

Guidance:
Photographing specimens in
natural history collections



ATLAS OF **LIVING**
AUSTRALIA
sharing biodiversity knowledge

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Photographing specimens

1 Introduction

This guide aims to document what was learned during Atlas of Living Australia (Atlas) supported digitisation projects at the South Australian Museum (SAMA) and Australian Museum (AM) during 2011-12.

Digitisation of natural history collections is a challenging but necessary step for museums all over the world. The approach to, and progress towards, getting collections photographed and, in some cases, published online is greatly varied across institutions.

The two projects that contributed to this guide were focussed on using volunteers to capture digital images of insect collections. SAMA imaged and databased terrestrial invertebrate holotypes, while AM focussed on capturing images of labels for whole families of invertebrates and historic archival material. AM images were later published in the Atlas volunteer portal to allow the labels and other documents to be transcribed.

This guide is intended to be a basic overview, describing our procedures and experience on these projects and giving some tips for solving common problems in specimen photography. A reference list at the end of this document provides links to some detailed resources.

This guide should be read in conjunction with Atlas's guidance on strategic approach to digitisation. Planning for any digitisation project should commence with an understanding of the range of specimens, the kind of images you are aiming to capture and your arrangements for storage and management of those images.

2 Micro- or macrophotography?

The range of entomological specimens likely to be imaged varies in size from 200mm, for some large phasmids, moths and cicadas, down to slide mounted thrips or springtails that can be 200µm in size. Different types of imaging equipment and set ups are needed for different types of specimens. These types basically fall into the categories of microphotography and macrophotography.

2.1 Microphotography

Microphotography is capturing an image using a camera and a microscope or another form of magnifying device. SAMA has found that it is best suited for specimens smaller than 15mm or particular features of larger specimens.

In a museum environment, microphotography is useful for biological specimens as well as geological specimens, materials used in cultural artefacts and for conservation purposes.

High magnification images of parts of insects, such as antennae, tarsal segments or dissected material, can be used for identification. High magnification shots of insect parts also make spectacular images for display purposes.

2.2 Macrophotography

Macrophotography is capturing an image using a camera and a macro lens – a lens in which the ratio of the subject to the size of its image on the camera sensor is 1:1 or higher.

Generally a digital camera, using a macro lens, can capture specimens down to the size of the sensor. While sensor size varies between cameras, it is easiest to think of these based on the old 35mm film standard. This means that specimens up to about 35mm in length can be captured at life size, in a 1:1 ratio.

Putting greater distance between the camera and subject increases the field of view, and both SAMA and AM used digital SLR (DSLR) cameras with macro lenses to capture large insects and their labels, including moths, cicadas and other insects between 15mm and 200mm in length. Image stability is aided by mounting the cameras on copy stands.

3 Technical issues in photographing specimens

3.1 Getting adequate depth of field

One of the challenges of high resolution macro- and microphotography is the limited depth of field in such images. Depth of field refers to the area of the subject that is in focus in an image. In close-up photography this is very shallow. This shallow depth of field can be used for dramatic effect or to highlight a particular area of an image, but can also pose a challenge. Two solutions to this challenge are available: stepping down the aperture and focus stacking.

3.1.1 Stepping down the aperture

It is possible to increase the depth of field by decreasing the lens aperture. The aperture is the size of the iris in the lens that controls the amount and angle of light passing through the lens onto the image sensor and in doing so determines the depth of field. The settings for aperture are called 'f-stops' and are a standard set of numbers: f2.8, f5.6, f8, f11, f16, f22, etc.

Each step up in aperture value (larger number) halves the amount of light reaching the sensor and increases the depth of field. Each step down in aperture value (smaller number) doubles the amount of light and reduces the depth of field.

AM found that they were able to capture suitable images of specimens and labels using the following settings with their Canon 550D set up:

- copy stand height: 44.5 cm
- shutter speed: 1/200 sec
- aperture: f16
- ISO: 200
- white balance: custom, set using a digital grey card, according to manufacturers' instructions
- flash: 1/16 power setting on Metz Mecablitz flash unit. Note that E-TTL capable flash units might not require a reduced power setting, but you can experiment with flash exposure compensation to get the lighting right, eg SAMA boosts the exposure compensation by 1 to 2 f-stops to compensate for a diffusion ring.

3.1.2 Focus stacking

Narrowing the aperture will achieve greater depth of field, but decreases the amount of light that reaches the camera sensor or film, which risks a loss of sharpness (especially at small apertures) or the introduction of 'noise' into the image due to diffraction of the light around the aperture.

Focus stacking overcomes the limited depth of field. It involves capturing a series of images of a specimen at different distances, then stitching together the parts of those images that are in focus to create a sharp image through the complete depth of the specimen.

Digital photography pioneers performed focus stacking through a painstaking, time-consuming process of slight shifts in the camera/microscope and careful image manipulation using photo editing software. However, affordable equipment and software solutions have made focus stacking a far simpler process today.

Photographing specimens

Focus stacking programs can be purchased for less than \$300. These programs ingest a series of tiff, jpg, or, in some instances, RAW image files and automatically create a montaged image within minutes. (Links to some of these programs are provided at the end of this report.)

But capturing the stack of images in the first place is still challenging. It is best done using a focussing rail, which allows small movements of the camera along the axis requiring the increased depth of field. Manual focussing rails are available, but a Stackshot automated focussing rail makes focus stacking a great deal easier. Priced around US\$600, the device automatically moves and then fires your camera. There are various settings, but the most straightforward allows the operator to set the highest and lowest points and the number of steps in between. The device then moves the camera evenly through that distance, allowing the vibration to settle after each movement before firing the shutter for each step. The device is simple and easy to use, but very precise. It can move in increments as small as 2 μm and can also be used in microphotography.

Microscopes are also available with automated z-axis stepping to facilitate focus stacking. Motorised focus drives are able to shift the optics carriage and capture images at different points along the z-axis. In SAMA's experience, specimens in the range 500 μm to 15mm require between 15 and 60 images to get a focussed picture. Some very small specimens can require up to 120 images.

Some microscopy suppliers have integrated licensed versions of montaging programs into their proprietary software so that capturing the images and montaging can be performed in a continuous operation. For instance, the Leica Microsystem used at SAMA captures the images and montages them automatically in less than seven minutes. Alternatively, a series of tiff or jpg image files can be exported from the microscope camera and run through a stand alone montaging program.

3.2 Managing light

Light is central to all types of photography and mastering light is essential in producing effective images.

3.2.1 Light in macrophotography

Macrophotography employs either flash or fixed lights to illuminate specimens.

3.2.1.1 Flash

Both SAMA and AM use flash to photograph specimens. The key benefit of flash is that the short burst of light minimises the exposure time, thus minimising the risk of vibration in the image. Also, quick bursts of light overpower ambient light, which can compensate for less-than-ideal studio environments.

SAMA uses two flash units controlled by a wireless transmitter, which adds versatility as the light can be directed as best suits the specimen and required image. The flash is diffused by placing a ring of tracing paper around the subject and in front of the flashes (Figure 1). This prevents flares of over-exposure on reflective surfaces and distinct shadows appearing on the subject and/or background.

To shoot single shots of specimens and labels from above, AM use a single flash unit mounted on the camera.

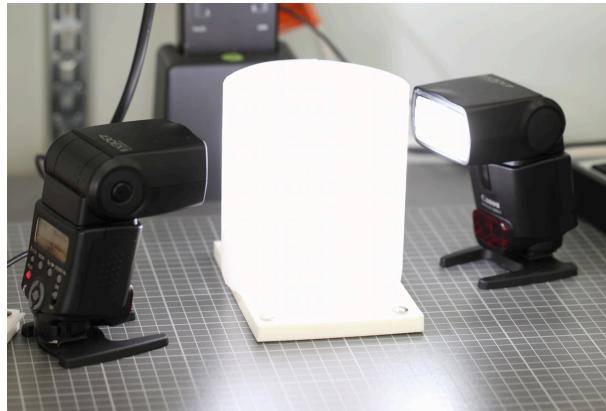


Figure 1 Wireless flash units diffused with tracing paper ring

3.2.1.2 Fixed light

To photograph their collection registers, AM uses a different lighting setup: fixed lights. These free standing, cold fluorescent lights ensure even lighting across the subject, which is the flat double page spread of a museum register about A2 in size. The lights were available to the project at the time of establishment and their even, consistent colour temperature and intensity has proven appropriate for capturing archived documents and register pages.

The register pages are photographed using the settings:

- shutter speed: 1/80 sec
- aperture: f14
- ISO: 200.

The different lighting situation and different demands of the subject naturally call for different settings from the specimen images (described in section 3.1.1).

3.2.2 Light in microphotography

Microphotography uses either reflected (incidental) lighting or transmitted light. To date, the SAMA and AM projects have only conducted reflected light microscopy.

3.2.2.1 Reflected light microphotography

Reflected or incidental light microscopy refers to the illumination of a specimen from the side or above and is traditionally done with light generated by an illuminator that is delivered to the specimen using moveable fibre optic arms.

Well-positioned fibre optic arms can highlight important taxonomic features or illuminate an otherwise darkened feature of a specimen. But they can also create flare and hotspots, obscuring features of the specimen, and distinct shadows, which compromise the quality of the photograph. The colour temperature of the light can also vary greatly. Traditional halogen lit fibre optic arms can create a very warm, or yellow, light while LED lights can create a very cold, or blue, light.

Illumination problems can be overcome by adding a diffuser to the light source. For occasional microphotography, diffusers can be fashioned from a range of materials, including polystyrene foam cups, plastic bowls, white paper and ping pong balls! Don't be afraid to experiment with some polystyrene foam, a pair of scissors, gaffer tape and some

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creativity to solve this problem. Fix the diffusion mechanism between the specimen and arms, keeping it clear of the microscope's objective and move the arms around until satisfied with the light and then capture the shot. (An example of this can be found in the case study at section 8.5.)

For high volume microphotography it is worth investing in an equipment-based solution. Microscopy suppliers have worked on this problem and come up with several options.

Lighting hood

A popular solution is the lighting hood. The hood creates even light all the way around the specimen, diffused by the fact that the LEDs are directed at the hood and reflected onto the subject (Figure 2).



Figure 2 Leica lighting hood in action

SAMA has found that while the light is diffused, some highly reflective surfaces on specimens still reflect the light from the Leica hood, creating a ring-like pattern on the surface (see Figure 3). This can be limited or avoided by making a small collar of black cardboard to go around the specimen, allowing the reflected light to come in from the top, at a single angle, but stopping the reflection of the white rubber hood.



Figure 3 Lighting hood reflected off the back of a highly reflective beetle

Ringlight

Many microscopy suppliers offer a ring light as an illumination solution for microphotography. These either work as a fitting that directs the light from a microscope's fibre optic light source to form a ring around the microscope objective, or a group of LEDs on a basic frame that sits around the microscope objective, often contained within a plastic frosted diffuser. Examples of both are available on eBay, or from microscopy suppliers. They can cost as little as \$50 (on eBay, for a very basic LED ringlight) to more than \$1000 (in the form of attachments for traditional microscopy bifurcated light sources).

3.2.2.2 Transmitted light microphotography

Transmitted light microscopy refers to illumination of the specimen from below and is used to examine very small slide mounted specimens or dissected material. Light is usually reflected through the specimen from an angled mirror, the angle of which can be adjusted for 'brightfield' or 'darkfield' microscopy.

4 Improving image interpretation

Unfortunately, an image does not always stand on its own; it may need some aids to assist the viewer to fully understand what is represented in the image. Typical aids are:

- a scale bar
- a colour patch
- administrative information
- multiple views of a specimen.

4.1 Adding a scale

Using scale bars is important in scientific photography. It is impossible to anticipate all the environments in which a photograph will be viewed or used. A guide to the scale, even if only approximate, helps indicate the size of the subject to the viewer.

If photographing a new or recently acquired specimen, it might be possible to mount the specimen on a card marked with measurements and frame the image so that the measurements are clearly visible.

There are methods for calculating and adding a scale bar to images after capture, provided the magnification, size of the camera's sensor and other elements are known. One method is described on Charles Krebs' microphotography site where he provides a spreadsheet for making relevant calculations (<http://www.krebsmicro.com/> accessed 11 April 2012).

But it is best to add a scale bar during the imaging process.

4.1.1 Scales in macrophotography

There are a couple of ways of providing an indication of scale in macrophotography. At SAMA, images are captured with a small 5 or 10mm square reference in the frame (Figure 4), which is later used as a guide to create a standard scale bar with Photoshop (Figure 5).



Figure 4 Unprocessed image from SAMA macrophotography equipment. Note 5mm reference bar at top of image



Figure 5 Processed SAMA image. Note that the scale reference, after being used as a guide to create a 5mm scale bar, has been removed from the final image

At AM, images are captured and archived with a scale bar in the image (Figure 6). This is quick and effective for their single shot of each specimen with its labels.



Figure 6 AM image with scale bar inserted at time of image creation

4.1.2 Scales in microphotography

Adding a scale bar is more of a challenge in microscopy as the field of capture is very small, with little room to insert a physical guide.

Microphotography systems from major suppliers may integrate software that can automatically apply a scale bar to the image at the time of capture. This is an excellent development that simplifies what could be otherwise a complicated process. Note that this may be an additional module/function on top of the basic microphotography software, so it is worth inquiring about extra cost when investigating a new system.

SAMA's integrated Leica system applies a scale bar at the end of the montaging process, as illustrated in Figure 7.



Figure 7 Scale automatically added to a SAMA image created with a Leica microscope

The Harvard Museum of Comparative Zoology's E-Type project provides instructions on how to insert a scale bar in microphotography using image processing software (<http://insects.oeb.harvard.edu/etypes/Imaging7.htm> accessed 11 April 2012).

4.2 Adding colour patches

Cameras, monitors, printers and scanners all interpret and display colour slightly differently and the photographer cannot anticipate the environment in which the image will be viewed. To overcome this situation a colour patch is often included in an image.

A colour patch (or colour checker or colour calibration patch) is a small reference bar of fixed colours. The colours vary depending on the brand and size of the patch but typically include values for blue, green, red, cyan, yellow, magenta, black and white. It is used to provide an indication of absolute colours within an image and to assist in manipulation and reproduction of an image

As shown in Figures 6 and 8, AM includes a colour calibration checker in their images.



Figure 8 Colour calibration checker used in AM images

4.3 Adding administrative aids

Depending on the eventual use of the photo, you might want to include administrative information in the image. This is one way of making sure that information is always associated with the picture. It might be useful to include the specimen database number, name of your institution or relevant collection or a barcode. Note that there are systems that make it possible for the barcode in an image to be read, but they cannot be read by a simple laser scanning barcode reader.

Some institutions also include a copy of the organisation's logo, which can serve as reminder of the copyright ownership of digital images once they are distributed electronically.

4.4 Specimen views

Planning in digitisation needs to consider what views should be captured based on the project's capacity and objectives. Taxonomists are able to provide guidance on which views of a specimen are taxonomically useful. The Museum of Comparative Zoology also provides a useful online summary of taxonomically useful images for different insect families (<http://insects.oeb.harvard.edu/etypes/specificimages.htm> accessed 11 April 2012).

A pragmatic understanding of the capacities of the organisation and the immediate requirements must impact that decision as well. For instance, the SAMA and AM projects took different kinds of photographs, reflecting the different goals of their projects.

SAMA type digitisation project

SAMA decided a standard set of three views – dorsal, lateral and labels – would be sufficient and ensure that all type specimens could be photographed in a timely fashion. Additional taxonomic views would be created on request and the images added to the image library. In this way, the collection of images of specimens that are being used and are of interest to users will grow in response to demand.

Pinned specimens are photographed on a white background, which is made up of a sheet of good quality white tracing paper pinned over a piece of foam. Very light coloured specimens are photographed on a piece of grey card. The longest part of the specimen is aligned with the longest part of the frame, with its head to the left of the frame. (In most Lepidoptera photos the longest part of the specimen is across the wings, so the head faces 'upwards').

AM rapid digitisation project

As this project was focussed on rapid capture of data and the rapid generation of images for label transcription via the Atlas's volunteer portal, a single view (dorsal, including specimen and labels in a single shot) was deemed sufficient.

In this way, AM has very quickly created a library of thousands of images of whole families of insects for uploading into the volunteer portal.

5 Assisting discovery of images

Creating large numbers of digital images leads to a new challenge: how to record and manage metadata for those images. Metadata refers to the information necessary for finding and using an image, such as the file name, subject, photographer, copyright status and date of creation.

Managing images can be as simple as a well thought out file-naming and folder structure for storing images on a shared or external hard drive or can be a high-end digital asset management system (DAMS).

What metadata is captured and recorded depends very much on the requirements of your image management system, eg each DAMS has its own metadata requirements that can usually be customised to the needs of the organisation.

All digital cameras embed metadata within an image, eg time and date created, exposure details. Some cameras also allow user-defined metadata to also be embedded, such as copyright or photographer.

Irrespective of how images are stored and managed, the following information, as a minimum, should be recorded somewhere:

- photographer
- date and time of image creation
- place of image creation or specimen collection as appropriate (if off-site, latitude and longitude, map datum, accuracy of location)
- copyright and licence conditions
- restrictions on use of the image
- subject or image description, eg species name, specimen database number or LSID, subject identifier
- image reference number (if used).

If allowed by the image management system, keywords should be used as a good way to document subjects or relevant information for finding and using an image.

If more than one person is recording the metadata consider using a controlled vocabulary so that everyone uses consistent terminology.

At SAMA, the images created by the volunteer digitisation project are organised into folders based on their taxonomy (ie divided into Orders, then Families), and metadata (the photographer, copyright information and date of creation) are added to the specimen record.

6 Choosing equipment

The choice of imaging equipment essentially devolves to choosing either an integrated or adapted system.

6.1 Integrated systems

Integrated systems refer to equipment that is usually purchased as a package, with a microscope, camera and peripheral equipment designed to work together. Most major microscopy suppliers are able to supply cameras and other useful equipment for capturing and processing digital images. SAMA's Leica Microsystem (Figure 9) is an example of an integrated system.



Figure 9 SAMA's Leica Microsystem

In a typical integrated microphotography system, a microscope camera is fixed on the trinocular tube of a stereomicroscope. Photographs are staged, captured and processed on a permanently attached computer. The system can include a motorized z-axis drive and in-built montaging software, which makes focus stacking easier. Generally, lighting can come from bifurcated fibre optics or other solutions such as a ringlight or lighting hood (see section 3.3 for further details).

Visionary Digital provide another model of integrated system with their BK systems. These handle macro- and microphotography using the same equipment, by attaching a custom built microscope objective to a DSLR camera. Some models include camera lift and integrated image capture, montage and post-processing software.

In integrated systems, an operator usually controls the preparation and capture of an image through the computer. In a focus stacking capable system, the operator finds and sets the top point of focus for the image, the bottom point and might suggest a number of steps, or this may be governed by the software, which captures all the needed images.

In a system without a motorized z-axis drive, the operator might narrow the aperture of the microscope to get greater depth of field, ensuring that the area of the specimen they want to capture is in focus, and capture a single shot. Alternatively, the operator may perform manual focus stacking. To do this, the top point of focus is found, an image captured and then either the focus on the microscope or the height of the specimen stage is moved slightly before the next image is captured, and so on until there is a set of images with all parts of the specimen in focus. The resulting images can then be run through a montaging program to get a fully-focussed image.

6.2 Adapted systems

Adapted systems refer to a user created combination of microscope and readily available consumer digital photography equipment, where all the gear is not specifically designed to be integrated.

Examples of this in microphotography include:

- using a compact digital camera through the removable eyepiece of a microscope
- attaching a DSLR body to a microscope's C-mount
- using a DSLR camera with a microscope objective, usually attached through bellows or an extension tube.

An adapted system can be a more affordable solution than an integrated system, but it requires more technical input from the user.

SAMA's macrophotography set-up (Figure 10), like AM's specimen and archives workstations, are adapted systems. Full lists of the components of those systems, including the cost and other tips are available in the final reports from those projects, available at <http://www.ala.org.au/about-the-atlas/digitisation-guidance/> accessed 11 April 2012.



Figure 10 SAMA's adapted macrophotography system

Other examples of adapted systems include those at the Harvard Museum of Comparative Zoology:

- JVC KY-F7OB mounted on Leica M12.55 microscope
<http://insects.oeb.harvard.edu/etypes/Imaging4.htm> accessed 11 April 2012
- Nikon Coolpix mounted on Leica MZ7.5 microscope
<http://insects.oeb.harvard.edu/etypes/Imaging5.htm> accessed 11 April 2012

6.3 Equipment tips for macrophotography

Most retail photographic suppliers are happy to advise on a solution that is suitable for your budget and needs. Be aware that they will want to know the size of specimens that you will be photographing, the nature of your workspace and the expected use of the resulting photographs.

When choosing macrophotographic equipment consider:

- A sturdy copy stand or tripod is essential; you won't be able to conduct a large scale digitisation project without this equipment. Be aware that there are very few good quality copy stands available and they likely need to be imported.
- Tethering the camera to the computer helps with image quality and workflow. Cameras from major brands are supplied with remote view software for free or a small cost. This also helps save images directly to a backed up hard drive.
- Flashes, flash transmitters and camera bodies all require batteries. Spare rechargeable batteries and easy-to-use, accessible chargers are essential.
- Processing high resolution images can be challenging for standard desktop computers. To prevent bottlenecks in opening, manipulating and saving high resolution files, seek advice from IT staff on the specifications for computers. Small increases in memory or processing power can make this process much easier.
- Remember to budget for the cost of non-photographic equipment. This can include network cabling, forceps, labels, trolley for movement of specimens, spare pins, foam for stages, scissors and labels and chairs for operators.

7 Basic procedures for photographing specimens

Figure 11 shows a generic process for imaging specimens.

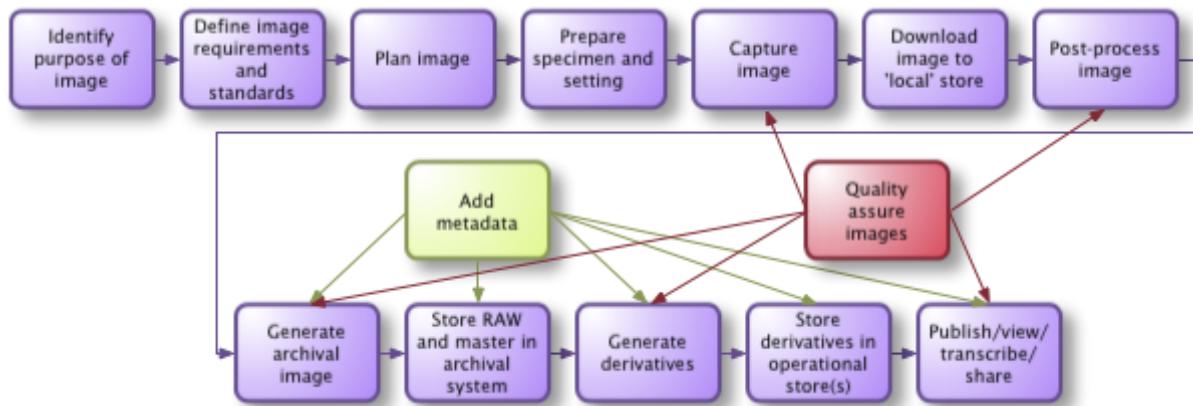


Figure 11 Generic process for imaging specimens

The basic procedure below is modelled on the SAMA Volunteer Digitisation Project.

1. Prepare the specimen

Carefully remove the specimen from its drawer or unit tray and position it ready for imaging. When photographing large numbers of individual specimens, the consistency of images is helped by establishing a standard setting for the specimen. Such a series of photographs also aids comparison. Ensure that your scale measure is in the frame.

2. Capture the image

This might involve focus stacking or using the live view on a tethered camera to capture the image direct to the computer. If you are capturing the image on an un-tethered camera, a remote trigger or a delay on the shutter will help avoid vibration as you press the shutter.

3. Check (quality assure) the image before removing the specimen from the frame

Check that the image is in focus, the light is appropriate and the colours represent what you see before you.

4. Save the image using a pre-determined naming convention

Consistent and sensible file naming is the first and simplest step to managing digital images. If you have a DAMS, this may not be a major issue. Without a DAMS, it is worth putting thought into a model of naming files and structuring folders that is sustainable, expandable, human-readable and documented, to aid in the longevity and usefulness of your images.

At present, SAMA does not have a DAMS. Image file names include the specimen's database number, genus, species and captured view. The file is saved in nested folders on a shared and backed up network drive that indicates the order and family of the specimen.

All images should be backed up. In large organisations this might happen on a shared network drive. Without that kind of infrastructure, it is worth purchasing a portable hard drive and regularly backing up files on it (1TB of storage costs around \$100 at the time this was written, which should store up to 10 000 mid to high resolution images).

If your image is for reference purposes or not for immediate publication, you are finished once it is saved in an appropriate manner. If it is for publication in print or online, you might want to continue to Step 5.

5. Post-process the image using a photo editing program

SAMA carefully optimises images using Photoshop, without manipulating them too much. The images are cropped to a consistent aspect ratio, Levels are adjusted to brighten the background, and the image is cleaned and sharpened very slightly. At this stage the scale reference is removed and a scale bar with an indication of measurement is applied to the image.

6. Metadata

As discussed in section 5, certain metadata must be recorded about the specimen and the image. Adherence to agreed standards such as Darwin Core and Dublin Core helps make the image and specimen record more useful, and aids sharing.

At SAMA, a second operator records specimen data from the picture of the specimen label in a custom Darwin Core compliant Microsoft Access database. Image metadata such as the photographer and date of capture are recorded as additional fields in the specimen record.

8 Case studies

8.1 South Australian Museum ALA Volunteer Digitisation Project

Equipment: Leica Microsystem

A team of volunteers has been working on the SAMA ALA Volunteer Digitisation Project using the Leica Microsystem. The Leica 205c microscope with a 1.0x objective has a highest magnification of 165x. The DFC500 multishot microscope camera captures true colour and the 5000HDI illuminator is a lighting hood, providing 360° diffused LED lighting. The motorised focus drive and Leica Application Suite (LAS) software integrate capturing and montaging a stack of images.

A volunteer removes the individual specimen from its unit tray and carefully removes the labels, setting them aside. They then pin the specimen on a piece of weighted foam and position it under the microscope for a dorsal shot. The specimen is centred in the field of view with its head facing to the left.

Looking at the live view on the screen in LAS, the volunteer adjusts the zoom so that the specimen fills approximately 80% of the frame and brings it roughly into focus, using the focussing knob on the Smarttouch controller.

They then draw the hood down over the specimen, being careful not to touch the foam stage, which can easily be knocked out of position.

The volunteer adjusts the exposure time using LAS, which increases or decreases the amount of light that is captured, making the image 'brighter' or 'darker'. Once they are satisfied with the brightness of the image, the volunteer uses the focussing knob to bring the uppermost point of the specimen into focus and indicates that as the start of the focus stack. They then move the focus to the lowest point of the specimen and indicate that is the end. The software recommends a number of steps based on the known depth of field at that magnification. The volunteer clicks the button 'Acquire MultiFocus' and the software instructs the camera to start capturing images and move the microscope carriage through the stack. The software automatically commences montaging once all the images are acquired; it can also be instructed to automatically sharpen or enhance the image in other ways.

If the volunteer is happy with the result, they insert a scale bar, which is a simple 'four-click' process, and 'exports' the final image to the networked data storage. They repeat the process to capture a lateral view of the specimen, which is made possible by pinning the specimen on its side into a cork that is attached to a weighted piece of white foam.

The tiff files that make up the stack are saved onto the local hard drive and the volunteer deletes them at the conclusion of their shift. The resulting images are between 20 and 30MB in size and around 2675 x 2005 pixels in dimension.



Figure 11 *Podotenus callabonnensis*
(Photographer: Kim Wendell)

8.2 South Australian Museum ALA Volunteer Digitisation Project

Equipment: Canon EOS 5D Mk II, Stackshot automated focussing rail, Zerene Stacker montaging software

SAMA's Canon EOS 5D Mk II is a 21MP full frame DSLR camera with a Canon L series 100mm macro lens. The Stackshot allows for the automated capture of focus stacks.

A volunteer removes the individual specimen from its unit tray and carefully removes the labels, setting them aside. They then pin the specimen on a piece of foam covered with a slip of high quality tracing film and position it under the camera for a dorsal shot. The specimen is centred in the field of view with its head facing to the left. A small 10mm square scale marker is positioned near the specimen, but at enough distance that it can be cropped out.

Looking at the Live View on the screen in EOS Utility, they move the camera up or down using either the Stackshot controller or the handle on the copy stand until the specimen is as large as it can be, up to approximately 80% of the frame. Due to the 100mm working distance on this lens, it is sometimes impossible to fill the frame with a small specimen. In such circumstances, the image will be cropped later using Photoshop. The camera is set to manual focus.

The volunteer looks at the specimen to detect the highest and lowest points then, using the Live View window on screen and the Stackshot controller, moves the camera so that the highest point is in focus and fixes that position. The volunteer then moves the camera to where the lowest point is in focus and fixes that position. The controller is usually set to capture 10 shots, which is enough for most specimens.

The volunteer positions a diffuser ring, made from high quality tracing film, around the specimen, checks that it is not visible in the frame, closes the Live View window and starts the Stackshot. Stackshot moves the camera from the start to the end points, stopping and firing the shutter at evenly spaced steps along the way.

Canon Digital Photo Professional is then used to convert the RAW images to tiff before montaging them using Zerene Stacker. As the images are captured on the highest RAW image setting and quality is maintained in the conversion, this creates a 120MB tiff image. The series of images that contributed to the stack are deleted and the final image is saved to the shared drive.

This image is optimised using Photoshop. In our project this is done by a different volunteer, who opens the montaged image. They draw a 10mm line using the reference marker as a guide. The volunteer then crops the image, brightens the background using the Levels function, cleans up any blemishes using the spot healing brush function and sharpens the image mildly. The volunteer then adds the text '10mm' above the scale bar, flattens the image and saves it to the shared drive using LZW lossless compression. This results in a 16 bit tiff file between 50 and 90MB, depending on the size of the specimen.



Figure 12 Rhinotia semipunctata
(Photographer: Ben Johnson)

8.3 Australian Museum specimen workstation

Equipment: Canon EOS 500D or 550D, ring flash

Contributed by Rhiannon Stephens, Australian Museum

Each workstation is set up with a camera on a copy stand, a specimen drawer, a stage and a computer with dual screens (Figure 13). Volunteers remove a specimen from the supplied drawer and prepare it for imaging by removing its labels and placing it in the stage box. After capturing an image of the specimen and its labels, they fill relevant fields in the database, put the labels back on the specimen and return it to the drawer.



Process and considerations

Two volunteers operate each workstation. One volunteer (the Specimen Handler) handles the specimen and the other (the Digitiser) captures the image.

Before starting a new drawer the Specimen Handler and Digitiser:

- identify any potential difficulties in handling specimens due to their fragility and curation
- note species changes
- note the approximate number of specimens in each species
- identify whether a specimen already has a new registration label, which indicates that it has already been databased.

This process helps the Digitiser avoid errors when entering data into the database. All family, subfamily, genus and species names are entered into the database before starting a new drawer.

The Specimen Handler then:

- places the stage box in front of them and always work within it
- removes the specimen from the drawer using fingers or forceps, bracing fingers on the edge of the drawer or unit tray to assist in pulling the pin out in a controlled fashion
- marks the position of the specimen within the drawer using a labelled pin
- removes the labels from the pin using forceps
- positions the labels on the left hand side of the label block with the label closest to the specimen at the top and subsequent labels below. If there is writing on the underside of a label, it is photocopied, cut out and the copy placed with the other labels
- places a new 'K' registration number below the lowest label that was set on the pin, directly above the colour bar and scale
- positions the specimen with the head in the same orientation as the label block.

The specimen is now ready to be imaged.

The Digitiser performs the next steps:

- places the stage box under the camera 'upside down' in the same orientation as the camera. This saves the image being rotated after it has been taken
- using the camera's remote Live View, ensures that the specimens and labels are in the camera frame. The specimen and label should be centred and take up as much of the field of view as possible
- moves the labels to the foam 'pinning' block alongside the label block and pins them in the order that they were removed from the specimen, ie starts at the top and works down with the new 'K' number pinned underneath the specimen labels. The labels are aligned with the body or wings of the specimen. The new registration label is pre-pinned for easy attachment and, if possible, offset so that the last three numbers can be seen from above
- once satisfied with the final image, enters the label information into the database
- passes the stage box back to the Specimen Handler who replaces the insect in the original pin hole in the drawer.

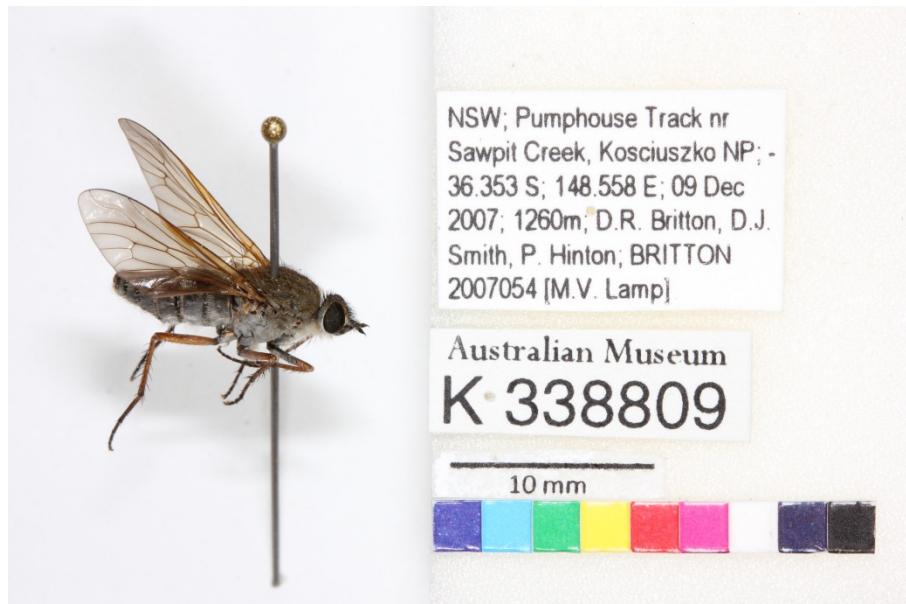


Figure 14 Specimen from Australian Museum

8.4 Australian National Insect Collection

Equipment: Visionary Digital Big Kahuna (BK) camera system

Contributed by Chris Manchester, Australian National Insect Collection

The BK imaging system developed by Visionary Digital is the main imaging workhorse for ANIC. From large insects with lots of fine hairs to very small shiny species and even genitalia, specimens of all shapes and sizes from all parts of the collection have been imaged using this system (Figure 15).

ANIC's BK system employs a Canon 5D MkII DSLR camera body and a variety of lenses mounted on a vertical focussing rail. The system has a pair of flash heads and a directional light source with three arms. Combinations of these light sources are used to create the exact lighting needed for each specimen. The flashes light the subject at the colour temperature of standard sunlight, showing the natural colours of the specimen. The camera body is attached to the rail, which is all controlled from the computer. The operator uses a specially designed program (Camlift v 2.05), to view the specimen under the camera to get the correct angle, lighting and focus. Camlift has calculated steps optimised for each lens to make sure you capture the most efficient number of images for focus stacking.

A specimen is chosen for imaging and the equipment set up. This means choosing the specific focal length lens needed to get the best magnification for each specimen. A 'cheat sheet' of the best lens for different sizes of specimen makes this choice easier. Once the lens has been chosen and attached, the specimen is placed underneath (generally with the labels removed) and the camera and Live View mode in Camlift is turned on. The specimen is positioned at the required angle and the lighting is set up. Due to the many options available, it can take a bit of time to find an optimum lighting solution, but once found it does not need to be changed. To aid workflow, operators usually photograph sets of specimens that involve the same equipment set-up. After a few test shots to check the lighting, Camlift is used to set up the focus stacking option (when required). In Live View, the top and bottom points of a specimen are found and recorded. Then the 'Auto Run' feature is selected, using the optimised step sizes mentioned above. The system captures a set of images, starting at the top and stepping down to the bottom.

All images are automatically imported into Adobe Photoshop Lightroom, an image management system, where they are available for processing. Slight adjustments to the exposure, colour, rotation and crop can all be applied in this program. The files are then ready for export to a stacking program, such as Zerene Stacker. Zerene aligns the images and merges the focussed area of each image, creating one file with the whole of the specimen in



Figure 15 *Apiocera Mullewa*,
inset: genitalia from *Apiocera rieki*

focus. This program allows for retouching any area that it was unable to correctly calculate. Once the user is happy with the image created, the image is saved for further use or processing.

Any further processing, including file conversion and adding scale bars, is done using Adobe Photoshop and the finalised images are saved into a backed up shared drive.

8.5 South Australian Museum researcher Dr Mark Stevens

Equipment: Nikon stereoscopic zoom microscope SMZ1000, fibre optic light source, Nikon D5001 DSLR camera body, Live View and Helicon Focus software

Contributed by David Hollingworth, South Australian Museum

Dr Mark Stevens has developed the following system and he, other researchers and volunteers, use this equipment in his office/laboratory.

Equipment

We use the Nikon SMZ 1000 stereoscopic zoom microscope with a Nikon camera body mounted on an LV-TV beam-splitter. The system includes the following: Nikon Plan Apo 1 x WD70 lens, Nikon Plan Apo 1.6 WD24 lens and eye pieces C-W 10 x B/22.

A World Precision Instruments Novaflex light source is used to illuminate specimens that are pinned or in an ethanol solution. This device has two fibre optic arms that can be manipulated to give optimum lighting (Figure 16).

We use a paper deflector and sit the power/light housing on vibration absorbing material to avoid the problems of vibration and drafts. It is important that the flexible arms of the light source don't come into contact with the microscope.

Illuminating the specimens is critical in achieving the desired result; we have devised a series of diffusers that can reduce the impact of high-lights especially on curved surfaces.

Polystyrene cups cut into rings can be very effective when imaging specimens at a low magnification and high quality tracing paper is also very effective. Specimens in ethanol can prove challenging, as the specimen often moves during image capture, usually caused by evaporation of the ethanol. The team has developed various techniques for keeping the specimen still, including crucibles made from dental wax, cotton wool, and women's stockings in various colours. Pinned specimens are more straightforward, but need still need careful handling.

Images captured using the Nikon D5001 camera body can be 22–32MB RAW or 9.3MB jpg files or less if storage space is limited.



Figure 16 Workstation including tools

Software and capture procedure

The camera is connected to the computer via a USB cable, the image being displayed live on the monitor's screen using Nikon Camera Control Pro 2. The microscope's fine focusing is used to bring the image into focus.

Nikon Camera Control Pro 2 software controls include the following options: exposure, shutter speed, aperture, exposure compensation, focus point, ISO sensitivity, white balance, image quality, image size and record to camera. The software can also control mechanical features, such as the release mode (eg single to continuous) and some image processing functions, such as picture control and colour space.

If you want to montage a stack of images, they can be captured by shifting the microscope's fine focus and moving the point of focus until the area of interest is captured. These images are stored in a designated folder and can be montaged using Helicon Focus v5.1.

The Helicon Focus interface is very friendly. Selected images are imported and stored in a holding area. The operator then hits 'RUN', the software montages the images and the final result can be previewed before being saved.

All final images are stored on a high capacity hard drive and backed up to an external drive.



Very good results can be achieved with due diligence at a moderate cost, as most laboratories have such equipment on the laboratory bench.

Figure 17 Unidentified bee specimen
(Photographer: David Hollingworth)

9 Resources

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