Digital Insect Specimen Photography with 'Helicon Focus 6' Computer Stacking Software and Rotating Specimen Stages.

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1. INTRODUCTION

Previous papers have described the development of photographic work stations, initially for use with film cameras and later for use with digital cameras. Ref 1,Taylor,2001, Ref 2, Taylor,2004, Ref 3, Taylor,2012.

The latter work station incorporated an angled camera and specimen stage, a large fluorescent tube equipped overhead light source and angled side mirrors for directing 'fill in' light onto the lower parts of the specimen. Specimens were photographed against a background of Jessops photographic grey card.

Fine focussing of specimens was achieved by using a manually pre-selected camera focus distance together with fine fore and back movement of the specimen in relation to the camera position by means of the 'Y' axis microscope stage rack and pinion movement.

The recent development of computer stacking software requires the taking of quantities of images in a controlled manner of small increments in subject to camera distances, a capability already incorporated into the existing work station design.

It was clear that some further development work was required to facilitate the taking of photographs of specimens from many different angles of view in order to maximise the choice of visualisation of the range of diagnostic characteristics of different species. Plan and inverted views together with oblique views often being required. It was considered important to try to achieve flexibility of view point without the need for repeated manual repositioning of specimens using forceps. Undue repeated handling of 'type' material being undesirable because of the dangers of specimen damage.

2. ADAPTATION OF THE WORK STATION FOR USE WITH COMPUTER STACKING SOFTWARE

Improvements have now been developed with respect to the attachment of specimens to the specimen carriers placed at the camera end of the microscope stages.

In developing the new attachments I have taken into account the following conditions:-

2.1 As with the previously developed work station only 'micro-pinned' specimens were to be catered for. Accommodating specimens directly pinned onto long staging pins would have seriously compromised the flexibility of, ease of use of and the compactness of the arrangement. However the equipment is capable of being modified to cater for photography of long pin-staged specimens when only these are available.

2.2 Helicon Focus 6 stacking software was to be used.

2.3 An Olympus E410 camera and 35mm Digital Macro Lens was to be used. This represented a widely available typical low cost genre of camera and lens combination.

2.4 Large numbers of series of images of each specimen would need to be taken to cater for the many direction of view selections possibly required. The developed equipment had to be versatile enough to cater for both top and side-pinned specimens.

3. DESCRIPTION OF THE UNIVERSAL STAGE CARRIERS AND ADAPTORS, THE DEVELOPED ROTATING STAGES AND THE NON-ROTATING STAGE

3.1 UNIVERSAL STAGE CARRIAGES

The upper stage carriage was made by attaching some aluminium alloy angle section pieces to the end of the microscope stage using epoxy resin. See Figs 1 & 2. The stage carriage contains a vertical plate for engaging the stage adaptor slot when setting up for photography. The lower stage carriage was assembled using a nut and bolt.

Fig 1

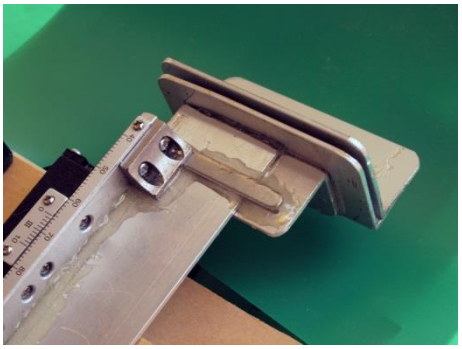

Fig 2

3.2 STAGE ADAPTORS

The stage adaptors were made from plywood and aluminium alloy angle section joined using epoxy resin. See Fig 3. The stage adaptors contain a vertical slot for engaging the photographic grey card background, see Figs 4 &5.

Fig 3

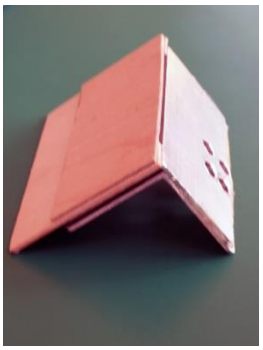

Fig 4

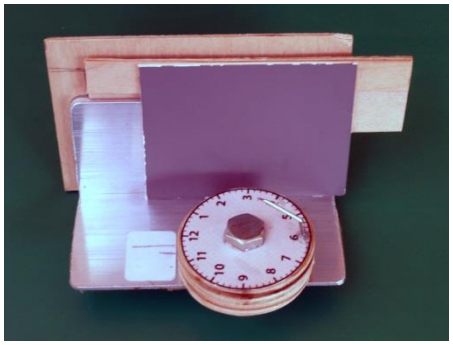

Fig 5

3.3 SPECIMEN STAGES

Following experimentation, rotating specimen stages were developed to cater for side, top and oblique views of upper and lower surfaces of side and top-pinned specimens. Another simple non-rotating stage was developed to allow both plan views of top-pinned specimens and side views of side-pinned specimens to be photographed.

3.3.1 ROTATING STAGE WITH SPECIMEN 'MICRO-PIN'RECEPTOR

Following experimentation I found that 4cm veterinary syringe needles fixed to the stage bases via a small diameter hole drilled through the base and fixed with epoxy resin made a simple and effective receptor for the point of the specimen 'micro-pin'. A box of 12 veterinary syringe needles was purchased on E-bay for £ 9, Fig 6. They were particularly effective because the shallow angled slanted terminal needle cut made the delicate 'docking' manoeuvre of the specimen onto the stage easily achievable, see Figs 7.

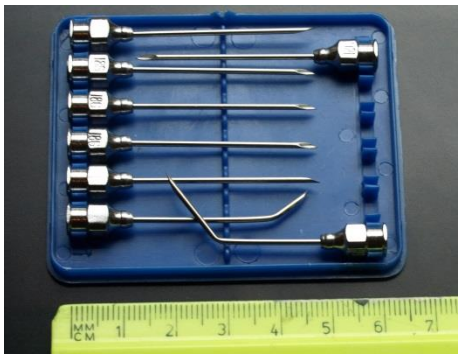

Fig 6

Fig 7

Fig 8

To restrain the specimen from making unwanted rotations within the receptor during sequence photography, particularly necessary with specimens on small diameter pins, the specimen pin needs to be wedged firmly into position in the 'throat' of the syringe needle. After some experimentation it was found that a cactus spine was best as they are robust, sharply pointed and finely tapered. See Fig 8, *Stenopogon junceus* . Wied 1820, head and thorax.

The circular rotating stage base, 40mm in diameter, was trepanned from 13mm thick 9 layer plywood. The 'clock-face' cover design was downloaded from a 'Google search' for 'clock face'. An attachment bolt was fastened through the centre of the 'clock face' using epoxy resin.

Two versions of rotating stages were developed:-

a) one with a vertically positioned syringe needle point, cranked to ensure that the specimen, once 'docked', was placed centrally over the pivotal anchor bolt.

b) one with the terminal half of the needle bent at an angle of 45^{o} to allow for oblique views of both side and top-pinned specimens, again ensuring that once 'docked' the specimen was placed centrally over the pivotal anchor bolt. See Fig 9.

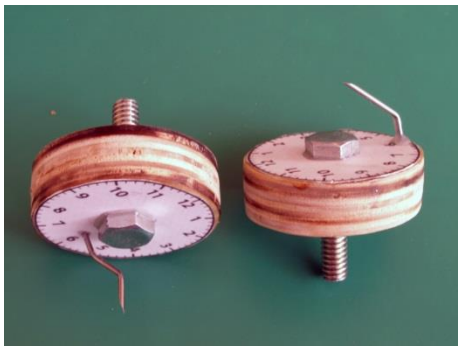

Fig 9

Fig 10

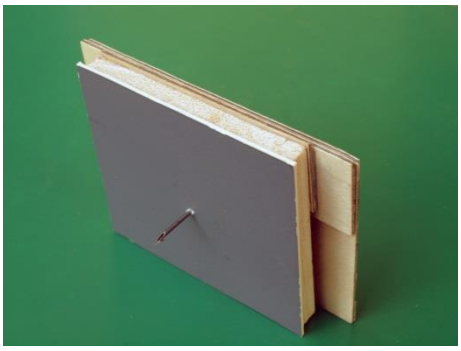

Fig 11

3.3.2 NON-ROTATING STAGE

This was made from a plywood and balsawood sandwich backing for the 'Jessops' photographic grey card through which the hole for the veterinary syringe needle 'micro-pin' receptor was drilled then fixed with epoxy resin. See Fig 10 & 11.

4. OPERATION OF THE DEVELOPED EQUIPMENT

Figs 12, shows the developed equipment set up to photograph a micro-pinned insect specimen, a female *Promachus leoninus*.Loew 1848, (Diptera, Asilidae), Span 29mm, length 17mm, using a vertical micro-pin receptor.

Figs 12

As the maximum fore and aft adjustment distance on the Y' axis microscope stages are 50mm the need to cover a range of working distances (distance between specimen and front of lens) lead to the incorporation of two microscope stages into the equipment. The upper stage is used for working distances of between 30mm and 60mm, covering fields of view from 60mm x 45mm to 30mm x 22mm. The lower stage is used for close-up photography of detail, or for the smallest specimens. See Figs 13 & 14.

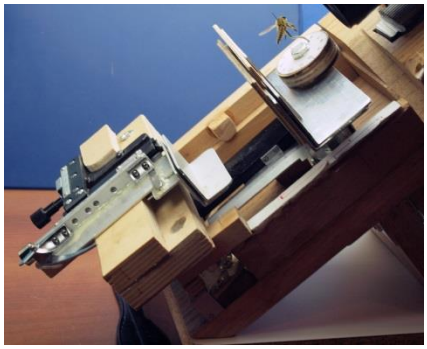

Fig 13

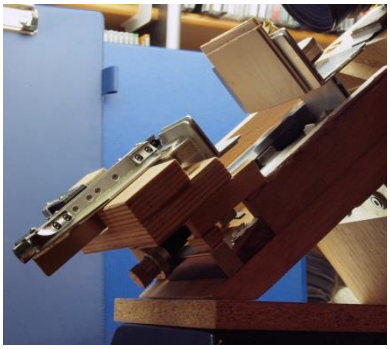

Fig 14

When taking a full sequence of images of a specimen at each of twelve angular positions, 'clock face stations', about twenty images at each station will take about one hour to complete.

Post photography operations, a) Helicon Focus 6 Rendering using Method 'B', followed by, b) Rotating image, cropping and Auto Colour Correcting in Photoshop take about one further hour.

With my 'set up' the lens to specimen distances are normally between 75mm and 85mm which cater for insects around 15mm to 35mm in wingspan. Exposures using ISO 100 speed are around 1/6th sec @ f22. The maximum fore and aft adjustment distance on the 'Y' axis microscope stage is 50mm, thus covering most insect specimen photographic requirements.

When commencing photography of a specimen, pre-set the lens focus manually and turn the 'Y' axis stage screw to place the part of the specimen nearest to the camera just out of the fixed focus distance just selected. Then rotate the 'Y' axis stage screw half a turn which, due to the gearing of the stage rack and pinion, brings the specimen 1mm closer to the camera, take a photograph, turn the screw a further half a turn, take another photograph……. and so on until 20 or so images have been taken. During this sequence all parts of the specimen have passed through the lens focal point selected.

5. SPECIMEN QUALITY

5.1 Existing Specimens

In many cases existing specimens need to be photographed, some can be of great age and have often suffered from damage or have gathered unwanted dust and debris of various kinds. If the debris does not obscure the view of desired critical detail then the best option is to photograph the specimen 'as is'. With type material minimum cleaning should be the aim.

Cleaning is best done with a binocular microscope using some small tools including fine pointed artist's brushes, fine tweezers and dampened cotton buds.

5.2 Specimens Collected for Photographic Purposes

I have tried several methods of killing specimens over the last 60 years including: -

	METHOD	COMMENTS
A)	Ethyl acetate	'Rigor mortis' problems, setting difficult.
B)	Crushed Laurel leaves	Availability, reliability.
C)	Cyanide	Dangerous material, availability.
D)	Rapid Heating	Wing membrane distortion.
E)	Rapid Freezing	Relaxed specimens, some increased fragility of tibia/tarsi joints when the specimen is dried.

I now frequently use rapid freezing using the freezer compartment set at -20^{o}C.

When collecting specimens they are put into glass or plastic tubes 'in the field' and placed in a small cooler box containing a cold cooler block. This keeps them inactive thus avoiding damage until I return home.

Most Diptera, my main Order of interest, are dead within two hours of placing into the freezer compartment, however I usually leave them in the freezer for three days.

I had an experience a few years ago with a batch of male *Merodon lydicus.* Vujic et al, 2007,(Diptera, Syrphidae), collected in the third week of March on the Greek island of Chios. Of the six specimens placed in the freezer compartment of my fridge four were dead after one day. The remaining two were reviving at room temperature and so were placed back in the freezer. After another day one was dead but the last one revived. It was placed back in the freezer and was dead the day after, three days in the freezer in total. I asked Ante Vujic, for whom I had collected these specimens whether an early emerging species like *M.lydicus* contained some antifreeze in their blood!!!

I have found that summertime emerging Diptera are dead within about two hours in the freezer. Sometimes it is necessary to use the minimum time in the freezer compartment. For example, in photographing the eye patterns of Tabanidae and Asilidae whose colour patterns rapidly fade after death. Figs 15, 16 and 17 show photographs of *Tabanus exclusus* Pandelle 1883, (Diptera, Tabanidae). Female, length 13mm. This specimen was photographed using the vertical rotating stage after 90 minutes in the freezer. A specimen of *Promachus leoninus.*Lowe 1848 (Diptera, Asilidae) received similar treatment for the same purpose. Neither of these specimens showed subsequent signs of recovery. Both specimens were finally set immediately after initial photography.

Fig 15

Fig 16

Fig 17

There are three main advantages of killing specimens by freezing:-

1) The specimens can be left in the freezer for a few days prior to thawing out and setting, without any detriment.

2) The specimens thaw out rapidly at domestic room temperatures and setting can commence in ten to fifteen minutes. Depending upon room conditions occasionally some slight bloom of condensation might appear on the cuticle. However it usually evaporates in a few minutes causing no detriment to specimens which are always of low thermal capacity.

One of the main advantages of killing by freezing is that there is no 'rigor mortis'. The legs and wings are perfectly relaxed and easily placed into the desired position, including ensuring a clear side view of the head, for photography. For the 'set ups' for top and side pinned specimens see Figs 18 and 19.

I generally use micro-pins Size F2 (0.42mm dia x 20mm long) though from time to time I use E (0.31mm dia) or G (0.45mm dia).

Fig 18

Fig 19

3) Specimens killed by freezing are ideally suited for subsequent DNA molecular analysis, having no chemical contamination.

There is one drawback due to the tendency for increased fragility in some of the more delicate species with respect to tibia/tarsi joints. Once aware of this potential problem specimens can however, with care, be photographed without damage.

I would not recommend rapid freezing for potential type material. Extra non-type specimens can be taken and frozen rapidly for photographic purposes.

APPENDIX

The Appendix gives three enlarged examples and 'thumbnail' views using both vertical and angled rotating stages of side-pinned and top-pinned specimens of a selection of specimens. These images illustrate the potential view selections available..

REFERENCES

Ref 1. Taylor M.J. 2001. The Development of a Practical Technique for Achieving Realistic Photographic Images of Set Insect Specimens. BJENH.**14**: 193-206.

Ref 2. Taylor M.J. 2004. The Digital Photography of Set Insect Specimens. BJENH.**17**: 25-33.

Ref 3. Taylor M,J, 2012. Insect Digital Photography Workstation. www.miketaylornaturalist.co.uk www.benhs.org.uk

APPENDIX

Note the large photographs have had the micro-pins removed in 'Photoshop'.

Merodon lydicus Hurkmanns (Diptera, Syrphidae). Male.

Promachus leoninus Loew 1848 (Diptera, Asilidae). Female, length 17mm.

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Stenopogon junceus (Wiedemann) in Meigen, 1820. Female, length 22mm.

SELECTION OF SPECIMEN ASPECTS USING ROTATING STAGES

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