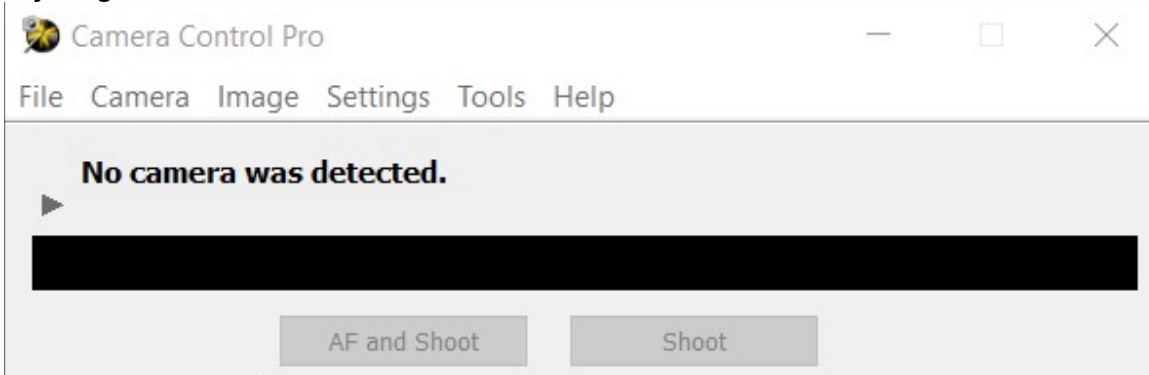
Unplug than replug in the camera (a common problem)

When the camera connects you should get a window that looks like this...

We are not going to mess with the exposure/storage/processing tabs, we will just leave it as the default first one.

11. Click on the camera tab on the top to reveal the dropdown
Click on the "Live View" option (which I have here highlighted)
This will allow you to control the camera from the computer and take images without having to look at the screen on the top of the physical camera

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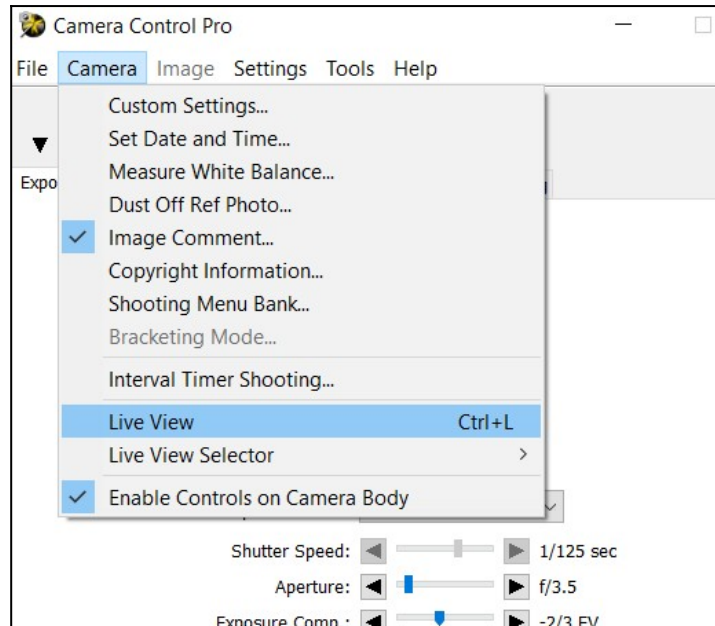
A window will pop up that looks like this...
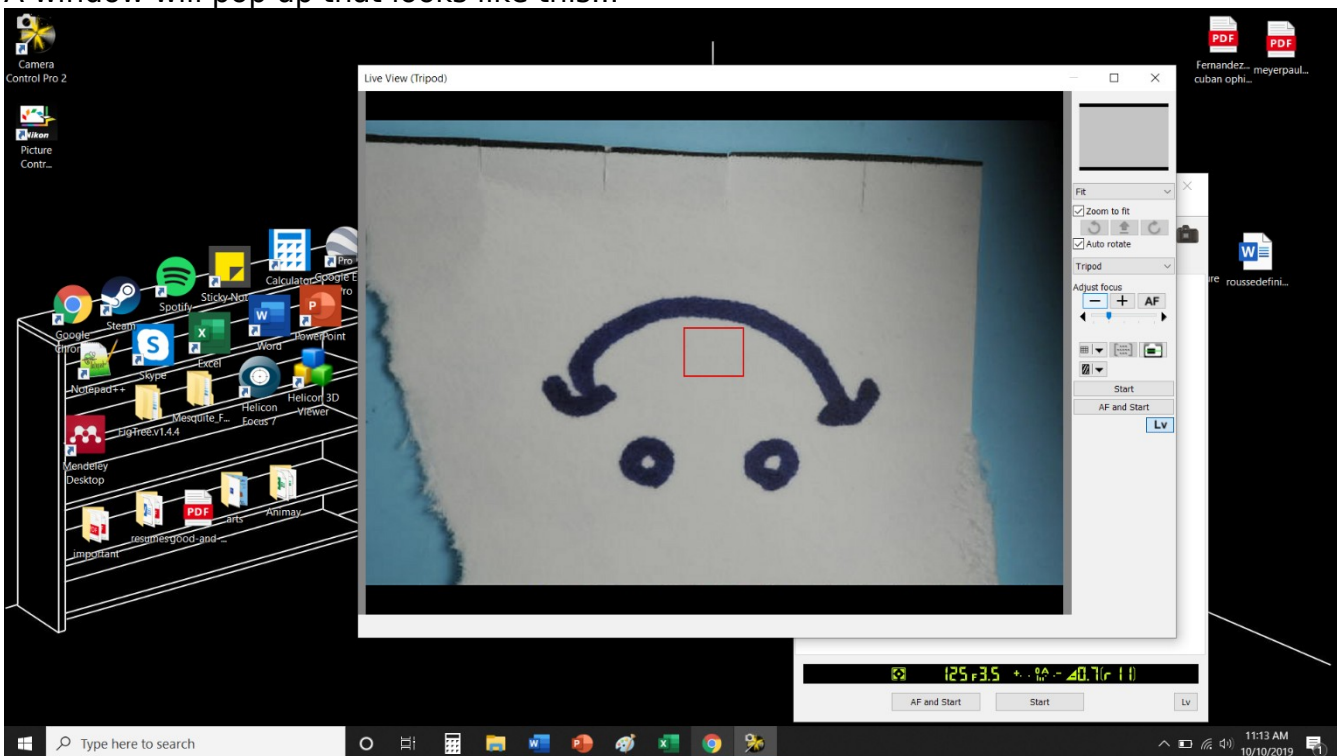
The contents of the window will display what you have on the pedestal but upside-down (this is fine we will edit it later)

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**12. Switch to 2nd to last focus option** (will have to do this each time you load the program)
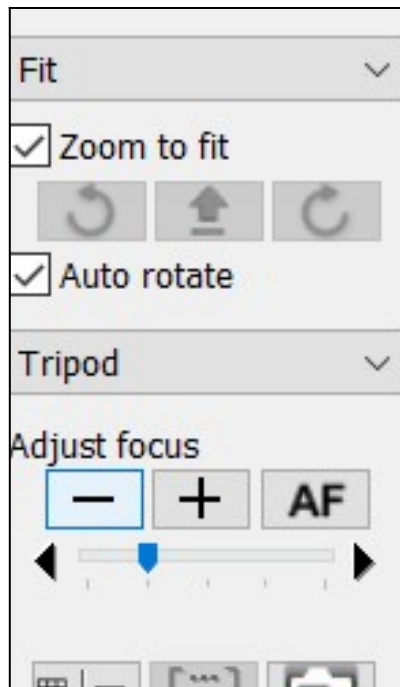
HOW TO IMAGE

1. Zoom to either the lowest or highest point of the specimen where a part is in focus (I prefer to start at the top and work my way down)

 For dorsal shots this will be the top of the pin of either the specimen or ruler…
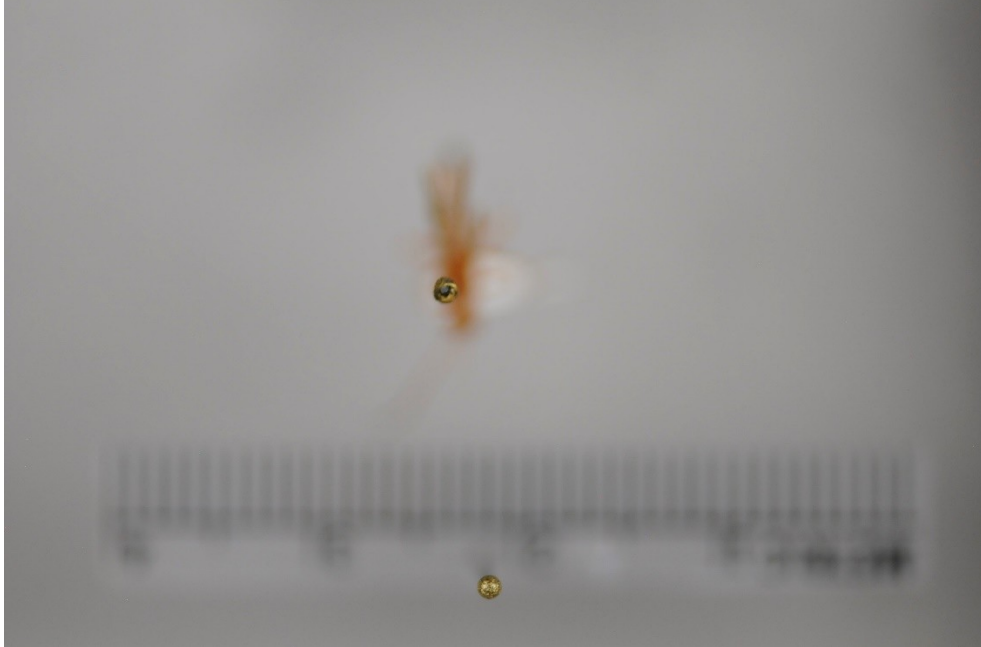
As you can see, everything EXCEPT the top of the pin is out of focus

For lateral shots this is the top of the pin through the ruler…

As you can see, everything EXCEPT the top of the pin is out of focus
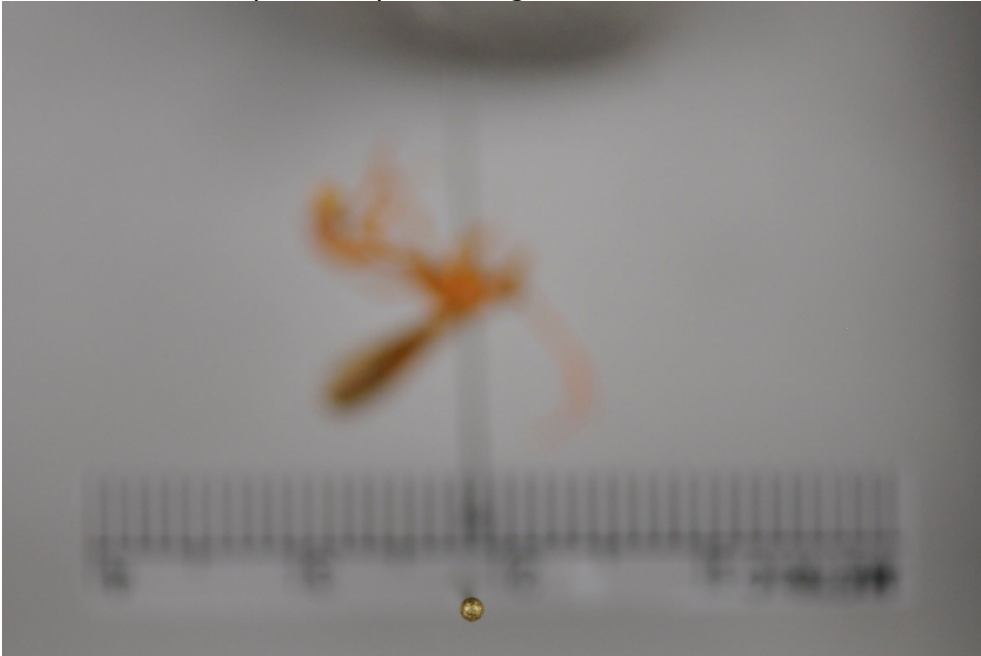
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2. To take an image click the start button (the image will be saved into "My Pictures" by default)
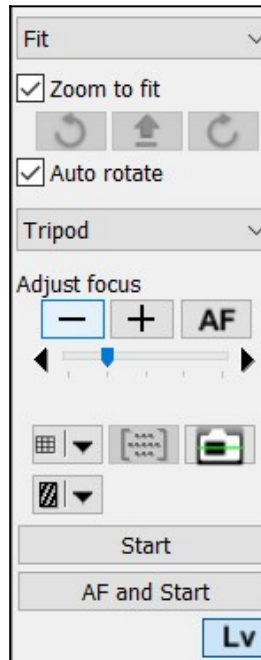
3. Gently grab the textured part of the lens and rotate the lens clockwise in <u>TINY TINY TINY</u> intervals, taking an image (clicking start) **each time**

Seriously the smaller the interval the better because the more pictures you have for a stack the more in focus and clear the completed stacked image will be.

I personally like use the lens screen to check how little I can move the lens for it to change

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In this example, I have rotated the lens 3 times, using the upside-down 1 ¼ as reference. In an image it looks like it hasn't even moved, but in reality, it is shifting the depth of field in small increments as I progress downwards.

3.Continue imaging and rotating in small increments until the last part is in focus (the bottom of the box)
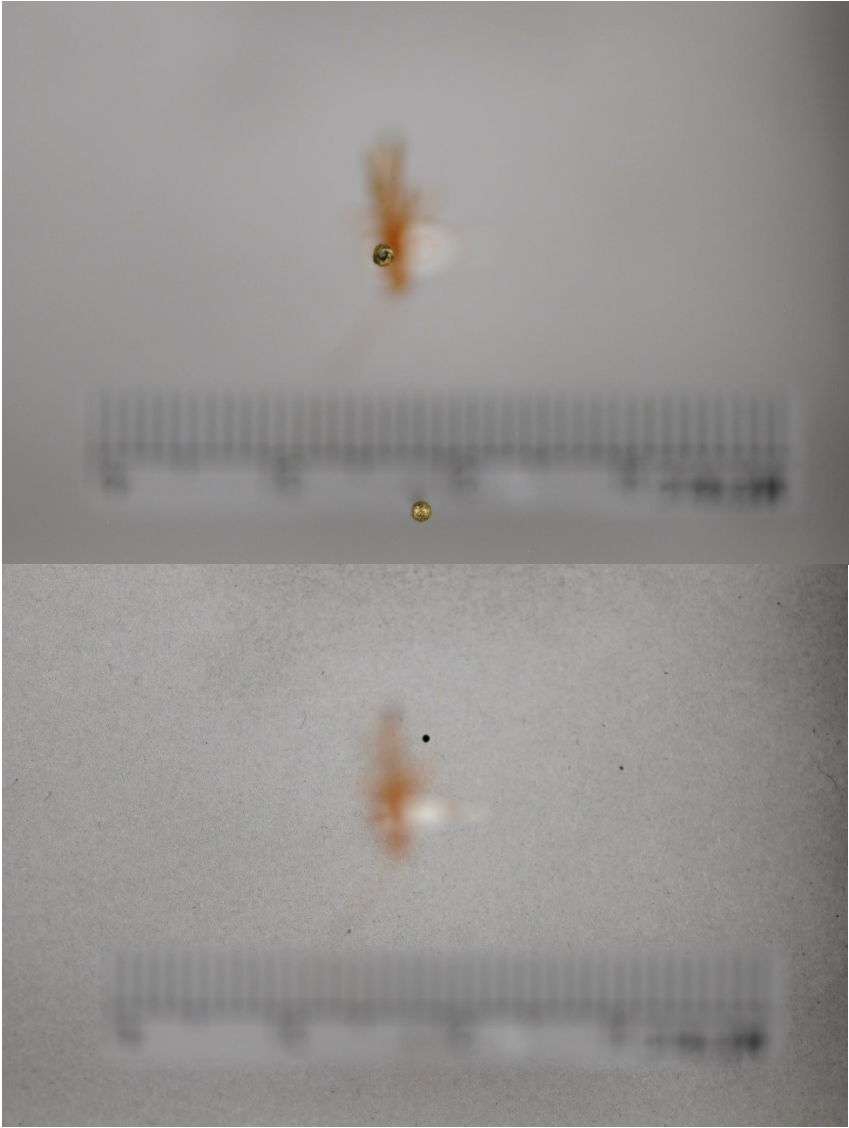
For lateral:
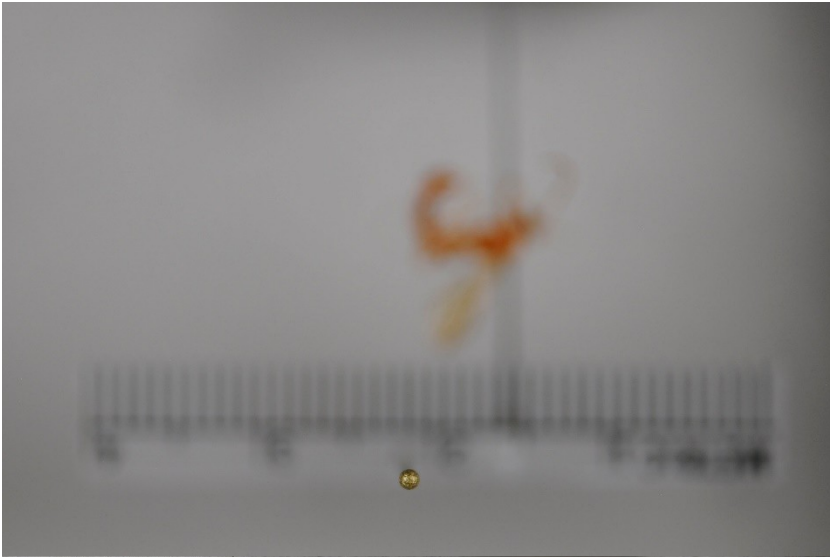
The first picture, starting from the highest depth of field (the pin point)
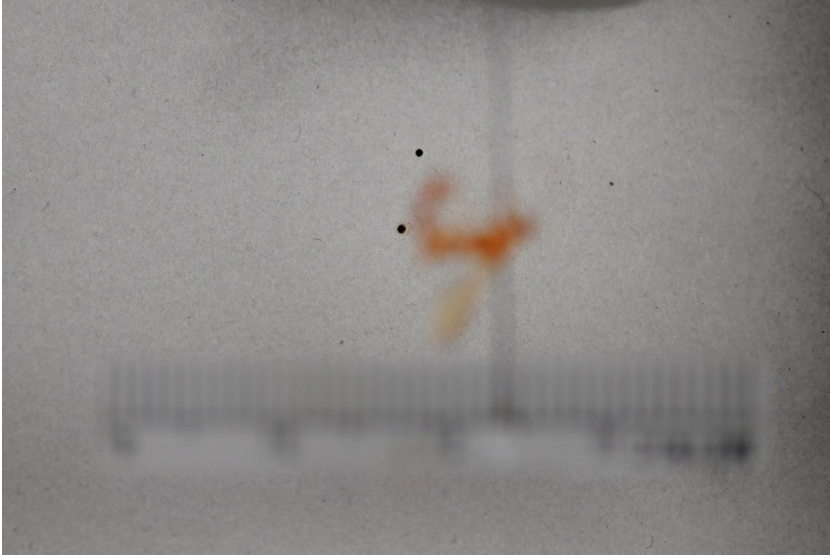
The last picture, ending at the lowest depth of field (the bottom of the box)

For dorsal:

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The first picture, starting from the highest depth of field (the pin point)

The last picture, ending at the lowest depth of field (the bottom of the box)
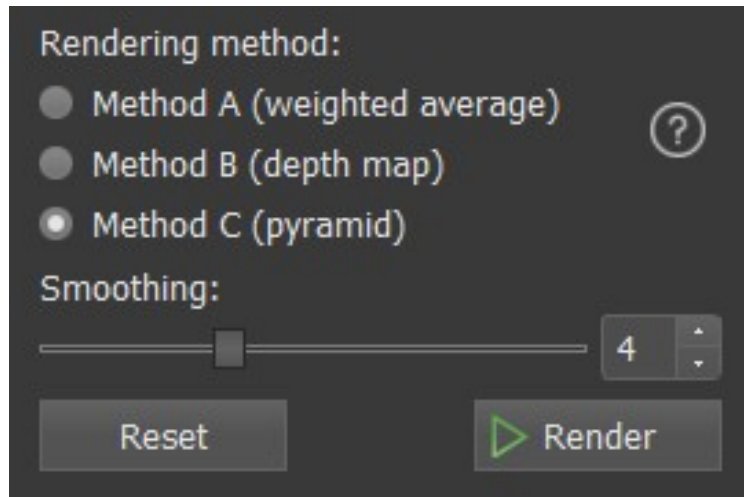
Once you've made your way through, you will have a series of images saved that look like this...

D. How to Stack
1. Open Helicon Focus 7
This window will pop up...
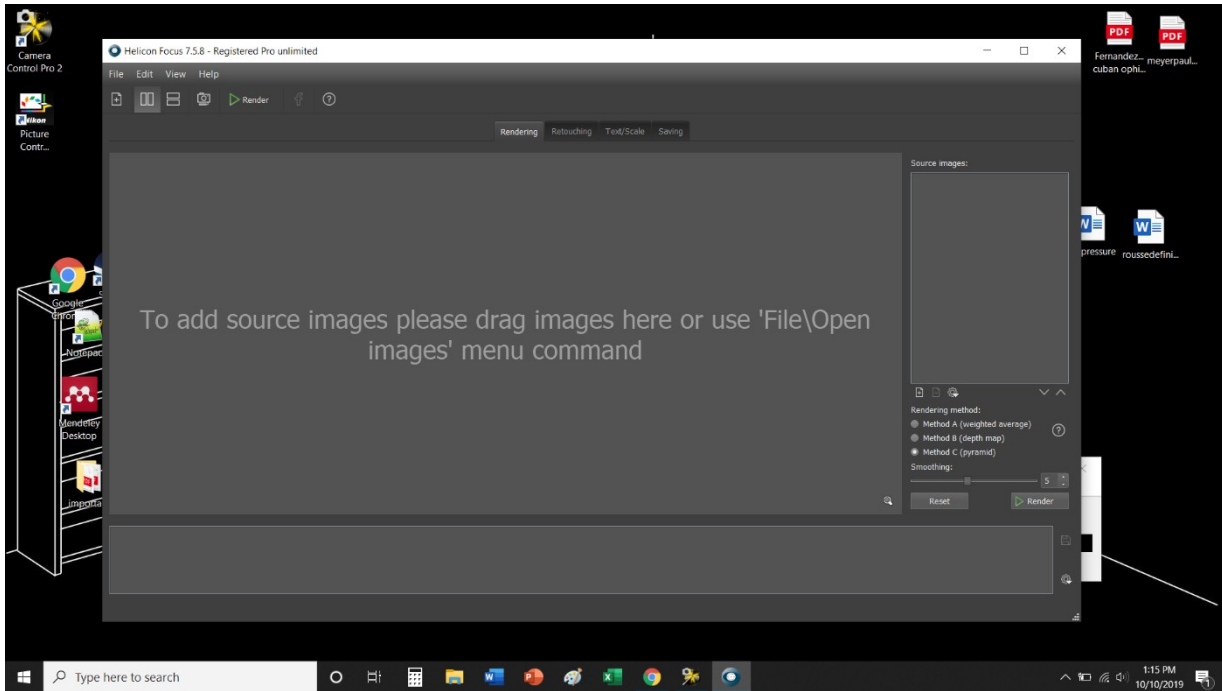
For the settings make sure it is set to Method C(pyramid) and a smoothing of 4

2. Click on the File tab in the top to display the dropdown and click on the first option "Open Images"

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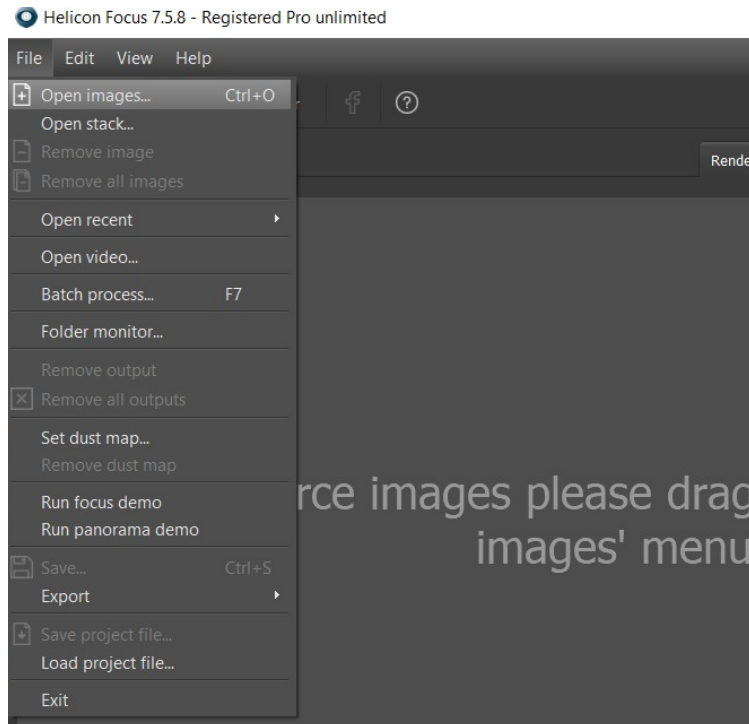
3.Highlight all of the images to be stacked then click "open" to load them in
Do one position, one specimen at a time (in this case dorsal for 1 specimen)
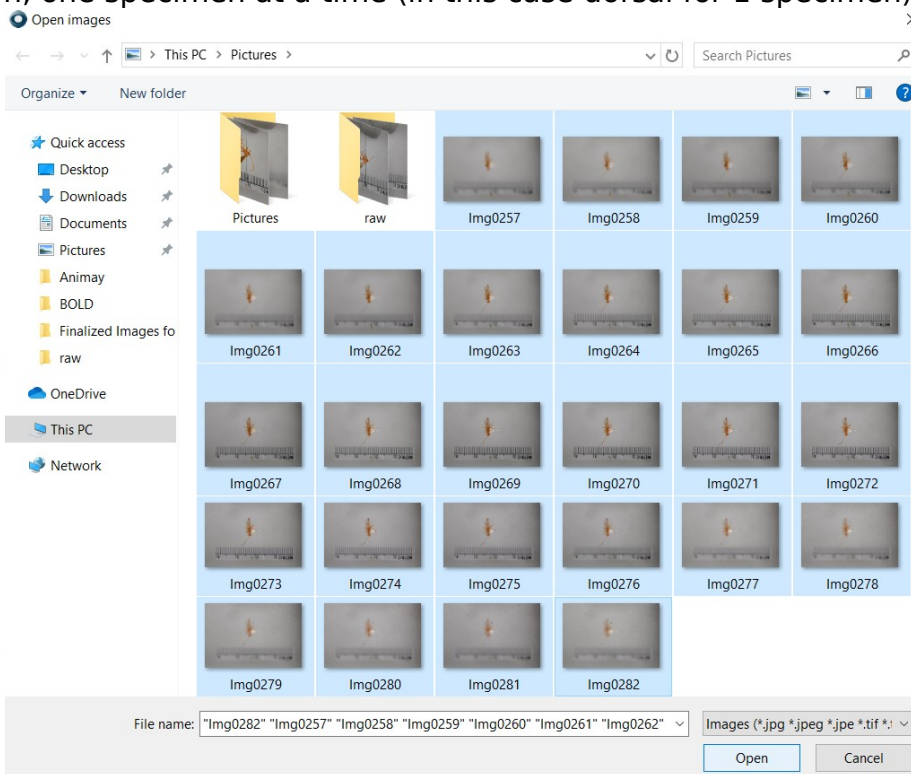
Once you load in the images, they will all appear in the right-hand source image window, and the first image will be displayed in the large window.

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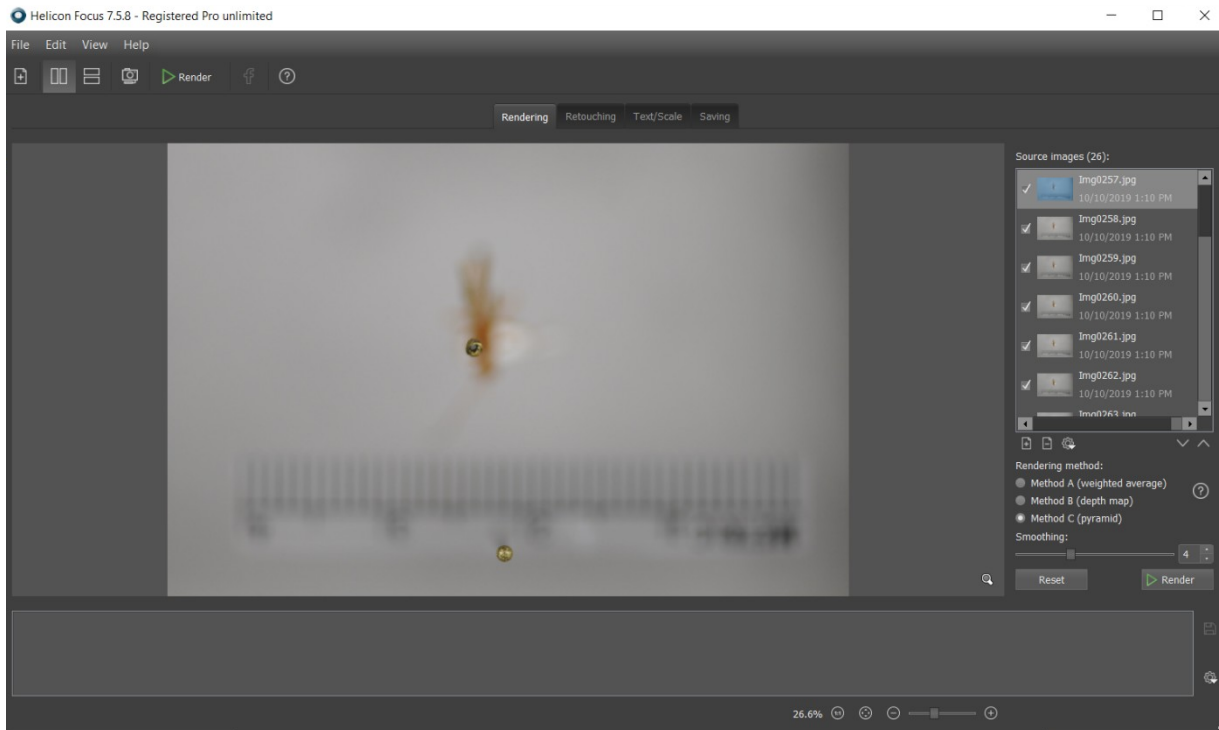
4.Stack the images

Make sure all the images you want to use are highlighted in the source image window (should be automatically) and then click <u>Render</u> in the bottom right
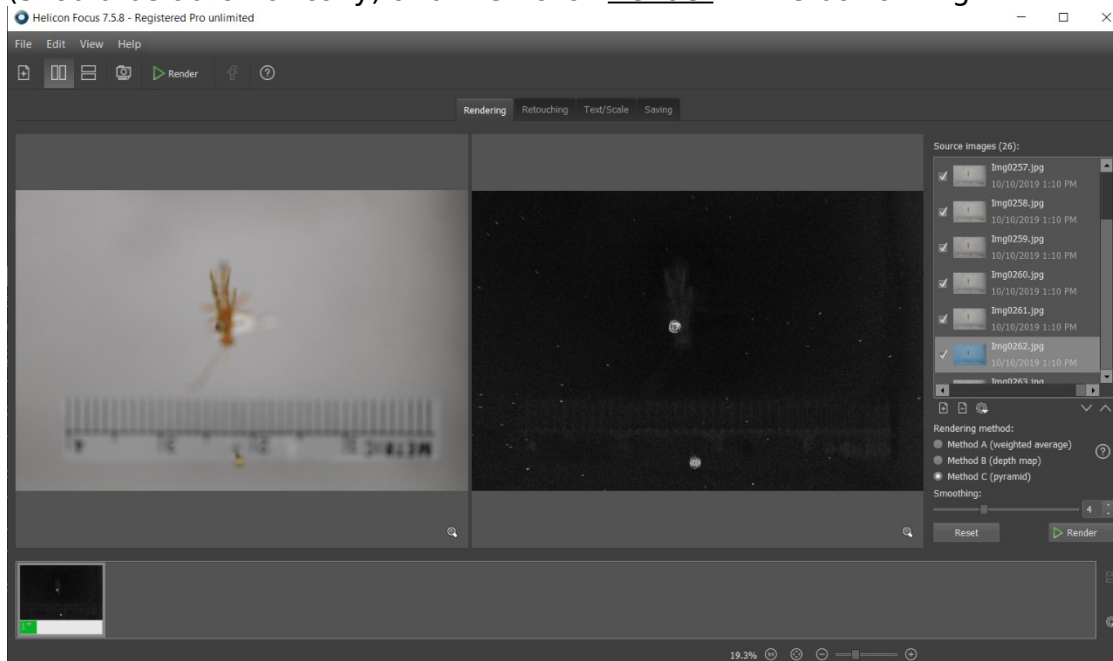

The program will go through each source image at a time (on the left) and then add them on top of eachother on the right

When the stacking is done, your final stacked image will be displayed on the right and also in the tray below...

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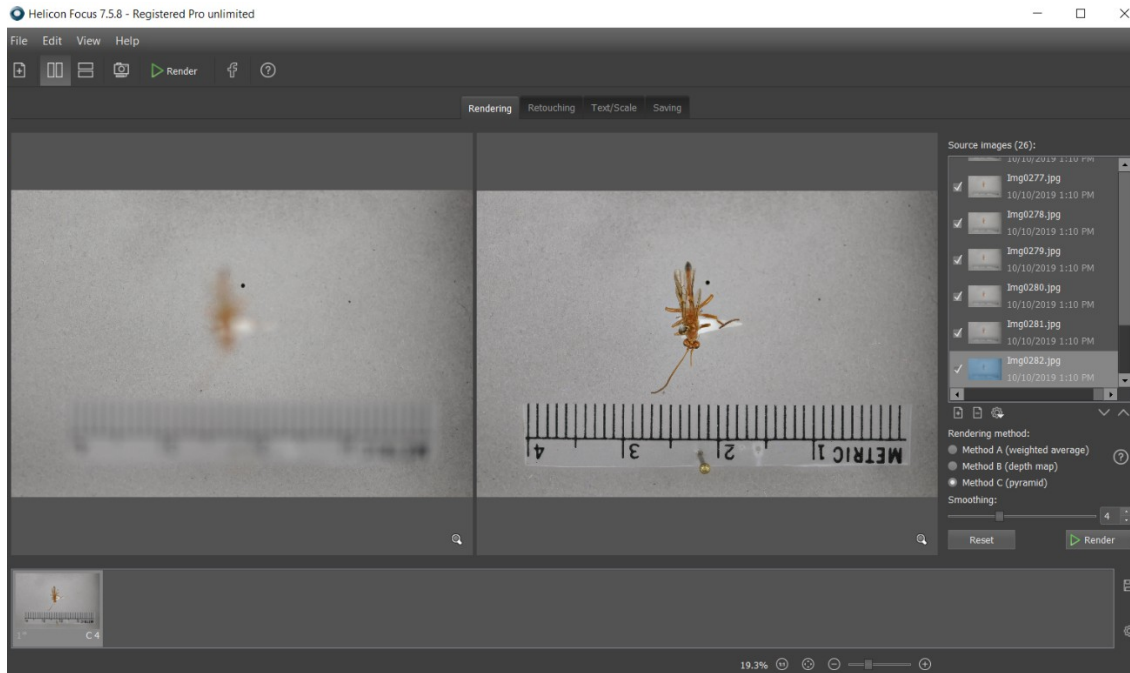
If the image is not clear enough or doesn't meet the standard of what is needed you will have to start from scratch and try again with the imaging (will happen often)

NOTE: Make sure your images are in order when they are uploaded into Helicon and also not to mix dorsal/lateral or diff specimens or else you will end up with an abomination like this...

5. Save the stacked image

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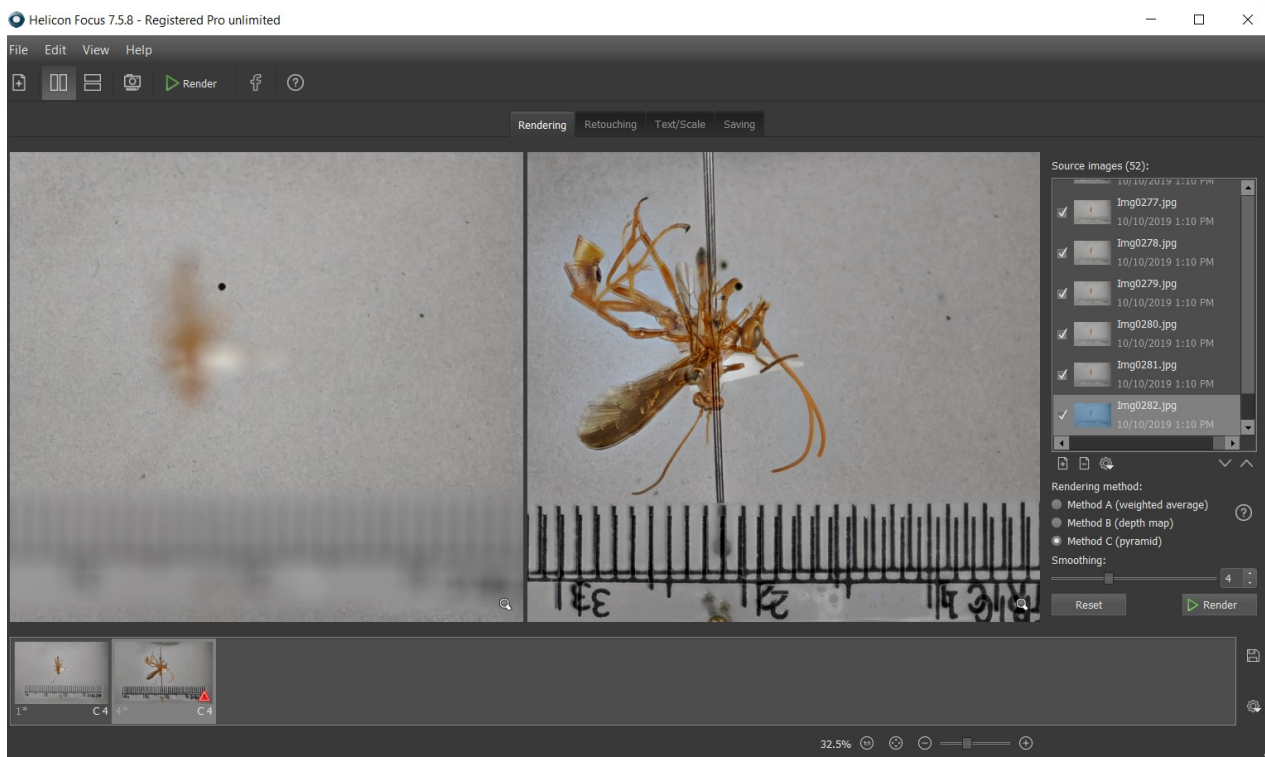
Right click on the desired image in the bottom tray and click the option "save"
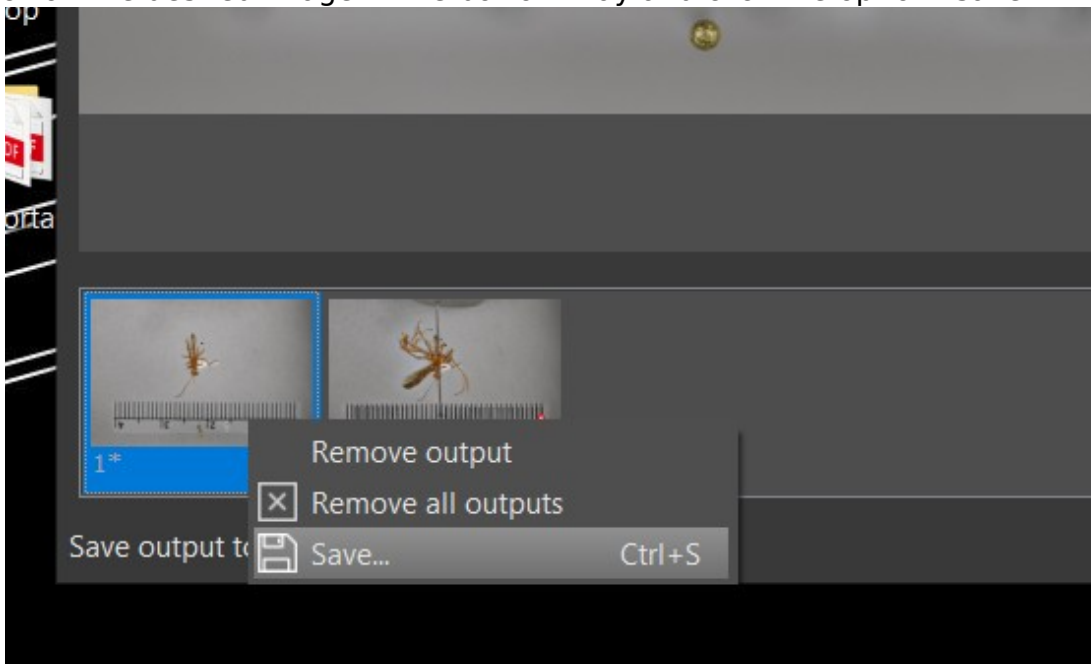
The default name of the stacked images that Helicon assigns are awful so we will rename each image
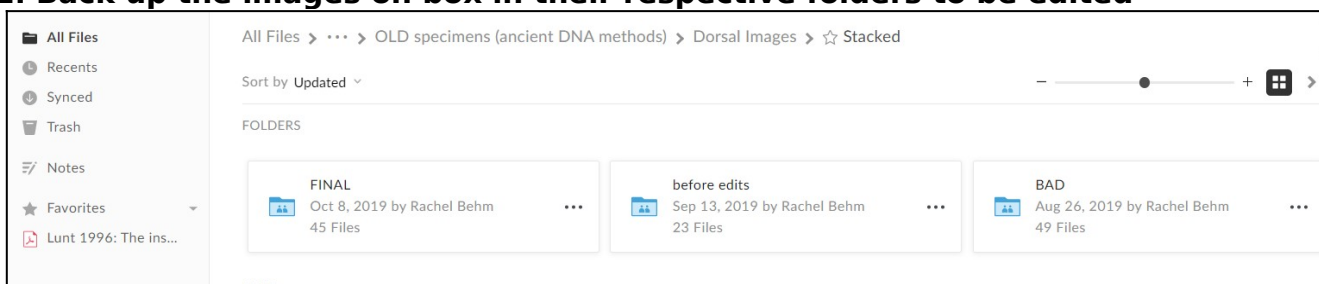

The new name will combine the barcode# of the specimen along with whether it was lateral (lat) or dorsal (dors) so that they will be easy to find and organize
(This can vary based on the project)
<u>Example:</u>

CLEAN UP

1. Back up the images on box in their respective folders to be edited

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2. Turn off lights and unplug camera from computer

3. Put away specimens to their respective place

4. Remove camera from tripod, by holding camera securely with one hand and lifting the large lever on the right with the other (make sure the latch on the left side is in the upwards position)

5. Remove lens from camera
Place camera on a safe flat surface

With <u>one </u>hand, press the hemispherical button next to where the lens attaches to the camera to unlock the lens

With the other hand, firmly grip the lens and gently rotate and lift it off of the camera

6. Put the protective lids back on each piece

<u>The lens:</u>

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The camera:

7. Gently place the lens into the case and return it to the cabinet in the key room

Remember to zip it completely so the lens is safe and secure

Make sure to sign back in the lens before locking up the cabinet and returning the key.

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