Apoidea (Bee) Types Digitisation workflow

About the collection

The Apoidea (Bee) Types project regards the digitisation of the synoptic collection of bee type specimens. The collection consists of two parts: a) series 17a and b) series 17b. The main distinction between the two series is based on the taxonomic family the specimens belong to, with all the specimens of the *Apidae* family in series 17b and the rest of the families in series 17a. Each type specimen has a unique number, the Primary Type Number (PTN), based on the position of the specimen in the synoptic collection. A relatively small number of specimens already have a specimen record in EMu, either with a NHMUK barcode or a BMNHE number, but the majority of specimens do not have a record.

Prior to imaging the specimens, the collection was prepared by assigning the required IRN tags to the respective specimens. Each tag contains three types of information: a) the TAX IRN, b) the LOC IRN (that is the original location of the specimen in the main collections, not in the synoptic collection), and c) the PTN. The specimens that were identified to have specimen records were given tags with the TAX IRN barcodes removed. This way the original taxonomy assigned to the specimen record will not be overwritten when the EMu ingest takes place.

The imaging tasks

Each specimen has to be imaged in three ways (if possible). We need:

a) A dorsal view of the specimen with the labels on the side,
b) A lateral view of the specimen, and
c) A close up image of the face.

We additionally image the reverse of labels if data are present.

As a result of the variety of images required, the imaging of the specimens is split in three parts:

A. Imaging the dorsal (all specimens) and lateral view of the specimens (specimens ≥1cm).
B. Face shots.
C. Close up lateral images for specimens ≤1cm.

As the specimens are types, it was thought necessary to create good quality images and therefore image the specimens by stacking.

Important:
Each part has different camera settings and different image processing requirements to follow.

Part A1: Dorsal and Lateral views: Imaging

Settings

Additional camera equipment: Stackshot, 100 or 90 mm lens.

Camera settings.
Shutter speed: 1/60
Aperture: f/7.1
ISO: 200

Software programs: Helicon Remote.

Helicon Remote Software preferences.
Small step: 1
Medium step: 5
Big step: 10

Size of focusing steps: 400 microsteps (0.2 mm)

Output folder: C:\SBILPictures\SlideDigitisation\processing\1_Helicon Remote\1_Processing

Imaging (step-by-step)

1. Turn on stack shot.
2. Turn on the camera.
3. Open Helicon Remote.
4. Check that you have the correct camera settings.
5. Use the bee type tray for imaging. Pick a specimen, remove its labels and place it on the straw for the dorsal image. The labels should be placed in the correct order on the stage next to the specimen.
6. Check if a specimen already has a barcode or if it has a BMNHE number. If it does, see in '*Specimens with a record'* further down for what to do in that case. If it doesn't, add an NHMUK barcode.
7. Place IRN tag above the labels, leaving a bit of space in between, with the 3 barcodes fully visible. The rest of the tag is not necessary to be in the picture. The tag is going to be cropped out after renaming the image (in *Processing*).
8. Set points A and B and desired interval (<5 focusing steps). Start Shooting.
9. Check for data on the reverse of the labels. If so, take an additional dorsal image with the reverse labels.

10. If the specimen is larger than 1cm (>>1), place the specimen on the other side of the stage for the lateral image.
11. Bring the camera as close as possible, but with the barcode visible.
12. Set points A and B and desired interval (5 focusing steps). Start Shooting.

If the specimen is smaller than 1cm (<<1cm), skip imaging the lateral view and see in part B how to image additional close up images.

The images are saved in separate subfolders in the folder *'1_Processing'* (folder path: C:\SBILPictures\processing\1_Helicon Remote\1_Processing)

Part A2: Dorsal and Lateral views: Image Processing

Settings

Software programs:
Helicon Focus, BardecodeFiler, command prompt, Bulk Rename Utility, Lightroom.

Helicon Focus Software preferences.
Rendering method: Method C (Pyramid)
Smoothing: Level 4

Output folder: C:\SBILPictures\SlideDigitisation\processing\BardecodeFiler\input

Summary

After imaging the dorsal and lateral views, the images have to be rendered (Helicon Focus) and renamed (BardecodeFiler). Then we need to generate a filelist for the dorsal images (command prompt) in order to associate each UID with the correct PTN. After this is done, we remove the PTN from the name of the image (Bulk Rename Utility) and we crop the images to remove the IRN tags and the dead space (Lightroom). After we perform the quality checks, we move the images to the final folders and we copy them to the EMu shared drives/DCP unified. Finally, we delete any raw copies ("Helicon Remote/1_Processing", "original_processed", Lightroom import folders).

This is the workflow for rendering and renaming the images at the same time overnight. It requires setting up the output folders of the various software to streamline that process.

Image Processing (step-by-step)

At the end of the day:

1. Turn off camera and stackshot.
2. Open BardecodeFiler. Optional: open 'input' folder, '1_Dorsals' and '3_Additionals' (Lightroom import folders).
3. Open Helicon Focus.
4. Set rendering method C (pyramid) and the smoothing level at 4.
5. Choose Batch Processing from the File menu.
6. Click on Add Multiple Folders and select all subfolders of the "1_Processing" folder.

7. Make sure they are going to be saved as JPEGs (**Quality 100%**) and that the output folder is set as the input folder of BardecodeFiler
8. (folder path: C:\SBILPictures\SlideDigitisation\processing\BardecodeFiler\input).
9. Click Render.
10. Check that the stacked images are going through BardecodeFiler and are renamed and saved properly in the correct output folders.
11. Leave to run overnight.

The next day:

1. In the morning check that the rendering has been completed and you have the expected number of images. The '1_Dorsals' folder will have the dorsal and lateral images. The '3_Additionals folder will have the reverse images.
2. In the '1_Dorsals' folder search for *__* (double underscore). This should bring up all the lateral images. Select all and move them to the '2_Laterals' folder. Check that the number of dorsal images is the same as the number of lateral images.
3. Open command prompt (cmd) and create filelist. Name filelist with the date of the day.
4. Move filelist to the 'Filelists' folder. Also copy the contents of the filelist to the bee spreadsheet, sheet 'Filelists'.
5. Open Bulk Rename Utility. Select '1_Dorsals' folder. Set to crop the image names after the dash (-). Rename images.
6. Repeat name cropping for the images of '2_Laterals'. Reverse labels do not need renaming because they are named as 'UID_additional'.
7. Open Lightroom. You may need to *Synchronise* your import folders to show your current images.
8. Crop and Export Dorsals, Laterals and Additionals separately (see Lightroom section).
9. Create final folders.
10. Move images to respective folders (dorsals to final / laterals and additionals to final_reverse).
11. Check that all images look alright.
12. Copy folders to the EMu shared drives.
13. Back up folders and filelist on the DCP unified drive.
14. Delete any raw copies ("Helicon Remote/1_Processing", "original_processed", Lightroom import folders).

Part B1: Face shots: Imaging.

Settings

Additional camera equipment: Stackshot, MPE-65 lens, ring flash.

Camera settings.
Shutter speed: 1/200
Aperture: f/5.6
ISO: 100

Software programs: Helicon Remote.

Helicon Remote Software preferences.
Small step: 1
Medium step: 10
Big step: 50

Size of focusing steps: 100 microsteps (0.05 mm)

Output folder: C:\SBILPictures\SlideDigitisation\processing\1_Helicon Remote\1_Processing

Imaging (step-by-step).

1. Turn on stack shot.
2. Turn on the camera.
3. Open Helicon Remote.
4. Check that you have the correct camera settings.
5. Use the ento-ball for imaging. Place the specimen on the ento-ball without removing its labels.
6. Position the ento-ball so the head is facing upwards. The flat part of the face has to be parallel to the camera view. Place the ento-ball under the camera.
7. Set magnification. Prefer 5x if possible.
8. Set points A and B and appropriate interval (see Guidelines for Interval).
9. Set appropriate flash intensity. Start Shooting.
10. After imaging rename the folder with the the NHMUK number of the specimen and the magnification that was used.

11. If a mid-way magnification was used, remember to take a photo of the scale bar at that magnification.
12. Proceed to the next specimen.

Part B2: Face shots: Image Processing.

Settings

Software programs:
Helicon Focus, Bulk Rename Utility, ImageJ.

Helicon Focus Software preferences.
Rendering method: Method C (Pyramid)
Smoothing: Level 4

Output folder: **Same as source file!**

Summary

After imaging the face shots, the images have to rendered (Helicon Focus) and renamed (Bulk Rename Utility). Then we may need to rotate the images and paste a scale bar according to the magnification that was used (ImageJ). After we perform the quality checks, we move all the images to the final_reverse folder and we rename them to 'UID_face' (Bulk Rename Utility). We copy the folder to the EMu shared drive/DCP unified. Finally, we delete any raw copies ("Helicon Remote/1_Processing", ImageJ import folders).

Image Processing (step-by-step)

At the end of the day:

1. Turn off camera and stackshot.
2. Open Helicon Focus.
3. Set rendering method C (pyramid) and the smoothing level at 4.
4. Choose Batch Processing from the File menu.
5. Click on Add Multiple Folders and select all subfolders of the Imaging folder.
6. Make sure they are going to be saved as JPEGs (**Quality 100%**) and that the output folder is set **the same as the source folder**.
7. Click Render.
8. Leave to run overnight.

The next day:

1. In the morning check that the rendering has been completed and you have the expected number of images. Check also that you don't have any 'fuzzy' or black images.
2. Search for 'img' in the '1_Processing' folder. This should bring all the raw images. Select all and move them to a separate folder or delete them. Now the original subfolder have only their rendered image.
3. Open Bulk Rename Utility. Rename the rendered images after their respective folder name.
4. Search for *.* in the '1_Processing' folder and select all the images. Move them to the ImageJ import folders.
5. Group images in separate subfolders according to magnification used. Check that all the images have the same orientation.
6. Open ImageJ and run the macros for pasting the scale bar on the images.
7. Check the images in the ImageJ output folder. If the scale bar is not fully visible on an image you may need to delete it and paste again the scale bar manually on a different corner of the image.
8. Create final_reverse folder move there all the images.
9. Open Bulk Rename Utility. Select all images and remove the magnification from the name. Add '_face' as a suffix.
10. Check that all images look alright.
11. Copy folder to the EMu shared drive and the DCP unified.
12. Delete any raw copies ("Helicon Remote/1_Processing", Scale bar input folders).

Part C1: Close up Laterals: Imaging.

Settings

Additional camera equipment: Stackshot, MPE-65 lens, ring flash.

Camera settings.
Shutter speed: 1/200
Aperture: f/5.6
ISO: 100

Software programs: Helicon Remote.

Helicon Remote Software preferences.
Small step: 1
Medium step: 10
Big step: 50

Size of focusing steps: 100 microsteps (0.05 mm)

Output folder: C:\SBILPictures\SlideDigitisation\processing\1_Helicon Remote\1_Processing

Imaging (step-by-step).

1. Turn on stack shot.
2. Turn on the camera.
3. Open Helicon Remote.
4. Check that you have the correct camera settings.
5. Use the ento-ball for imaging. Place the specimen on the ento-ball without removing its labels.
6. Position the ento-ball for imaging the lateral view of the specimen. Place the ento-ball under the camera.
7. Set magnification(1x - 3x). Prefer 2x if possible.
8. Set points A and B and desired interval (see Interval Guidelines).
9. Set appropriate flash intensity. Start Shooting.
10. After imaging rename the folder with the the NHMUK number of the specimen and the magnification that was used.
11. If a mid-way magnification was used, remember to take a photo of the scale bar at that magnification.
12. Proceed to the next specimen.

Part C2: Close up Laterals: Image Processing.

Settings

Software programs:
Helicon Focus, Bulk Rename Utility, ImageJ.

Helicon Focus Software preferences.
Rendering method: Method C (Pyramid)
Smoothing: Level 4

Output folder: **Same as source file!**

Summary

After imaging the laterals, the images have to rendered (Helicon Focus) and renamed (Bulk Rename Utility). Then we need to paste a scale bar according to the magnification that was used (ImageJ). After we perform the quality checks, we move the images to the final_reverse folder and we rename them to 'UID_lateral' (Bulk Rename Utility). We copy the folder to the EMu shared drive/DCP unified. Finally, we delete any raw copies ("Helicon Remote/1_Processing", ImageJ import folders).

Image Processing (step-by-step)

At the end of the day:

1. Turn off camera and stackshot.
2. Open Helicon Focus.
3. Set rendering method C (pyramid) and the smoothing level at 4.
4. Choose Batch Processing from the File menu.
5. Click on Add Multiple Folders and select all subfolders of the "1_Processing" folder.
6. Make sure they are going to be saved as JPEGs (Quality 100%) and that the output folder is set **the same as the source folder**.
7. Click Render.
8. Leave to run overnight.

The next day:

1. In the morning check that the rendering has been completed and you have the expected number of images. Check also that you don't have any 'fuzzy' or black images.

2. Search for ‘img’ in the ‘1_Processing’ folder. This should bring all the raw images. Select all and move them to a separate folder or delete them. Now the original subfolder have only their rendered image.
3. Open Bulk Rename Utility. Rename the rendered images after their respective folder name.
4. Search for *.* in the ‘1_Processing’ folder and select all the images. Move them to the ImageJ import folders.
5. Group images in separate subfolders according to magnification used. Check that all the images have the same orientation.
6. Open ImageJ and run the macros for pasting the scale bar on the images.
7. Check the images in the ImageJ output folder. If the scale bar is not fully visible on an image you may need to delete it and paste again the scale bar manually on a different corner of the image.
8. Create final_reverse folder move there all the images.
9. Open Bulk Rename Utility. Select all images and remove the magnification from the name. Add ‘_lateral’ as a suffix.
10. Check that all images look alright.
11. Copy folder to the EMu shared drive and the DCP unified.
12. Delete any raw copies (“Helicon Remote/1_Processing”, Scale bar input folders).

Software tips.

BardecodeFiler: Settings and how to set it so it renames while you render.

Import settings:
‘Primary Types _Entom NHMUK_NO memory between images_UID_LOC_TAX-PTN_REG_v2’

Set input and output folders:

Input folder: this is the usual input folder we use in our projects - no need to change
(C:\SBILPictures\SlideDigitisation\processing\BardecodeFiler\input)

Output folder: the Lightroom import folder for the dorsal images
(C:\SBILPictures\processing\Lightroom\1_Dorsals)

Exception folder: the Lightroom import folder for the additional images.
(C:\SBILPictures\processing\Lightroom\3_Additionals)

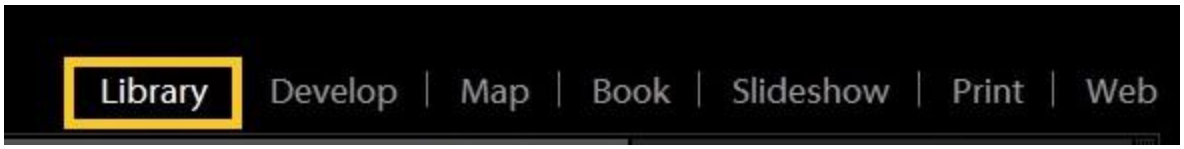
Processed folder: the usual processed folder we use in our projects - no need to change
(C:\SBILPictures\SlideDigitisation\processing\BardecodeFiler\original_processed)

Lightroom: Suggested folder structure and how to batch crop.

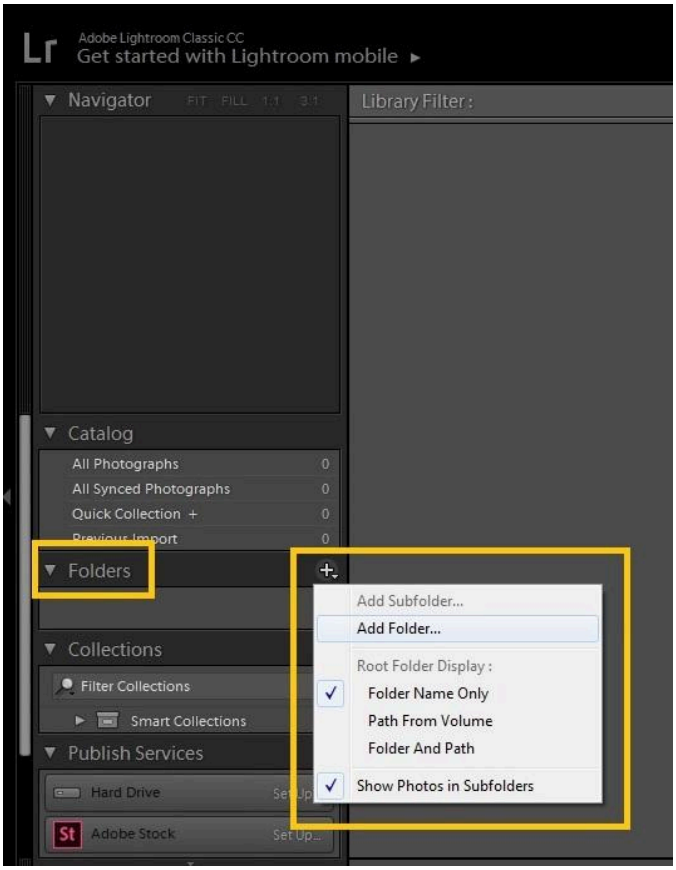
- Create folder, i.e. ‘Lightroom’ in C:\SBILPictures\processing.
- Within folder ‘Lightroom’ create subfolders ‘1_Dorsals’, ‘2_Laterals’, ‘3_Additionals’.

The first time you use Lightroom, you will have to add the folders you will be using for the import to your Lightroom Library.

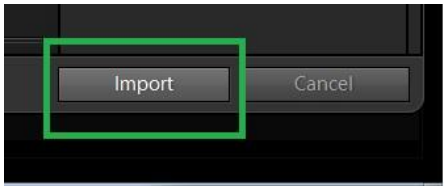
- Go to Library.



- Click on the plus button next to the Folders section.
- Select Add Folder…



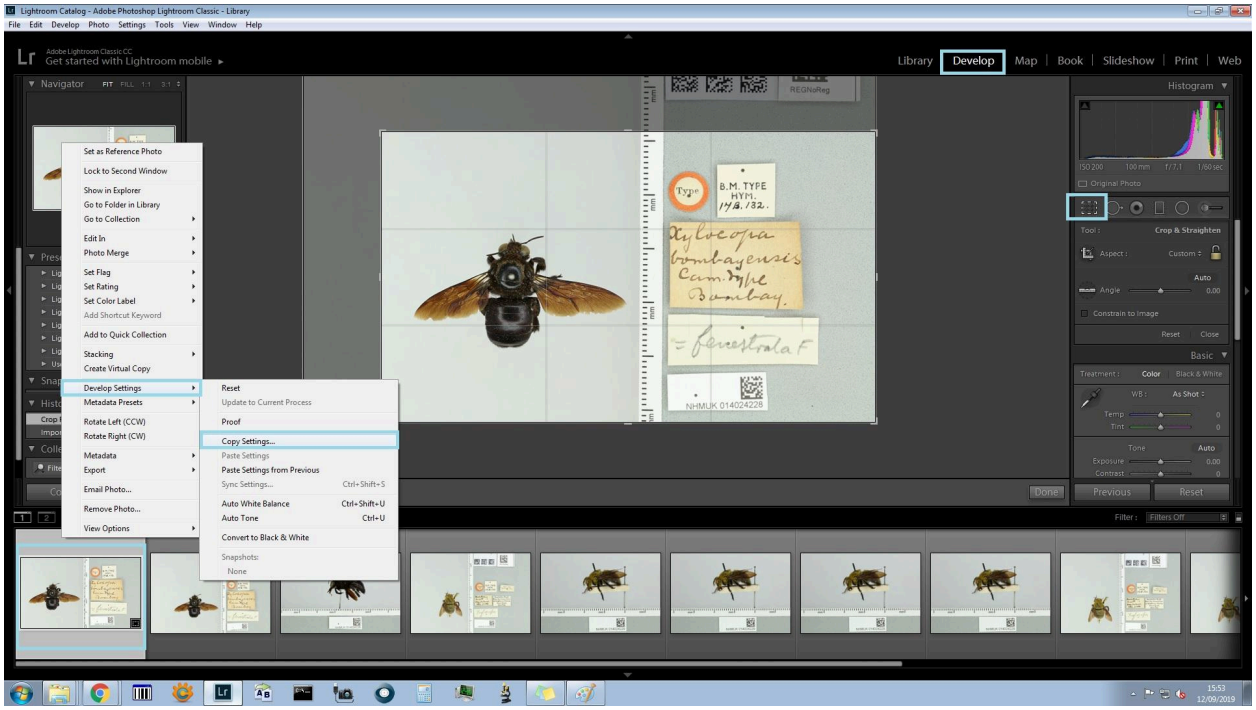
- Choose your import folder.
- Click Import.



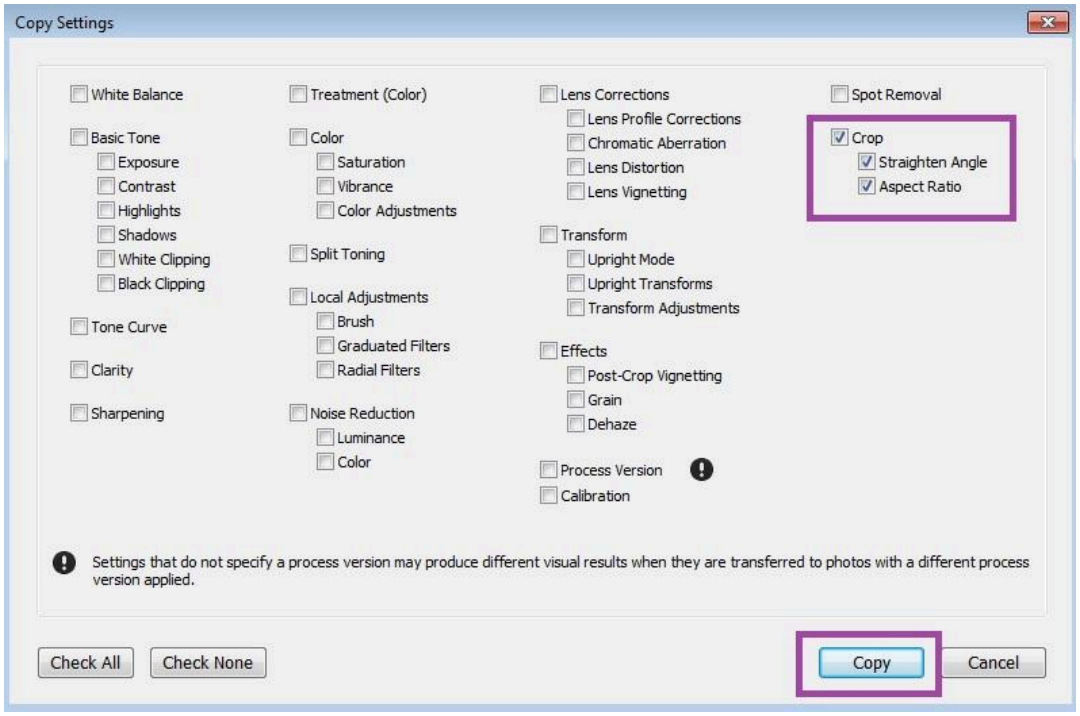
You will only need to do this once. Lightroom will remember your import folders for the next time.

To batch crop your images:

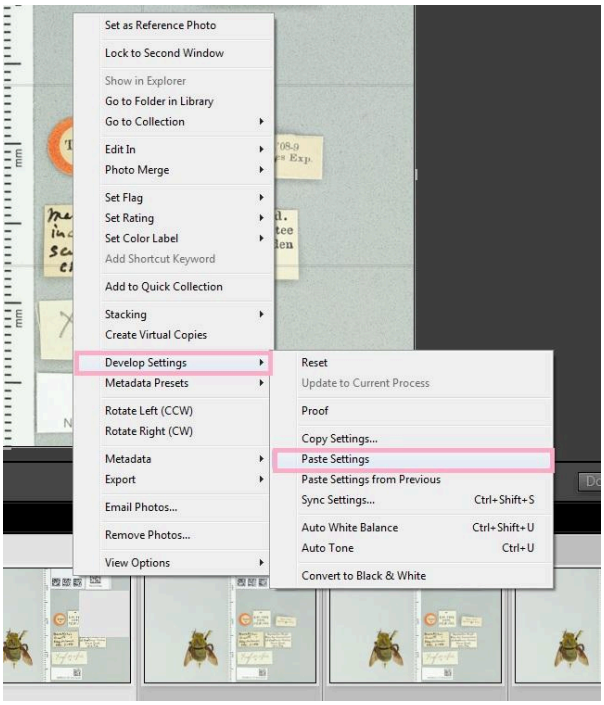
- Go to Develop.
- Crop your first image to remove the IRN tag.
- Then right-click on the thumbnail of your image on the bottom of your screen.
- Select Develop Settings and then Copy Settings…



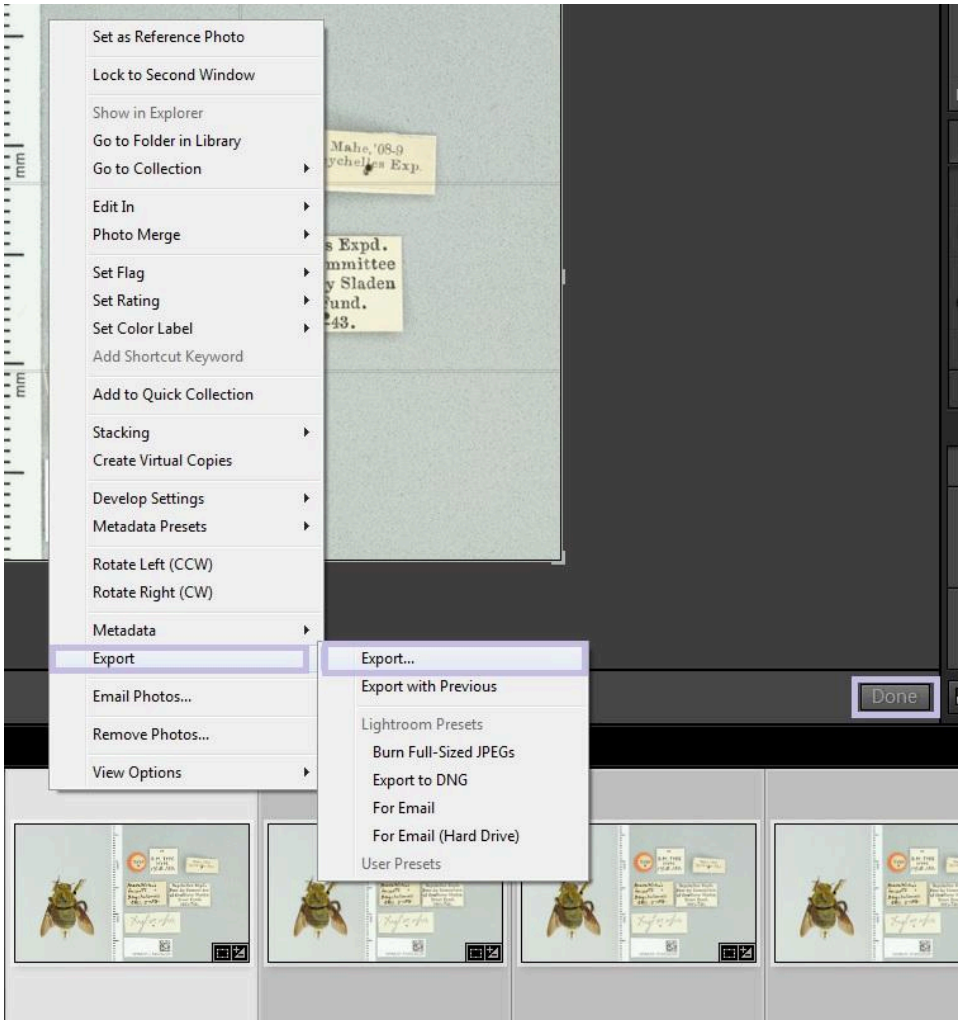
- Check only the Crop section (Crop, Straighten Angle, Aspect Ratio) and click Copy.



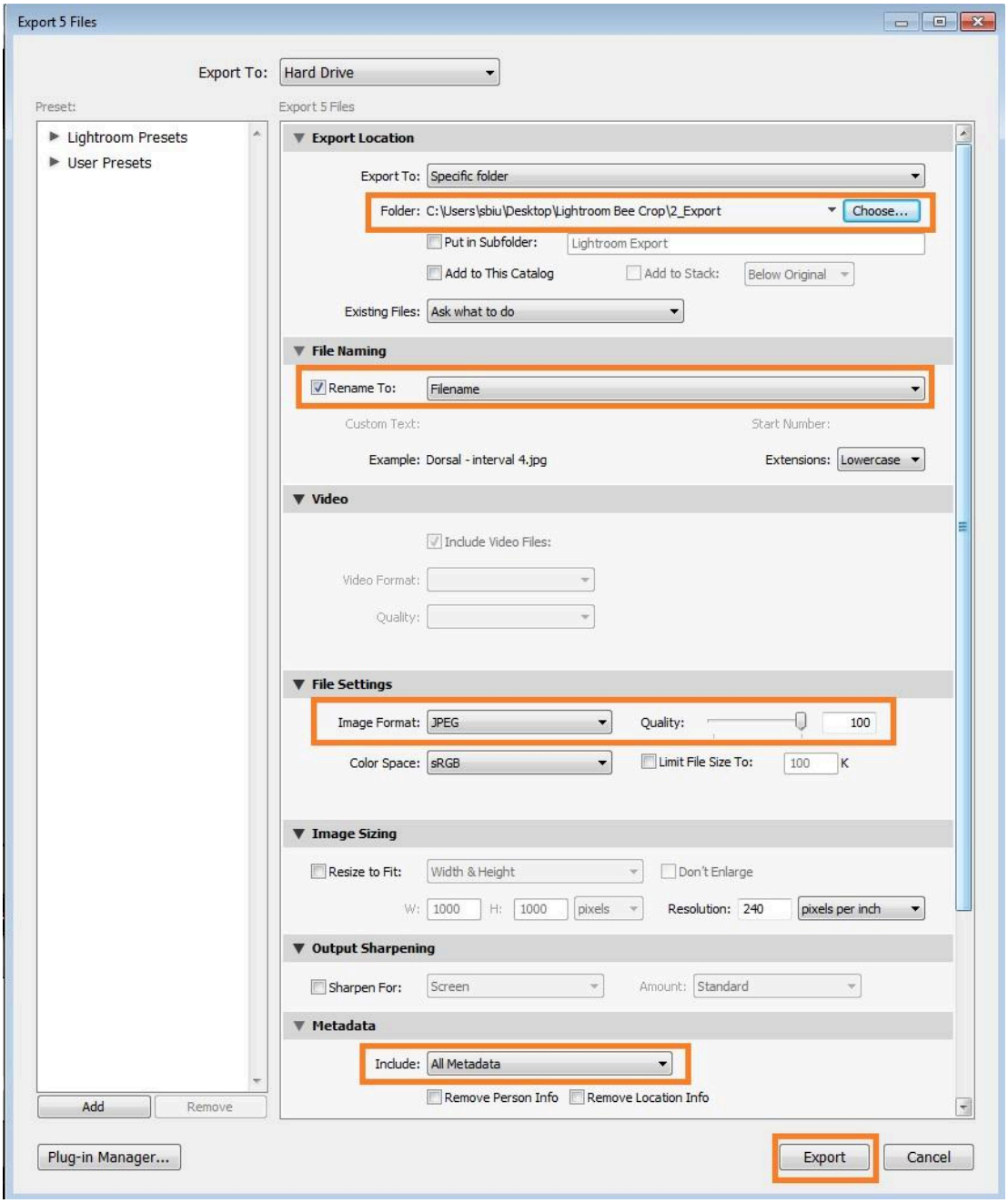
- Select all your image thumbnails at the bottom of your screen (Ctrl + A).
- Right-click on one of the images.
- Select Develop Settings and then Paste Settings…



- Check that all of your images have been cropped alright.
- Adjust any images if needed.
- When all are ok, click Done.
- Select all your image thumbnails at the bottom of your screen (Ctrl + A).
- Right-click on one of the images.
- Select Export and Export…



- Choose your export folder.
- Set Image Format as JPEG and the Quality to 100 (maximum).
- Click Export.



Note: Everytime you open Lightroom you will need to **Synchronise** your folders! (Right click on folder in Lightroom Library, then Select “Synchronise”.)

ImageJ: Suggested folder structure and what every Macro does.

- Go to C:\SBILPictures\processing\1_Helicon Remote\Scale bar.
- Create subfolders for every magnification (1x, 2x, 3x, 4x, 5x).
- Within each magnification folder, create subfolders ‘input’ and ‘output’.
- Download scale bar macros for every magnification and save them in their respective folders.

There are two general macros for every magnification level, one with the rotation command and one without.

As the face imaging is usually capturing the face upside down, the scale bar macros for the face images are also rotating the image. The close up lateral images do not need to be rotated, so you have to use the macro without the rotation command.

For close up lateral images taken in magnification 1x, it is better to use the macro that pastes a longer scale bar (2mm).

Useful instructions

Things that might go wrong (in random order)

- Forget to set the appropriate interval.
- Forget to change IRN tags.
- Forget to image the reverse of the labels or the lateral view.
- Have the wrong camera settings for the view you are imaging.
- Flash going to sleep mode.
- Forget to name a folder after the UID of the specimen (face/close up lateral imaging).
- Forget to specify magnification on the name of the folder when it is needed.
- Part of the specimen cut off from the image after rendering.
- Shutter speed changing to 1/125 (Helicon Remote bug).

Guidelines for the labels

The labels of a specimen might not be attached in the correct order on the pin of the specimen, but they have to be imaged and re-attached in the following order:

1. Round type label.
2. PTN label.
3. The rest of the labels in chronological order.
4. NHMUK label.

At the end make sure that the number on the PTN label and the barcode of the NHMUK label are fully visible.

Tips when imaging or processing

- It helps a lot with the cropping later if you have a mark on the computer's screen for the middle of the live view and align the specimen with that.
- Mark a spot in the lightbox where the tray usually sits when you image the dorsal view.
- When using the ento-ball, remove capsules if it is needed to make room on the pin. Place barcode sideways if it is getting in the way.
- When using the flash, make sure that it hasn't gone to sleep mode.
- In the beginning of the day, after checking that the rendering is complete and that all images are properly saved, it is best to restart the computer otherwise it can be very slow for the rest of the day.
- Save Bulk Renaming Utility instructions as templates.

Specimens with a record

In this project we are trying not to overwrite information on existing records (with NHMUK) and also not to create duplicate records (specimens with BMNHE numbers).

NHMUK barcodes.

You might come across a specimen that **already has a barcode**. In that case there are two possibilities:

a) That specimen was discovered during the preparation of the collection. There is a note on the lid of the drawer indicating that it has a specimen record, as well as a comment on the spreadsheet. The TAX IRN should be cut off from the tag, so the original information on the EMu record will not be overwritten.

➔ Check the notes on the spreadsheet to confirm that and make sure that the tag is cut off.

b) The specimen was **not** noticed during the preparation for digitisation.There isn't any note on the lid or a comment on the spreadsheet and the tag still has the TAX IRN barcode.

➔ Check the NHMUK number in EMu.

- If the specimen record exists, cut off the TAX IRN from the tag before imaging. Make a note on the spreadsheet and add its barcode number on the NHMUK column for that specimen.
- If the specimen record doesn't exist, image specimen as normal with the all the IRNs on the tag.

BMNHE labels.

You might also come across specimens that have **BMNHE** labels. These specimens are very likely to have specimen records. In that case there are two possibilities:

a) That specimen was discovered during the preparation of the collection. It already has a NHMUK barcode and there is a note on the lid of the drawer indicating that it has a specimen record, as well as a comment on the spreadsheet. The TAX IRN should be cut off from the tag, so the original information on the EMu record will not be overwritten.

➔ Check the notes on the spreadsheet to confirm that and make sure that the tag is cut off.

b) The specimen was **not** noticed during the preparation for digitisation and it doesn't have a NHMUK barcode. There isn't any note on the lid or a comment on the spreadsheet and the tag still has the TAX IRN barcode.

➔ Check the BMNHE number in EMu.

- If the specimen record exists, add a barcode to the specimen and update the record with the NHMUK number in EMu (do not erase the BMNHE number). Cut

off the TAX IRN from the tag before imaging. Make a note on the spreadsheet and add its BMNHE and barcode number in the respective columns.

- If the specimen record doesn't exist, image specimen as normal with the all the IRNs on the tag.

Multiple specimens

- Give one barcode per specimen.
- Make sure it is clear which barcode corresponds to each specimen (written on the barcode, examples: male/female, a/b/c, type etc.)
- Image as many times as the specimens, each time with only one UID visible (the other ones reversed).

Guidelines for Interval

Stackshot image calculator.

Set number of shots depending on magnification, aperture and size of object using the table below.

	Shots per mm @ each aperture					
Magnification	**f/2.8**	**f/4**	**f/5.6**	**f/8**	**f/11**	**f/16**
< ×1 (100mm lens)	3	2	2	**1**	**1**	**1**
×1 (MP-E 65 lens)	3	3	2	**2**	**2**	2
×2 (MP-E 65 lens)	7	6	**4**	**3**	3	3
×3 (MP-E 65 lens)	12	**9**	**6**	5	5	5
×4 (MP-E 65 lens)	16	**12**	**9**	6	6	6

×5 (MP-E 65 lens)	21	**15**	**12**	8	8	8

Optimum apertures highlighted in green.

To use this table multiply the estimated depth of the object by the table value at the selected aperture and magnification.

Example: A 3mm deep object photographed at x5 and f/4 would need at most 45 images (3mm x 15 = 45 images).

Taking excessive images (>100) will wear out the camera and flash. Taking >60 images per stack will not improve quality, even for large stacks.

Summary of Quality Checks and more

- Check that you have the correct camera settings for each workflow.
- Check if a specimen already has a barcode or if it has a BMNHE number.
- Make sure you place the specimens in their corresponding positions (PTN of specimen and unit tray label matching).
- In the morning check that the rendering has been completed and you have the expected number of images.
- Check also that you don't have any 'fuzzy' or black images.
- If no laterals were skipped, check that the number of dorsal images is the same as the number of lateral images.
- Check that all images look alright before sending them to EMu.
- If you are using the MPE-65, make sure you have renamed your folders with the NHMUK number of the specimen and the magnification that was used.
- Remember to rename face images with the suffix '_face' and the close up lateral images with '_lateral'.