



South Australian Museum
Atlas of Living Australia Volunteer
Digitisation Project
Procedures Manual Supplement:
Microphotography
(version 7: July 2015)





© South Australian Museum 2011
This document is licensed under Creative Commons 3.0 Australia: Attribution.

Contents

1	Preparing the specimen	5
2	Leica Application Suite	5
3	Preparing to capture photograph - settings.....	8
4	Using Multifocus to create a stack of images	11
5	Applying a scale bar.....	13
6	Saving your image.....	14
7	Photographing labels.....	15
8	Copying label images from the Nikon Camera	16
9	Delete all your files	16
10	Troubleshooting.....	17

1 Preparing the specimen

Choose the next suitable specimen from the drawer of insect specimens provided. If a specimen looks too delicate or complex, feel free to leave it and move onto the next available specimen.

Carry it in its unit tray to the microphotography area.

Look in the box to see if there are any hazards. If there is an extra pin stuck through the labels in the floor of the box, remove that first. Are there any broken parts of the specimen lying on the floor of the box.

Prepare a spare piece of foam to work on.

Hold the head of the pin with your thumb and forefinger and carefully remove the specimen from the box. Check that your fingers are far enough away from the specimen that you aren't at risk of touching it.

Use the pointed forceps to carefully remove the labels from the pin. These might be very brittle so hold them as close to the pin as possible, and remove one at a time. Try to preserve the order of the labels on the pin.

Once all the labels are removed, pin the specimen in either of the two dorsal or lateral stages ready for photography.

Lay the labels out neatly on the labels stage, ready to be photographed using the DSLR.

2 Leica Application Suite

Open the program Leica Application suite, by double clicking on the logo labelled LAS v3.7

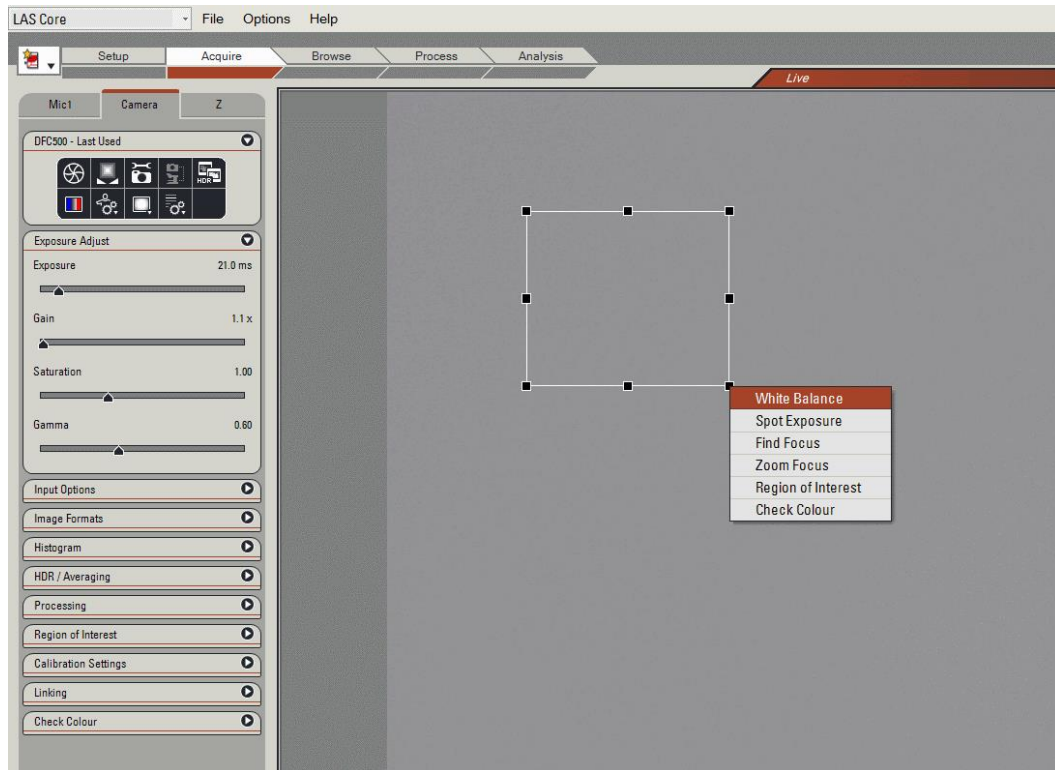


LAS will open a live view of the area under the microscope.

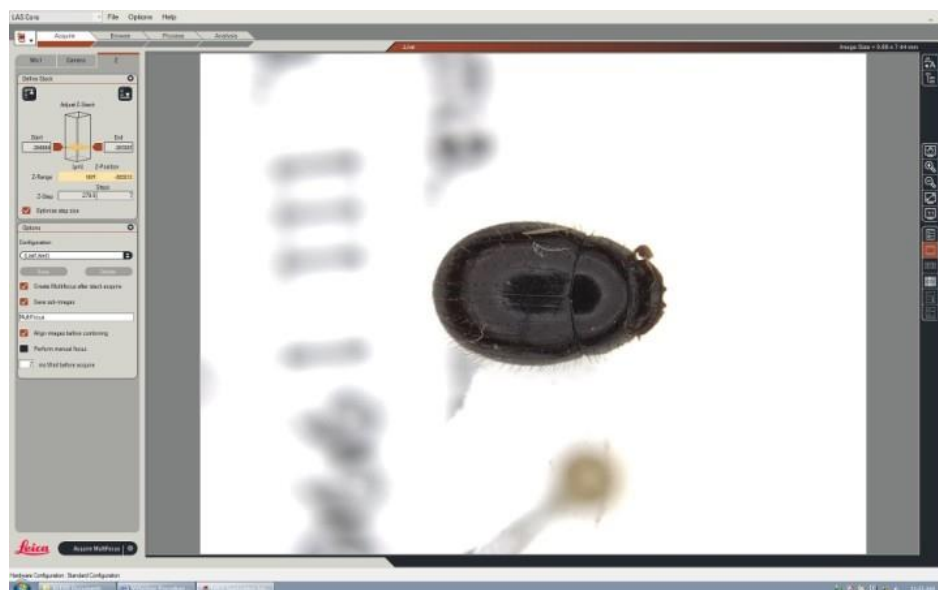
SA Museum ALA Volunteer Digitisation Project

The camera and software are prone to shifting the colour, so that the pictures come out a bit yellow or pink.

The best way to avoid this is to 'white balance' the setup at the start of each shift. Find the digital grey card and put it under the microscope, pull the lighting hood down and click and drag a square on any part of the screen, and click on **White balance**. This should instantly correct the colour.



Now set your specimen up under the microscope.



Check that you can see three tabs across the control panel on the left, titled **Mic**, **Camera**, **Z**.

If there is no **Z** panel, click on this icon  to change the Acquire mode to **Montage**.

Check that the body or main part of the specimen is horizontal, parallel with the foam pad.

The specimen should be oriented with its longest side parallel to the longest side of the frame. Move it so that it is centred, appears straight and fills approximately 80% of the frame.

Specimens' heads should be pointing towards the left of the frame.



If you're having trouble finding the specimen in the frame, zoom right out so that you can see more of the area under the microscope. Then centre the specimen, and zoom back in. If you need to look through the eyepieces turn the black knob on the left side of the microscope to 'VIS'. The knob is circled in the picture below. This knob switches your view between the computer and eyepieces. (VIS = eyepieces, DOC = computer screen).



Use the **Zoom** knob to make the specimen larger in the frame (marked below).



3 Preparing to capture photograph - settings

Bring the specimen roughly into focus before starting to adjust any settings. This means that you will be better able to see the impact of what you are doing.

To bring the specimen into focus use the knob on the Smarttouch controller. The top knob is coarse focus, and the outer knob is fine focus.



At high magnifications, you'll be able to see that the depth of field gets shallower.

Once you have part of the specimen in focus you can start adjusting the exposure and other settings.

Click on the **Acquire** tab across the top of LAS, and then click on the **Camera** tab to adjust camera settings.

You will need to set the exposure setting correctly. The Leica Application Suite software can show you if your image is under or over exposed. Clicking on the button with the red and blue pattern ('Show under/over exposure in the viewer')

The red areas are a bit over-exposed, and the blue areas are a bit under-exposed. It's impossible to get this perfect. Optimum exposure is as close to no colour as possible, but if the choice is between red and blue, a bit of red is better than blue. Don't panic if the background is red, while crevices on the insect are blue, this is normal.

Adjust the **exposure** by sliding the marker left and right on the **Exposure** bar. This marker is very sensitive, so you might find it easier to use the left and right arrows on the keyboard to control it. The optimum settings are usually **between 10 and 25ms**.

Saturation should be 1.0 and **Gamma** should be close to 0.6. Don't change them.



When you are satisfied with the orientation and exposure, it is time to choose the low points and high points for focus stacking.

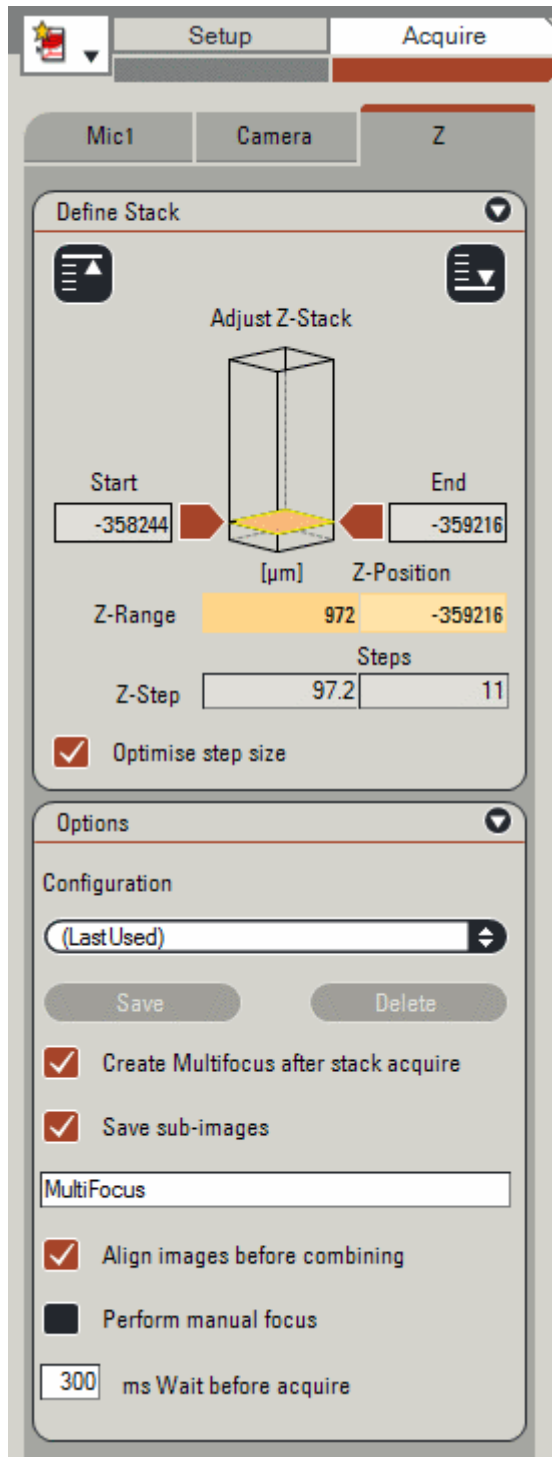
If you have difficulties with any other camera settings, please see the Troubleshooting section of this manual.

TIP: The 5000HDI illumination hood is made up of two LED rings, at the top and bottom. If the specimen is very reflective (usually shiny beetles), you can use the Smarttouch screen to adjust which of these rings are switched on, and the intensity of their light. This can help minimise the reflection of the light off the back of the beetle. It isn't a good idea to adjust the light too much, but it can help if you have a lot of glare. If the specimen is very shiny, sometimes it helps to keep the hood raised, but increase the exposure settings to compensate for the decreased light.

4 Using Multifocus to create a stack of images

Click on the tab marked **Z** to set up your stack.

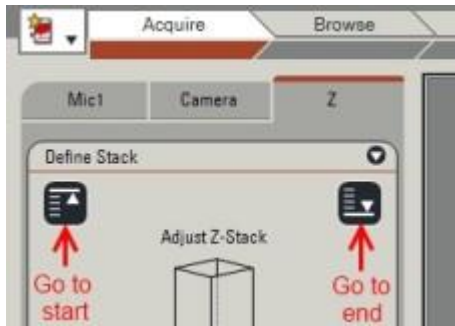
Leica Application Suite lets us go either from the bottom of the stack to the top or the other way around. The software will work out which direction to go, once you have set the start and end points. .



To select a starting point, click on the red arrow near the word **Start**. It will turn black. Use the focus knob on the Smarttouch controller to bring the lowest point on the specimen into focus.

Once you are confident that you've found the lowest point, click on the black arrow. This sets the starting point. It is difficult to be precise with this equipment, so if you're struggling to find the bottom point, find what you think is in focus, and go a bit past it.

Click on the red arrow near the word **End**. It will turn black. Use the focus knob on the Smarttouch controller to bring the highest point into focus. When it is in focus, click the arrow again, and it will turn red again.



The specimen will change in size as the mechanism goes through the steps, and it will also move from right to left across the screen. You can check that the whole specimen is in the frame at the top and bottom of your stack by clicking on the **Go to start** and **Go to end** buttons (shown left)

Tick the box marked **Optimise step size**. LAS will suggest a number of steps depending on the depth of the specimen (often 20 to 30).

Under **Configuration** make sure that **Create multifocus after stack acquire**, **Save sub-images**, and **Align images before combining** are all ticked. Also allow 30ms wait before acquire. These settings should only need to be set once. You should only need to check these settings once.

Once you're ready to capture your images, click on the black bar marked **Acquire Multifocus**.



This will take a couple of minutes.

Once the pictures have been captured, the Leica Application Suite software will montage them into a complete image. This will open automatically under the **Process** tab.

TIP: How is your image looking? You can sharpen it slightly by clicking on **Montage Enhance** and under **Enhance Multifocus Image**, select **Whole Image: Sharpen (Med)**. Click on **Enhance Images** to sharpen your picture.

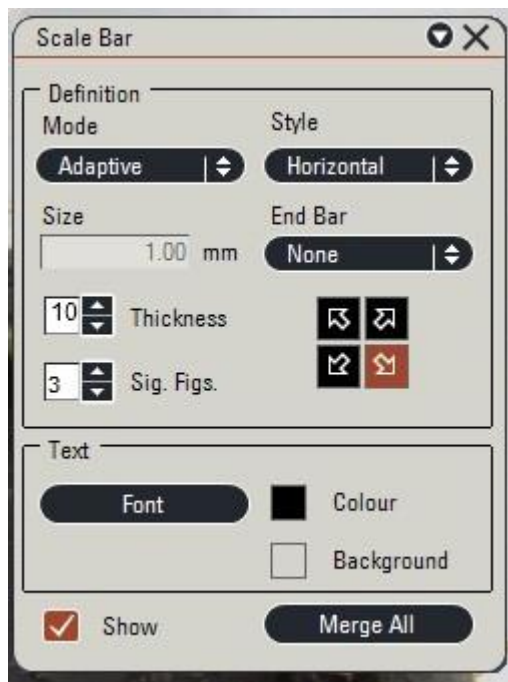
I recommend then clicking on **Montage Undo/Redo** and 'undoing' then 'redoing' the sharpening process to check whether you think it is an improvement. This is helpful for specimens that have tiny hairs or textured surfaces, but isn't recommended for *all* images.

5 Applying a scale bar

If it isn't already selected, click on the image titled Montage.tif.

The scale bar window should already be open. If so, you just need to change the **Thickness** setting to 10, and click **Merge All**.

If it is not open, click on the **Annotation** control in the panel on the right hand side of the screen



Click on **Scale bar** and a Scale bar window will open. Tick the box marked **Show**.

The settings should be:

Mode: Adaptive

Style: Horizontal

Thickness: 10 (this will vary depending on the size of the specimen, use your judgement)

End bar: None

Sig fig: 3

Position: arrow pointing to the bottom right

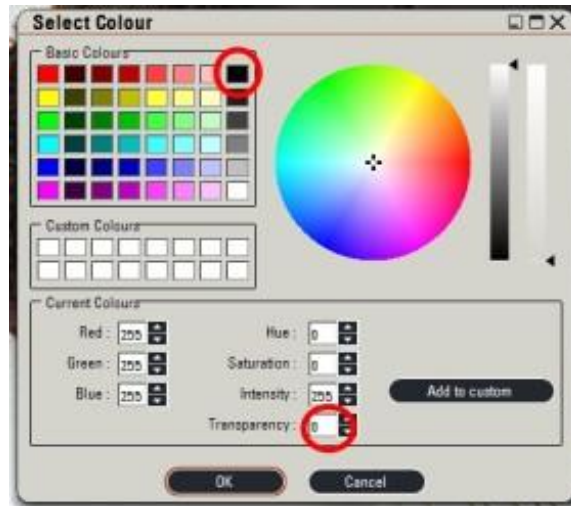
You might need to adjust the colour of the bar, background and transparency.

To do so, click on the coloured square next to the word Colour, and it will open a colour 'picker' window.

Select the black square in the default colours for the **Colour** setting. Then click **OK**.

Repeat the operation for the square next to the word Background, and adjust the transparency to 0. Then click **OK**.

Both are marked on the image below, but you will need to do them one at a time.



Once you are happy with the settings, click **Merge all**.

LAS will ask if you want to replace the stored image, or create a duplicate. Select **Create a duplicate** in case you need to re-do this process.

LAS will create a file in the bottom of the window called 'Copy of Montage.tif'.

6 Saving your image.

Click the right mouse button on your final image (Copy of montage.tif) and select **Export**.

Choose where it is to be saved under **Select export** – it should be the appropriate holotypes folder on the shared drive

(in R (**Digital assets**):\ALA Digitisation\IMAGES\HOLOTYPES)

You should only need to set this once, but remember that it will need to be changed if we are working on a new Order of insect.

Click **Rename image** and type in the title of the image.

(e.g. 99-99999_Genus_species_dorsal.tif or 99-99999_Genus_species_lateral.tif)

Repeat the full process to capture a lateral view, by mounting the specimen on its side in the cork.

If its legs or antennae appear too delicate, don't put them at risk by mounting it on its side, but note that you haven't been able to take a lateral photo on the Job Record Sheet.

7 Photographing labels

Carefully carry the stage with the labels laid out on it to the area under the Nikon D70.

Look through the eyepiece and check that they are reasonably neatly laid out, and centred.

To set the timer delay, press and hold the shooting mode button (pictured below)



And wind the ridged wheel on the right hand side once notch to the right (towards the windows).



Take your hands off both button and wheel, press the shutter halfway to focus, and then the full way to take the picture. Take your hands away from the camera. There should be short beeps for 2 seconds and then you'll hear the shutter. This makes sure that the picture isn't blurred from vibrations when you press the shutter button.

8 Copying label images from the Nikon Camera

At the end of a shift or before finishing with the microphotography set up, you will need to copy the label images that you've taken with the Nikon D70 camera onto the shared drive, and reformat and relabel them.

Plug the cable that connects the camera to the computer in. This cable remains connected to the computer all the time. The camera should be switched on. When it is plugged in, it will display the letters PC on the display screen.

On the computer, open **Computer** and find the Nikon D70.

Find the DCIM folder (this should be two levels down)

You will find a number of jpg files with names like DSC001.jpg

Right click on the first file, choose **Open with**, and then choose **Paint.NET**.

The file will open with Paint.NET (Microsoft's free photo editor).

Choose **File** in the top left corner, then **Save as**. Find the folder where you've been saving the specimen images.

(Usually **R:Digital Assets/ALA Digitisation**

Project/IMAGES/Holotypes/Coleoptera/Unprocessed Coleoptera)

Move the saving window so that you can see the database number in the image. Change the file type to .tiff in the **Save as file type** field. Start typing in the database number and the system should start suggesting the file names for the specimen. Select one of these and overwrite the last section of the file name (i.e. change 25-006435_Genus_species_dorsal.tiff to 25-006435_Genus_species_labels.tiff). Click on **Save**.

Once you've saved all the labels files, delete them from the camera by selecting all the files in the folder and pressing **Delete**.

9 Delete all your files

We don't want the memory on the computer to fill up with all the stacked files. At the end of a shift, go to the **Microphotography** folder on the desktop. Select all files and then press delete. The computer will ask you if you're sure you want to move these files to the **Recycle Bin**. Agree, then **right click on the Recycle Bin** and choose **Empty recycle bin**.

10 Troubleshooting

10.1 I've broken the specimen.....18

10.2 How do I turn everything on?18

10.3 What should the settings be?20

10.4 Where are my files?23

**10.5 The end result doesn't look right, it is soft and fuzzy
and seems out of focus. What can I do?23**

**10.6 Our problem isn't covered in this 'Troubleshooting'
document, what can we do?24**

10.1 I've broken the specimen.

Stop what you're doing immediately. It is imperative that we do not cause any further damage. If the Project Manager is available, draw it to her attention immediately.

If the Project Manager is not present, collect all parts, return them to the unit tray, and return the unit tray to the glass covered drawer. Make a note of the incident to bring to the Project Manager's attention as soon as possible.

We will need to return the specimen and *all* of its parts to the Entomology Collections Manager for inspection. He may decide to re-attach the broken part, or to re-organise the parts in the unit tray to prevent any further damage. Be aware that there may be multiple small parts that have broken off and should be collected (e.g. look for tiny antennal or foot segments as well as the main part of the broken antenna or leg. The number or forms of certain segments are diagnostic characteristics of certain species.)

10.2 How do I turn everything on?

To conduct microphotography you need to turn on the **computer**, **microscope** and **illuminating hood**.



When you turn the computer on the small light on the front of the microscope camera will become red:



The switch for the microscope is on the back of the bottom of the support column on the right hand side. When you switch this on, the Smarttouch controller will also come on.



The Illuminating Hood has an on/off button on the front of it, or can be switched on and off on the Smarttouch controller.



10.3 What should the settings be?

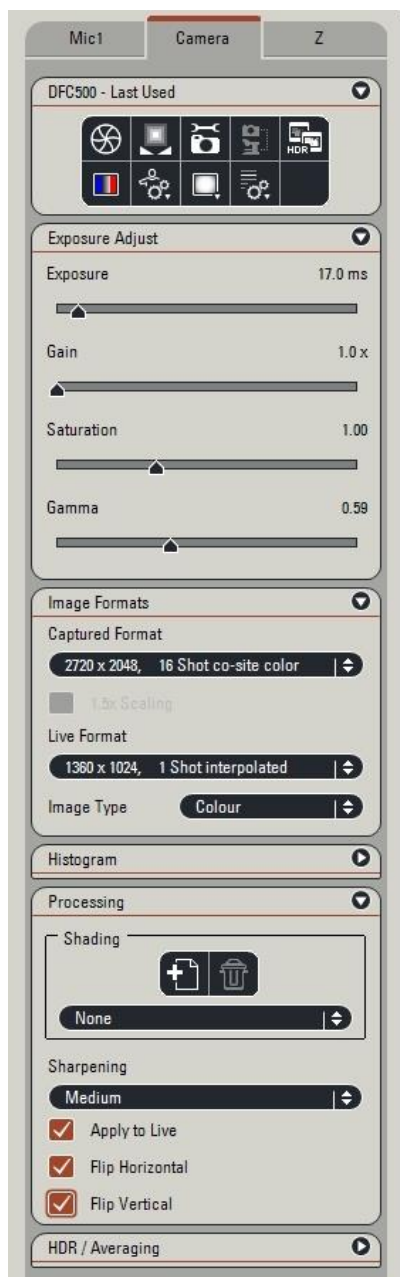
There are a lot of settings that will not need to be changed from one image to the next. If your image appears differently from what you were expecting, you might want to check these settings.

Basic settings under **Options, Preferences**:

Click on the **Image** tab and choose:

16 bit tiff, and **350 dpi**

Under **Acquire**, then **Camera** tab:



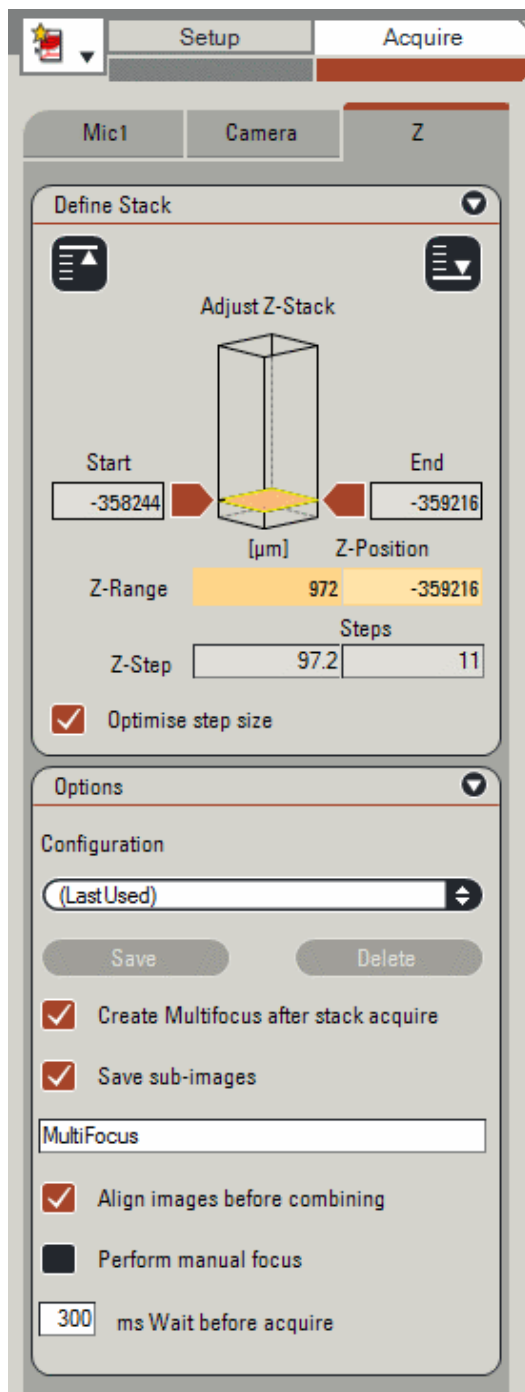
Note:

Captured Format:
2720x2048, 16 shot co-site colour

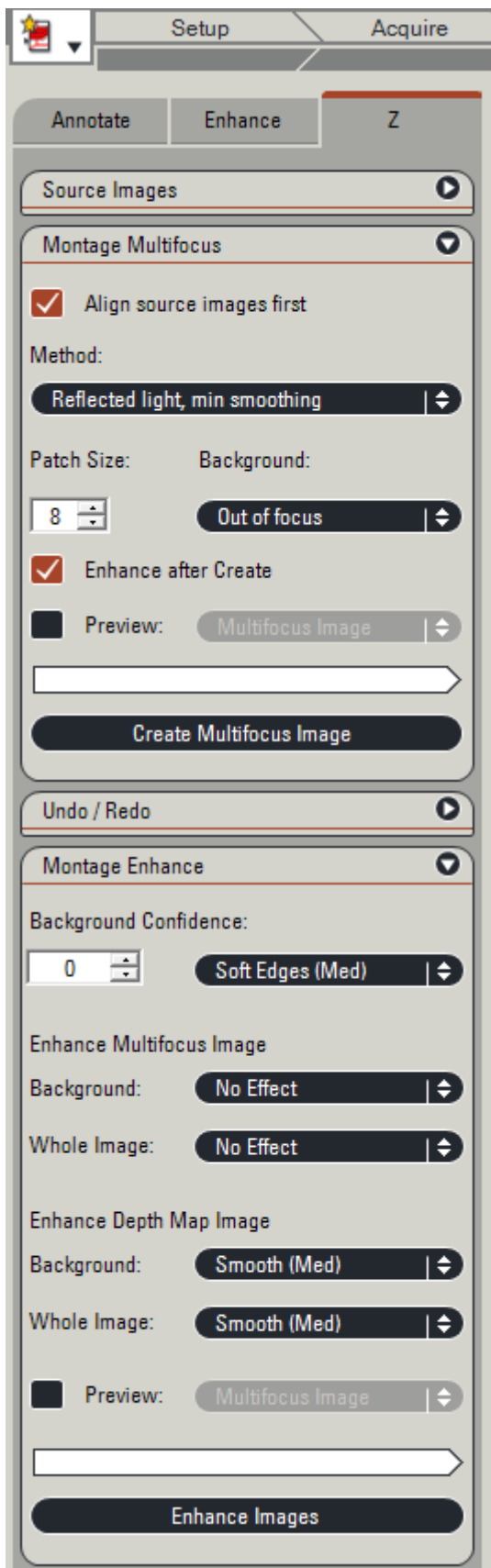
Sharpening:
None

Apply to Live; Flip Horizontal, Flip Vertical all ticked.

Under **Acquire** then **Z** tab:



Under **Process** then **Z** tab:



10.4 Where are my files?

- **TIFFs created by the LAS Multifocus (before being stacked):**

C:/Users/alexist/Desktop/Microphotography/Multifocus0012

(Please insert your username where it says "alexist" in this file path, and the number will change for each job).

- **Final Images** – after they have been stacked by Leica Application Suite

R:\ALA Digitisation\IMAGES\HOLOTYPES

Inside that folder will be folders for the relevant orders that we are presently working on, and for the families of insects that we are working on.

10.5 The end result doesn't look right, it is soft and fuzzy and seems out of focus. What can I do?

There are a couple of steps you can take to fix this.

10.5.1 Check for movement in the source images

It might be that the specimen moved while the stack of images was being taken.

Click on the 'Browse' tab in the top row.

Click on the 'Stack Images' icon and press the play button (with an arrow ►)

Watch the screen as it plays your source images like a video. Is the specimen moving around the screen? Are there certain pictures that show either **jagged** edges or edges that are very **cross-hatched**? If so, the specimen has moved during the capture of the stack of images.

This can be because the foam is sitting too close to the lighting hood and it has touched it as it moved down the stack. Check that the specimen is in the centre of the foam, and that the foam is in the centre of the staging area. Then re-take the photo.

If the positioning of the foam and specimen seems correct, it might be because the bench was knocked during the capture process, or the card or insect shifted on its pin, or there was a serious gust of wind. Re-take the pictures being careful not to knock the bench while doing so. Sometimes these movements are a bit inexplicable.

10.5.2 Try running the set through Zerene Stacker

Occasionally, especially with very small images, Leica persists in giving fuzzy montaged images. In this circumstance, it can be helpful to run the set of images through Zerene Stacker to check if you can get a better result.

In the **Process** tab on Leica Application Suite, go to the **last** image in the stack (usually called something like z_0056.tif). Put a scale bar on that image.

Minimise Leica Application Suite. Open Zerene Stacker. Open the **Macrophotography** folder on your desktop. Open the last **Multifocus** folder. Click on **Type** to sort the images by type.

Scroll down until you can see the tiff files. Select all of the ones that start with **z** and the one called **Copy-z_0056.tif** (this is the one with the new scale bar).

Drag them into the **Input Files** box of **Zerene Stacker**.

Click **Stack** and select the first option **Align & stack all (PMax)**. This will take a minute or more.

Have a look at the resulting image and compare it to the image that you got in Leica Application Suite. If it is better, save the image from Zerene Stacker.

Click on **File**, then **Save output image(s)** and enter the appropriate file name.

Use the usual folder for saving:

R:\ALA Digitisation\IMAGES\HOLOTYPES

10.6 Our problem isn't covered in this 'Troubleshooting' document, what can we do?

In the first instance, chat with the Project Manager. If that person isn't around or can't resolve the issue, the manual for the Leica Application Suite is saved in the folder S:/Collections Management/ALA Digitisation Project/Manuals.

Sections of this manual are also printed and spiral bound on the shelves.