DEC 01, 2022

WORKS FOR ME 1

Image processing to generate first-level product

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.8epv5z5b6v1b/v1

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ABSTRACT

Details could be found in the Supplementary Information of "A high-throughput multispectral imaging system for museum specimens"

DOI

dx.doi.org/10.17504/protocols.io.8epv5z5b6v1b/v1

PROTOCOL CITATION

Wei-Ping Chan 2022. Image processing to generate first-level product. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5z5b6v1b/v1

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CREATED

Jul 14, 2021

LAST MODIFIED

Dec 01, 2022

PROTOCOL INTEGER ID

51553

Img Synchronization

1 Setting up rclone

rclone is a cloud file-manager operable at the command-line level which is used here to copy image files uploaded to and stored on Google Drive to a directory on a computing cluster. Once uploaded to the cluster, these image files may then be used in downstream high-throughput analyses.

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1.1 Setting up the connection to the Cloud Drive by following [the official rclone documentation]

1.2 rclone works under a CenOS6 environment. Load the environment for rclone before loading the rclone module (see step 1.3)

Command

rcolne works under this environment (centos 6)

```
module load centos6/0.0.1-fasrc01
```

(Note that this is s step implemented specifically for the cluster at Harvard since rclone is available on the CentOS6 environment)

1.3

Command

load rclone (centos 6)

```
module load rclone
```

2 Synchronizing images from the cloud drive. A cron job launching this command can also be established to monitor the cloud drive regularly so the newly uploaded images will be synched to the target directory automatically. Note that if the token for the cloud drive is password-protected, the interactive bash mode may be more secure as the cron job would then require you to embed your password in the script.

Command

Synchronizing all taken drawer images to the cluster (linux)

```
srun --pty -p PARTITION_NAME -t TIME --mem 20000 rclone --transfers=8 --checkers=16 -c copy
GOOGLE_DRIVE_NAME THE_DIRECTORY_ON_THE_CLUSTER
```

(All variables in CAPITAL LETTERS should be specified by the user)
GOOGLE_DRIVE_NAME: The name and directory of the linked Google drive
THE_DIRECTORY_ON_THE_CLUSTER: The destination where the images go to

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3 Preprocess the images

3.1 Prepare the corresponding specimen label file

A	B	C	D	E	F	G
Drawer_name	1	2	3	4	6	...
Drawer_1	### [Specimer	###	###	###	###	...
Drawer_2	###	###	###	###	###	...
Drawer_3	###	###	###	###	###	...

Generalized structure of the specimen label file. It is essential that the file be formatted according to the specifications above, as downstream programs will use the values in the Drawer_name column to name drawer batch image files and the specimen IDs (denoted as ### above) to match with and name specimen images. If specimens are mislabeled in this CSV label file, they will also be misidentified in downstream analyses.

	A	B	C	D	E	F	G	H
1	Drawer_name	1	2	3	4	5	6	7
2	EU_1-1	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069
3	EU_1-2	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069
4	EU_1-3	MCZ-ENT00192	MCZ-ENT00192	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00073
5	EU_1-4	MCZ-ENT00071	MCZ-ENT00071	MCZ-ENT00071	MCZ-ENT00071	MCZ-ENT00183	MCZ-ENT00071	MCZ-ENT00183
6	EU_2-1	MCZ-ENT00073	MCZ-ENT00072	MCZ-ENT00072	MCZ-ENT00072	MCZ-ENT00072	MCZ-ENT00072	MCZ-ENT00072
7	EU_2-2	MCZ-ENT00183	MCZ-ENT00183	MCZ-ENT00072	MCZ-ENT00072	MCZ-ENT00072	MCZ-ENT00072	MCZ-ENT00072
8	EU_2-3	MCZ-ENT00073	MCZ-ENT00073	MCZ-ENT00073	MCZ-ENT00073	MCZ-ENT00050	MCZ-ENT00050	MCZ-ENT00071
9	EU_2-4	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00069	MCZ-ENT00073	MCZ-ENT00073	MCZ-ENT00073

Example of a specimen label CSV file filled according to the formatting specifications above. Note: green and purple arrows correspond to the ordering and orientation of specimens in drawer images (see below).

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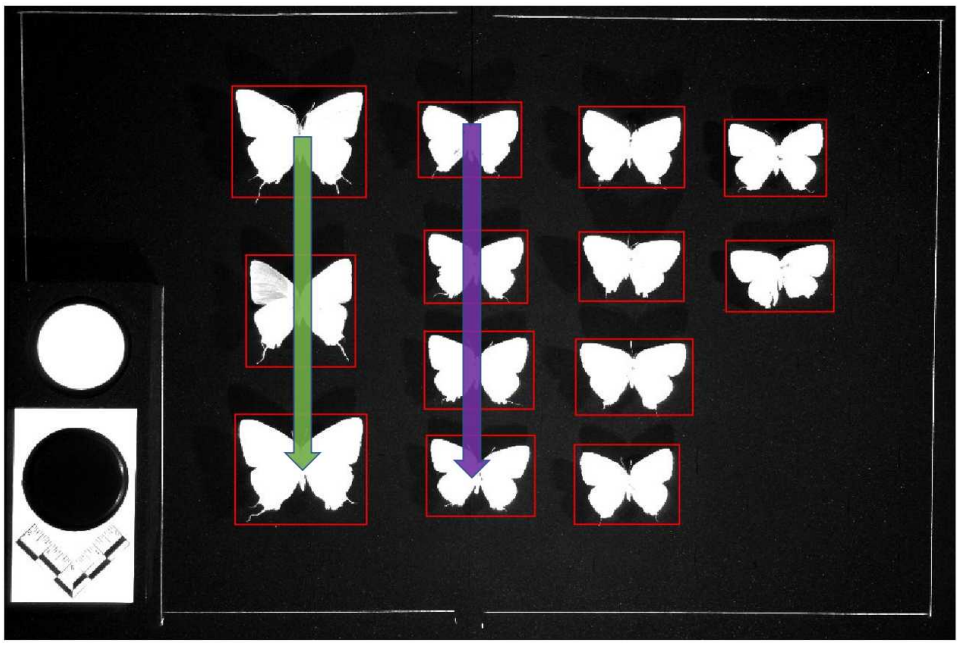
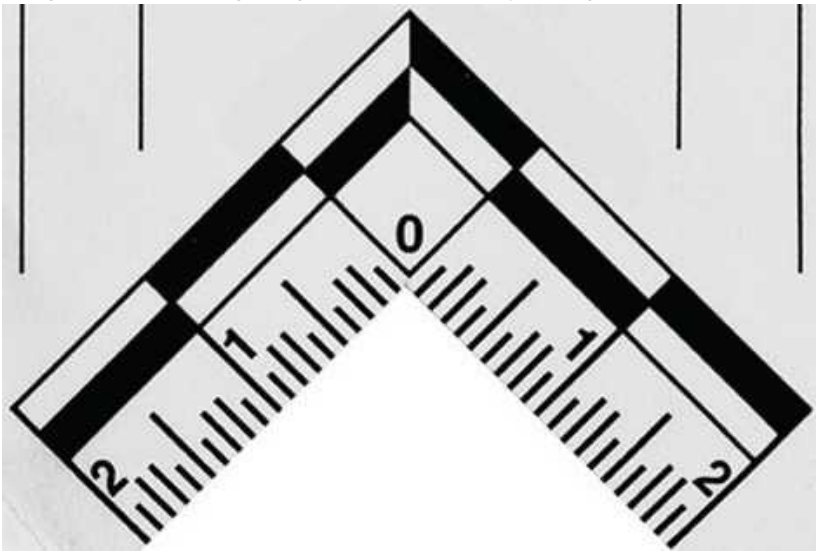


Starting in the top left-hand corner and moving top-to-bottom and column-wise from left-to-right across the imaging stage (see diagram above), specimens are logged accordingly into the specimen label spreadsheet within the corresponding image row (see green and purple arrows in the table and image above).

3.2 Download the MatLab scripts from the GitHub repository to the **MatLab code library.** Drop a clear image of the scale bar into the provided **MatLab code library** so it can be used to identify the scale in the images. The following image is the one corresponding to the scale used in our imaging system.



Adhesive forensic evidence labels (2 CM measure; A-6243) link

3.3 Download the suitable dcraw here and put it into a directory on the compute cluster.

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For **Linux** and **Unix** systems, downloading and compiling the "dcraw.c" file is suggested.

Command

compile dcraw.c

```
gcc -o dcraw -O4 dcraw.c -lm -DNODEPS
```

The job of running dcraw is integrated in the shell script in 3.7

For **Mac** or **Windows**, downloading the precompiled version is suggested.
If the user wants to process the image through dcraw, the following command can generate linearized 16bit tiff images as the default input formats for our Matlab scripts.

Command

run dcraw

```
cd NEF_IMG_DIRECTORY
DCRAW_DIRECTORY/dcraw -4 -T -w -o 0 -v *
```

DCRAW_DIRECTORY: directory containing dcraw program files
NEF_IMG_DIRECTORY: directory containing input raw .nef images for processing by dcraw

3.4 Reset all corresponding file/directory permissions for the user who is going to launch job scripts in downstream imaging processing steps.

3.5 Check all required MATLAB 2021a software and toolboxes have been installed on the cluster:

- Mapping Toolbox
- Image Processing Toolbox
- Statistics and Machine Learning Toolbox
- Curve Fitting Toolbox
- Computer Vision Toolbox

3.6 Copy all MatLab scripts provided into a directory designated the **MATLAB_CODE_LIBRARY**
(These scripts do not include the Toolbox listed above)

3.7 All-in-one Image Pre-processing

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Command

all_in_one_img_analysis.sh linearizes reflectance of all channels in the input images and saves them in 16-bit .tiff format (a rarely used format for image analysis). batch_img_analysis.sh will be launched automatically after this process finishes.

```
sbatch all_in_one_img_analysis.sh DCRAW_DIRECTORY NEF_IMG_DIRECTORY TIFF_IMG_DIRECTORY MATLAB_CODE_LIBRARY RESULT_DIRECTORY CORRESPONDING_LABEL_CSV LEGACY_OR_NOT
```

(All variables in CAPITAL LETTERS should be specified by the user)
DCRAW_DIRECTORY: directory containing dcraw programs files
NEF_IMG_DIRECTORY: directory containing input raw .nef images for processing by dcraw
TIFF_IMG_DIRECTORY: directory where linearized output .tiff image files will be saved (one .tiff file will be created for each corresponding raw .nef image input). Once fully processed by the script above, .tiff images will automatically be moved into a subdirectory "done."
MATLAB_CODE_LIBRARY: directory containing MatLab program files
RESULT_DIRECTORY: directory in which another directory "Drawer_result" will be created (architecture shown below). Processed .jpeg images of drawers and individual specimens -- organized drawer-by-drawer into individual sub-directories -- will be saved in "Drawer_result." In addition to these processed outputs, "Drawer_result" will be populated with 4 additional directories "drawer_inspection," "spp_inspection," "spp_matrices," and "log_history."

- drawer_inspection: contains .jpg drawer images (naming convention: DRAWER_NAME_drawerSpecimenBoxes.jpg) for manual error-inspection of boxes drawn by the MatLab algorithm around individual specimens
- spp_inspection: contains 940nm band .jpg images of each specimen (naming convention: SPECIMEN_ID_940_demo.jpg) as cropped out of drawer batch images by the MatLab algorithm. These are used for manual inspection of mask-drawing around each specimen outline, as 940nm band images provide the most contrast for accurate quality inspection.
- spp_matrices: .mat MatLab matrices for each processed specimen.
- log_history: Log output files for each drawer image processed by the job script. (a legacy folder that can be activated if the user would like to)

(below) Architecture of the RESULT_DIRECTORY

```
RESULT_DIRECTORY
├── Drawer_result
│   ├── drawer_inspection
│   ├── log_history
│   ├── spp_inspection
│   ├── spp_matrices
│   ├── Drawer_1_dorsal
│   ├── Drawer_1_ventral
│   ├── Drawer_2_dorsal
│   ├── Drawer_2_ventral
│   ├── ...
|_
```


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CORRESPONDING_LABEL_CSV: Label file (format outlined above, see **3.1**)
LEGACY_OR_NOT: Legacy denotes those images with brighter backgrounds and shadows. Legacy 0 represents NO, 1 represents YES. (i.e If the background is super dark without small patches other than specimens, we say it is not the legacy and specify 0. Otherwise, we specify 1 for this variable to better overcome the interference of background.)

3.8 If the all_in_one_img_analysis.sh script didn't finish properly and all images in .nef format have been successfully converted into .tiff formats (this can be checked by cross-referencing the number of images in the **TIFF_IMG_DIRECTORY**), one may simply run the script batch_img_analysis.sh

Command

batch image analysis

```
sbatch batch_img_analysis.sh TIFF_IMG_DIRECTORY MATLAB_CODE_LIBRARY RESULT_DIRECTORY LABEL_FILE_NAME LEGACY_OR_NOT
```

The definition of each variable is the same as those in the previous step (**3.7**).

4 Drawer inspection, manual correction, and rerun

4.1 Download all .jpg files in the **drawer_inspection** directory (under [**RESULT_DIRECTORY]/Drawer_result**) from the cluster to a folder on your local machine. As outlined in **3.5**, these image files should be suffixed with "_drawerSpecimenBoxes.jpg"

4.2 Manually inspect and flag drawer images for specimen boxing errors

As the algorithm used to identify and create cropping boxes around individual specimens in each drawer image is bound to make some mistakes, manual human inspection is required to identify and correct those errors before downstream image processing may continue. Below are given criteria for identifying **problematic** boundary boxing errors along with illustrative examples of common boxing errors (Fig. **1**) and an **acceptable** drawer image (Fig. **2**).

An image should be regarded **problematic** if:

- A specimen lacks a bounding box
- A box encloses more than one specimen
- A box does not completely enclose or inappropriately crops out part of a specimen

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- Multiple boxes enclose one or multiple specimens

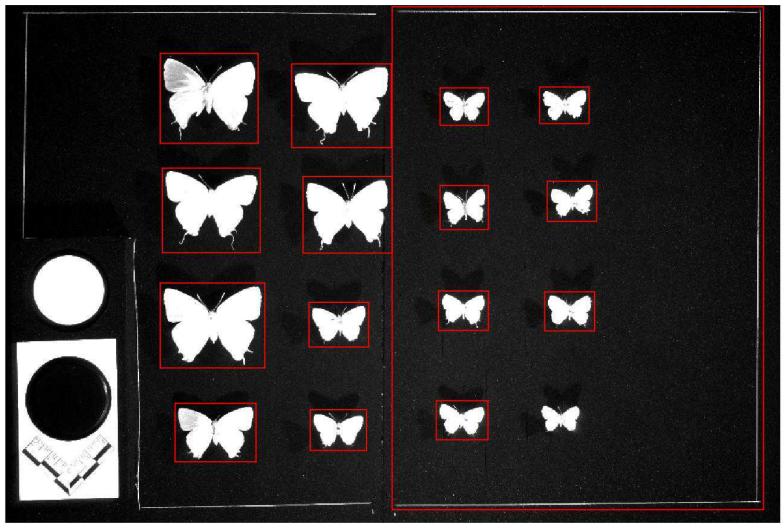

Figure 1: Problematic specimen boxing; three criteria above are met.

Figure 2: Acceptable specimen boxing; all specimens are contained within their own distinct bounding box.

We suggest either of two methods for manual inspection: one uses the file managing system native to your computer's OS to preview and sort through image files, whereas the other streamlines the process using Bridge, a file-managing interface by Adobe. Both of these methods are outlined below, the second of which (employing Adobe Bridge) is more time-efficient and highly recommended if processing many image files at once.

Create a folder titled "problematic" within that containing the .jpg files for inspection. This will be used to

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house all images with specimen boxing errors.

1. Manual drawer inspection using the **native file managing system** on your local machine:

- Previewing .jpg thumbnails as "large icons," simply navigate through all drawer images in the folder, making sure that all meet the proper criteria for successful specimen boxing (see description and examples above).
- If any drawer images are problematic, move them into the "problematic" folder

2. Manual drawer inspection using **Adobe Bridge**

- Open Adobe Bridge and navigate to the folder containing the .jpg images for manual inspection
- Using the large preview pane in Bridge, navigate sequentially through the images in the folder
- Flag any problematic drawer images using the Bridge "label" feature (keyboard shortcut: Cmd (Mac) / Ctrl (Win) + 6-9)
- Filter for and select "labeled" files only. Move labeled images to the "problematic" folder (**PROBLEMATIC_DIR**)

4.3 Generate a list of problematic image file names using any available script. The text format in the file should look the same as follows. (They are corresponding to the image name, but the tails indicating wavelength bands were removed.)

Note

Drawer-1_dorsal
Drawer-1_ventral
Drawer-2_dorsal
Drawer-2_ventral
Drawer-5_ventral
Drawer-9_ventral
(An empty row is required at the end of the list)

Note that an extra empty row is necessary in the list, or there will be some images being left over in the processing procedure. Here, we provide an example script written in Mathematica.

Note

```
inspectionDir =
  "PROBLEMATIC_DIR";
imglist = FileNames["*.jpg", inspectionDir];
problemlist =
  Join[Table[
    StringSplit[StringSplit[imglist[[n]], "\\"][[-1]],
      "_drawerSpecimenBoxes.jpg"][[1]], {n, 1 ,
     Length[imglist]}], {""}];
```


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```
outname =
 inspectionDir <> "\\" <>
  StringSplit[StringSplit[imglist[[1]], "\\"][[-3]],
   "_drawer_inspection"][[1]] <> "_problem_list.txt";
Export[outname, problemlist, "List"];
MatrixForm[problemlist]
```

- The resulting .txt file can be found in the **PROBLEMATIC_DIR**

4.4 Upload the list of problematic drawer images to the cluster and convert to UNIX system format by running dos2unix

Command

converts DOS to UNIX-type files

```
dos2unix PROBLEM_LIST.TXT
```

4.5 Prepare drawer images for manual correction

Command

A command to prepare required drawer images (.tiff) for manual correction. It also prepares the TIFF_IMG_DIRECTORY for the run after uploading the manually identified bounding boxes.

```
sbatch prepare_files_for_manual_analysis.sh PROBLEM_LIST_DIR PROBLEM_LIST 940
TIFF_IMG_DIR/done TIFF_IMG_DIR MANUAL_DRAWER_CORRECTION_DIR
```

(All variables in CAPITAL LETTERS should be specified by the user)
PROBLEM_LIST_DIR: directory containing the text file list of problematic image filenames (generated in step **4.3**)
PROBLEM_LIST: name of the list of problematic images
TIFF_IMG_DIR: directory containing the drawer images in .tiff format (output of **3.5**). These images were moved into an automatically-created folder named "done" within this directory upon completion of the all_in_one_image_analysis script.

- Note: those drawer files named in the problematic_list will be moved out of the "done" folder by this script into the directory specified here. This is to prepare for the rerun of image analysis (following manual corrections) using batch_image_analysis.sh (step **4.12**), which will rerun image analysis on all image files matching the names of those in this tiff directory (outside of the "done" folder); thus, it is essential that all files, once transferred from the "done" directory, remain untouched. All files will be retransferred to the "done" folder following completion of

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batch_image_analysis in downstream steps.

MANUAL_DRAWER_CORRECTION_DIR: cluster directory into which 940-band problematic .tiff drawer images will be copied for manual processing in later steps. Only the 940-band images are used as they provide a high degree of contrast between specimens and the dark background which ensures both ease and accuracy during the manual partitioning of specimens.

4.6 Download the following 3 components to the local machine to proceed with manual bounding box correction:

1. .tiff images found in **MANUAL_DRAWER_CORRECTION_DIR**. Note: this may take some time and requires adequate disc space as the image files are rather large.
2. Corresponding bounding box parameters in the **manual_boxes** directory, found within **MATLAB_CODE_LIBRARY**: these are MatLab matrices (.mat) prefixed with the names of drawers analyzed**.**
3. Manual bounding box correction tools from Github (https://github.com/weipingchan/Drawer_img_manual_define_bounding_boxes).

4.7 Create a folder to organize materials for manual bounding box correction on the local machine. Suggested folder architecture is provided below, and will be referenced as such in subsequent steps of this protocol. Various folders and subfolders are defined below.

```
manual_drawer_correction
├── 940_tiffs_for_correction
│   ├── done
│   ├── Drawer_1_dorsal.tiff
│   ├── Drawer_1_ventral.tiff
│   ├── Drawer_2_dorsal.tiff
│   ├── Drawer_2_ventral.tiff
│   ├── ...
├── matlab_code_library
│   ├── manual_boxes
│   ├── label_file.csv
│   ├── import_img.m
│   ├── manually_def_with_record.m
│   ├── manually_def_without_record.m
│   ├── ManuallyBoxDefine.m
│   ├── ManuallyFindCoraseOutlines2.m
│   ├── README.md
|_
```

940_tiffs_for_correction: contains .tiff files needing correction which have been downloaded from the cluster. During the correcting process, manually corrected .tiffs will be moved into the **done** subfolder also created within this local folder.
matlab_code_library: houses the following three components needed for manual correction:

1. **manual_boxes:** MatLab bounding box parameters corresponding to each downloaded .tiff file (which was copied from the cluster). Note that this folder *must* be placed in the

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matlab_code_library along with the MatLab package files in order for the manual correction protocol to run smoothly

2. **label CSV file** (as described in **3.1**)
3. **MatLab package files** for manual correction from Github

4.8 Open Matlab and run the script **ManualBoxDefine.m**

Specify paths in script header prior to running:

```
Img_directory ='path_to/940_tiffs_for_correction'; %path to .tiff-
containing directory
Code_directory='path_to/matlab_code_library'; %The path to the MATLAB
script library
Result_directory='path_to/results'; %Directory to which the results of
correction should be written
labelfileName='label_file.csv'; %The corresponding CSV file that
containing label information (see Examplar file for data structure in
section 3.1)
```

Note: requires user to have or install 'struct2dataset' package in the Statistics and Machine Learning Toolbox for MatLab

4.9 Guide to using the manual box-bounding interface:
*The program will cycle through specimens in top-to-bottom, left-to-right order (see drawer schematic in **3.1**) and ask you to confirm the bounding box of each one individually or adjust if errors are present*

- A window displaying a problematic drawer image will be opened automatically by the program
- Press the "down" arrow TWICE to confirm a specimen's bounding box and move on to the next
- If the bounding box shown for a given specimen appears problematic, press the "left" and "right" arrow keys to move through box options and select the preferred bounding box. Confirm the selection and move on by pressing TWICE the "down" arrow key
- Alternatively, drag a bounding box from a similarly-sized adjacent specimen over a specimen that appears to have been skipped (use "left" and "right" to view and select)
- Use the cursor to resize a box if needed by dragging the edges of the red outline
- Press "r" if you want to redo this entire image before it is closed
- Press 'f' if you want to finish the process or if the panel doesn't close by itself (i.e. No corresponding record found.) after defining the box for the last specimen on the stage. (Must do it right after defining the last one! [pressing twice the "down arrow" for the last one.])

4.10 Reinspect the drawers once fully manually processed (see methods outlined in **4.2**).

4.11 Reupload the altered bounding matrices files (from **manual_boxes**) from the local machine to **manual_boxes** within the **MATLAB_CODE_LIBRARY** on the cluster, overwriting those of the same name already present on the cluster. Easily identify manually-corrected matrices by sorting the files in the **manual_boxes** folder by descending time of modification.

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4.12 Run batch_image_analysis.sh to re-process manually corrected image files.

Command

batch image analysis

```
sbatch batch_img_analysis.sh TIFF_IMG_DIRECTORY MATLAB_CODE_LIBRARY RESULT_DIRECTORY LABEL_FILE_NAME LEGACY_OR_NOT
```

(All variables in CAPITAL LETTERS should be specified by the user)
see **3.7** for variable definitions.

5 Specimen outline inspection and manual correction

*This process resembles step **4** (drawer inspection and correction) in many of its steps and aspects; however, there are some salient procedural differences* that *warrant the detailed, though at times redundant, protocol description below.*

5.1 Download all .jpg files in the **spp_inspection** directory (under [**RESULT_DIRECTORY]/Drawer_result**) from the cluster to a folder on your local machine. As outlined in **3.5**, these image files should be named accordingly: "[specimen_ID]_[dorsal/ventral]_940_demo.jpg"

Note: some files generated from previously problematic drawers during the first round of batch_image_analysis will be prefixed with "TempLabel." These may be deleted post-manual drawer correction (section 4) so as not to conserve duplicates

5.2 Manually inspect and flag individual specimens for specimen outline errors using either the local machine's native image previewing system or Adobe Bridge (methods outlined in **4.2**). Move problematic images into a separate subfolder named "problematic" (**PROBLEMATIC_DIR**). For guidelines regarding how to evaluate specimen boundaries, see the Image Criteria section of the Manual for Specimen Inspection and Correction)

5.3 Generate a list of problematic specimen files to extract (using a script like that used in step **4.3** to do the same for problematic drawer files). The text format in the file should look the same as follows. (They are named according to specimen name, but the tails indicating wavelength bands were removed.)

Note

Specimen-1_dorsal
Specimen-1_ventral
Specimen-2_dorsal
Specimen-3_ventral
Specimen-5_ventral
Specimen-9_ventral
(An empty row is required at the end of the list)

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Again, note that an extra empty row is necessary at the end of the list, or there will be some images left over in the processing procedure. See below (or step **4.3**) for an example Mathematica script which will automatically generate a list of correctly formatted filenames:

```
inspectionDir =
  "PROBLEMATIC_DIR";
imglist = FileNames["*.jpg", inspectionDir];
problemlist =
  Join[Table[
    StringSplit[StringSplit[imglist[[n]], "\\"][[-1]],
      "_940_demo.jpg"][[1]], {n, 1 , Length[imglist]}], {""}];
outname =
  inspectionDir <> "\\" <>
   StringSplit[StringSplit[imglist[[1]], "\\"][[-3]],
     "_spp_inspection"][[1]] <> "_problem_list-" <>
   FileNameSplit[inspectionDir][[-1]] <> ".txt";
Export[outname, problemlist, "List"];
MatrixForm[problemlist]
```

The above Mathematica script takes one input (the path of the problematic specimen image directory; under **PROBLEMATIC_DIR**) *Note: Mathematica path hashes take the form "\\"*
The resulting problematic_spp_list.txt file will be written to the PROBLEMATIC_DIR

5.4 Transfer the list of problematic specimen images to the cluster and convert to UNIX format using the command:

```
dos2unix [list_file]
```

5.5 Create a series of directories on the cluster to organize files for manual correction as they are pulled using the list of problematic images generated in step **5.3**. Suggested directory architecture is specified below:

```
spp_manual_correction
└── spp_matrices_history
    ├── spp_matrices_remask
    └── spp_matrices_remask_tiff
```

spp_matrices_remask: MatLab matrices (.ma) specifying boundary-drawing parameters for each problematic image specified in the problematic_list.txt will be copied here (see **5.6**)
spp_matrices_remask_tiff: TIFF images of problematic specimens will be copied here (see **5.7**).

5.6 Extract specimen MatLab matrices according to the filenames specified in the list of problematic images.

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Command

copy_spp_matrices_according_to_list.sh

```
sbatch copy_spp_matrices_according_to_list.sh PROBLEM_LIST_DIR PROBLEM_LIST_NAME
SPP_MATRICES_DIR/ SPP_MATRICES_REMASK_DIR
```

(All variables in CAPITAL LETTERS should be specified by the user)
PROBLEM_LIST_DIR: directory housing the **problematic_spp_list.txt** specifying filenames to be pulled
PROBLEM_LIST_NAME: .txt file specifying the filenames of specimens whose boundaries were misdrawn by the algorithm and classified as "problematic" upon inspection (generated in step **5.3**)
SPP_MATRICES_DIR: Directory containing MatLab specimen boundary parameters for every specimen imaged. Located and created in the RESULT_DIRECTORY during all_in_one_image_analysis (see **3.5**) [note: the directory name *must* be followed by a '/' in order for the files to be correctly sourced from the folder
SPP_MATRICES_REMASK_DIR: Directory to which problematic matrices will be copied (see previous step)

5.7 Extract TIFF files for manual correction.

Command

batch_matrices_to_shp_correction_tiff.sh

```
sbatch batch_matrices_to_shp_correction_tiff.sh SPP_MATRICES_REMASK_DIR MATLAB_CODE_LIBRARY
SPP_TIFF_REMASK_DIR
```

(All variables in CAPITAL LETTERS should be specified by the user)
SPP_MATRICES_REMASK_DIR: Directory to which problematic matrices were copied (see previous two steps)
SPP_TIFF_REMASK_DIR: Directory to which problematic TIFFs corresponding to matrices in SPP_MATRICES_REMASK_DIR will be copied (see step **5.5**)

Note: problematic TIFFs for manual correction are pulled and copied by the script according to the filenames of matrices in SPP_MATRICES_REMASK_DIR (pulled in previous step). Thus, it is important that matrix files are not renamed or moved into a subdirectory prior to running the above script. Matrices will automatically be moved to an autogenerated subdirectory within SPP_MATRICES_REMASK_DIR ("done_matrices") once their corresponding TIFFs have been copied.

5.8 Use rclone to upload the **spp_manual_correction** folder from the cluster to the cloud. You can then download them to your local machine.

(Direct download from the cluster to local disk is also fine, but the large file transportation requires a stable internet connection. A secure SSH/FTP server such as Bitvise is advised for file management directly

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between the cluster and a local machine.)

5.9 Use Adobe Photoshop or GIMP to adjust the mask belonging to each problematic image. Instructions and guidelines for manual mask correction are specified in the Manual for Specimen Inspection and Correction.

5.10 Copy the images with redefined masks (*.tiff) to the cluster
Upload those .tiff files to the cloud drive and use rclone to clone them to the cluster, or -- as mentioned previously in **5.8** -- simply use SSH/FTP software to upload the .tiffs directly from your local machine.

5.11 Run batch_update_manually_remasked_spp.sh to update matrices for manually corrected images

Command

batch_update_manually_remasked_spp.sh

```
sbatch batch_update_manually_remasked_spp.sh REMASKED_TIFF_DIR
SPP_MATRICES_REMASK_DIR/done_matrices MATLAB_CODE_LIBRARY
CORRECTED_MATRICES_SAVE_DIR
```

REMASKED_TIFF_DIR: directory containing manually remasked specimen image files (.tiff)
SPP_MATRICES_REMASK_DIR/done_matrices: directory containing matrices previously extracted for remasking (see **5.7**)
CORRECTED_MATRICES_SAVE_DIR: directory to which the program should save corrected MatLab matrices

5.12 Copy all original spp_matrices under **Drawer_result** to a new directory named '**final_spp_matrices**'.

Copy updated (remasked) matrices from their directory (**CORRECTED_MATRICES_SAVE_DIR**) to **final_spp_matrices,** overwriting those outdated ones. Now we have a final specimen matrix database.

For copying files, a simple 'cp -r' command or 'rclone' should both work. If one wants to submit a bash script, the following bash script may be useful:

Command

copy specimen matrices from one directory to another

```
sbatch copy_spp_matrices_to_somewhere.sh ORIGIN DESTINATION
```

ORIGIN: the directory containing specimen matrices (*AllBandsMask.mat)
DESTINATION: the destination to which those files will be copied

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6 Manual fore- and hindwing segmentation

6.1 run batch_matrices_to_beautiful_RGB.sh to generate .jpg images for fore-hindwing segmentation.

Command

Export RGB images for each specimen matrix

```
sbatch batch_matrices_to_beautiful_RGB.sh SPP_MATRICES_DIR MATLAB_CODE_LIBRARY RESULT_DIR REMOVE_BACKGROUND IMG_EXTENSION RESOLUTION
```

SPP_MATRICES_DIR: Directory containing all finalized/manually checked and corrected specimen image matrices
MATLAB_CODE_LIBRARY: Directory containing MatLab program files
RESULT_DIR: Directory in which the output images should be placed
REMOVE_BACKGROUND: Remove background according to the mask (0: no background removal; 1: background removal; for fore-hindwing segmentation, 0 is suggested)
IMG_EXTENSION: Image format (e.g. jpg, png, pdf, etc... 'jpg' is suggested for the fore-hindwing segmentation)
RESOLUTION: Resolution of the output images. (e.g. 150 [dpi]; decent resolution [>=150] is suggested for the fore-hindwing segmentation. However, higher resolution means larger image size)

6.2 Copy newly generated .jpg images in **RESULT_DIR** from the cluster to the local disk

6.3 Set up aworking environment for fore-hindwing segmentation and begin the segmentation by following the rules listed in the tutorial document & video.

6.4 Upload the .json files in the folder named '**segmented**' to the cluster

7 Download the MatLab scripts from the GitHub repository to another **MatLab code library** to prevent potential conflict.
Run the shape analysis script to segment the specimens

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Command

Uses manually segmentation data contained in .json files as well as data compiled in specimen matrices to analyze myriad morphological features (size and shape of wings, tails, antennae etc.) of imaged specimens.

```
sbatch batch_morph_preprocessing.sh SPP_MATRICES_DIR MATLAB_CODE_LIBRARY JSON_DIR RESULT_DIR SPHINGIDAE
```

SPP_MATRICES_DIR: Directory containing all finalized/manually checked and corrected specimen image matrices (note: do not include '/' at the end of the path)
MATLAB_CODE_LIBRARY
JSON_DIR: Directory containing .json files created during manual wing segmentation. Found in folder named 'segmented' and uploaded to the cluster in step **6.4**
RESULT_DIR: Directory to which segmentation results should be written
SPHINGIDAE: (Options: 0,1) If the pre-posed-wing image is desired desperately for Sphingidae-like specimens, manually change this to 1. Please note that the resulting Sphingidae shape analyses may be very error-prone.

8 Landmarks inspection and manual correction

8.1 Download the images in **Shape_analysis/wing_segmentation** to local disk

8.2 Use Adobe Bridge to do the inspection and put problematic ones into a subfolder labeled "problematic"

8.3 Generate a list of problematic specimen names and upload the list to the cluster.

Note

Specimen-1_dorsal
Specimen-1_ventral
Specimen-2_dorsal
Specimen-3_ventral
Specimen-5_ventral
Specimen-9_ventral
(An empty row is required at the end of the list)

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See below for an example Mathematica script which will automatically generate a list of correctly formatted filenames:

Note

```
inspectionDir =
 "path_to_[problematic_folder]";
imglist = FileNames["*.jpg", inspectionDir];
problemlist =
 Join[Table[
   StringSplit[StringSplit[imglist[[n]], "\\"][[-1]],
    "_wing_segmentation.jpg"][[1]], {n, 1 , Length[imglist]}}, {""}];
outname =
 inspectionDir <> "\\" <>
  StringSplit[StringSplit[imglist[[1]], "\\"][[-3]],
   "_wing_segmentation"][[1]] <> "_problem_list.txt";
Export[outname, problemlist, "List"];
MatrixForm[problemlist]
```

8.4 Convert the PROLEM_LIST to UNIX format using the command

```
dos2unix [list_file]
```

8.5 First, run copy_files_according_to_list.sh to copy morph-seg.mat files corresponding to those specimens in the list of problematic images generated above (step **8.3**) to a new, separate directory.

Then, copy this directory containing problematic matrices (*morph-seg.mat) to the local disk.

Command

copy_files_according_to_list.sh

```
sbatch copy_files_according_to_list.sh PROBLEM_LIST_DIR PROBLEM_LIST_NAME morph-seg.mat
*/Shape_analysis/wing_shape_matrices-seg/ DESTINATION_DIRECTORY
```

PROBLEM_LIST_DIR: directory in which the list of problematic specimens resides on the cluster
PROBLEM_LIST_NAME: name of the .txt file listing all problematic specimens
MORPH-SEG_DIR: Directory containing all morph-seg.mat matrices, which describing detailed segmentation information. It's usually in the following path '*/Shape_analysis/wing_shape_matrices-seg/'
DESTINATION_DIRECTORY

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8.6 Copy json files (*.json) to the local disk.

(Note: These files may already be in a directory on your local disk if using the same machine used to manually segment specimens -- see Step **6.3**)

8.7 Download the required script here, and check if all necessary MatLab toolboxes are installed properly according to the list below:

required_toolboxes.txt

8.8 Open MatLab and open the script named: manually_calculate_morph.m
Specify the required arguments and paths in the heading and run the script.

Arguments & paths for specification [in the script header]:

```
Code_directory=''; %Directory in which MATLAB morphometric segmentation
scripts live
morph_mat_directory=''; %Directory of morph-seg.mat files pulled for
problematic images; It can also be the dir of allBandsMask.mat
spp_json_directory=''; %Directory of JSON files created during manual wing
segmentation process
Result_directory=''; %Directory to which you would like to write shape
analysis results
addpath(genpath(Code_directory)) %Add the library to the path
SphingidaeOrNot=0; %If the pre-posed-wing image is desired desperately for
Sphingidae-like specimens, manually change this to 1. Please note that the
resulting Sphingidae shape analyses may be very error-prone.
antTrimPx=10; %Adjust this to extract bold antenna if necessary (20-30 for
big antenna; 5-10 for thin antenna; <5 for tiny specimens)
jumboAntenna=0; % set to 1 for the jumbo antenna which have multiple forks
(jumbo mode will dilate a little to remove those forks)
```

8.9 Manually correct shape landmarks. See the following quick guide:

Using the cursor, click and drag misidentified landmarks (red points) to the desired location.

Plot order moves in a clockwise manner around the specimen image, identifying morphometric landmarks in the following order:

1. Left corner dividing Fore & Hing Wings
2. Left corner dividing Forewing & Body
3. Right corner dividing Forewing & Body
4. Right corner dividing Fore & Hing Wings
5. Right corner dividing Hindwing & Body
6. Left corner dividing Hindwing & Body
7. Left forewing tip

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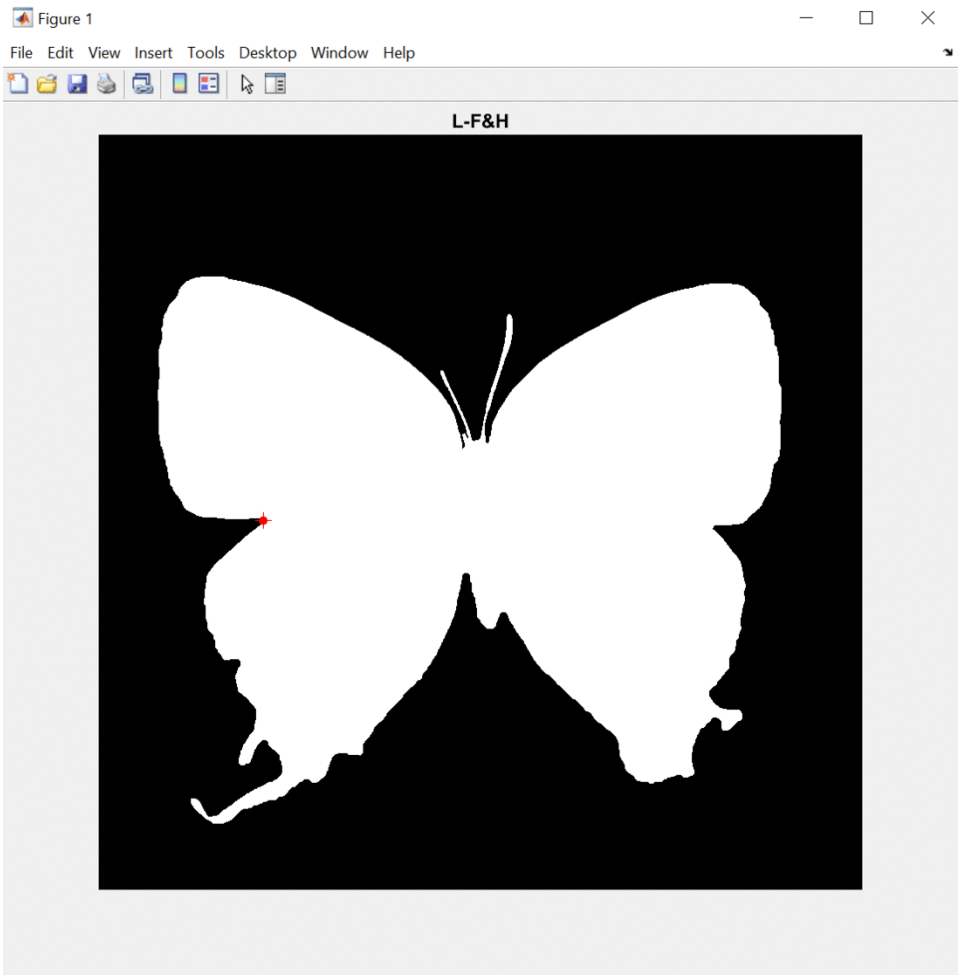
8. Right forewing tip



Workflow and clockwise ordering of landmark point correction within the application for manual correction.

Press ENTER to confirm using the point shown on the screen. This will also move the correction process forward to the next landmark.
Press 'r' to redo the manual process
Press 'f' to force-exit the entire manual process

Note: once ENTER is pressed, the point will move to the nearest detected white/black edge boundary; for precise and rapid point placement, merely re-position landmark points just close enough to the desired location to enable the program to automatically detect the correct and nearest edge at which to place the landmark.

8.10 Copy the output folder (***\Shape_analysis**) to the cluster

8.11 Update the original shape results with manually corrected ones.

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Command

update_shape_analysis_folder.sh

```
sbatch update_shape_analysis_folder.sh UPLOADED_SHAPE_ANALYSIS_DIR
ORIGINAL_SHAPE_ANALYSIS_DIR
```

UPLOADED_SHAPE_ANALYSIS_DIR: shape analysis folder containing manually corrected results
ORIGINAL_SHAPE_ANALYSIS_DIR: shape analysis folder generated in **7**

9 For all images, matrices, and results, change owner or open permission for group members

Command

Change directory & file permissions

```
chmod -R 754 */Shape_analysis
```


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