ALICE Workflow

Imaging, File Renaming, Cropping & Scale Bar Stamping

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Image Capture

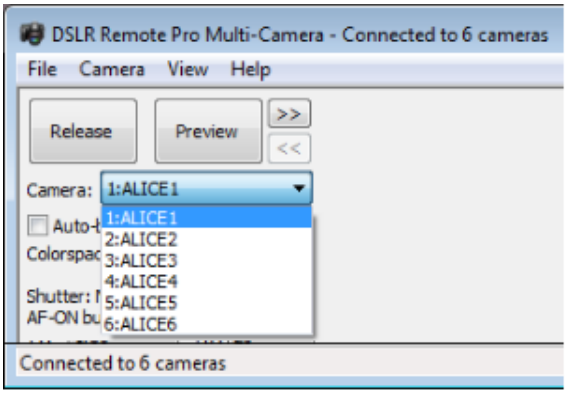
1. Turn on imaging equipment

a. Switch on the top LED light (switch located at the top of the lightbox)
b. Plug in the additional bottom LED lights
c. Turn on all 6 cameras

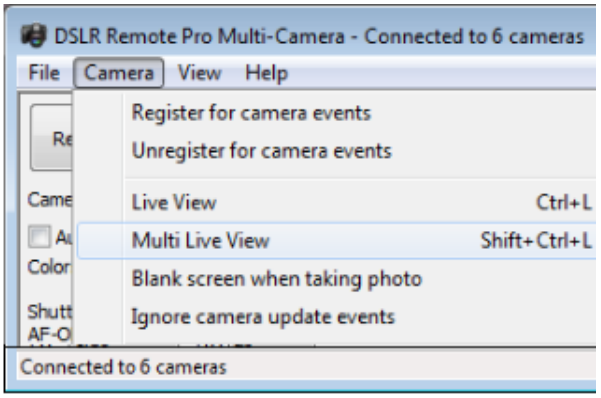
2. Software 

a. Open the DSLR Multi Camera software:
b. Check all 6 cameras have connected
   - check status in the programme window or number of cameras in drop-down list - example 1
c. check the camera settings by selecting each camera - example 1 (camera set to manual)
   - **ALICE1** = F20; ISO 400; ss 1/80, **ALICE2** = F14; ISO 400; ss 1/50; **ALICE3-6** = F20; ISO 400; ss 1/50
d. Open live view for either a single camera (Live View) or all 6 cameras (Multi Live View) - example 2

Example 1:



Example 2:



3. Image the specimens

At the start of each session, the software creates a date folder within the '1_ALICE image capture' folder in the folder 'processing' (only after the first image is taken). All subsequent images taken in a session are saved in this folder.

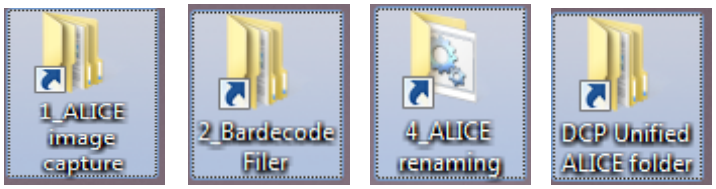
***Note**: To take an image, click 'Release' or use the spacebar*

a. <u>Calibration:</u> Before any specimen images are taken, image the calibration square (these will be needed for any future label transformation)
b. <u>Specimens:</u>
   i. insert the corresponding location and taxon IRN label(s) for the specimen in the top left hand corner of the ALICE1 image (raised foam platform in template)
   ii. Place the specimen in the centralised hole
      - use the right hole for specimens where the pin is through the side of the label(s)
      - use the left hole for specimens where the pin is through the centre of the label(s)

***Note:** Position folder window so the set of six images align with the next six to identify irregularities.*

Daily File Processing

There are four main folders that will be used whilst processing (shortcuts on the right hand side of the desktop screen):



At the end of the day:

1. Calibration Images

1.1 File transfer

a. **MOVE (cut and paste)** the 6 calibration images from '1_ALICE image capture' folder to the ALICE folder on Unified - shortcut on the desktop:

* Folder structure: desktop \ DCP Unified ALICE folder \ "project" \ YYYY_MM_DD \ calibration

2. Rename Specimen Image Files via BardecodeFiler

- **ALICE1 (dorsal):**

2.1. File Transfer

a. Separate the dorsal images from the lateral and label images:

i. Within the day's session folder in '1_ALICE image capture' use the folder search function to lookup 'ALICE1'

ii. **MOVE** the ALICE1 dorsal images from '1_ALICE image capture' folder to the 'input' folder in '2_BardecodeFiler' (desktop shortcut):

* Folder structure: desktop \ 2_BardecodeFiler \ input \

2.2. BardecodeFiler

a. Using the BardecodeFiler software rename the ALICE1 (dorsal) images

- click 'Run'

(use the .ini settings = 'ALICE_Entom NHMUK_NO memory between images_UID_LOC_TAX')

- leave this to run overnight - *continue the renaming the following day (see 2.4 and 2.5)*

- **ALICE2-6 (lateral & label):**

2.3. File transfer

a. **COPY** 'ALICE2'-'ALICE6' (lateral & label) images from '1_ALICE image capture' to the 'ALICE2-6' folder in '4_ALICErenameing' (desktop shortcut):

* Folder structure: desktop \ 4_ALICErenaming \ ALICE2-6 \

***Note**: ensure that the "ALICE2-6" folder is empty before copying the new set of images*

***Note:** copy the files rather than moving them so there is a backup if the renaming needs to be repeated*

Following day:

- **ALICE1 (dorsal):**

2.4. <u>BardecodeFiler</u>

a. **'renamed' folder:**

The BardecodeFiler software will transfer the renamed files to the BardecodeFiler 'renamed' folder:

* Folder structure: desktop \ 2_BardecodeFiler \ renamed \

- image files will be renamed with the original filename followed by UID_LocIRN_TaxIRN
  i.e. 0001_ALICE1-UID_LocIRN_TaxIRN

Files with issues:

- image files missing one or more of the three barcode values will be renamed with the original filename followed by the barcodes that could be read
- check the image files for short filenames:
  - most common error is UID not read (obscured by specimen / not found)
    i.e. 0001_ALICE1-_LocIRN_TaxIRN
  - manually add the missing information to the filenames of these images
    (use the hand barcode scanner to prevent manual renaming errors)

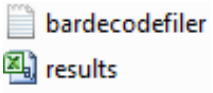
b. **'Exceptions' folder**

Check BardecodeFiler to see if any exception images have been created, these can be found in:
desktop \ 2_BardecodeFiler \ exceptions \

- if there are images:
  - rename them manually and move them to the 'renamed' folder

Note: *if we are not using the location and taxon temporary IRN label then the filename must include the* ***double underscores****, otherwise the renaming script and EMu ingestion script will not work: i.e. 0001_ALICE1-UID__*

c. Delete both the 'bardecodefiler' text file, and 'results' Excel file



Note*: these may affect the renaming script if copied over with the images*

d. Once the renaming is complete **COPY** all the dorsal images from 'renamed' to the 'ALICE1-Dorsal' folder in '4_ALICErenameing' (desktop shortcut):

* Folder structure: desktop \ 4_ALICErenaming \ ALICE1-Dorsal \

Note*: ensure that the "ALICE1-Dorsal" folder is empty before copying the new set of images*

Note: *copy the files rather than moving them so there is a backup of the initial renamed ALICE1 images.*

e. Bardecodefiler may now be closed

- **ALICE 1 (dorsal) and ALICE2-6 (lateral & label):**

2.5. <u>Batch Renaming - Script</u>

a. Ensure the images are in the correct folders:

- 'ALICE1-Dorsal' = dorsal images
- 'ALICE2-6' = lateral and label images

b. Renaming

- double click the "ALICE-rename_script" file (.bat) (use latest version)
  - script location: desktop \ 4_ALICErenaming \

c. All files will be renamed = see script explained (next page)

Script explained:

using a renaming script 1) the lateral and label images will be renamed using the corresponding dorsal image as a reference, and 2) the dorsal image will have the original file name removed:

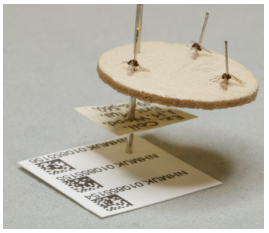
File	Start	End
ALICE1	0001_ALICE1-UID_LocIRN_TaxIRN	UID_LocIRN_TaxIRN
ALICE2	0001_ALICE2	UID_lateral
ALICE3	0001_ALICE3	UID_additional_1
ALICE4	0001_ALICE4	UID_additional_2
ALICE5	0001_ALICE5	UID_additional_3
ALICE6	0001_ALICE6	UID_additional_4

- **Quality Checks**
  a. **Dorsal** filenames
  check for the following:
    i. any suffixes i.e. _1 or (1)
    ii. misread or duplicate UID barcode values

  b. **Lateral** and **label** filenames
  check the files for the following and correct any issues:
    i. files that have not been renamed
      ○ rename these manually using original images for verification (dorsal in 'renamed' folder)
    ii. unusually suffixes i.e. '_additional_5', additional_6' etc

Unusual pinned specimens: multi-barcode specimens require special renaming

Some multi-specimen pins may be assigned multiple barcodes. The specimens will need to be imaged three times, and as a result will show up in both the **dorsal** and **lateral and label** folders as misread and will need to be manually renamed.
***Note**: pay attention to which plane each 'additional_#' is assigned to when renaming via the table above (eg: The direction of view of '*additional_1*' in one specimen should equal the same direction of view in another).*



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3. File Processing

Dorsal images:

i. if the location and taxon temporary IRN label was present in the initial dorsal image then these images must be cropped - see Stage 3.1.
ii. if no temporary labels in the initial dorsal image i.e. only the specimen, then no cropping is required:
- **MOVE** the dorsal images to the 'final' folder:
  * Folder structure: desktop \ final \ YYYY_MM_DD \ images

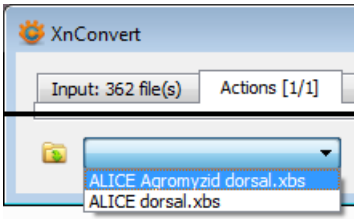
Lateral and **label** images:

i. if you wish to add a scale bar to the lateral images - see Stage 3.2.
ii. if you do not wish to add a scale bar:
- **MOVE** the lateral and label images to the 'final_ReverseSide' folder:
  * Folder structure: desktop \ final_ReverseSide \ YYYY_MM_DD \ images

3.1. Cropping - Dorsal Images

3.1.1. XnConvert

a. For the dorsal images in the 'ALICE1-Dorsal' folder crop out the location and taxon IRN label from the final image using XnConvert:



   i. drag and drop the images from the folder into the XnConvert 'input' tab
   ii. select the appropriate project settings from the dropdown list (bottom left - next to the folder symbol)
   iii. click 'Convert' to crop the images
      - a pop up window will appear asking if you wish to replace the files, select "yes to all"

***Note**: some images may need different requirements for cropping - these images can be manually cropped by dragging the individual files to Input, and choosing the required settings in Actions. Finish these images before batch-cropping the rest of the files.*

b. Once cropped **MOVE** the dorsal images to the 'final' folder:
   * Folder structure: desktop \ final \ YYYY_MM_DD \ images

Dorsal image processing is now **complete.**

3.2. Scale Bar - Lateral Images

3.2.1. File transfer

a. Separate the lateral and label images:
   i. in the folder 'ALICE2-6' use the folder search function to select 'additional'
   ii. **select** and **MOVE** all the label images to the 'final_ReverseSide' folder:
      i.e. UID_additional_1, UID_additional_2, UID_additonal_3, UID_additional_4
      * Folder structure: desktop \ final_ReverseSide \ YYYY_MM_DD \ images

b. You should be **left with** just the lateral images in the 'ALICE2-6' folder
   i.e. UID_lateral

3.2.2. ImageJ

a. Using ImageJ stamp a 5 mm scale bar on the lateral images using a macro script
   i. open: Plugins ⟶ Macros ⟶ Run
      - "Run Macro or Script" - select the macro 'ALICE_800D 60mm_scale bar_5mm_Lateral'
         * Folder structure: SBILPictures \ documents \ ImageJ \ macros
         - use the  to find the documents folder (3 clicks)
      - "Choose the input folder!" - select the images folder 'ALICE2-6'
         - choose the desktop option to find the folder
      - "Choose the output folder!" - select where to save the new images ('ALICE2-6' folder)
        ***Note:** by saving to the same folder the macro will overwrite the original images*
      - The macro will begin running
        ***Note:** there is no indication when the macro has completed - check the final image*
        (help document Add scale bar using ImageJ)

b. **MOVE** the lateral images to the 'final_ReverseSide' folder:
   * Folder structure: desktop \ final_ReverseSide \ YYYY_MM_DD \ images

Lateral and label image processing is now **complete.**

Daily File Cleardown

a. Once the image renaming and processing is completed **delete** the following copies:
   i. '1_Alice image capture' folder area
      - original lateral and label images (also delete the capture date folder)
   ii. '2_BardecodeFiler' folder area
      - 'original_processed' folder = original (non-renamed) images
      - 'renamed' folder = dorsal renamed ALICE1 images
      - 'exceptions' folder = dorsal renamed ALICE1 images

File Transfer

1. FLOWS

a. **Copy** the date folders with the images daily to the corresponding EMu import shared folder - daily:
   i. **"final"** copy to \\dfs-ctdb\emu-import-Slides_SLR_X
   ii. **"final_ReverseSide"** copy to \\dfs-ctdb\emu-import-Slides_SLR_X_Reverse

   Note: *the Data Manager will be notified about the images and, once in EMu, the FLOWS script will automatically clear down the images and date folders in* ***"final"*** *and* ***"final_ReverseSide"***

2. Future Label Extraction and Transformation (label images)

a. **Copy** the lateral and label images (additional_1 to _4) to the ALICE folder on Unified where the calibration images were saved - shortcut on the desktop:
   * Folder structure: desktop \ DCP Unified ALICE folder \ "project" \ YYYY_MM_DD \