A Guide to Digitizing Insect Collections Using MANTIS and Following the Protocols of the

Harvard MCZ Entomology Type Image Project

By Sarah Ashworth and Jennifer Fogarty

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Introduction

This guide is offered to help anyone wanting to database and / or digitally image their collections. It is written in very simple terms since this work should not require a computer expert.

This guide is based on digitizing a type collection so it is more rigorous and careful than may be necessary for other collections, particularly the archive protocol. The user should take or leave whatever information they feel is necessary. However, if the guide is to be used for a type collection, it is recommended that the degree of rigor, if not the actual protocol, be matched.

Three different imaging setups are described, from a very inexpensive solution to the top of the range. These descriptions are not intended to prescribe the best or only setups, but to inform others about the setups we are using now as a result of over 5 years research—much of this with limited budgets. We developed these under the guidance of Dr. Piotr Naskrecki, with early and continued assistance by Dr. Gary Alpert and Dr. Brian Farrell.

In a similar way we describe a Filemaker Pro implementation called MANTIS. It is one of many database solutions for managing taxonomic information, but is recommended since it is easy to use, runs on both PC and Mac and is can be downloaded free from the web. It is also the creation of Piotr Naskrecki.

Part 1: Databasing with MANTIS

Database Absolute Basics



The database:

Think of the **database** as a filing cabinet, containing and organizing a set of folders and files in such a way that is it very quick and easy to find individual records.



The Files:

(In other database software these are called tables). The database, like a filing cabinet, contains a set of folders or **files** that separately hold the data for each category. In MANTIS, you have one **file** for the species data, one file for specimen data etc.



The Records:

Inside the **files** are sets of records. Imagine them as identical forms filled with different data. Each form carries the information for each genus or species or specimen etc. and they are all formatted in the same way. These forms are called **records**.

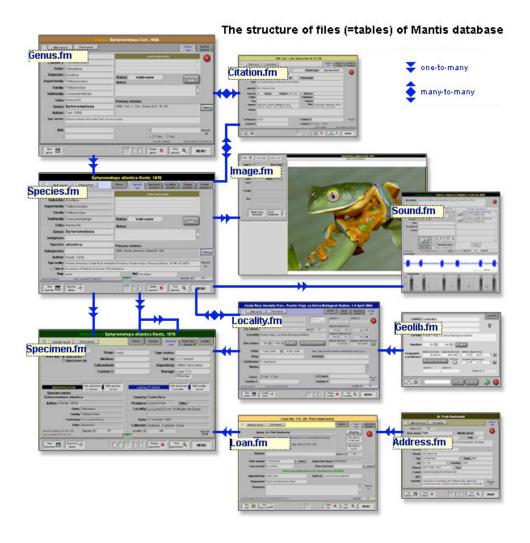
Species: erraticus

The Fields:

Each record is formatted like a form, with boxes for you to enter information. These boxes are called **fields**. You have the same fields for all the records in the same file (e.g. in the species file) and you enter different information into them.

Structure

The MANTIS database is a relational database. This means it is made up of many separate files, each of which could be databases in their own right. They are separate files but in order to make them useful to us (e.g. to be able to look at a species and then move straight to the specimens for that species without having to search again) they need to be linked together. The database does this by **key fields**. The key field makes a connection by having the same field in 2 separate files, so that when, for example you are looking at a particular species, the database has a direct connection to its key field in the specimen file that you can go to straight away. For an example see **Navigation** section: **The Key Field.**



To look at more details of the structure of the database click on the "About MANTIS" button and then "Structure". You can click on the different pages to view additional detail about each file including the key fields.

Navigation



Open the folder called MANTIS and double click on the Species file. You can open any of the other files, but the species file will open all the files. The page that opens is called the **Menu**Page and is where you instruct the database what to do, depending on whether you want to find records, browse records or add new records.



Begin by familiarizing yourself with MANTIS.

If you are ever "lost" in the database you can always click the MENU button (usually on the bottom right corner of every page) to take you back to where you started

1. Browse

Click on the **Browse records** button on the left side of the front page, then click Genera. This will open the genus file for you to browse. You will be looking at the first record in the genus file. Notice at the bottom of the page, in the middle, there is a square gray box with gray arrows either side. Try clicking the arrows to take you to the next and previous records, the far right and far left arrows will take you to the last records and first in the file. Try clicking the gray box. A different page appears, giving you the same records as before, except in a list format, so you can see them all at once.





Genus

2. Moving from one file to another

Still in the browse mode of the Genus file, from a single record (not the list) notice the species tab on the top right side. If the tab has a green square, this means there are species present, if red, no species are present for that genus. In this database the tab should be green, since there should be no genera entered without species. Click the Species tab and you will be taken from the Genus file to the Species file, and the species for that Genus will be displayed. If there is only one species you will see the full page view of it, and if there are any more you should see a list.

From within the Species file, when you select a single record from the list, you

should see a new set of tabs. Try clicking the Specimen tab and you will be taken to the specimens for that species. Experiment with clicking from one to another to get an understanding of how it works.

3. The Key Field

As explained in the **Structure** section, the files (species, genus, specimen etc.) are connected by a key field. These are what allow you to move from a species directly to the specimens for that species, or any related records in different files. An example of a key field is the Species ID field. Browse the species file and look at an individual species record. Look for the field **Species ID** and memorise the



number in the field. Then tab to the Specimen file and check one record to see how the Species ID field will contain the same number.

Finding Records

Access the find commands from the **Find records** button on the MENU page. You can use any piece of information that you have, type it into the appropriate field and click **Perform search**. You can use more than one piece of information entered into different fields if you want to narrow your search further. You can search in any of the files; genus, species, specimen etc. but since they are linked, you can access one file from another by clicking the tabs at the top of the page to move to other files. When you do this you will always tab to records that are related to the records in the current field. If you are unsure of the spelling it is best to only type the first few letters and then select the record you want from the



found set. You can also use the **All taxonomy** field as a quick search shortcut. Follow the steps below if you'd like to be guided through these find methods using an example in the newly downloaded database.

To find Sphyrometopa atlantica

- 1. Click Find records then Species
- 2. Type *atlantica* into the species field and hit **Perform Search**



Say you were interested in the genus Sphyrometopa and you were looking for species that Rentz had described you could type *Sphyrometopa* and Rentz into the appropriate fields and come up with Sphyrometopa atlantica

OR

If you weren't sure of the spelling of the species name you could type in *atla* into the species field

OR

If you weren't sure whether *atlantica* was a generic or a specific name you could Type in *atlantica* into the **All Taxonomy** field

The Found Set

Whenever you find more than one record, this is referred to as the found set. For example:

1) Click **MENU**, **Find species**, type Agraeciine into the Tribe field and hit perform search. Your found set will be 4 species and they will appear as a list.



2) Click on any of these species, browse through them with the arrows and whenever you click the square gray box to take you to the list, your found set will still be there until you perform a new search. To quick check your status look in the bottom right corner of the page above the menu button displayed like: record 1/4. This tells you that you are currently looking at record 1 of a found set of 4.



Record: 1/4

Creating Records

confusing at first.

The easiest and most systematic way of entering new data is to begin at the **Genus** level and work down the taxonomic hierarchy, clicking the tabs from right to left adding data. You can easily enter data starting from other files, and work from that point, creating records in other taxonomic levels from there, or link back to higher taxonomic levels that already exist in the database, but this can be

Example: Entering a new **specimen** into the database starting at the higher taxonomic levels:

Click on the New Records button on the menu page, then click Genus

Last

searches

Browse records

New

1. **Enter data for Genus.** Type in all the information you have about the genus species, fill as many fields as you can but don't worry if you can only fill a few,

Type species: This genus already exists in the database. If it is a homonym, select 'Homonym' in the status field. Otherwise, delete it WITHOUT deletion its species

you can always add more information later.

N.B. If, when you type in the genus, a red message appears saying this already exists in the database you should follow the prompts and delete this record (button delete genus) and when prompted choose NOT to delete its species. You should then find the genus which already exists in the database and work from there. If, when about to enter a new specimen / species, you suspect the genus already exists in the database, you should try to **find** the genus record first, then tab to the species record (continue steps from 2)

Duplicates

The importance of avoiding record duplicates: One of the purposes of a database is to remove the need to duplicate data. Data is stored in one place only and is accessed from other places, so that any updates/changes are always true in all places. More than one record for the same piece of data not only compromises time and accuracy in terms of data entry, but also confuses the way the database works since they may be recognized as different in the database. The database will pick up and help you correct some duplicates you create by mistake, as explained above, and will find certain errors when you perform "Database Maintenance" (see section). But many will be seen as new records and you will have to find the errors and correct them yourself. The best solution is to get into good habits of checking to see what is in the database already before creating new records.

In the case of **Collection event** data, it is particularly easy to build up duplicates which will hinder any analysis (e.g. mapping) you may want to do in the future. See "**Collection Events**" number 4 in this section.

- 2. **Enter Data For Species**. From the genus you have just entered/found click on the species tab in the top right hand corner.
 - a) If you have no species entered for this genus, the tab will be red:

 Click on the red tab and you will get a dialogue box asking if you would like to create a new species. Click OK and enter data for species, filling as many fields as you can.
 - b) If you already have species entered for this genus, the tab will be green:

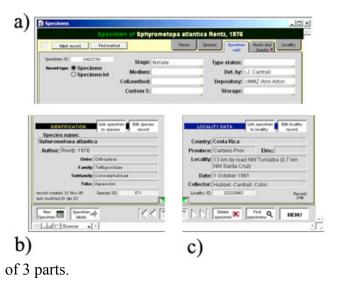
 Click on it and you move to either the single species record as a page, or a list of species depending on how many species have already been entered.

If it is a list, click the **Add New Species** button above the list. On the single page click the **New Species** button at the bottom left. You can then enter data into the fields for the



species.

3. **Enter Data for Specimens.** Once you are finished entering data for the species you can tab over to the specimen file. As before, follow the prompt to add a new specimen, or click the **Add new specimen** button. The specimen page is made up



- a) The top section is for the specimen information that you should fill now.
- b) The bottom left corner is already filled in with the taxonomic data drawn from the species file.
- c) In the bottom right corner are fields from the Collection Event file, which you will fill next.
- 4. Collection Events are a set of data about the specimen that includes the location, collector and date. If you want your locality data to be useful for analysis such as mapping, it is important to follow the "Process of entering collecting events" protocol below. The Collection events file contains another file called the Geolibrary. The Geolibrary holds all the locality data you enter and serves as a look up for the same locality entered at a different time. Think of both the Collecting Events and Geolibrary files as you would your species, genus, etc. files in terms of avoiding duplicate records, (see Duplicates this section) always look for existing records rather than typing in the data on your specimen label straight away. The database cannot warn you when you are typing in a duplicate collecting event as it does with the genus file. It is easy to build up a set of data containing repeated information, information that will not be useful for things like mapping until it is cleaned up, which means extra work. Follow the protocol below to avoid this.

5. Process of Entering Collecting Events

- a) In the c section (illustration above) of the specimen file, click the button Link Specimens To Event. This will take you to the Collecting Event Lookup.
- b) Type a word, or the first few letters of a word for your specimen locality into the appropriate field and click **perform search.**



If the lookup comes up with nothing, click the **New event** button and enter all the data in here you can.



c) If you have many results, it helps to sort the list by clicking the titles of the columns (in blue-see first arrow illustrated). Click to sort by date, then locality. If you can see the exact collecting event, click on it and it will be entered into your record. Use the blue dots at the end of the line (see second arrow) to go to the quick view window (below) without entering them.



- d) If you can see the same locality, but not the same date, click on the blue dot and then click the **Clone event** button from the quick view window. The locality information will be entered without the date and collector, which you can enter now
- e) If you find no matching locality, click the New Event button and enter all the data in from scratch.

Deleting Records

Single Delete

On any page in any of the files there is a **delete** button at the bottom. By clicking it you will delete the current record in view.

Children, parents and orphans When deleting a record you should be aware of the records that are linked to it. If you delete a genus with a species linked to it in the database you will be asked whether you want to just delete the genus, but also the species attached to it. If you just delete the genus you will be left with an "orphan" a child or children (e.g. the species) without the parent (e.g. genus). To make the database complete you will need to go back to that species and assign a new genus to it. If you say that you want to delete the species attached to it you won't have the problem of orphans but you must be sure that you don't want those species records.

Multiple Delete

Whenever you are in a list format you can delete them all by clicking the **Delete this set** button. For example, if you wanted all the species in the genus *subria* deleted, you could first find them, then **Delete this set**.

Importing Data

If you wish to import data from an older version of MANTIS or any other MANTIS database, go to **Database maintenance, Import old data** and follow the instructions.

Importing data from other sources

Before you import any data or do anything major with your database, you should **first make a copy of your whole database** incase of mistakes. Copy the whole MANTIS folder and store it with the date of backup.

Mantis will import:

FileMaker Files (*.fp5;*.fp3;*.fm)
Tab-Separated Text Files (*.tab;*.txt)
Comma-Separated Text Files (*.csv;*.txt)
SYLK Files (*.slk)
DIF Files (*.dif)
Lotus 1-2-3 Files (*.wk1;*.wks)
Basic Files (*.bas)
Merge Files (*.mer)
Folder...
dBASE Files (*.dbf)
Microsoft Excel Files (*.xls;*.xlw)
ODBC Source...
XML Source...

1. The first and most important step before importing into MANTIS to understand the structure of the database and how the files link together. (See **Structure** pg. 4

and the key field, in Navigation pg. 5). Now click "about MANTIS" on the MENU page then click Structure. Take a few minutes to understand the relationships within the database and how the import you wish to make involves those relationships. Click on the file images in this Structure page to discover the key fields. These will be important for your import.

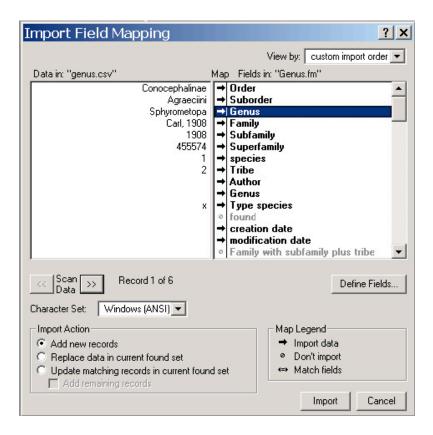
For example, I am about to import data that includes information both in the Genus and the Species files. Looking at the database structure map I see there is a relationship between the two. Clicking on the individual file images I find that the key fields for the files are "species_ID" and "Genus". The



Genus field appears in both files so will be the key field that forms the relationships between the files.

- 2. Inspect the data you wish to import and match it to the fields in the MANTIS database. Renaming them helps avoid confusion. If your data needs to go into more than one file, separate the data into separate import files making sure both have data for the key field. No other data should appear in both files.
- 3. Clean your data so there are no unnecessary characters, columns, etc. And remove data that is not going into this database.

- 4. Import beginning with the higher taxonomic level. Open the file in MANTIS (e.g. browse genus). Go to menu **File**, **Import records**, **File**
- 5. The import field mapping window (illustrated) will appear. The data you are importing appears on the left and the MANTIS fields on the right. You must match the two together by selecting the fields on the right side of the window with your mouse and dragging them to the correct position. If you don't want one of the fields to import click on the arrow so that it displays a "don't import" symbol.



- 6. Select your character set and be careful to check the correct **Import action**. Click import.
- 7. If you are importing e.g. a Species file after a Genus file import, as long as you are including the key field (in this case the Genus) each species will link to the correct genus record.

Exporting Data

This command will copy the records and fields of any of your database files and produce an export file of your chosen type

- 1. Selecting the appropriate file, find the records you wish to export. If you wish to export them all **Find all**
- 2. Click the **export records** button if available or go to menu **File**, **Export records**. Give the export a name and choose what format you wish the export to be. If you want the data to be imported into another MANTIS or FileMaker database be sure to select the FileMaker format.
- 3. In the Specify Field Order for Export window the file you are exporting from is on the left and the file export you are creating is on the right. Either **Move all** the fields from the database for the export, or **Move** selected the fields you wish to have exported. Select the character set and output and click export.

Database maintenance

There is a database maintenance button on the top left corner of the MENU page. This is a very useful tool which helps keep your data clean and free of duplicates and orphans. You can also run the database maintenance function to update the All taxonomy search field.

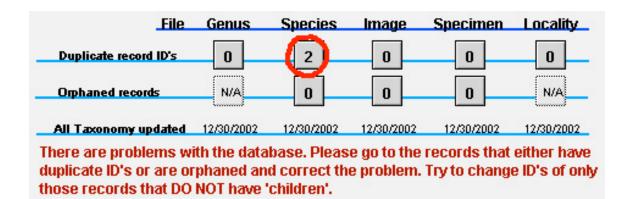


To use it click the Database maintenance button, from option 1 check the boxes you wish to be checked/updated and continue. The procedure will take seconds to minutes depending on the size of your database.

Illustrated below is an example of a problem found in the database. Clicking on the duplicate or orphan found takes you to the records that need



correcting. You will need to inspect the duplicates for children before deleting any so that you can decide which to delete see **Deleting records** pg. 11. For orphans you will need to find out the higher taxonomic data for that orphan and either re-link it if it is still in the database, or re-enter it by working back up the taxonomic hierarchy from the orphan. Alternatively you can delete it and re-enter the data from the beginning





The remaining options in the Database maintenance window:

Custom fields. If you browse single records in any of the files in the database you will notice a green arrow located at the bottom of the record. Click

this and you are taken to an expanded view where there are additional fields including the custom fields. Click continue in option 2 and rename any of the custom fields whenever you need fields specific to your project. Also you should rename the project name here now unless you want to stick with Tettigonioidea.

Web Publishing Read the next section for details of how to put your database onto the web

Customize loan Form MANTIS allows you to generate a loan form which you can customize here and add your own logo if you want.

Import old data This function is for entering data from previous versions of MANTIS.

Recovering Damaged files

Power outages, computer system crashes and leaving Filemaker open while transferring files may damage files. Most of these things are avoidable so please try and adhere by these suggestions:

- 1) If your work place suffers from frequent loss of electricity, purchase a battery backup supply for your computers. This will usually keep your computers running for 5-10 minutes after a loss f electricity so you can properly shut down the computer and save all unsaved information.
- 2) When transferring MANTIS files over a network make sure that Filemaker and MANTIS are NOT running on either computer while you are making the transfers. Do not open MANTIS or Filemaker while a transfer is running

If you are unable to open one of your files it may have become damaged. You can try and recover the damaged file. Usually however, all information entered since your last save will be lost and you will need to reenter the information.

- 1. Go to File....Recover
- 2. "Open Damaged File" box should appear
- 3. Locate the damaged file (for example "locality.fm") and open the file
- **4.** It will then rebuild the file and a new recovered file will appear (for example "Locality Recovered.fm")
- **5. DELETE** the damaged file and **RENAME** the new recovered file to exactly the same name as the old deleted file.
- **6.** Close the database and open it again and the file should open properly.

Web publishing with MANTIS

In order to publish your data on the web using MANTIS you need (1) a copy of FileMaker Pro 5.5/6 Unlimited (it has to be an "Unlimited" version of FM, other versions are limited to 10 visitors in a 12 hour period), (2) a server computer with a static IP address, and (3) a basic understanding of HTML (to modify the HTML templates provided with MANTIS). There are also a few very important ground rules that you must follow. First, there can only be **one** copy of MANTIS running on the server computer. Second, a server is a computer that cannot be used for any other purpose, it has to be dedicated to data serving only. Third, you should **never** work on the copy of MANTIS being served. Instead, maintain at least two copies of MANTIS, on two separate computers. One will be your working copy where you add and modify your records, the second is the server version, updated occasionally to reflect recent changes (you can even update it daily.)

On the MANTIS website I provide some sample HTML templates that you can use and modify for your purposes.

Ouick Start

You can quickly test the web publishing features of MANTIS using FileMaker Pro 5.5 or higher (you don't need FM Pro Unlimited for this limited test.) Here is what you do:

- 1. Make sure that you have downloaded the sample templates ("MANTIS" and "Types") and placed them in the "Web" folder of your FileMaker Pro program
- 2. Open the Application Preferences for FileMaker Pro and click on the Plug-Ins tab
- 3. Check the Web Companion check-box and click "Configure"
- 4. Uncheck the box "Enable Instant Web Publishing"

- 5. Make sure that the TCP/IP Port Number is 80 (if you get a message that this port is already taken, disable any other web servers currently running on your computer)
- 6. Go to the main menu of MANTIS and click "Database maintenance"
- 7. On the next screen continue to "Web Publishing"
- 8. Click the "Prepare for Web" button
- 9. Select an HTML template
- 10. Click the "Test Web Server" button

A script will launch your web browser and connect you to your MANTIS database. **Note:** Computers running Mac OS often have problems resolving the "localhost" domain and you may need to replace it with the actual IP address of your computer (e. g., http://localhost/MANTIS will have to become http://localhost/MANTIS will have to become http://x.x.x.x/MANTIS, where "x.x.x.x" is your IP address to be found in the TCP/IP or Network control panels.)

Part 2: Imaging

What camera setup do I need?

Small specimens: If your specimens are less than about 2.5cm it is best to use a camera mounted on a microscope. With the option of attaching either a X0.5 or a X1 lens to your microscope, photographs of specimens from the range of less than a millimeter to 3 or 4 cm can be taken with the same system. Read the following setup solutions to help decide. **Very small specimens:** If your specimens are less than 5mm or require close-ups that aren't flat, you will probably need Auto-Montage. Read about Auto-Montage and setup solutions 2 and 3

Larger Specimens: If your specimens are larger than 2.5 cm and don't require close ups you can do without a microscope and use a digital SLR camera with good macro lenses, see setup solution 4

Auto-Montage software

When you take pictures at high magnification there is a problem with the depth of focus. This means that your picture will only show one section of the image in focus, and this one section might not be enough to display the characters you need to show in the picture. Auto-Montage software can solve this problem by reconstructing all the in focus sections of a series of pictures to make one picture completely in focus. Auto-Montage software costs \$3900.

1. The interfaced JVC/Auto-Montage setup

If you need to use Auto-Montage on a day-to-day basis this setup is highly recommended if you can afford it. It is the same in effect as the Coolpix and Auto-Montage software setup (below) except the interfacing of the camera and computer allow pictures to be taken with far greater ease and speed. A very good live image lets you take the pictures as you see the specimen and as they will turn out as pictures. Immediately after capturing the images the software reconstructs the image. One of the main advantages is that if the image is unsatisfactory, you can immediately retake the set while the specimen is still there under the microscope. This is compared to the Coolpix, where images are taken with a 1.5 inch monitor, so it is hard to tell if the pictures are good enough until they are downloaded after shooting is done. Therefore if the images turn out to be no good you will either have to cut your losses and make do with an unsatisfactory image, or go back, hunt for the specimen, get it out, remove its labels and start over again.

The full set up including interfaced camera, IBM Workstation computer and an automated z-stepper (focus drive) costs \$42,000. The automatic focus drive is not essential but helps if you are taking very many pictures or those at very high magnification (e.g. above X80) since the focus drive cannot be moved by hand without some vibration to the camera. Although the company, Syncroscopy who makes Auto-Montage, prefers not to sell incomplete systems, they can be negotiated and the absence of a z-stepper takes about \$8000 off the bill. The minimum system, of just the camera, interface board, software and color corrected c-mount costs under \$19,000. This is the

setup outlined here in this manual. Caution is advised when buying this system, since peculiarities of computers can cause problems with the interfacing. An IBM Workstation is recommended for problem free functioning, although we use a Compaq with no trouble. You will also need a 17 inch monitor in order to view the capture screen properly.

This setup is recommended for those on a budget, and is perfectly adequate if you are

2. Nikon Coolpix 990 / 4500 with microscope

taking fewer pictures (e.g., for publications), and are excellent for portable uses. The Nikon 990 is no longer manufactured but can often still be purchased online through resellers at very reasonable prices (\$300-500). The Nikon Coolpix 4500 is the newest version of the 990. Both have good optics and excellent macro capabilities (focusing down to 2 cm), so they can be used without the aid of a microscope for larger specimens that don't require high magnification close-ups (also see http://www.perspectiveimage.com/ for Coolpix attachments that increase the capacity of the camera without using a microscope). These models also have the advantage of a 30mm lens which is very close or the same diameter as dissecting microscope oculars and the attachment adapters available (the Coolpix 5000, for example, requires an additional stepdown adapter). Several eyepiece adapters are available ranging from \$200-\$400 (e.g., MVIA.com). You will also need a tripod and a remote control (for these Coolpix camera, the Nikon MC-EU1) to prevent shake and/or good lighting. Any microscope can be used, although the better the optics the better the pictures. Fiber optic lighting is also recommended. Prices for the camera vary. Expect to pay between \$600-\$700 for the camera, plus AC adapter, remote shutter control. You'll also need to purchase a Compact Flash card to store the images and a card reader is recommended although not necessary.

The camera can produce surprisingly good results for the price. On the down side, it takes practice to get consistently good results. The camera cannot be connected to a computer while capturing images, so there is a delay between capturing and viewing. At time of capture viewing the specimen is done through the 1.5" monitor at the back of the camera rather than through the eyepieces, this means accurate focusing can be a problem. There is a certain amount of guessing and hoping that the pictures will turn out well, and back tracking if they do not.

3. Coolpix with Auto-Montage

The camera you choose to buy may be governed by what you can afford. Auto-Montage was designed to be used with a camera and microscope setup up provided by Synscroscopy but this setup up is expensive. It is possible to use the Auto-Montage software with the Coolpix, which, if you are on a tight budget may be one of the only ways you can afford to see the benefits of Auto-Montage. Assuming you already have a microscope, you can get set up for just over \$5000. However, since the Auto-Montage software is not designed to work with the Coolpix the procedure you need to follow can be difficult and painstaking, as well as time consuming and sometimes frustrating.

Pictures are taken with the Coolpix, they are downloaded onto your computer, renamed so that Auto-Montage can read and finally process them.

4. Digital SLR cameras

For insects on the larger end of the scale here are brief outlines of 2 good options: At the MCZ we have extensively used the Nikon D1X, one of the best digital SLRs with an AF Micro 105mm lens and a 60mm lens. The camera now costs about \$3800 and the lenses: \$5-600 for the 105mm and \$350 for the 60mm. Set on a tripod it can be used with normal desk lamps and longer exposure. A high quality flash system is recommended for optimal detail.

The Canon 10D (new as of March 2003) is also strongly recommended and is comparable in quality to the Nikon D100 (and much cheaper than the D1X: see KenRockwell.com for information).

Canon 10D \$1500

2 lenses to cover the full range of macro shooting:

MP-E65mm (super macro mag from 1-5X) \$800

Canon EF 100mm macro \$470

As with the D1X you can use desk lamps and a longer exposure you but will need a remote cord or this highly recommended flash:

Canon Flash MT-24EX Macro flash \$600

Taking Pictures and Handling specimens

Here are some suggestions about the general procedure of taking pictures and handling specimens. For specific instructions go to the sections for the Coolpix or JVC.

1. The Specimen

a) Before taking any pictures an expert in that particular group should be contacted and asked which views of the specimens are most useful to have photographed.

If you are dealing with type specimens you need to take great care with your handling procedure. It is best to get into good habits straight away, here are some tips:

- **b)** Remove the specimen from its drawer by taking its box with it. Don't take any more than one box out at a time and always replace the lid of the drawer immediately.
- c) If you are dealing with a choice of specimens (e.g. a syntype series) select a good-sized specimen with important features visible e.g. legs not obscuring areas of interest. You may need to look at a few under the scope to decide.

DON'T try to move any parts of a specimen if it is a type, and only attempt to move parts of non-types if you are experienced and authorized to do so.

2. The Labels

Remember that without the labels the specimens have no value, so treat them with as much care and **never** remove the labels from more than one specimen at the same time!

Remove labels with fine or preferably flat forceps and place the labels in the order of removal onto a putty block so:

- a) That the labels can be returned to their pin in the same order that they were found
- b) That the labels are more easily positioned for photographing and they can be put back onto the pin more easily
- c) When handling the labels always pin the specimen back into its box so it is safe and identifiable. A specimen pinned anywhere else is quite invisible and very vulnerable.



3. Align the specimen:

- a) Laterally, dorsally and ventrally in perfect parallel to the lens so that as much of the body is in focus at once. Even when you use Auto-Montage, a badly aligned specimen will make the specimen appear distorted once it is put all in focus.
- b) Frontal views should be as requested for the particular group e.g. whether the shape of the top of the head is important or whether the mandibles are more important. Always have the head symmetrically in view, use the position of the eyes to judge. If antennae come into view easily with the headshot, include at least one. Use your judgment; sometimes it is easier to get the antennae in on the dorsal or lateral view.

4. Lighting

a) Place the light diffuser, (see illustration in the following section) around the specimen and check to see what the specimen looks like again. Adjust the exposure time in the capture window. The light diffuser is very important for even, soft lighting that maximizes the detail on the specimen

Adjust the position of the lights or the exposure time trying to even out the lighting. Try to avoid glaring white reflective spots by pulling the fiber optic arms back. By covering up each spot light in turn with your hand you can see where problem reflective spots are coming from. If the image becomes too dark increase the exposure time to compensate.

The illustration demonstrates how careful lighting improves an image.

d) Labels need to be photographed with a X0.5 lens and will require a much shorter exposure time.

Careful positioning, focusing and lighting is what makes good images, so it's worth taking time over this







Auto-Montage Software

The following is a brief outline of some of the features that we use regularly in Auto-Montage and suggested settings for use in imaging entomological collections. It is highly recommended that you contact Syncroscopy customer support for further details on how to optimize your particular setup or for more detailed explanations of how the software works.

Auto-Montage can be used in conjunction with a camera to capture images. The software also can combine a series of images each taken at different focal distances, with different areas in focus and merge them together, so that the end result is a single image entirely in focus. Several parameters can be adjusted during this process to obtain better results.

You want a final image that is accurate and free of noise created by the montaging process. Noise refers to artifacts which appear as patchiness, blank spots and blotches, sometimes within the specimen and often in the background. These can often be eliminated or at least reduced by optimizing the montage parameters.

Here is a brief outline of some of the functions we use.

Scan Montage Preview Montage Cancel Method: Fixed Blended Weighted Exponentially Weighted Compound Weighted Precision Patch Size: 10

The Scan Montage command

When you go to montage a series of images, the Scan Montage box will appear. You will have a choice of montage method and optimization and patch size.

Methods

Fixed is the default mode. It is also the quickest mode. The 'Fixed' algorithm selects the single source image plane which is in best focus at each pixel location in your image. It's best to try this first and see how things look, then adjust if necessary.

Blended takes into account the effects of two or more in-focus planes at any one pixel location. The resulting depth map values may be fractional, which shows slopes within the sample more accurately. Syncroscopy recommends this method for biological specimens, but we find although it can make images appear more "smooth", it also can make them look slightly hazy when compared with the fixed method.

Weighted takes into account multiple in-focus planes at any one pixel location. This is not of much use when photographing insects. Images look nothing like the specimen.

Exponentially Weighted is similar to Weighted depth, but biased more strongly towards the planes of best focus. Also of no use in our work.

Compound Weighted is a combination of Exponentially Weighted and special post-processing. It is particularly effective on biological samples under incident illumination, according to Syncroscopy. We have found it to work on some difficult specimens but the image does have a "soft focus" look.

Optimize

Speed uses an extremely fast calculation of best focus. This is the default setting. It is best to try this first combined with the Fixed method to get an initial result.

Precision is significantly slower than Speed (can take several minutes) and can generate cleaner images but the difference is barely perceptible. Generally we use this only for images to be used for print and publication purposes.

Patch size

The 'patch size' is the diameter (in pixels) over which you might expect the surface of the specimen to remain relatively continuous.

The default value is 10. We first run a scan with patch size 10 and see how it comes out. If it looks good, we'll leave the patch size as is

Larger values (>10) may be required when noise is visible within the specimen The noise should be reduced or may completely disappear, however, larger values will tend to "smooth" the image, losing fine detail and making the image appear less sharp.

We have found that most of our insect specimens work best with a patch size within the range of 10 to 30. Try scanning the same image with different patch sizes and comparing the results if you are unsure which patch size to choose.

Generally speaking, use the lowest patch size which does not contain noise within the specimen. If noise is found solely within the background areas of the image, then a better option may be to use the Enhance tools and increase the confidence level (see section on Enhance).

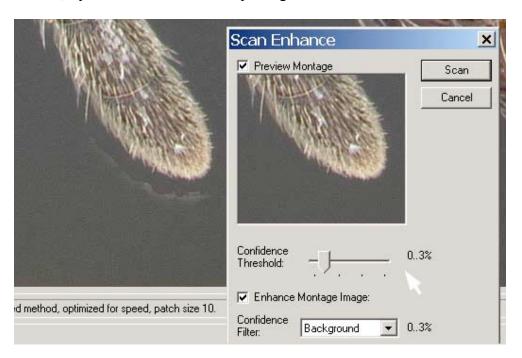
Scan Enhance command

The scan enhance command allows you to make changes to the image after the montage has been performed.

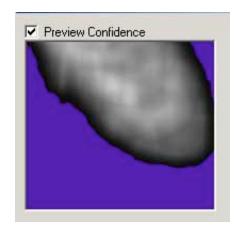
A useful feature for eliminating noise in the background is to set the confidence filter to the background and increase the threshold.

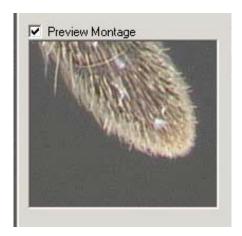
The confidence filter allows you to apply various effects to the montage image but only within the low confidence region selected by the confidence threshold. When the filter is set to background, then within the low confidence region, the last sources image is copied to the montage image.

A quick protocol for this enhance command is to move the Confidence Threshold to 0.3 or 0.4, see how it looks, use the **purple arrows** to compare with the previous version, try another value then compare again.

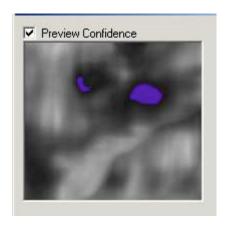


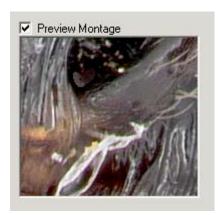
A more in depth understanding: To determine what confidence threshold to use, use the preview window to view what you would like to eliminate and slowly increase the confidence threshold. Care must be taken, however not to eliminate information from within the specimen. Areas of the specimen which are dark or obscured (ie. Anywhere the computer would have trouble determining what's there) may be within the low confidence region, and could be eliminated should you place the threshold too high. To avoid this, preview the confidence levels. This will appear as a black and white x-ray like image, where areas of high confidence are lighter and areas of low confidence darker. As the threshold is increased, areas which will be replaced or "ignored" will turn purple. Be sure that no purple areas appear within the specimen boundaries. Conversely, to get the best results, the majority of the background of the image should turn purple, meaning that these areas will be replaced with the last source image of the series, resulting in a nice smooth background





Example of the background being ignored (area in purple) by increasing the confidence threshold (this is what you want)

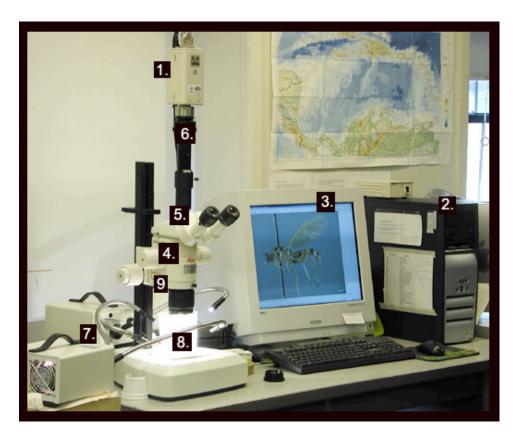




Example of a patches within the specimen being ignored, something you don't want!

The Syncroscopy/JVC camera with Auto-Montage

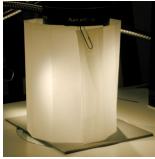
These guides are for the JVC KY-F7OB Camera interfaced with the computer by a bus mastering system developed by Syncroscopy specifically for Auto-Montage.



Details of our particular setup, refer to illustration

- 1. Camera: JVC KY-F7OB
- 2. Computer: Compaq Evo Pentium 4 Desktop
- 3. Monitor: NEC FE1250 16 inch. This size is important for Auto-Montage to view properly
- 4. Microscope: Leica MZ12.5
- 5. With Leica Trinocular Video/Phototube And we tried 2 options for a c-mount:
 - The cheaper version uses no optics; a photo port and a c-mount adapter for Leica (not shown here)
- 6. The more expensive version uses a 0.45X color corrected chip from Diagnostic instruments (\$1160) or priced by Syncroscopy at \$1487 plus the same photo port plus an L clamp.
 - We tested them against each other and found a crisper image, more light and better color. Also more field of view since it is 0.45X 1 rather than just 1. But this also means the magnification power is reduced and since it is priced at over \$1000 the slight increase in quality might not be considered worth it.

- 7. Lights: We use 2 Fostec light boxes with fiber optic adjustable arms
- 8. Diffuser: We use 2 types of diffuser, both are easy to make.
 - a) Vellum diffuser; the light is diffused by passing through the vellum and then bounced around the chamber. Use light-weight vellum (so that enough light can pass through it) cut a strip a little higher than the distance between your lens and microscope stage. Make folds so that the vellum can stand upright as a cylinder. Wrap it around the lens and specimen and point the lights at it as illustrated



b) Styrofoam cup; the light is sent in over the edge of the rim of the cup and is bounced around inside the chamber. Make one by cutting the bottom off a Styrofoam cup and placing it over the specimen. There should be enough space between the lens and the cup to shine light into the chamber. The aim is not to point any light directly at the specimen, direct it at the edges of the top of the cup

Generally the vellum diffuser is easier to use since it takes less manipulation to get the required effect. The styrofoam, since it is reflecting and bouncing very efficiently, needs less light to achieve the same illumination. This is good if your lights aren't very powerful and because the styrofoam distributes light so well it sometimes helps give definition which improves Auto-Montage results. However it can be difficult trying to avoid the harsh look of direct light if you are not careful with the way you position the lights.

9. Sliding axial microscope carrier: This enables the camera to be shifted so that it is positioned directly over the specimen rather than slightly to one side as with any stereo microscope. The axial position improves the results of Auto-Montage since the images stack on top of each other rather than each going to one side. Since Auto-Montage has such a good align feature, this axial carrier is not essential, but results are improved. To slide the carrier over, grip the binocular tube with both hands and physically slide the whole scope and camera over to one side until it clunks into place.

Fine focus is necessary for high magnification where the focus increments between images is very narrow

Photographers neutral gray card is necessary for color balance and improves the general look of images when used as background. From any good photography store (not seen in diagram, under the diffuser).

Neutral Gray Plastercine (Children's play putty type material) for holding specimens and labels in place.

Camera Settings

Install the camera, software and bus mastering system according to directions by Syncroscopy.

Any problems with the camera not responding etc. Run through the Section "Auto-Montage troubleshoot" in the Appendix. If there are still problems contact Syncroscopy.

1. Check for correct settings

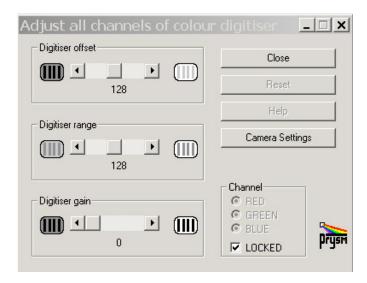
- a) Turn on the camera
- b) Open Auto-Montage
- c) Click on the capture icon in top left of Auto-Montage page



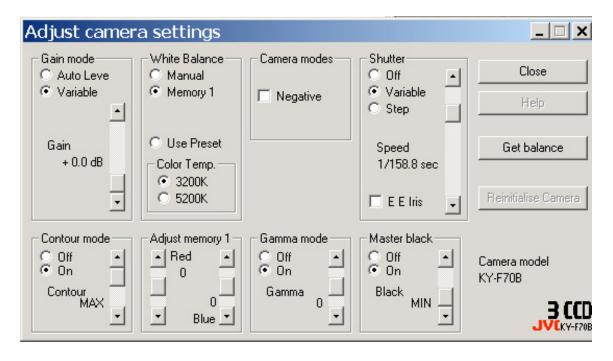
d) Click Settings in capture window



e) Click Camera settings in Adjust All Channels of Color Digitizer window



f) Adjust the settings if needed to the following:



Click close to save settings and exit

2. Setting white balance

For the first time set up you must do this since all light sources are different. For the most accurate color, adjust the white balance whenever the lighting in has changed. Adjusting once before you start each day is good practice.

a) Make sure the dials on the light boxes are at max.

- b) Put the 1.0x lens on the scope and place a piece of neutral gray board under the lens. Insert a medium sized specimen under the camera as if you were about to take a picture with the light diffuser in place and the lights arranged to give as best lighting for the specimen, all as normal.
- c) Follow the steps above to arrive at adjust camera settings window
- d) In the **adjust camera settings** window click **get balance**. A signal saying **Autowhite OK** should appear on the gray screen

3. Creating calibration files

A useful function in the Auto-Montage software is that you can add a scale bar to the image. The scale bar tool needs to be calibrated to your microscope and lenses. If you are setting up your system from scratch, follow the steps below on how to save a series of templates, one for each magnification. If you are unsure whether your setup is already calibrated, you can check it by taking a picture of a mm rule and adding the scale bars as explained in section **Taking pictures no. 5 this section**

- a) Beginning at the lowest magnification, take a picture of a ruler with mm sections (Follow instructions as in **Taking pictures no. 2 this section**)
- b) Click on the calibrate button or find the same icon in the measure menu



- c) Use the mouse to draw a line over the units on the ruler, type the amount into the X graticule box and select the correct units. N.B. The more units you cover the more accurate the calibration
- d) Go to menu File, Template, Copy from document
- e) Go to menu **File, Template, Save as** make a folder e.g. named with the lens magnification, and name the file with the magnification at which the picture was taken
- f) Repeat the process for all magnifications and lenses

To add the scale bars see **Capturing Images with the JVC** (following section)

Capturing Images with the JVC/Auto-Montage Setup

1. Preparing for image capture:

- a) Switch on the JVC AC Adapter
- b) Turn on the lights (remember to set them to the same light intensity as you did when performing your white balance).
- e) Insert the pin of your specimen into a specimen stand under the scope. If you are taking a dorsal or ventral shot it is best to use a gray putty block so as the background is a consistent gray color, the putty works well for all shots if you do not have a stand.
- f) Open Auto-Montage and click on the capture icon. The live image should appear, maximize it if necessary.
- g) Reduce your magnification to minimum so that you can locate the specimen easily. Adjust the exposure time on the capture window so that you can see the specimen. Don't bother spending time on this since it will have to be changed again when you put the diffuser in place. (If you are using an axial microscope carrier and you like to use the microscope eyepieces push the PHOTO/VIS lever of your microscope to VIS and locate and maneuver the specimen here, you will need to push the lever back to PHOTO position before taking your pictures)

2. Taking a single picture in Auto-Montage.

If your image does not require depth of focus reconstruction, e.g. a label shot, a large or a flat specimen. You can take and save one image:

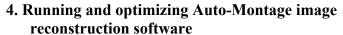
- a) From live image, when you are happy with the focus, positioning and illumination (adjust exposure time from capture window) click the capture button in the capture window.
- b) Click close in the capture window then right click on the image and select export image. Name and save to a place on your hard drive.
- c) Select the file format tiff since it is the most stable format for archive files. You can generate web-optimised jpgs from them later, see next **Section Processing images** for more details. When you close the image it will ask if you want to save it. This is because you saved an export, select no to saving it again.

3. Taking an image series for Auto-Montage

To make a clear image from a number of shots you need to minimize any differences between the images. The only changes should be the focus. This means setting the position and the light exactly how you want them from the start, not touching anything but the focus and trying to minimize movements made to the camera while adjusting the focus

- a) In live image position, align and illuminate the specimen as explained in **Taking** pictures and handling specimens, numbers 3 to 5
- b) Take the focus to above the highest point of the specimen
- c) Position yourself so that you have one hand on the fine focus drive and the other on the mouse
- **d)** Bring the focus down slowly until the topmost point is just in focus then hold the position
- e) Click capture

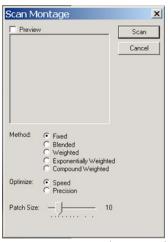
- f) While watching the focus in live image, move it down slightly until the focus has moved to a new area. Make sure you are not missing any areas in between the last capture and this position. (Don't try to move the focus back up if you have gone too far down, it is better to start afresh)
- g) Click capture then move the focus down a little again. Keep moving down the focus and clicking capture until you are through the specimen making sure you are perfectly still during capture



With your set of images open (as after 3 above) click on the Montage button in the toolbar



Continue from **Chapter 2 Auto-Montage** starting from the point this Scan Montage window appears









- 5. Scale bar: Once you are happy with your picture you can add a scale bar to it. This is best done on a lateral shot and requires that you have calibrated the software to your magnifications and lenses. If you have not calibrated for your setup, see section on Creating calibration files in First Time camera Setup no.

 3
- a) Go to menu **File, Template, Open** (Shortcut: Hold down Alt key while keying in sequence F, T, O)
- b) Find the correct file for the magnification and lens you are using and double click to open it
- c) Go to menu **File, Template, Copy to document** (Shortcut: Hold down Alt key while keying in sequence F,T,T)
- d) If a scale bar does not appear, right click on the image and select Show scale bar

e) You can alter the appearance of the scale bar, the units displayed and the position of the scale bar in **Options.**



- **6. Saving images.** As described in this section no. 6, save your final Auto-Montage image by right clicking on the image and choosing **Export image** and select tiff file format (see no. 6). Sometimes you may want to save the whole series of images as well as the final product, e.g. so that you can demonstrate the Auto-Montage process in the future, or if you think you may want to go back and rework the image. This can be done as you close the window, simply follow the prompt to save it, but be aware that the files are very large and will take up a lot of space. Most often you will only want to save the final product export, so normally select no to the prompt.
- **7. Folders and names for images:** The folder is what gives the images their identification so it is very important that this is correct, particularly the identification number if there is one. It is also important to name each file individually since as they go through the chain of processing it is possible for them to become displaced from their folders. Work out a system so that you can do this quickly and without mistakes.

At MCZ we search for the specimen in the database and have created a special field in the database which combines the first letter of the generic name, followed by underscore, followed by the species name, followed by the ID number. e.g. L_gibba20136. We copy the field and use this to name the folder. Then we use the name, still on the clipboard to name each image file for that specimen as we capture them. Each views is differentiated by a 3 figure suffix e.g. hef = head, frontal view

Using the Nikon Coolpix 990 and Coolpix 4500

The Nikon Coolpix 990 and 4500 can be attached to a microscope with the use of an adapter. We use a through-the-eyepiece adapter. One end threads onto the lens of the Coolpix and the other end slides into the eyepiece tube. In the past we have used adapters from three manufacturers:

- 1) **LM-Scope** Digital Adapter for Coolpix 990/4500 (<u>www.lmscope.com</u>) About \$400. Excellent quality and optics, but expensive.
- 2) **MVIA** adapter for Coolpix (<u>www.mvia.com</u>) about \$300. Very good quality, we found this was the best balance of optics and price.
- 3) Adapter from **Mark Simmons** about \$200 (http://www.perspectiveimage.com/) the adapter wasn't as robust as MVIA but visit his site for other Coolpix attachments for various needs. He responds well to e-mail questions.

You will also need to purchase these additional items:

- 1) **Nikon Coolpix remote control**—this remote shutter control has proven to be somewhat temperamental but it's the only one available for use with Coolpix cameras. The remote is necessary to avoid camera shake while taking pictures, as often the shutters speeds are very slow.
- 2) **AC adapter** much preferable to batteries, which will need to be replaced/recharged often.
- 3) White plumber's tape this is a stretchy, non-sticky PVC tape that can be found at most hardware stores. A small portion of tape is wrapped around the portion of the adapter which slides into the eyepiece tube. This helps create a good snug fit so that the camera can be positioned horizontally. Without the tape, the camera often will slide into a vertical position, making viewing the monitor and taking pictures awkward.
- 4) **Compact Flash card** we use a 128MB card and recommend a mimimum 64 MB card. The Coolpix 4500 also supports the IBM 1 GB microdrive (the 990 does not).
- 5) Card Reader The Coolpix comes with a cable which allows direct transfer to your computer however the cable and the remote share the same jack on the camera. Constant unplugging and replugging has lead to some excessive wear on one of our cameras and so we recommend a separate card reader.

For our microscope we use the **Leica MZ 7.5 stereomicroscope** upgraded with fine/coarse focus knobs and a longer 500mm stem. The longer stem increases the maximum focal length between the specimen and camera, allowing us to take photos of larger specimens (10-15mm) under the microscope. These is very useful as often specimens in this size range do not photograph well without some magnification. If you are planning to use this setup with Auto-Montage we also recommend using a microscope withj fine and coarse focusing as it's very useful when taking a series of photographs with only very slight changes in focus, which is what you'll need to do to get good results with Auto-Montage.

Coolpix Camera Settings

In order to familiarize yourself with the camera layout and menus it is highly recommended that you read the camera manual before using the camera for the first time.

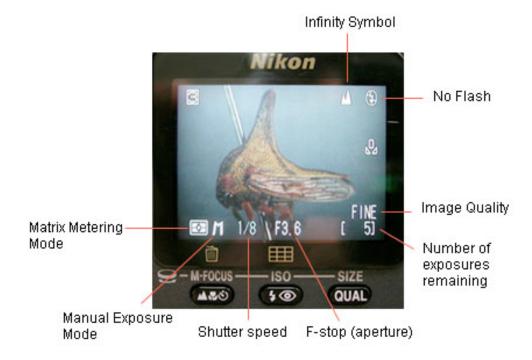
Adjust the power and lens settings

The camera will normally switch off if not used for more than 30 seconds This can be irritating if you are in the middle of setting up a shot. This can be changed so that the camera will remain on for longer by going to the **SETUP** menu, and changing the **AUTO OFF** function to a higher time limit, such as 5 or 30 minutes.

The first time you attach the camera to the adapter and look through the monitor some vignetting may appear around the edges of the viewable area. Zoom in using the T (telephoto) button until the vignetting disappears.

Normally the camera lens will revert back to its starting position (no zoom) each time the camera is turned on. This will make it very difficult to add accurate scale bars to your images. Once you have adjusted the lens to eliminate any vignetting, it is best to adjust the settings so that the lens remains fixed, by going to the **SHOOTING MENU, ZOOM OPTIONS** and choosing **fixed aperture**.

Here is a general view of what the screen should look like when the settings are adjusted. (Camera shown is Coolpix 990, the Coolpix 4500 is virtually identical.)



- 1. Make sure the power settings is continuous, and does not shut off after 1 or 5 min.
- 2. If you plan to add scale bars later on, set lens to remain fixed, and not return to the starting position (see adding scale bars for more information).
- 3. Plug in the remote control cord, make sure AC adapter is plugged into camera and turn the camera on.
- 4. Half push the remote button down until the monitor is active. You may have to hold the remote button down for several seconds before this happens. If it doesn't work try turning off the camera, unplugging the remote, plugging it in again, then turning it on and trying again
- 5. Adjust the settings if necessary as follows:
 - a. **Exposure mode: Manual**. An **M** should be visible in the bottom left corner of the monitor. If not, press and hold down the **MODE** button while rotating the command dial, until an **M** appears.
 - b. The **Infinity symbol** (two peaks of a mountain) is showing and not a flower or clock. If it is not showing press the button on the back of the camera (nearest you) with these symbols on it until you get the infinity symbol.
 - c. Check that the **No flash** symbol is showing (the lightning symbol with a line through it). If it is not press the button to the right of the previous button, the one with the flash/anti-red-eye symbol until the no flash comes up.
 - d. **Image Quality** and **Image Size:** should be set to highest levels to get best results. The Coolpix 4500 allows files to be saved in TIFF format for the highest quality results. However there is a significant loading time between shots when saving in TIFF format. For our purposes we use FINE mode and save images as JPEGs. The compression is minimal and it saves time between shots, when the camera is saving the image to the card.

Setting white balance

For the first time setup you must do this since all light sources are different. For the most accurate color, adjust the white balance whenever the lighting has changed. Adjusting once before you start each day is good practice.

- e) Make sure the dials on the light boxes are at max.
- f) Put the 1.0x lens on the scope and place a piece of neutral gray board under the lens. Insert an average sized specimen under the camera as if you were about to take a picture with the light diffuser in place and the lights arranged to give as best lighting for the specimen, all as normal.
- g) Press **MENU** button on the Coolpix. The Shooting menu should appear. White balance is the first item on the menu. Choose **White balance preset**.

h) Choose measure white balance. You should hear the shutter click and the camera beep. Now return to the normal live view.

Capturing Images with the Coolpix

- 1. Make sure the settings are adjusted. Turn on the lights that will illuminate the specimen (remember to set them to the same light intensity as you did when performing your white balance).
- 3. If you are planning to add scale bars to you images and have not already created the scale bar files, create them before you take photos of your images. (See **Creating Scale bars for Coolpix Images**)
- 2. Insert the pin of your specimen into a specimen stand under the scope. If you are taking a dorsal or ventral shot it is best to use a gray putty block so as the background is a consistent gray color, the putty works well for all shots if you do not have a stand.
- 3. Reduce your magnification to minimum so that you can locate the specimen easily.
- 4. Making sure you're in manual mode (M), adjust the f-stop on the camera to the lowest available. To do this, press the MODE button. Each time you press the MODE button you will toggle between exposure and f-stop. You can tell which one is active because the display for each will change from white to green in color. Activate the f-stop and then using the dial rotate so that the lowest f-stop number is showing. This usually is around 3.6.
- 5. Now press **MODE** again to adjust the shutter speed. When trying to adjust the shutter speed an exposure display will appear. This tells you whether the image will be over or underexposed at a chosen shutter speed (also taking into account the f-stop value you have chosen). You want the white marker to remain as close to the CENTER of the graph as possible. This is the camera's suggested shutter speed for proper exposure. When the marker moves to the left, the camera is suggesting that the image will be underexposed, when the marker moves to the right, your image may appear overexposed. Generally the camera's suggestions are correct so it's best to try and keep the marker in the center.
- 6. Once the shutter speed is chosen, press the remote to take a picture. The camera will beep. A green card symbol may start flashing in your monitor. This is telling you that the camera is storing the image to the card. Wait until this symbol disappears before taking another picture.
- 7. If you intend to add scale bars you will need to maintain a log book for each specimen you photograph including the bar code number of the specimen and for each image or series of images, the view (lateral, dorsal, head), lens used, (1.0 or 0.5 and magnification on the microscope.

Using Auto-Montage with the Nikon Coolpix 990 or 4500

Once you have finished taking your images and transferred them to the computer you will first need to organize them and in some instances rename the images. The easiest way to sort your images is to use an image browser. A program called ACDSee, which can be downloaded from the internet for a small fee, is recommended for PCs, I-View is recommended for Mac. If you're computer is running on Windows XP you can also use the thumbnail view to organize and rename files.

Organizing and renaming files

If using ACDSee, open the program and find the folder containing your images. View the images as thumbnails. The easiest way to organize your images is to create a new folder for each specimen labeling the folder with the first letter of the genus followed by and underscore, following by the species name and bar code number.



For specimens which are undetermined, name the folder with the bar code (or other code) number only.



Select and drag the images for that specimen into the newly created folder. The folder which contained all the transferred image files should now contain a series of folders, each containing the images for that specimen only.

Images which are part of a series to be montaged together using Auto-Montage must be renamed using the following format:

- a) The images contained in each series should be named with numbers which follow sequentially with no gaps in the sequence. This sequence must be formed with numbers from 1 to 255. Auto-Montage will not recognize numbers over 255.
- b) If there is more than one series of images in the folder (for example, both the head and lateral shots need to be montaged) then each series must follow a separate sequential numerical sequence so that Auto-Montage treats them as two groups and not one. This can be done by placing a gap in the sequence between the last number in the first sequence and the first number in the second sequence.

For example you have the following images in a folder:

Name given by Coolpix	Type of image	Renamed file
DSCN2307	label	*
DSCN2308	lateral shot 1	1
DSCN2309	lateral shot 2	2
DSCN2310	lateral shot 3	3
DSCN2311	lateral shot 4	4
DSCN2312	lateral shot 5	5
DSCN2313	lateral shot 6	6
DSCN2316	head shot 1	20
DSCN2317	head shot 2	21
DSCN2318	head shot 3	22
DSCN2319	head shot 4	23
DSCN2320	head shot 5	24
DSCN2321	head shot 6	25
DSCN2322	head shot 7	26

^{*}The label will not be montaged, so it does not need to be renamed. The lateral and head shots need to be renamed for two reasons:

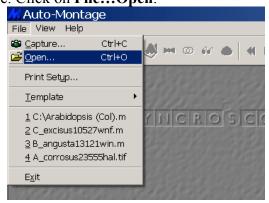
- 1) the original numbers are above 255 (ie. DSCN2307, 2308, 2309 etc.)
- 2) there needs to be a gap in the sequence for Auto-Montage to distinguish the images as belonging to two separate series.

If there is only one sequence for Auto-montage it is often easiest to delete all the characters before the last 2 e.g. DSCN2308 becomes 08, DSCN2309 becomes 09. It doesn't matter what numbers they are, as long as they are sequential.

Running Auto-Montage

You should look at the section: **Auto-Montage Software** before continuing with this protocol

1. Start Auto-Montage. Click on File...Open.



2. The **Open** dialog box will appear. Locate the folder you want to work on. The folder should contain one or more series of files (depending on how many views needs to be montaged) renamed as described above, so that Auto-Montage can read them.

Note: The **Open** dialogue box will initially appear empty. That is because the default **Files of type** is set to **Montage files** not **Image Files**. Switch to **Image files**.

- 3. Click on the first file of a series and click on the **Open** button. Auto-Montage will automatically open the remaining images in the series.
- 4. Auto-montage will now preload the source images. After a second or two the **Source Images** box will appear and the first image in your series will be visible. Maximize the box if necessary and adjust the zoom (located in the upper right corner of the screen) so that the entire image visible on your monitor.
- 5. If you are using a stereomicroscope without a sliding carriage adapter, your source images will not be aligned properly. To correct for this, click on the align source button. Align Source Images box will appear. Leave the other settings as they are and click Scan. Afterwards you should notice at the bottom of the screen that the alignment of the X (and to a lesser extent the Y) axis has changed to compensate for the movement of the image when taking the photos on a stereo microscope. Now you are ready to montage the images.
- 6. Click on the montage symbol The Scan Montage box will appear. The default settings are Method: Fixed, Optimize: Speed and Patch Size: 10. The Method and Optimize setting should be left as they are. The Patch Size, however, can often be adjusted to help minimize patchiness in the montaged image (see Auto-Montage Software section). The patch size is a parameter to the Scan Montage operation, and corresponds to the size of detail you are attempting to focus on. For our purposes we have found that most insects scan best with a patch size between 10 and 30.
- 7. It is best to first montage the image with a patch size of 10. Click on **Scan** to start the process. If you watch the monitor you will gradually see the full image come into focus.
- 8. Now look at the montaged image. Do any parts of the specimen appear patchy? Do any parts of the background appear patchy? There are two ways in Automontage to help reduce this patchiness. One is to **increase the patch size**. This is often useful when the patchiness occurs within the specimen. For example there may be patchiness in darker areas of the specimen. Patchiness within the specimen can sometimes be eliminated, or at least diminished, by increasing the patch size, for example to a patch size of 20 or 30.

The other sort of patchiness occurs in the background of the image. If your specimen looks good but the background looks patchy, using the **Enhance** operation may be a better option. First let's look at increasing the Patch size.

- 9. Repeat the process from step 6, but increase the patch size. Using the **purple**arrows

 you can toggle back and forth between the different montaged images and decide which one looks best. Look at the bottom left of the screen to determine the patch size of the image you are currently looking at. Remember, that by increasing the patch size you will lose a small amount of overall detail, so don't increase any more than necessary to smooth out patches in your specimen.
- 10. If the patchiness in your image is only in the background of your image, then the **Scan Enhance** command may help reduce this without reducing the image quality of the specimen. Press the Enhance button will appear. Slowly increase the Confidence threshold to 0..3 or 0..4, click **Scan** and see if patchiness goes away. Use the **purple arrows** to toggle back and forth between images and adjust as necessary. (refer to The **Scan Enhance** section of the **Auto-Montage Software** chapter p.25 for more in depth instructions).
- 11. Once you are happy with your image, click File... Export... Single Image to File.
- 12. Name the file, save the file as a JPEG with minimal compression, in the appropriate folder. Click **SAVE**

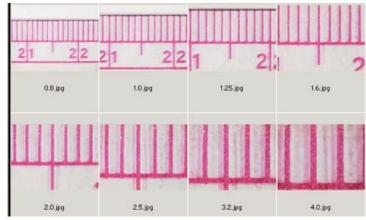
Creating Scale bars for Coolpix Images

When using the Coolpix, scale bars are created separately and then added to the specimen image in Photoshop before being processed for the web.

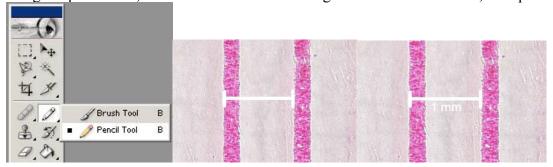
Scale bars are created by first photographing a ruler under a microscope at each magnification. Then these images are opened in Photoshop and a scale bar is created for each magnification of the microscope, using the millimeter markings on the ruler images as a guideline. Each scale bar is then saved, named after the magnification it represents and placed in a folder which can be accessed while processing the images. The images should have a notation as to what magnification they were taken at, and the digitator can then place a copy of the appropriate scale bar onto the image.

The following protocol explains in more detail how to create the scale bars for use with the Coolpix camera:

- 1) Turn on the camera. Take a look at the live image on the monitor of the camera. Make sure the zoom on the camera is adjusted so there is no vignetting around the edges. Be sure that the camera is set so that when it is shut off and turned back on, it will maintain this zoom position. NEVER TOUCH THE ZOOM AGAIN. This is VERY important. If the zoom on the camera is changed, your scale bars will be inaccurate and you will have to create new ones (very time consuming). You can try and make a note of where the zoom is positioned by looking at the W/T line but this is not a very accurate and it will be hard to get things back exactly as they were if the zoom is accidentally moved.
- 2) Place a ruler with millimeter markings under the microscope. Try and use a good quality ruler with crisp marks straight millimeter marks or even better, a stage micrometer. As you go up in magnification, the accuracy of your scale bar will depend on how accurately you can measure from one mark to another.
- 3) Starting with the lowest magnification, focus on the millimeter markings and take a picture. Then move to the next magnification, focus and take a picture. Do this for all the magnifications. Make sure at the higher magnifications at least two of the millimeter marks are in view, as you will need to measure from one mark to another to create your scale bars.



- **4)** Once all the images are taken, transfer them to a computer and rename them with the magnification and lens that they represent. For example for the 1.0 lens name the scale bars 1.0x1.jpg, 1.25x1.jpg, 1.6x1.jpg.ect and for the 0.5lens 1.0x.5.jpg, 1.25x.5.jpg, 1.6x.5jpg, etc.
- 5) Open up the first image and create a new layer (CTRL-SHFT-N).
- 6) Click on the **Pencil** tool. Set the brush size to 5 and color to white. In this new layer, using the pencil tool, click and hold on the left edge of one of the markers, then press



SHIFT and drag the mouse across to the left edge of the next marker, creating a straight white line as you go. Now create shorter lines at each end of the bar to make it appear as a scale bar. Click and hold where you want to start the line, then press shift, and drag the mouse to create the line. Always click and hold first and then press **SHIFT**. You can use Photoshop guides to help make the shorter lines of the scale bar the same height.

- 7) Click on the type tool \mathbf{T} , choose the font Arial font size 14. Now click just below the line you've made and type "1 mm".
- 8) Press **CTRL-E** to flatten the type layer onto the scale bar layer. You should now have two layers, the background and the scalebar and 1mm.
- 7) When you need to place a scale bar on an image, open both the specimen image and the scale bar files, click on the **MOVE** tool in Photoshop. Click on the scale bar and drag the scale bar onto the specimen image and place close to the specimen, usually just below the specimen. Now close scale bar file. It will ask you if you want to save the changes made to the file. Click **NO**. This will keep the file as it was when you opened it, so you can use it over and over again.
- 8) Your specimen image now has two layers. To merge the layers, press **CTRL-E**. Now you can process the images for the web.

Processing images for the web

This section will take you through the manual sequence of processing images ready for the web, then it will show you how to make and run scripts which will speed up your processing time and ensure consistency in your images.

The MCZ database images first appear as 500 pixels wide (300 for labels) and clicking on them enlarges them to 900 pixels wide. All are set at a resolution of 72 dpi for placement on the web. These files are all generated from one original raw file which itself is archived in it's original state. All our images are in three forms:

- a) Raw tiff (or sometimes raw jpg depending on the camera)
- b) Large jpg 900 pixels wide (500 pixels wide for labels)
- c) Small jpg 500 pixels wide (300 pixels wide for labels)

This process demonstrates an efficient way of creating these 3 file formats

Preparing the images for processing

- 1. Check through your images using and image browser such as ACDSee (PC) or I-View (Mac). Delete any extra files, check the file names and make any other changes that may be necessary. You are about to duplicate your files, extra or uncorrected files create extra work after duplication.
- 2. Duplicate your folder of raw images (Cm-D Mac, Copy and Paste PC) and label the duplicate "Large jpg." This step is essential, it means you are free to make mistakes since they can always be corrected by going back to the original raw file.

Processing manually

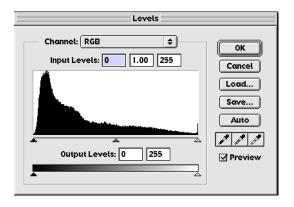
Image processing is done mainly in Photoshop. Even if you don't know how to use Photoshop these instructions should be comprehensive enough

N.B. If you have Auto-Montage files that need cleaning up, go to "Cleaning up Auto-Montage Images with PhotoShop Tools" in this section before processing for the web.

- 1. Cropping: From the tool bar select the crop tool.

 If you are using Photoshop 5, hold down the rectangular marquee tool until the crop tool appears. Crop the image close to the specimen. You should have the specimen taking up all the space of the picture. Unnecessary background makes the resized images of the specimens smaller than they need to be. With lateral shots try to keep all near side legs and near side antennae (if not in other shots) in frame (legs are less important). With head shots keep at least one antennae in frame (the one most in focus).
- **2.** Levels: Select menu image, adjustments, levels (Cm + L for Mac Ctrl + L for PC). The levels histogram will appear. The shape of the histogram represents the light balance of your image. You should try moving the 3 slider arrows at the bottom of the histogram to see the effect it has on your image, this is how Photoshop help explains it:

"You can set the highlights and shadows in an image by moving Input sliders to the first group of pixels on both ends of the Levels histogram. This maps these pixels--the darkest and lightest pixels in each channel--to black and white, increasing the tonal range of the image. The corresponding pixels in the other channels are adjusted proportionately to avoid altering the color balance. You can



use the middle Input slider to change the intensity values of the middle range of gray tones without dramatically altering the highlights and shadows".

You should experiment yourself but I find that a good way to adjust levels quickly is to bring the side arrows in to the edges of the histogram and if the image still looks too light or too dark move the center arrow to the right or to the left slightly. **Don't rely on levels to make your image look good. Your image should look good before you apply levels and the levels should enhance it.**

3. **Image Size:** Select menu **image, image size.** Enter 900 pixels (MCZ Types) into the width, or 500 for labels. This will be your large image for the web. You can generate your small image from this one later. N.B. If the image, once cropped is smaller than this (900 pixels wide), don't increase the size, since it will reduce the quality. Leave the size as it is and move on to the next step, see the example in **Using the history window**, below

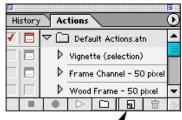
4. Unsharp Mask:

This will crisp up your image a little. Select menu **filter, sharpen, unsharp mask.** Experiment with the settings that look best to you. This is what we use: Coolpix: Set the amount to 200%, the radius to 0.5 and the threshold to 5. JVC: Mostly, sharpen doesn't improve the pictures from the JVC camera, if we sharpen at all, it is very little, such as: Set the amount to 100%, the radius to 0.3 and the threshold to 0.

5. **Save:** This will overwrite your raw file, so make sure you have duplicated all the raw files since they should be kept for archive

Writing a Script to Perform these Processing Tasks Scripts in Photoshop are simple so don't be afraid! It is simply a matter of recording whatever steps you want to be in a process (the above for instance) and then you can perform it again with only one key stroke (almost)

1. In Photoshop, select menu Window menu and choose Actions. In the Actions window click on the icon indicated by the arrow for new script. Give the script a name and assign a short cut key if you wish. Click Record.



Levels

- 2. Note the record button is now red which means that anything you do will be recorded to that script name. The steps to record are: Crop, Levels, Image Size, Unsharp mask and Save. In the actions window toolbar (at the bottom, see actions window image) reading from left to right are stop, record, play, new folder, new script buttons that you can use at any time.
- 3. Go through the above process (**Processing manually**) as normal. After save, click on the stop button on the actions window. The process has been recorded.
- 4. Certain steps will always need to be done by hand, e.g. not all specimens have the same light conditions, so need individual levels treatment. You can allow individual tweaking History Actions to any step by clicking on the icon indicated by the arrow. This will suspend the script at this ▼ Specimens F2-Cm step until you press return/enter. While ₹ Crop
- 7 Image Size 5. Make separate scripts for specimens and labels. Alternatively suspend your script at the image size stage, and change the width to 500 each time you process a label. You can run the script either by pressing the assigned shortcut key (assigned when you first create the script), or by clicking the play button in the actions window.

7

Using the history window

suspended you can alter the levels.

Access the History window by either clicking on the history tab (see the actions window image above). Or by selecting menu Window, History

This very useful function in Photoshop allows you to back track on the steps you have made, it is also why you shouldn't include close in your script because the history option is not open after closing.

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e.g. While using the script described above, you notice that the image has got larger instead of smaller. This means that the cropped image was originally smaller than 900dpi and the script is increasing it to that size. Images that have been enlarged by Photoshop are interpolated and will appear of reduced quality. You are trying to optimize the images so in this case you should back track a few steps.

- 1. Click on the History window tab
- **2.** With the mouse, select the step **levels** in the history window, your image will be restored to the state it was in after the levels were adjusted.
- 3. Tab back to the actions window and select the step after image size. Click the **play** icon at the bottom of the actions window

When the images are done, a final check by loading them into image browser software like I-view or ACDSee is a good idea.

If your images are tiffs, you need to convert the images to jpgs. Software such as ACDSee (PC) or Graphic Converter (Mac) can do this quickly. If you are given the option always save for high quality, large images.

Generating the Small jpgs

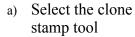
For the small jpgs we duplicate the large jpg folder and reduce the duplicate files to 500dpi wide (or 300ppi for labels).

- 1. Duplicate the entire Large jpg folder (Cm-D Mac, Copy and Paste PC) and label the duplicate "Small jpg."
- 2. Write a simple script in Photoshop to change image size to 500, save and close
- 3. Either:
- a) Open Photoshop and select menu File, Automate, Batch
- b) In the batch window, select your script, choose the folder you have just created (Small jpg), check box: include all subfolders and select destination none
- c) Click OK and all the images will be reduced as a batch
- d) Since the label files were already 500dpi wide we reduce these to 300dpi. We launch the label files from I-view or ACDSee (shell edit to Photoshop) so that all the label files are open in photoshop at the same time, stacked on top of each other, then we run the script on them from here by repeatedly clicking the shortcut key or play button.
- 4. Or:
- a) Launch all files at once in Photoshop and run the script on them from here, repeatedly clicking the shortcut key to run the script and running a different script each time a label file appears.

Cleaning up Auto-Montage Images with PhotoShop Tools

After Auto-Montage images often look messy and have distracting lines around the edges of the specimen. Much of this can be improved by using the **Enhance** command in Auto-Montage. See Chapter 2 Section 2 **The Enhance Command.** Improving your images after Auto-Montage is often a combination of the Enhance command and Photoshop. The enhance command, if not cleaning up the image completely, is quick and easy and can make clean up in Photoshop much easier. Since every image is different, each needs to be treated differently and there is no one prescribed way to do it. You also need to make a judgement between the time it takes to improve the image and how important it actually is to improve the appearance of say the background. Below are some tips.

1. The Clone Stamp: This works by sampling a chosen area on the image and copying that area onto another area chosen by your mouse. The sampled area stays the same distance away from your cursor and re-samples wherever you move it. So, for example, you want to get rid of the line around your image and you want the area to be replaced by the same background as that nearby so it looks natural.



- b) First you need to sample from somewhere, so move the cursor to the area you want to copy
- c) Hold down the
 Alt Key (PC) or
 Option key (Mac)
 and click the mouse



- d) Release the option key and move the cursor to the area you want to cover with the sample.
- e) You have to experiment to get the hang of it. Remember you can always undo; Cm/Ctrl+z or go back further by going to *window* then *history* and selecting a previous state.
- f) Try out different brush sizes and adjust the Opacity depending on the effect you require



Try not to interfere too much with the actual specimens (most of what you will need to do is for the background). Sometimes you will need to use the clone stamp to fill in areas on the specimen which have patches left by the Auto-Montage process. Try to be as true as possible to what was originally there

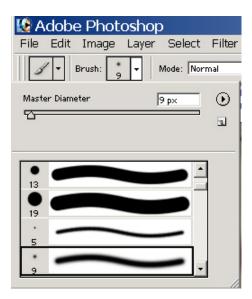
2. Sample and Airbrush: If the background has fairly uniform areas of one color, these tools are very useful. Or if you want to blend the edge of a hard line (see illustration)

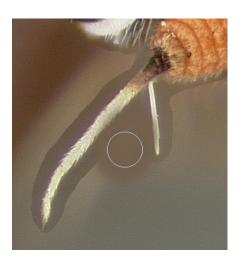


- a) Select the airbrush tool or in Photoshop 7 the brush tool
- b) Sample by Alt-click (for PC) or Option-click (Mac) a color from the image that you want to cover areas with.



- d) Go to the brushes window and select a suitable sized brush. Click off spacing if you want a clean edge to your airbrushing, click it on if you want a softer effect. Experiment with different brush settings. In Photoshop 7 click on the brush icon (illustrated) to check airbrush is on, and click on the brush size icon (illustrated) to get this brush window. You need to select a soft brush like the one selected in the picture and adjust the size with the "master diameter". Reduce "flow" for softer effects
- e) To even out large areas of background: Use a large soft brush, applying colors frequently sampled from the area
- To blend a line into the background:
 Use a large soft brush (turn up the spacing or down the flow) and sample from close to the specimen. (using a hard brush would just create a new artificial line).





Part 3 Backing Up and Archiving

At The MCZ we make backups of the database whenever new data is added or updated. As we work we make daily temporary backups of the images and ultimately each image is burned on 2 CD's and one DVD. The information about what image is stored on which CD is stored in a simple database, the updated version is copied onto each CD.

Backing up your data is a priority. Make sure you have all your data in 2 or preferably 3 places. Never rely on one hard drive. Someone could accidentally delete your folder, the hard drive could crash, the computer get stolen etc.

Get into good habits from the start: At the end of each session, think about how much work you have done that is not backed up somewhere else. Then imagine loosing it all. If this thought bothers you at all, you need to back up.

The Database

It is very important to be particularly careful about this. Not only is there a risk of loosing information through not backing up, but once you start making backup copies there is also a risk of overwriting data with the wrong version of the database. Overwritten data cannot be recovered, and since FileMaker (MANTIS) automatically updates as you go along, any changes are permanent.

Some ways to help prevent disasters:

- 1. Understand the importance of having one version of the database as the definitive one. This version should never be overwritten unless you are 100% sure that the one you are overwriting with has the same and more data as this one. Be meticulous about devising a system that is as fool-proof as possible.
- 2. Incase of overwriting and other errors occurring, as you add data it is recommended that you make a backup copy of the database at the end of every day that you add anything to the database. This means that whatever happens you can always go back to the previous day or the previous week if need be. At MCZ we do this by transferring the files to another computer via Timbuktu Pro (crossplatform file sharing software), but you must be careful to shut the database down before transferring these files since the database can be damaged. One way of managing multiple backups is to change the name of the database so that it includes today's date before transferring. This way the previous backups won't be overwritten. You still might need those previous backups if you suddenly notice something went wrong a week ago. If you don't have another computer and some means of transferring files, you can make CD copies, but this could get out

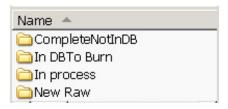
- of hand if you are backing up each day and it is not advisable to keep re-using rewritable CD's, they are not very safe. It might be worth while investing in an external drive for your computer.
- 3. Every week (or other suitable time depending on how often you update the database) you should make a CD of the database and label it with the name and date (write on the actual disk with a permanent marker). If you are making daily backups on your computer, devise a system of deleting previous versions of the database. e.g. When you burn a new disk, you can delete the files from two weeks before, freeing up space on your computer.

Before burning CD's or transferring files you should shut down your database to avoid possible damage to files.

Images

Making a copy on another computer

- 1. In a similar way as with the database, make sure you always have another copy of your image files. At MCZ we do this on a daily basis by copying to another computer.
- 2. When creating new image files and generating the smaller jpgs from them, create a good system for yourself so you know what stage your files are at. e.g. The files illustrated ensure that no one has to waste time checking which files have been imported (In DB) or burned. But you must remember to keep renaming the folders or moving the files as you work so you know where you are.



Burning CD's of Images

Unless you don't expect to have many image files, it is very important, particularly with types to have your saved images easily accessible in the future. It would be a very tedius and time wasting task trawling through 100 or so CD's sometime in the future looking for a particular specimen. So when you compile your disk you should work out a way of putting that information into some kind of database so that a simple search can find the CD you want.

To track the files we use a simple FileMaker database and a method of importing folder names. There are many software applications that will create CD tracking databases, it is worth taking the time to manage your archives. Below is just the method we use.

Making a record of what goes on the CD's

Using a simple FileMaker database

- 1. Create a new, blank FileMaker Document. Define 2 fields, one for CD number and one for specimen name
- 2. A disc safely holds about 650MB. First establish what you are going to put on the disc. Keep the small, large and raw files of the same specimen on the same disk and keep within 650MB.
- 3. Making sure all the folders have the same names, open one of the small, large or raw folders and make a copy of the folder names. In Mac simply select all, copy and paste to an Excel document (most text documents will work with FileMaker 5.5 and previous). In PC you need to download some free software called PrintFolder http://no-nonsense-software.com you can then copy and paste the folder names into Excel or a text file.
- 4. Save the document somewhere, named with the CD number you are about to burn
- 5. Open the FileMaker CD Catalogue you created. Select menu file, define fields. Click CD number then click Options. In Options, make sure the data box is ticked and enter your new CD number into the data box. Click OK then Done.
- 6. In FileMaker select menu file, import records, file. Find the Excel (or text) file you have just created and open it. In First Row option check data and OK
- 7. In **import field mapping**, check the species names are going to the right place and click **import**
- 8. In **import options** click **OK** (for auto enter options). Click **import** and **OK**. Your file names, the CD number and image type will be imported into the database. Close the database and quit FileMaker Pro

Burning the disc

- 1. Close the CD catalogue and add it to be burned on the CD along with the image files. If you want 2 copies on CD, burn another copy now (it is the quickest and easiest time to make a copy)
- 2. If you want to make a DVD copy you will need to wait until there are enough to fill the 4.7 GB. Put the files you have just burned into a new folder and label it with the CD number. Store the folder somewhere ready to by burned on DVD.
- 3. Once you have 2 CD's burnt and a 3rd copy waiting to DVD, find and delete all the other copies of these files generated through the imaging process from all other places. Disk space gets used up quickly with images, especially raw files and it is a very time wasting procedure trying to work out which files have and haven't been backed up at a later stage when you are trying to free up disk space.

Appendix 1

Automontage Troubleshoot

To check if the board is working correctly

Go to My Computer (KAKAPO) icon on desktop. Right click the mouse and select properties.

Select the Hardware tab

Click Device Manager

Click on + sign next to Imaging dervices

Double click on Synoptics Prysm grabber

Check device status (if it's working properly)

To select the grabber

Go to start menu, programs, synoptics, and open Iris

Select Synoptics prysm generic

Select KYF-70U RGB manual control

Close Synoptics

To configure in Automontage

Open Automontage

Click capture icon

In capture dialog box click select (if can't select click freeze)

Select prysm camera interface card

Click tab for Prysm

Select KYF-70U RGB