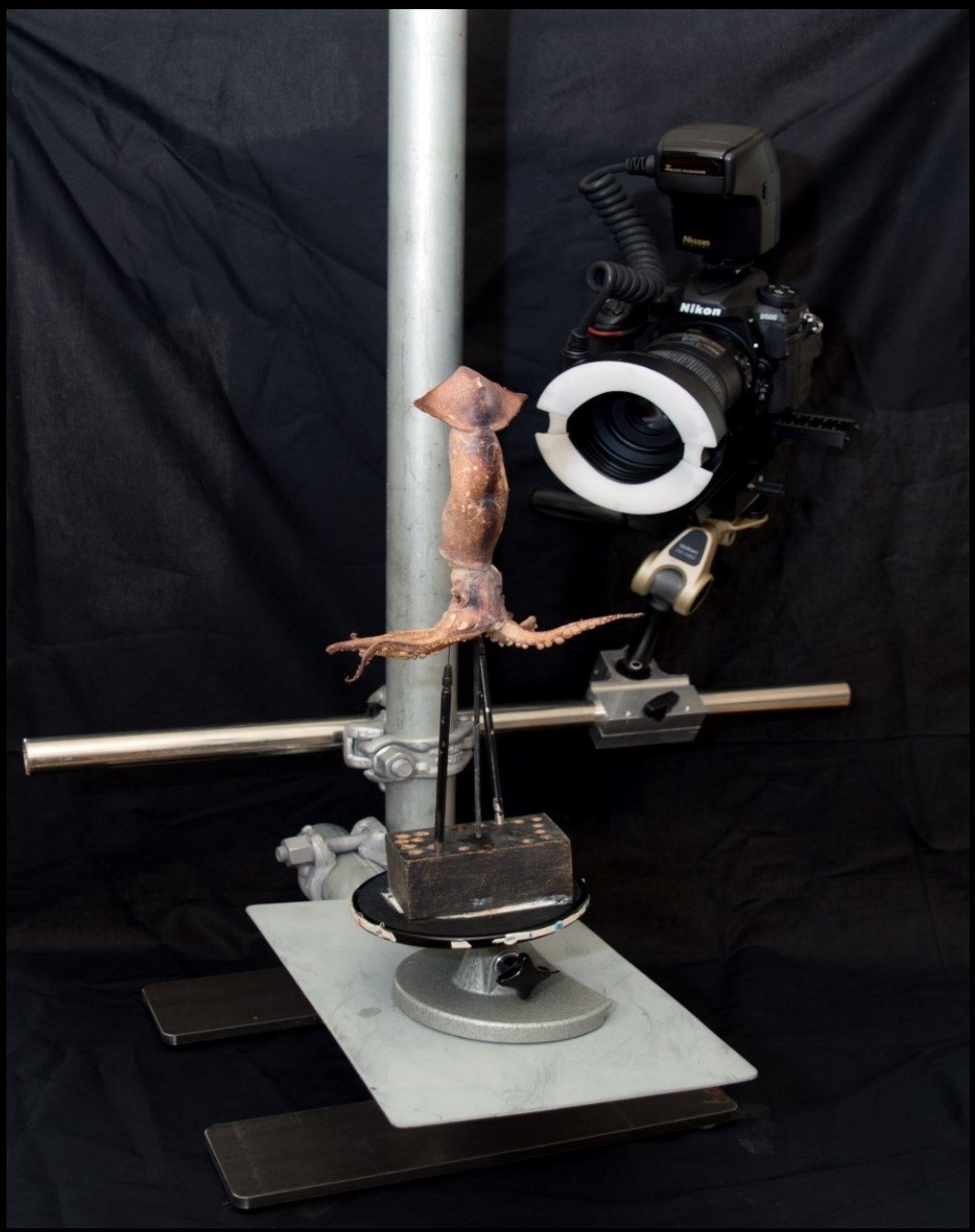
A 3D MODELLING GUIDE FOR SMALL ANIMALS

USING PHOTOGRAPHS



VIDAR ØRESLAND & GERT OXBY

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Divers and Scientists

West Coast Sweden

2022

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PREFACE

During our study of bobtail squids (Øresland & Oxby, 2021), we became interested in doing macro photogrammetry-based 3D models of them together with different organs including statoliths and otoliths. However, to our surprise, we found that 3D models of small animals and their organs were still not that widespread or commonly found on the Internet. More importantly, we could not find a suitable guide that included all the practical aspects of macro photogrammetry of small animals and their organs that we were looking for. We, therefore, decided to prepare one ourselves using a test specimen from a rubber, the digestive gland from a bobtail squid (*Sepietta oweniana*) and two cephalopods (the squid *Illex coindetii* and the octopus *Eledone cirrhosa)* as demonstration objects.

3D modelling using macro photos can be demanding since photos with different focuses have to be stacked together (z-stacking) in order to produce a photo that is sharp throughout. In addition, nearby photos must overlap to 80 %. Different specimens will therefore give rise to different photographic challenges all of which cannot be foreseen here; just one example of many is gelatinous aquatic animals that must be photographed in water or, e.g., clear gelatine. This signifies that there is a large potential for creative photography.

We used a microscope camera and the Nikon NIS-Elements software for z-stacking photos of the test specimen and the squid organ. The squids were photographed using a Nikon D500 DLRS camera equipped primarily with the DX macro lens AF-S Micro NIKKOR 85 mm and the software Helicon Remote and Helicon Focus for z-stacking. The z-stacked photos were then used for our 3D modelling which was carried out using the Agisoft Metashape Pro software, the use of which will also be described.

The aim of this guide is to enable beginners with no prior knowledge to obtain an understanding of the equipment and skills needed to make their own 3D models. We describe, step by step, the process from animal collection and fixation/preservation to the final 3D models and how to share them with others. The process can be a challenge for the beginner (as it was for us) but we hope that this guide may help to avoid our mistakes as beginners and make 3D modelling a fun and easy experience. Finally, one must not forget that taking optimal photographs is the major challenge in photogrammetry; once achieved, the Agisoft Metashape software is subsequently able to provide 3D models of high quality. The 3D models can be viewed in a number of software programmes like Agisoft Viewer, Adobe Acrobat, Power Point etc. Comments and suggestions are welcome (to be included in new PDF versions).

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PART I: WHY 3D AND WHAT YOU NEED

INTRODUCTION

Why should one bother to make a 3D model when one can take photos from different angles? This question needs to be asked before any 3D modelling project is started and the answer will depend on the audience and aim: what should they see, reflect upon, investigate, and understand? Providing a combination of 3D models and photos can often be the optimal solution for audiences.

There are many reasons for showing specimens as 3D models and /or as photos. 3D models for example could be preferable if one wants to attract attention during a PowerPoint presentation, or on the Internet thus enabling people to investigate and compare different models for themselves, as well as to estimate lengths, area and volume. Volume estimates can be used for many purposes, e.g., to estimate density (g/cm^3). Note that when dealing with organ density one should consider using dry instead of wet weight since the blood vessels and other structures contain fluids (which may vary between samples). On the other hand, photos could be chosen if the specimens are to be shown and comparted at a specific angle and position (a good example is Pedà C. et al. (2022)) or when comparing complete specimens or magnified small-sized features side by side along with your additional remarks. It is also possible to make remarks in a 3D model. In addition, photos may be sharper and allow for higher magnification. Note that correct length and area estimates in a 2D photo can only be achieved if it was taken at a 90-degree angle to the area of interest, something that is easily overlooked. However, in a 3D model, correct length and area estimates can be done regardless of viewing angle.

Before producing 3D models of animals, we strongly recommend the beginner to make a simple and durable training specimen (see **below**). Finally, the photogrammetry industry is developing quite rapidly and we recommend regularly checking for software upgrades. It is, for example, already possible to jointly process laser scanning and photos (included in Metashape, but not dealt with here), providing benefits from both sources and avoiding some of their shortcomings.

EQUIPMENT

Camera, lenses, flash, batteries and macro focus rail slider

In this guide we refer to the equipment that we ourselves used; however, this should not lead to any misunderstandings. We used the Nikon D500 APS-C DLRS camera (21MP, pixel size 0.0042 mm) with the DX macro lenses AF-S Micro NIKKOR 85 mm 1:3.5 ED and the AF-S Micro NIKKOR 40 mm 1:2.8 G. From the photo quality point of view, one does not need a “full-frame” camera for macro photogrammetry of small animals. The cost of APS-C cameras and DX lenses is much lower and there is also a second-hand market offering good prices.
We used the ring flash MS18 Macro with a LED light which is useful during focusing. This flash is fast, does not get overheated and is fully compatible with the camera. One should keep a number of loaded camera and flash batteries ready before starting the photography and two battery chargers for the camera and two for the flash. We used four camera batteries and six sets of flash batteries. Note that one might want to take thousands of photos in one day and charging batteries takes time.

For manual macro focus, we used the Neewer Pro 4-Way Macro Focusing Focus Rail Slider (**fig. 1**), which is both affordable and proficient. The camera is manually moved forward (to a

minimum) between each image so that the focus changes very little between them, as can be observed on the computer screen. The interval between two photos should be smaller than the depth of field (**Part II** describes the use of the camera rail slider). One should always use a manual camera trigger when the camera is not computer-controlled.

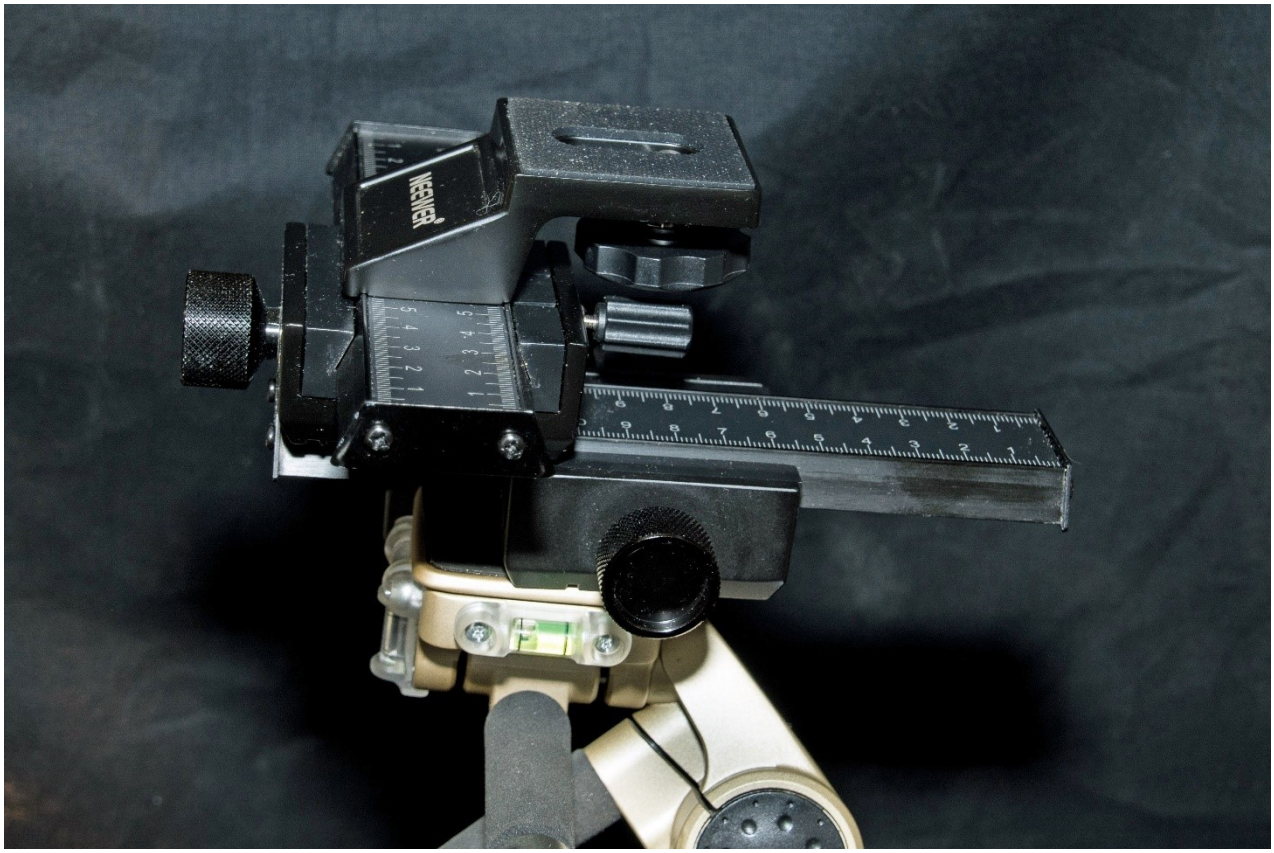


Fig. 1. Neewer Pro 4-Way Macro Focusing Focus Rail Slider. This is an inexpensive but good quality item that worked very well for us, with the camera and flash weighing 1.7 kg. The camera is mounted on top of the rail slider.

Camera stand, turntables and background

A very steady camera stand that allows for taking z-stacked macro photos from all possible angles is a prerequisite when making a 3D model of small animals. Therefore, we do not use a tripod as the legs make it difficult to take optimal macro photos from all angles and tripods can also become unsteady at certain photo angles due to the weight of the camera equipment.

Our inexpensive and easy-to-operate camera stand (**fig. 2**) was made from pieces of scaffolding, a 61 cm long metal pipe that was 3 cm in diameter, a moveable shelf, a heavy 1 cm thick iron foot and the upper part of a camera tripod (to which the rail was mounted) that was fitted into an aluminium block (**fig. 3**). A piece of rubber attached under each corner of the foot makes the stand, together with its weight, extremely stable. The height of the camera stand is 126 cm and its weight is 17.0 kg.

On the moveable shelf we place our turntables. **Fig. 4** shows a turntable for small specimens that we also use with stereomicroscopes. We also use a hand support (an old one made for stereo

microscopes) as a turntable (**fig. 2 and 5**) that can be rotated 360 degrees as well as turned backwards and forwards. Behind the camera stand, we have a movable and height-adjustable stand for a black cloth background.

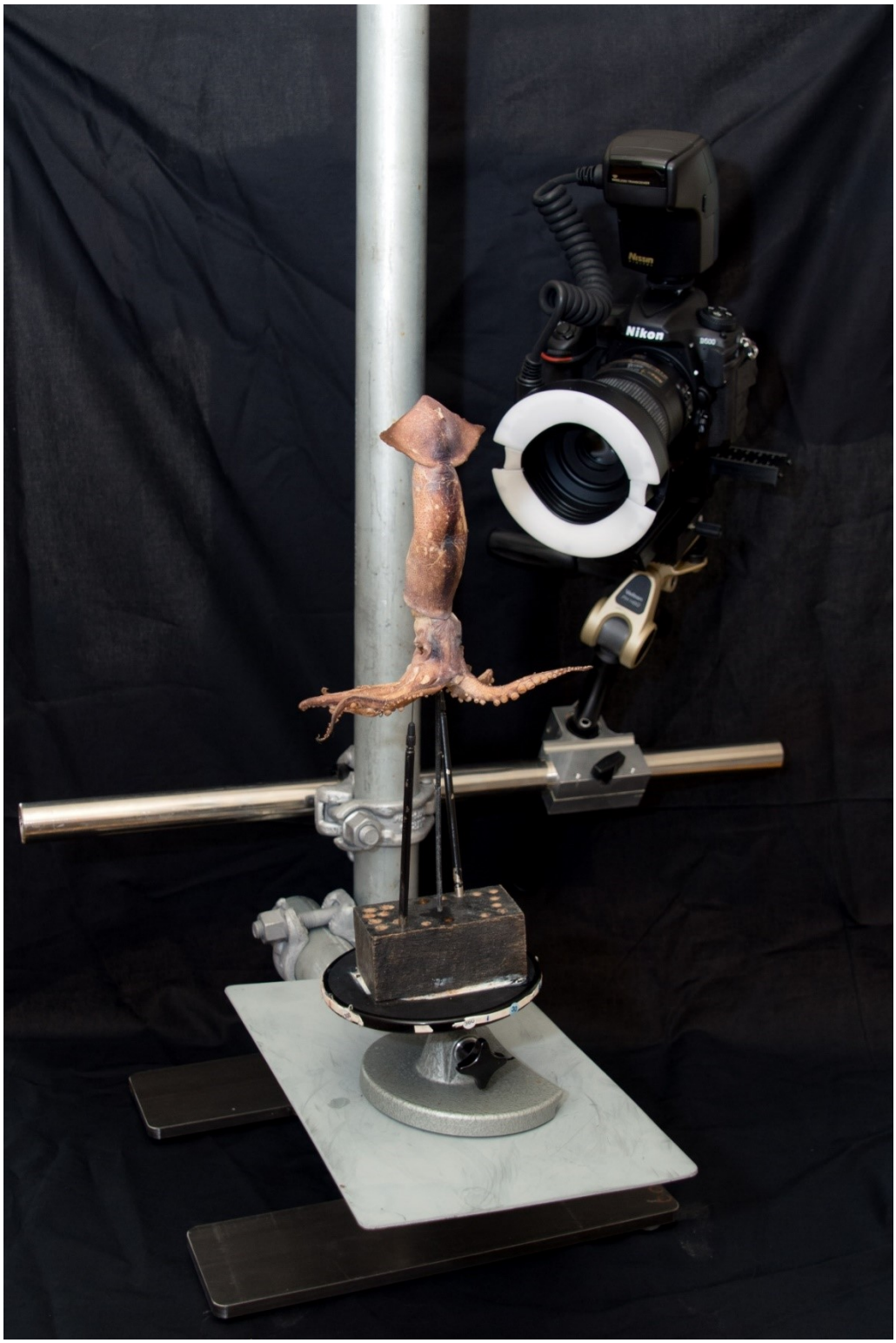


Fig. 2. Our camera stand and a mounted squid (*Illex coindetii).*

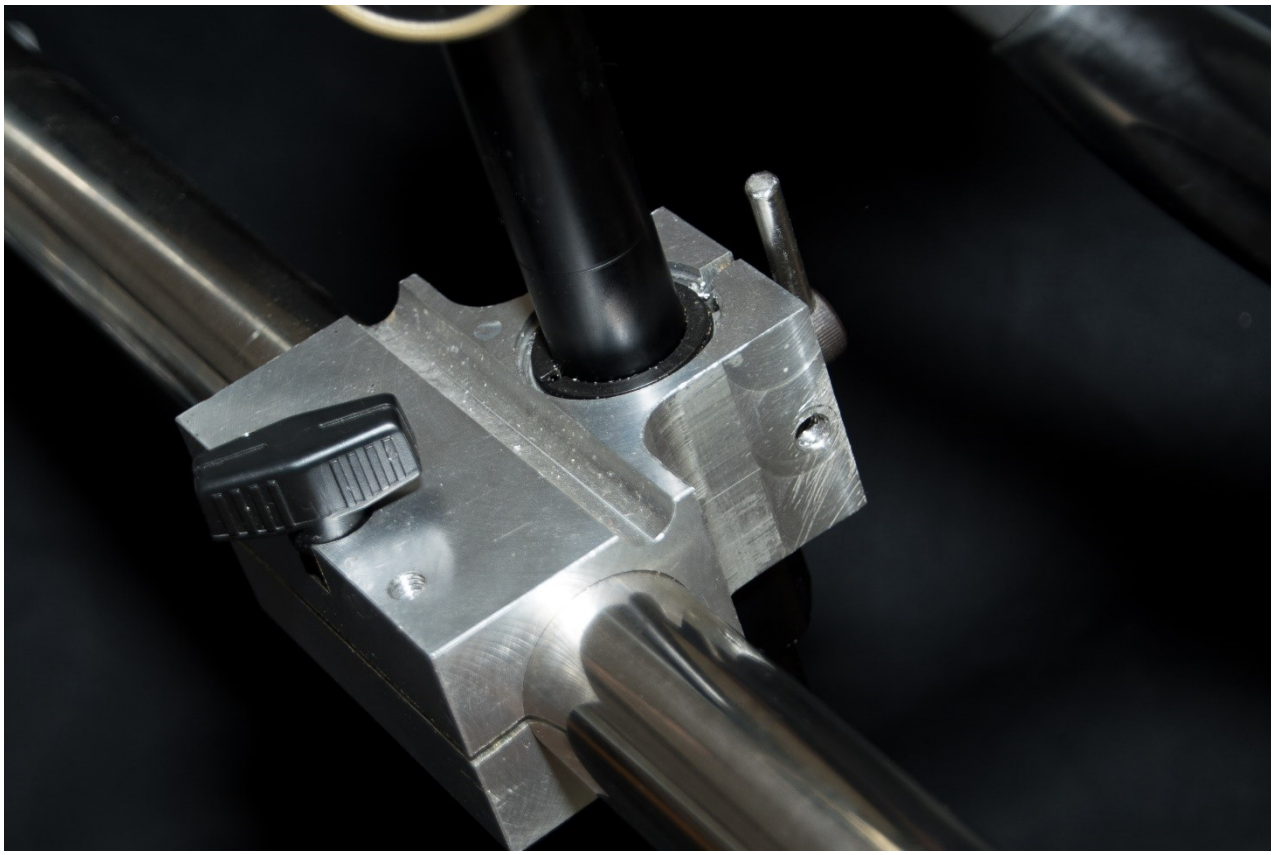


Fig. 3. This aluminium block can (after the release of its two locking mechanisms) be moved horizontally and rotated around the horizontal pipe (which in turn can be moved up and down and around the vertical scaffolding pipe).

Fig. 4. A turntable designed for small specimens often used with stereomicroscopes. Note that one can drill holes and attach needles for keeping a petri glass or a specimen in place during rotations (and at the same time place a black background photo).

Fig 5. We used a hand support for stereo microscopes as a turntable that can be rotated 360 degrees as well as turned backwards and forwards. This squid *(Illex coindetii)* was a test specimen that was used many times and therefore in bad condition. See **below, Mounting,** how to mount a squid.

Microscopes

When photographing smaller specimens, we used: the Wild M3Z stereo microscope; up to four movable stereomicroscope lights; and the microscope camera DFK33UX250 (5MB). One can use an oblique viewer (**fig. 6)** mounted on a stereomicroscope (which makes it possible to take photographs from the side) but this would have to be calibrated (see **Part II**) and used for all the photos that are part of a 3D model. It should be noted that our stereomicroscope Wild M3Z has a Type S holder attached (**fig. 6**) which makes it possible to take "one light beam " photos, an essential addition when creating 3D models (see **Part II, Microscope photography**). Tips for photography using microscopes and microdissection techniques can be found in Øresland & Oxby (2021). Note that an inverted compound microscope can be used for 3D modelling of small objects like statoliths and otoliths.

Fig. 6. An oblique viewer (type 404233) by Wild Heerbrugg. Our Wild M3Z is equipped with a Type S holder which is capable of providing a single light beam (see below, **Microscope photography**).

Computers

To improve data security and speed up the process of data processing, we used two computers: one with a good memory capacity and high screen resolution for photography, photo storage and 3D modelling. This computer (Windows 10, 64 bit) has 32GB of RAM which should, as a very rough guide, be considered a minimum (but worked fine for us), and an NVIDIA GeForce RTX 3060 graphic card. The other computer was used for data recording, the Internet, and for all other purposes. Photography and data collection are time and labour-intensive and will, over time, represent months and years of work. Therefore, we tried to avoid having our computer for photography connected to the Internet and used good protection software on both computers. It is important to have at least two backup memories and to have an additional backup in another location. Backups should be made daily. A tried and tested, easy-to-use, folder system should be utilised since data and photos will be extensive and diverse. More information about our folder system is found in **Part II** and **III**. A data table should be created where all the scientific information can be tabulated from left to right as soon as it is obtained.

Training specimen

It is advisable to make a simple and durable training model before commencing the 3D modelling. We made one from a rubber which we cut at different angles and coloured (**fig. 7**). A hard plastic or metal model of a specified volume and area, plus a mm scale, would be even better since the resulting 3D model could be checked more easily. One should make sure that the model has many identifiable feature points and do not create glares during the photography. It is important to ensure that the Metashape Pro settings are correct in order to produce accurate length, area and volume estimates (see **Part III, MEASUREMENTS**). There is no room for error if the estimates are to be utilized scientifically.

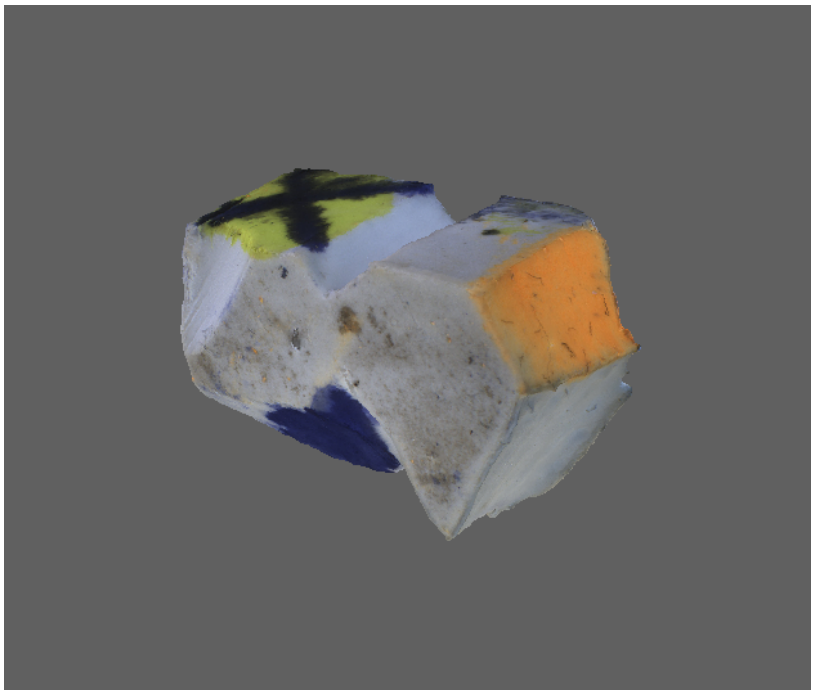


Fig. 7. Our test specimen was made from a rubber. The 3D model can be viewed in the PDF version using Adobe Acrobat Reader (click the figure).

SOFTWARE

Helicon Remote and Helicon Focus

We used the integrated Helicon Remote (3.9.12 W) and Helicon Focus (8.2.0) software that is easy to use and which together produce sharp z-stacked images (**Part II**). When connecting the Nikon D500 camera to Helicon Remote one will get computer control of its settings, with the possibility of direct storage of photos, without using a camera memory card, which will save considerable time. The principle behind z-stacking is that a number of photos are taken with sharpness in different planes, from the front of the specimen to the back or the other way around. Once completed, a click on the Helicon Focus icon (inside Helicon Remote) enables the images to be inspected and z-stacked into one. This sharp z-stacked image and all partially blurred initial images (often 20 - 60 images) should now be saved and it is possible to test different z-stacking settings, if necessary.

One should first make all the camera and flash settings needed, take a test photo, and **then** connect the cable between the camera and the computer. When photos are taken in the field without computer access, they can be imported to the Helicon Focus using the camera memory card. Note that images can be taken both manually and automatically in Helicon Remote, but we use only manually captured images in 3D modelling with Metashape (see **below** and **Part III**) since our rail slider is manual. The Helicon Remote Automatic focus bracketing cannot be used here since the 3D modelling requires a fixed camera focus (see **Part II**, **Choosing macro lens and focal distance**). When taking photos in microscopes we did all the above using the NIS - elements software (see **below** and **Part II and III**).

Nikon NIS-Elements

When doing microscope photography of small organs (including our rubber model, **fig. 7**), we used our **microscope camera** and the NIS-Elements software (5.02.03) to produce sharp z-stacked images. The NIS software is also used to estimate distances in both microscope photos and Nikon D500 camera photos (**Part II**). The NIS z-stacking is very easy to do and much quicker than using the Nikon D500 and the rail slider together with the Helicon software (**Part II, TAKING PHOTOS AND Z-STACKING).**

Agisoft Metashape Pro

The Agisoft Metashape comes in both a professional (we used version 1.8.4) and a standard edition (where one cannot make estimates of the distance, area, and volume and which also lacks a number of other options). However, the standard edition comes at a very reasonable price, is a good choice for beginners, and is a useful introduction to the pro edition since the overall structure is the same. Metashape is produced for a number of applications but here we are only concerned with its relevance to the 3D modelling of small animals. Once all the z-stacked photos are taken one can import the photo folder to Metashape and follow the instructions given in **Part III**. Additional photos can always be imported at a later stage. Additional software from Agisoft (free down load) that we used was **Agisoft Viewe**r (to visualize 3D data) and **Agisoft De-lighter** (to remove shadows from model textures). We also used **Adobe Acrobat Pro** to insert 3D models into PDF documents (**Appendix III**).

ANIMALS

Collection and preservation

Since 3D modelling of small animals is a labour-intensive task, it is important to obtain specimens in perfect condition. Collection, anesthetic, and preservation methods for invertebrate animals can be found in Lincoln & Sheals (1985), Øresland & Oxby (2021) and on the Internet. Marine animals can be obtained from, e.g., fish markets, fishermen, museums, research institutes, divers, or found on the beach, etc. Even if the models are not intended for research purposes one should always note important collection data such as date, exact location, collection method and the collector´s name (as a minimum). The data should be noted (using a soft lead pencil), on a waterproof label that is kept with the animal inside a bag or jar, directly after the collection.

1. Determine whether the specimen (a whole individual or an organ) is worth 3D modelling or being photographed for 2D image purposes: no external damage, lost parts or discolourations.

2. Give the specimen an individual ID code and tabulate all collection data in a general data file.

3. Decide whether the specimen should be photographed in water, air or gelatine, etc.

4. Does the specimen has to be preserved, stained, dissected, or fixed in a certain position?

5, **If possible**, the specimen should be fixed in its preferred position prior to the fixation/ preservation.

One can use fresh or live animals as long as they don't´ move or change appearance during photography, otherwise, the animals have to be killed and preserved or at least immobilized. What is the desired appearance of the specimen (standing, lying, attacking, open mouth, etc)? Sometimes one wants to model only a part of the animal, e.g., a snake's head. The animals can be anaesthetized until near death before preservation in order to reduce contraction. One can use 4% formaldehyde in freshwater (buffered to ~ pH 7 using borax ($Na_2B_4O_7 \cdot 10H_2O$) or 70-96 % ethanol as preservatives. However, there are a few things to consider before preservation can commence. Preservatives may affect the animal's appearance permanently (shrinking, bending, colour); some parts of the animal (e.g., squid arms, tentacle clubs, and fins) may first need to be fixed prior to preservation in order to prevent bending. If the specimen is already preserved and its position needs to be changed, one can put it in water for 24 hours (change most of the water 3-4 times) prior to the final fixation. The arms of a squid, e.g., may then soften a bit and can, **hopefully**, be stretched out in the preferred positions. Finally, and most importantly, one must be able to take photos from every angle after the position has been corrected.

Figs. 8 and 9 show our fixation tube for squids and how the squid arms can be fixed in optimal positions. First, a sewer pipe of appropriate length and width was cut and then we glued a piece of plastic at the top, with a central oval-shaped opening to fit the squid mantle and fins. Pieces of **Velcro** were attached to the **plastic** and to **two** half-moon-shaped **pieces of cork**, both of which have a half-circle opening that becomes a full circle when the cork pieces are put together. The squid is put through the plastic hole and between the cork pieces from above, leaving the arms and tentacles above the cork. The cork pieces are then moved towards the squid's head, and the cork pieces can be attached to the plastic piece. The arms and the tentacles can subsequently be fixed to the cork using pins and/or stripes of water-resistant field notepaper and pins. Before inserting the squid, its **fins** should be fixed between thin pieces of stiff **plastic** that are held together with tape. Finally, the fixation tube is put into a plastic bucket with 4 % formaldehyde and lead weights are put above the cork to keep the fixation tube in place. The squid in **fig 9** was already preserved and

then put in water for 24 hours to soften it prior to the preservation on the cork. As can be seen in **fig. 9,** we could not stretch out the arms as much as desired. Clearly, the best option is to fix the positions of **fresh specimens** before adding preservatives.

We use 4 % formaldehyde since it fixes the position of the arms and tentacles better than ethanol and does not deform the animals or their inner organs as much as ethanol can in some cases. Formaldehyde can affect one´s health and should be treated with caution, e.g., by wearing gloves and avoiding inhaling the fumes. After three days the specimen can be removed from the fixation tube and put in water overnight in order to remove the formaldehyde fumes before mounting (see **below**). When using ethanol, we started with 20 % for 24 h, then 40% for 48 h, and finally 80 % for seven days. This stepwise preservation makes the squid softer and not so deformed as is often the case when put directly into 80 % ethanol.

We definitely prefer ethanol (which is not dangerous to inhale) for our **reference collection** of squids as they are often taken out of their jars for examination and comparison with other individuals. Once animals are in their final ethanol concentration or 4 % formaldehyde they can remain there for years. However, preservatives may affect the colour and shape of the specimen, though different species and organs may react differently. It is therefore advisable to keep the specimens in the preservation fluid for as short a time as possible before starting the photography. If it´s uncertain when the work can begin, one should consider deep-freezing the fresh animal before fixation and preservation in formaldehyde or ethanol. Deep-freezing, can, however, dry out soft-skinned animals so they should first be put into a moistened plastic bag with a minimum of air. One can also test how different animals are affected by deep freezing in water and the following thawing.

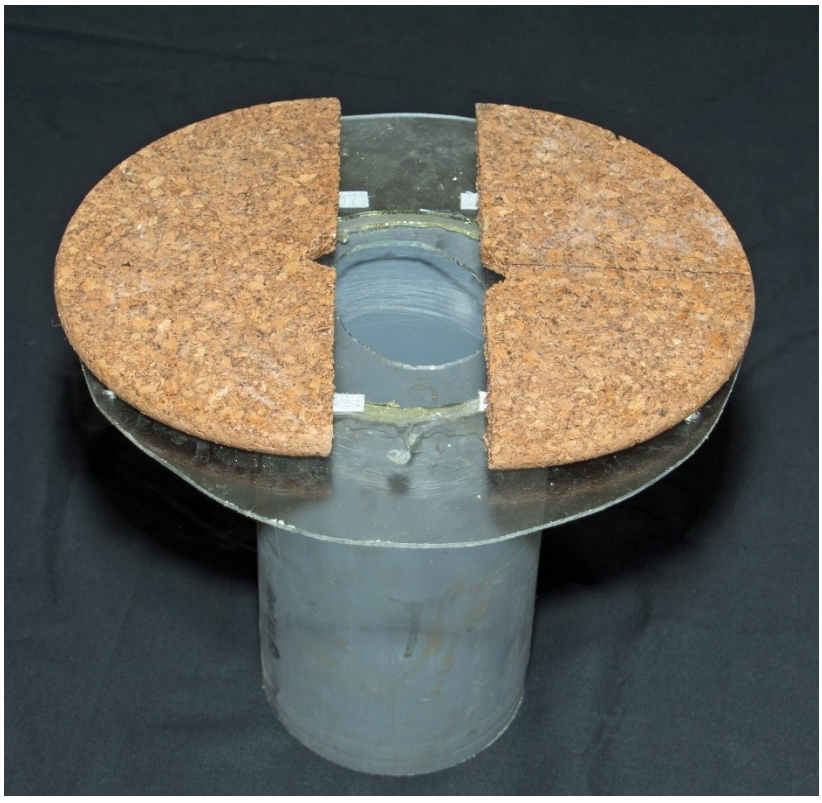


Fig. 8. A fixation and preservation tube for squids with the moveable cork pieces and the white pieces of Velcro between the cork and the plastic.

Fig 9. An eight-armed *Eledone cirrhosa* is fixed to the cork of the preservation tube using water-resistant field notepaper and pins. The specimen had been preserved in 4% formaldehyde prior to this and the arms could therefore **not** be stretched out in full in the preferred positions, despite being in water for 24 hours.

Mounting

Staining must be carried out prior to mounting and in some cases even before fixation depending on the dye used (see Øresland & Oxby, 2021). If one cannot move the animal around (e.g., on top of a turntable or in a petri dish) without affecting its shape, it must be mounted. **Fig. 5** shows a turntable with a woodblock glued to it and a squid attached to a black metal bar. Two other bars **with needles at one end** fix the position of the squid tentacles. Make sure that the metal bar thickness and length are optimal (too long and thin will cause vibrations and too short will make it difficult to take photos from underneath. Sharpen the bars to facilitate mounting. Note that a number of holes (drilled at different angles) in the woodblock makes it easier to fix the tentacles in

an optimal position. It is crucial that no parts of the animal can move during photography (this is why we prefer formaldehyde which makes the animals more rigid). If the photography session has to continue on another day, one must **not** remove the squid from the turntable! Instead, one should simply turn it upside down into the preservative, keeping the table in the air by resting it on two pieces of wood over the container opening. All the **arm and tentacle** photography should be taken during the **first photo session** in case their fixed positions change.

PART II: PREPARATIONS AND MACRO PHOTOGRAPHY

INTRODUCTION

We are now going to deal with the all-important preparations and photography before we make the 3D model **in Part III**. The two Helicon and the Nikon NIS-Elements software programmes are easy to use and we have chosen to concentrate on the necessary preparations and insights that will help to achieve an optimal z-stacked macro photo and a series of such photos, which together meet the requirements of a good Agisoft Metashape 3D model. The **crucial** importance of **optimal photos** should not be underestimated. Macro photography can be demanding but we hereby provide solutions for the commonly met problems (see also Øresland & Oxby, 2021). Finally, **it´s all about preparation and photography**!

PREPARATIONS

Folder system for photos and 3d models

There are a number of things that should be considered **before** starting photography. First of all, one should create, and test, a practical photo and 3D model folder system in order to easily save and find all files, as well as making a data table (we use Excel for tabulating all specimen data, from collection to 3D model analyses). **Note** that Metashape cannot locate the files if their file names have been changed during the 3D process. If folders have been renamed or their positions in the folder system have been changed, during, e.g., model copying, their new positions can be specified using the *Change Path* command. We will deal with this problem in **Part III**, **Saving models**. We show below a **general** folder system used for **all** our 3D projects.

D/3D PROJECTS/DATA/3D EXCEL DATA; PHOTOS/
Under 3D EXCEL DATA we save our Excel data file containing all specimen data and under PHOTOS there are five main folders:

A. MICROSCOPE CALIBRATION PHOTOS
B. NIKON D500 CALIBRATION PHOTOS
C. BLANK PHOTO FOLDERS TO BE COPIED TO D
D. 3D PROJECTS
E. TESTS

The main folders A and B contain all the microscope and Nikon D500 calibration photos for all 3D models and 2D photos used in **all** 3D projects.

The main folder C. BLANK PHOTO FOLDERS TO BE COPIED TO D contains two empty folders: ***SPECIMEN*** and ***ORGANS***. Under both these folders we have seven empty sub folders into which the photos and 3D files are saved:

1. FRESH AND PRESERVED. For 2D presentation photos of the whole specimen or organ.

2. ESTIMATING NO OF PHOTOS REQUIRED. For estimating the positions and number of z-stacked photos that will be needed (with 80% overlap).

3. 2D SCALE PHOTOS. For "**scale photos**" for the 3D model and 2D presentation photos that need a scale bar.

4. Z-STACKED PHOTOS. For the microscope and Nikon D500 z-stacked photos to be used in the 3D model.

5. Z-STACKED PHOTOSHOP. For copies of Z-stacked photos to be used for Photoshop adjustments. These images replace (when needed) the z-stacked photos used **just before** *Texture* (see **Part III**). **Note:** The file names (just a running number) must be identical to the original ones and the photos must not be cropped.

6. 3D MODELS. For 3D model in progress.

7. FINAL 3D MODELS AND 2D PHOTOS. For different final 3D models and 2D photos to be used for publication and other purposes, like PowerPoint, and Agisoft Viewer models etc. The **report** (see **Part III**) and the 3D project`s **data file** should also be saved here. Keep all files that are relevant to the 3D model in this folder.

The main folder D. 3D PROJECTS contains **all** our 3D projects, old ones as well as those in progress. Here one creates and name a project folder, using the **ID code**, and the **Specimen** and **Organ** folders (**including the subfolders 1-7** shown above) are copied from C. BLANK PHOTO FOLDERS TO BE COPIED TO D and pasted into the new project.

The main folder E. TESTS is used to save tests that may or may not be deleted after use. We also use it for **saving** the **original Helicon Remote photos** which can be **deleted** after z-stacking (see **below**, **Nikon D500 photography**).

ID codes. The 3D models, photos, Excel data, labels used for jars and vials etc. were each given the specimen ID code. However, the photos used for z-stacking (Helicon only) and the z-stacked photos were given a running number (to save time). How to create and use ID codes is very much a personal choice but they should be informative and consistent. Our ID codes consist of lowercase letters, numbers, dots and hyphens with no spaces between.

Photography room

1. Clean the microscope objective(s) and oculars: remove as much dust and dirt as possible with a blower or soft-bristled brush; apply a few drops of lens-cleaning solution to a lens paper or a cleaning cloth and using a circular motion, working from the centre outwards, to gently remove fingerprints, oil and grime from the lens surface. Never remove the camera from the microscope since it protects the inside of both from dirt that can be very difficult to remove! Make sure that the camera is tightly connected to the photo tube adapter. Always use a plastic hood for the microscopes and cameras when not in use!

2. Check the magnification calibrations of microscopes and stereomicroscopes against a glass slide micrometer using NIS-Elements (see **below, Microscope magnification calibrations)**.

3. Have available different solutions of dyes, sharp dissection tools, microscope lights, Petri glass dishes, distilled water and a black background, etc., ready for dissection and photography (see Øresland & Oxby, 2021).

4. Keep an ultra-fine spray bottle at hand to moisten the specimen (with distilled water) and use a paper to protect the flash, camera and microscope objective(s).

5. Have lens paper ready to absorb any excess water on the specimen.

6. Have black sprayed metal rods of optimal length and thickness ready for mounting the specimen.

7. Cut out small pieces of lead to support small organs from underneath when in a Petri dish during microscope photography. However, one should consider mounting the organ on a needle connected to a small turntable (**fig. 5**).

8. Have available a small flashlight/headlamp in order to see the camera settings and keyboards, etc., when the photo room is blacked out. Have no other lights on except for one computer screen in use (use blackout curtains and keep doors closed).

Microscope ocular adjustment

The most common microscope oculars used have a magnification of 10x. Adjust the stereomicroscope ocular appropriately (with or without glasses). Make a cross on a piece of paper with a graphite pencil and place it flat under the stereomicroscope. Set both oculars to zero and obtain sharpness, using the focus knobs, at the **highest** magnification. Then choose the **lowest** magnification, **without moving** the focus knobs, and adjust the oculars individually for optimum sharpness. One can thus change from low to high magnification with a minimum of focus adjustment. Tape the ocular scale settings. Compound microscope oculars are adjusted directly i.e., in accordance with one´s sight. We use the oculars primarily during dissection and when removing small parts that do not belong to an organ.

Microscope magnification calibrations

A compound microscope (not used in this guide) has objectives of different quality and magnifications (often 4x, 10x, 20x, 40x, 60x and 100x). A stereomicroscope can also have objectives with different quality and magnifications (0.5x, 1x, and 2x) and zoom capability to provide different total magnifications. The two **zoom knobs** have different numbers marked on them which, when multiplied by the ocular and objective magnifications, give different total magnifications. However, some stereo microscopes (such as our Wild M3Z) assume a 1x objective and a 1x ocular, in which case the zoom knob numbers give the total magnification directly. A 0.5x objective will give half the magnification. It is very **important** to set the zoom knob to "**fixed step zoom**" instead of a continuous zoom.

In order to estimate distance, area and volume in a 3D model (and distance in a 2D photo) one has to calibrate (in NIS) both the microscope magnification to be used and the Nikon D500 x/y frame size in mm for the chosen macro lens and focal distance (see **below**, **Nikon D500 frame size**). In **Part III** we will use the microscope magnification calibration and the Nikon D500 frame size calibration to estimate the distance between known points in "**scale photos**" (in NIS) and to make **scale bars** in 3D models in Metashape (see below, **Scale photos for 3D models** and **Part III, Bundle alignment and making scales**). Before the microscope calibrations starts: make sure that

the camera fits tightly to the photo tube adapter and set the stereomicroscope to "**one beam**" (see **above**, **Part I, Microscopes**). Make a "***quick calibration***" in NIS of the compound microscope objectives and the different "fixed" zoom magnifications of the stereomicroscope using a **glass slide micrometer** and the microscope camera. Save the calibrations on the NIS access bar with a shortcode: e.g., 40wild1.0x (**zoom knob number**, microscope name, and objective magnification) and remove old shortcodes. Obviously, one only need to calibrate the chosen compound microscope objective or stereo microscope "fixed" zoom magnification.

Make a distance measurement, using the relevant shortcode (check that the microscope magnification and the shortcode in NIS are in compliance), take a photo and burn the distance line and the estimated length data in to it and save the photo (**fig. 10**) in the main folder ***A. MICROSCOPE MAGNIFICATION CALIBRATION PHOTOS.*** The photo file name should consist of the date, microscope name, objective magnification and **zoom knob number** for stereomicroscopes (e.g., 08-08-2022wildm3z-obj1.0x-zoom 40). Note that **if an oblique viewer is used**, it must be calibrated for the selected magnification and viewer angle (to be taped!), which must be specified in the calibration photo file name.

Do not delete old calibration photos used for old photos since one may need to validate measurements made on them as well as making new ones (after making a new "***quick calibration***" shortcode using a slide micrometer). **Always check** the shortcode to be used against a **slide micrometer** before starting a photo session. It often happens that one must recalibrate! However, a small deviation can be due to an inadequate focus setting. Any distance in a microscope photo can now be estimated using NIS by choosing the correct shortcode (**see below, TAKING PHOTOS AND Z-STACKING).**

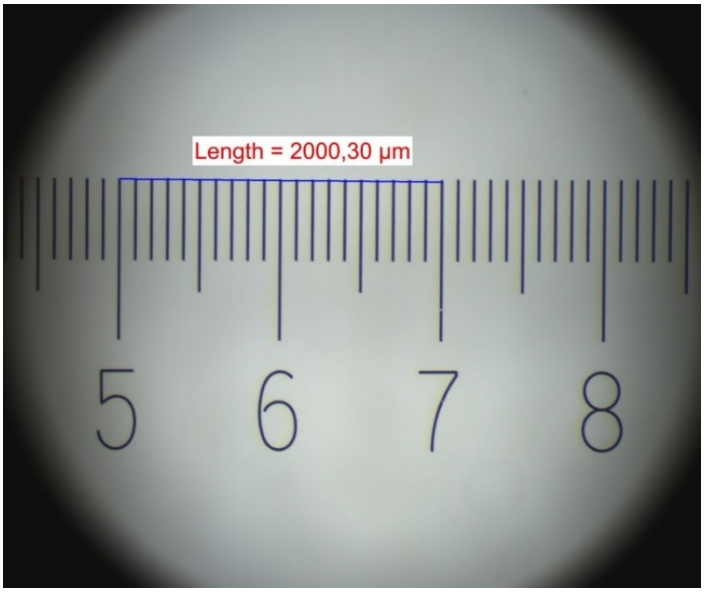


Fig. 10. A stereo microscope magnification calibration (08-08-2022wildm3z-obj1.0x-zoom40) was here tested by measuring a 2 mm long distance in a glass slide micrometer.

The Nikon D500 camera and flash settings

1. Clean both the camera lens and the camera sensor (the *wrench icon* in the camera menu).

2. Charge all camera and flash batteries before each new photo session.

3. Set the flash compensation to 0 (to start with). Use the TLL setting for automatic camera control of the flash and manual flash compensation.

4. Set the camera to: single shot; HDR **off**; mirror lock **up (MUP)**; matrix measurement (**not spot**); and lens and camera focus to **manual**. Automatic "picture rotation" and "rotate high" should be **off**. Set the ISO to **100** (**must not be changed** during a 3D project), the exposure time to **1/250 s**, and start with an aperture of **f /8** (see **sweet spot**, **below**). Set the camera exposure compensation to **0**. White balance settings; see **below**, **Light and natural colour.**

Choosing macro lens and focal distance

Here we compare a 40 mm macro lens and an 85 mm macro lens. The choice of macro lens and focal distance demands a compromise between the time spent on the photography and the sharpness and zoom/enlargement capability of the 3D model. For example, a 40 mm lens will give a large photo area, thus needing fewer z-stacked photos to cover the specimen/organ. However, an 85 mm lens gives a smaller photo area (with more pixels per area) that enables greater enlargement (which might be useful for PowerPoint presentations) but more z-stacked photos are needed. Since the z-stacked photos must overlap by 80 %, many photos have to be taken; the time saved by using a 40 mm lens can therefore be considerable. However, this can be avoided by using an 85 mm macro lens set to a larger focal distance providing a larger photo area (frame size). The 85 mm lens can thus provide the user with a higher degree of flexibility when it comes to macro photography.

Since Metashape requires that all photos depict the specimen with exactly the same magnification, frame size and x/y pixel numbers, the chosen focal distance on the macro lens must be fixed. The **focal distance** should be **taped** so that it cannot be accidentally changed. One must then manually move the camera forward (or backward) so that all images, each focused on different parts of the specimen, can be taken for z-stacking. This is achieved by the use of a macro focus rail slider (**fig. 1).**

A camera lens "sweet spot" refers to the aperture that will give a minimal amount of aberration and diffraction and hence produces the sharpest image. For most lenses, the sweet spot is around f/8. However, when taking macro photos for z-stacking one may wish to go somewhat higher than that in order to increase the depth of focus and thereby reduce the number of photos needed. Avoid using different f-settings in order to save time and effort.

Nikon D500 frame size

As shown previously, (**Microscope magnification calibrations)** we used the "*quick calibration*" in NIS to calibrate different microscope magnifications using a glass slide micrometer and making magnification shortcodes. However, when using "*quick calibration*" to make a magnification shortcode for a Nikon D500 photo, one must first estimate in mm the total horizontal and vertical distances that the photo will depict (frame size). The NIS **shortcode** is used later to calibrate "**scale photos**" to be taken during the Nikon D500 z-stacking photography (see **below, Scale photos for 3D models** and **Part III, 3. Bundle alignment).**

Here we use our Micro NIKKOR 85 mm macro lens as an example and tape the chosen focal distance at 0.35 m. The focal distance is that from the subject area in focus to the camera sensor. Using the camera stand, take **two** calibration photos, at a 90-degree angle from above, of a slide micrometer (or a ruler for large focal distances/large photo frame sizes) so that its scale covers the photo **width** (**fig. 11**) and then its **heigh**. In this case, these are approximately 45/30mm (always check that the width/height correlation equals that of the Nikon D500 camera Image sensor: 23.5 x 15.7 mm (CMOS sensor). Save the photos in the main folder **B. NIKON D500 CALIBRATION PHOTOS**. Name the calibration photo files with date, camera, lens, focal distance, and width (w) or height (h), e.g., 03-05-2022 Nikon D500 macro Nikkor 85 mm-fd0.35 w45mm.
Finally, we make a "*quick calibration*" in NIS by opening the "width photo" and calibrating it using the known width distance. Save the calibrations on the NIS access bar with a **shortcode**, e.g., NIK85mmfd0.35. Measure any distance using a glass slide micrometer or ruler in order to check the calibration.

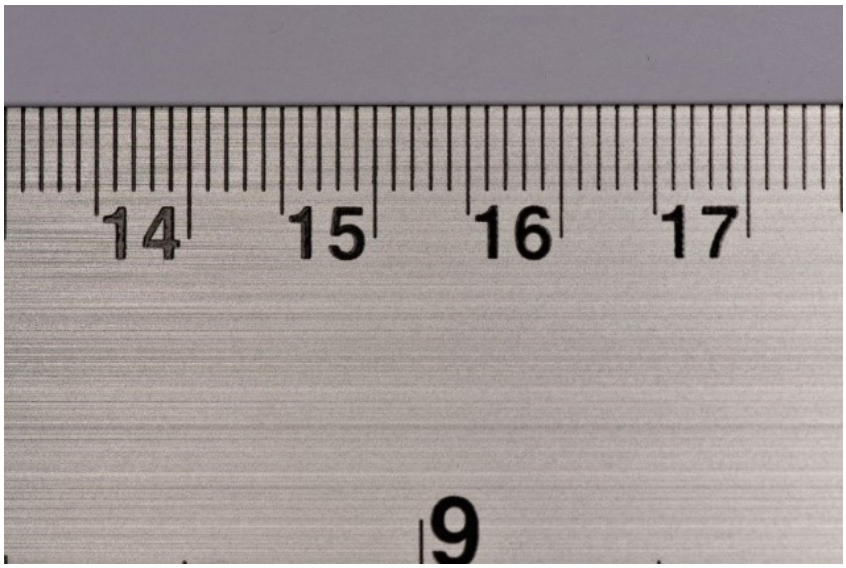


Fig. 11. A scale photo (03-05-2022 Nikon D500 macro Nikkor 85 mm-fd0.35 w45mm) taken of a metal ruler using a Nikon D500 camera and Micro NIKKOR 85 mm macro lens and a focal distance set to 0.35 m. The frame width is 45 mm in this case.

Light and natural colour settings

Lighting and images resolution affect the aligned photos result, 3D model quality and model texture and should be taken into account when planning the photography session. Take **test images** and check for uniform lighting and natural colours. Ensure that there are no too dark or light areas or any reflexions in the photo. During stereomicroscope photography we use stereomicroscope lights only (**some with simple modifications**, see Øresland & Oxby (2021)). Turn on the first extra light, reposition it as needed. Add more lights if necessary (we use up to four lights). During Nikon D500 photography we use primarily the flash but also stereomicroscope light when needed. Set the flash lighting compensation to **0.0**, increasing it if more light is needed. If the maximum flash compensation and extra light are inadequate one can increase the camera exposure compensation. If one side of the specimen needs to be brighter, the

flash ratio can be changed or one can try changing the photo angle. Do not forget to check and change lightning when needed during the photography in order to make all photos equivalent.

The lighting used can affect the colours of the photos. For example, a higher flash compensation value can remove yellowish shades. The Nikon D500 has different white balance settings that can be used for optimal colours:

1. Kelvin setting. Press the ***WB*** (white balance) button and check the control pane. The current setting is at the top and at the bottom there are small icons that can be selected by pressing the **WB** button while turning the **main command dial** (e.g., k = kelvin; if k is selected, then turn the camera **sub-command dial** to 5560 k (5600 k is optimal for MF18 Macro).

2. Select the **Menu button >** *camera* > *white balance* > ***auto*** > *OK;* press the ***WB*** and the letters WB A is shown in the control pane.

3. Select the **Menu button** > *camera* > *white balance* > *flash* > *right-click* the **multi selector***;* chose a colour compensation from the colour chart (if needed) > OK; press the ***WB*** and the letters WB and a flash symbol is shown in the control pane. We normally use this alternative as it enables the colours to be controlled manually. Check that the **shutter speed of 1/250 s** has not changed!

Estimating the number of photos required

In order to estimate the number of z-stacked photos (microscope or Nikon D500 photos) required for a 3D model, one can project the image frame size on photos of the specimen. One should take 6 photos around the specimen. With increasing experience, this kind of estimation should not be necessary.

1. Mount the specimen on a turntable or put it in a Petri dish.
2. Take a photo of the whole specimen then rotate the turntable (or the specimen in a Petri dish) 60 degrees and take another photo, repeat the process until 6 photos have been taken.
3. Save the photos in photo folder *2. FLAT SCALE PHOTOS* (File name: ID code +required photos+1-6).
4. Take a macro photo (using the correct lens and focal distance) which shows only the specimen surface (no background).
5. Open the first of the six photos in **Paint 3D** and use the macro photo above to make a rectangle that corresponds to the macro photo area. This is only a rough estimation so do not spend too much time to make it accurate.
6. Copy the rectangle and move it 20 % (to get an 80 % overlap) **fig.12**) and so on until the photo is covered with rectangles.
7. Repeat for the remaining photos.
8. Count all rectangles.
9. Decide whether or not to change macro lens or focal distance.

Use these photos, at least for the first models, as a guide when making the z-stacked photos. **Note** that more than 80 % overlap is required for photos of areas with sharp corners (like the outer part of fins).

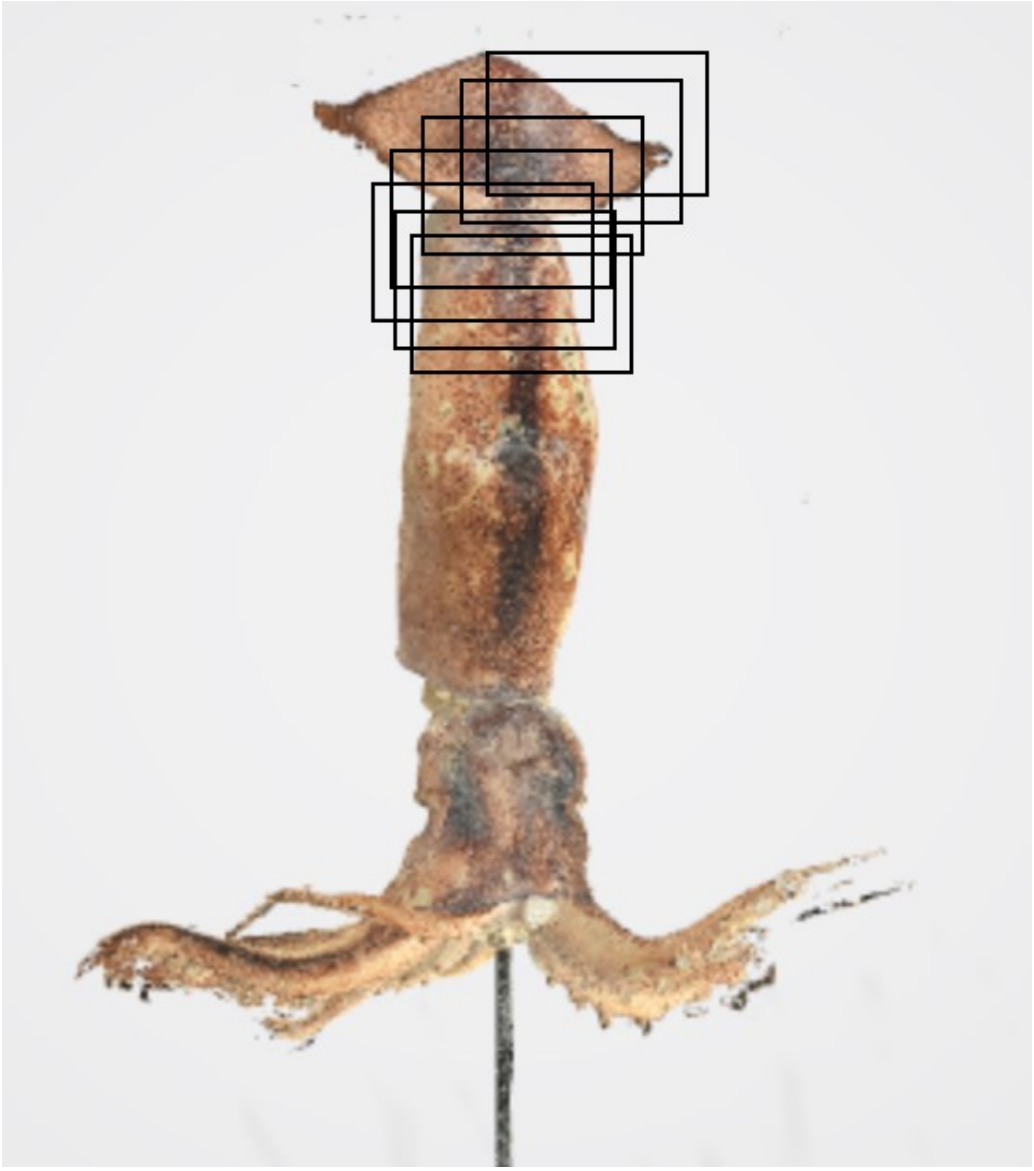


Fig. 12. One of 6 photos, each taken with a 60-degree rotation of the turntable. Here we started to place seven rectangles with 80 % overlap. The number of photos needed under and between the arms can be estimated by eye, or one may take more photographs and place rectangles on them as well.

In conclusion, check, via practical tests, that the chosen microscope magnification or Nikon D500 macro lens and focal distance will provide high-quality photos that allow for any necessary enlargement of the final 3D model. Finally, make a rough calculation of the required number of z-stacked photos. The number of photos needed for a z-stacked photo depends on the specimen's 3D appearance, the chosen lens, focal distance and aperture. Note that making z-stacked photos using a microscope camera and NIS-Elements is much quicker than using the Nikon D500 camera, manual rail slider and the Helicon software.

GENERAL WORKFLOW

Whole specimens

1. All field data should be written on a waterproof label (using a graphite pencil) kept inside the preservation jar (or plastic bag in deep freezer) and the ID code put on the outside.
2. The fresh specimen. Weigh and measure standard lengths and tabulate all specified data in a data table (e.g., ID code, species, sex, catch date, name of the locality, position, depth, collection method, collector, preservation method, weight, standard lengths, comments).
3. 2D photos for presentation purposes. Take at least two photos with the same frame size (using a microscope camera or the Nikon D500 camera) of the fresh (if possible) specimen against an appropriate background. The first photo must include a specimen ID code label and a glass slide micrometer or a ruler covering the width of the image (needed for making a length scale in the other photos, using NIS-Elements). Save the photos in folder 1. FRESH and PRESERVED.
4. Deep freeze the specimen if it cannot be fixed immediately in the preferred position in a preservative. Note that preservation fluid affects the specimen size and possibly colour.
5. The fixed and preserved specimen. Put the specimen in water 6 to 12 hours to eliminate the smell of formaldehyde.
6. Remove all particles, even small ones, that do not belong to the specimen (except for epibionts).
7. Weigh and measure standard lengths of the preserved specimen and tabulate them as above.
8. Is it necessary to use dyes (see Øresland & Oxby, 2021)?
9. If needed, mount the specimen at the appropriate height on a black metal rod so that photos can be taken from all angles.
10. Take **test photos** in order to optimize correct lighting and natural colours.
11. Take photos for z-stacking and save the z-stacked photo in photo folder *5. Z-STACKED PHOTOS*. At least five z-stacked "scale photos (at 90 degrees) have to be taken for later scaling of the 3D model.
12. Start Agisoft Metashape 3D modelling using the z-stacked photos (**Part III**).
13. Check if more z-stacked images are needed. If the photos are not overlapped enough one can get holes in the 3D model (see **below** and **Part III**), in which case no area and volume estimates can be made.
14. Save the final 3D model in folder 7. *FINAL 3D MODELS* AND 2 D PHOTOS and make **BACKUPS.**
15. The specimen can now be taken off the rod and put in a jar with formaldehyde for later dissection of external and internal organs (if not destroyed by the rod).

Organs

1. Dissect the formaldehyde-preserved specimen for all organs of interest (see Øresland & Oxby, 2021) and save them in formaldehyde in separate vials with an ID code label + name of organ

(inside and outside the vial). It is of course possible to dissect the fresh or thawed specimen, in which case the data for each organ should be tabulated also before preservation.

2. Put the organ into water until the smell of formaldehyde has disappeared.
3. Remove any thin outer membranes that may affect photography (especially light reflections) and remove all **small** particles that do not belong to the specimen (except for epibionts).
4. Take the measurements/weight of the preserved in accordance with to the data table specifications.
5. Take 2D presentation photos (as for the whole specimen) by either using a microscope camera or the Nikon D500 camera and save the photos in photo folder *1. FRESH AND PRESERVED.*
6. Is it necessary to use dyes (see Øresland & Oxby, 2021)?
7. Take **test** photos of the organ in a Petri dish in order to optimize correct lighting and natural colours.
8. Take photos for z-stacking and save the z-stacked photo in photo folder *5. Z-STACKED PHOTOS*. At least five z-stacked "scale photos (at 90 degrees) have to be taken for later scaling of the 3D model.
9. Start Agisoft Metashape 3D using the z-stacked photos (**Part III**).
10. Check if more z-stacked images are needed and if so, make a new 3D model.
11. Save the final 3D model in folder 7. *FINAL 3D MODELS* AND 2 D PHOTOS and make **BACKUPS**
12. The organ can now be saved for later dissection and new photography or for other reasons (e.g., dry weight).

TAKING PHOTOS AND Z-STACKING

Microscope photography

Photography should **not be started** before **all** preparations are done. When making a 3D model, the individual z-stacked photos should have (more or less) the **same number** of x and y pixels. This can be **checked** in **NIS**: Check *Image Attributes* once a photo has been taken or **File** > Open > Z-stacked photos > right-click a photo > *Properties* > *Information* or in **Metashape**: **Photos** > *Details* > *Size*. One can always *right-click* a photo > *Show Info* in order to see the number of pixels. This is not a problem when using a compound microscope or a Nikon D500 camera. However, z-stacked stereomicroscope photos may have very different numbers of pixels due to the fact that stereomicroscopes have two light beams (although less z-stacking will reduce this problem). All z-stacked photos will have **almost** the same number of x/y pixels when using a stereomicroscope which is capable of providing a single light beam. Our Wild M3Z is equipped with a Type S holder (**fig. 6**) where the microscope body can be moved to the left to select one light beam. Other stereomicroscopes may provide other solutions. **NOTE**: check, regardless of photo method, that the first three z-stacked photos, once taken, have approximately the same x/y

size before proceeding! Try to keep the difference in pixel numbers between all photos **to ~ 5 pixels** (reduce z-stacking if needed).

Provide an image path in order to quickly save the photos in the correct folder**.** Go to **NIS** > ***Edit*** > *Options* > ***General***: tick the box *Use fix path for images;* provide the path to the photo folder *5. Z-STACKED PHOTOS;* tick other relevant boxes; leave the "*Temp"* option as it is > *Apply* > OK. Then choose ***Edit*** > *Options* > ***Save Next***: choose the same image path as above in *Directory*; Prefix seq 5 digits; Tiff format; *Compression* None; Next file to **1**; **tick** *Skip already existing files*; choose among options in *Define image info* > *Apply* > OK. We use the **"Save as" option** (rather than the NIS Autosaving option) since we feel it provides a better control.

Z-stacking using NIS. When creating a z-stacked microscope photo the original photos used for z-stacking are **not saved.** In order to **z-stack photos**, we first choose **Acquire** > *Live fast* and then **Applications** > *Real Time EDF Manually* and **tick** the *Align Images* box. After clicking *Start,* and once the two photos are opened, we begin by focusing the front of the right one and then, **very gradually,** move to the back and click *Finish*. This takes approximately one minute. The photos that are taken automatically are continuously being z-stacked, which is shown in the left photo. If one is not satisfied with the result the whole process has to be redone. This software, with which we have a long experience, fulfills all our requirements regarding quality, functionality, and manageability; however, there are of course other similar software products available.

Start the photography early in the morning and try to take all the pictures within one day if possible since it is important for photogrammetry that the specimen does not change in appearance during the photography. Small specimens can be put in a Petri dish, supported underneath by small pieces of lead, or attached to a needle fastened to a piece of wood, which in turn is glued to a small turntable (**fig. 4**). Any unwanted large objects, like lead pieces, needles or metal bars, will be removed from the photo at a later stage. The specimen can then be rotated and turned over enabling the photos to be taken from all possible angles, especially if an oblique viewer is used (**fig. 6**). However, it must then be used for all photos. The order in which the photos should be taken may depend on the object and how it is placed, but essentially it is the same for microscope and Nikon D500 photography (see **below**, **Nikon D500 photography**). At some point during the photography, one should take at least **five z-stacked photos at a 90-degree angle** of a flat area so the 3D model can be scaled (see **below**, **Scale photos for the 3d model**). One should try to take these photos at some distance from each other, if possible.

Camera settings: NIS > **Acquire** > *Camera Setting: exposure time* to 100 ms; *Offset* to 0 and *Gain*, *Gamm*a and S*aturation* to 1.0. Prior to taking the photos, it is important to **check** whether or not the compound microscope magnification (**chosen objective**) or the stereomicroscope magnification (**chosen zoom knob number)** matches the chosen magnification **shortcode** in the NIS access bar. After the photo has been taken, one should **check this again** and **type OK** in the NIS photo's (right-click the photo) *Image Properties* > *Image Fields* > *Type* dialogue box to confirm that the information in the *Optics* box is correct. Otherwise, one should not to make a scale bar or any measurements in such photos!

Note: DON'T crop or geometrically transform (i.e., resize or rotate) **images**. Metashape operates with the original images. If done, it will affect the autocalibration procedure - the process of the automatic estimation of calibration and distortion parameters.

Scale photos for 3D models

In order to estimate the length, area and volume of the 3D model one must, during the modelling process, provide the distances between a number of known points in at least three flat z-stacked "**scale**" photos in order to create scale bars (in **Part III, 3. Bundle alignment).** The "**scale**" photos are to be taken at the appropriate time during the **ordinary microscope or Nikon D500 photo session** (and be given their correct running number). It is crucial that these photos are **aligned** (**Part III**).

1. **IMPORTANT.** At some point during the microscope and Nikon D500 photography, make at **least five** (from which we later chose at least the three best ones) z-stacked "**scale"** photos of different **flat areas** at a **90-degree angle** (for accurate distance measurement) and name the photo files with their **running number + "scale" +1-5.**

2. Save these "scale" photos in the photo folder *5. Z-STACKED PHOTOS*.

Nikon D500 photography

The LED lighting on the flash and external lights can facilitate the focus setting. The LED light automatically goes off briefly when the photo is being taken. However, we often turn it off manually after the first photo in order to save the batteries, with the hope that we can focus the next photos correctly by a **minimal** movement of the rail. One can also see on the computer screen that the area in focus is changing. During z-stacking one should be able to take a new photo within five seconds. Change the Nikon D500 battery as soon as the Helicon battery indicator turns yellow and the current z-stacking is finished. The flash batteries will last longer than the camera battery but can be replaced at the same time to avoid interruptions.

1. Insert the metal bar between the arms and the beak and the needles through the tentacles. Check that the specimen is in a fixed position and that all areas can be photographed.

2. Check that the rail slider allows for focus on both the front and the rear of the specimen.

3. Focus on the front of the specimen and start the z-stacking.

4. Take the z-stacked mages from around the specimen by rotating the turntable, noting where the first image was taken so that one knows when a 360-degree coverage (with an 80 % overlap) has been reached. The rotation of the turntable between photos should be about 10-15 degrees which correspond to 24 - 36 photos. If there is only one opportunity to take photographs (e.g., in the field) it is better to take them with even more overlapping, especially for areas with sharp corners.

5. Start taking photos from above and around the top of the specimen paying attention to all sharp edges and cavities. Lower the camera **slightly** (80 % overlap) and continue all around the specimen until the turntable needs to be raised above the camera in order to continue from underneath. Note that one can also turn the squid upside down on the black metal bar. In that case, one should make sure that the photos taken before and after turnover are overlapping and that the areas of overlap have not changed in appearance. Note that when the metal bar is inserted through the squid mantle it will be damaged.

6. Photographing the arms and tentacles is demanding and time consuming. We recommend that such photos should be checked for alignment and quality (above 0.5) in Metashape. Check the

thumbnail photos (*Show Cameras* icon on the tool bar) surrounding the model and look for gaps that might indicate missed areas. A simple 3D textured test model (without scaling and error corrections) could also be made in order to determine whether or not more photos are needed (see **Part III, Alignment**).

7. Do not forget to take five "scale photos" (see **above**).

Z-stacking using Helicon software. The Helicon software was introduced in **Part I**. The Helicon Focus help function is very good and provides detailed instructions.

In short:

Helicon Remote: First, under **File** > *Preferences* > *Image saving* > *Folder for images.* Choose the **TEST** folder and **these original photos can later be deleted** since we have no use for them after z-stacking. The **z-stacked photos** (only) are saved in folder *5. Z-STACKED PHOTOS*. We prefer to name the z-stacked photos with running numbers. Saving the project file is not really necessary. **Check** that the first z-stacked photo can be found. When taking photos manually, as we do, one should left-click on **Stack** on the toolbar and select ***Create new stack folder*** and previous photos are deleted from the Helicon Focu*s Source Images* list in Helicon Focus (see **below**). Closing and opening Helicon Focus will also empty the *Source Images* list.

Helicon Focus: First, under ***EDIT >*** *Preferences* > *Autoadjustments* one must **untick** "***Crop output automatically*"** otherwise the different z-stacked photos may get significantly different numbers of pixels. Other input values should be kept at 0 % and Depth map featuring to 3.

If the photos were taken with Helicon Remote, click on its Helicon Focus icon; otherwise, open Helicon Focus separately and import the photos from your computer or camera memory stick: **File** > *Open stack* > *double left-click* a photo and all photos appear in *Source images*|. Check the photos for consistent brightness and quality by clicking on them one by one or use Pg up and Pg down.

Below the *Source images*, there are three different rendering (z-stacking) methods (*A, B*, and *C* which should be tested in order to find the optimal one. They are often quite similar but will sometimes give different results (which is well explained in the help function). **Do not mix different rendering options in a 3D model**!

The radius and smoothing options come next: the *Radius* is usually set at to 3. Being closer to 0 provides the sharpest details but can give colours a helios effect at the edges. We set the *Smoothing* to around 3. Higher values give a darker image.

Quick comparison of photos and settings before rendering. Choose **Edit** > *Render preview* to see the enlarged z-stacked photo, brightness variations and misalignments etc. One can also choose different rendering methods and other settings and directly compare the preliminary results in real-time mode.

At the top of the screen, there are four options: *Rendering, Retouching, Text/scale, and Saving*, all of which are self-explanatory. Click on *Saving* and one will see the saving options to the right. Click on *Save* to save the z-stacked photo (**with a running number**) in photo folder *5. Z-STACKED PHOTOS*. One does not need to use the *Save project file* option.

MAKE several backups of all your photos to **external hard dries** of which one should be kept at an **external location.**

In **Part III**, using Metashape, we start checking whether more photos are needed during the alignment procedure. When 3D modelling a specimen such as a squid, which can become dry and change shape, it is important to keep it moisten (using an ultra-fine spray, with distilled water, at least every 10 minute). The specimen should **not be removed** from its turntable until the 3D model is ready.

PART III: 3D MODELLING

INTRODUCTION

The Agisoft Metashape Pro (used here) and Standard editions are advanced software that are used for a number of 3D applications. Note that markers, the scale and measurement tools are available only in the Metashape Pro edition. In addition, there are free standing software such as Agisoft Viewer and Adobe Acrobat, etc. that accept 3D models, which bypass the need for Metashape software in order to view the final 3D models.

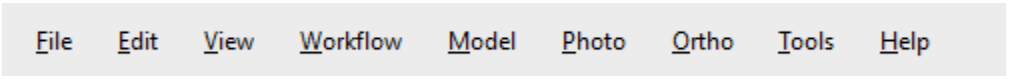
The latest Metashape manual can be downloaded from https://www.agisoft.com/ and should be used **together** with this guide. However, the manual is necessarily quite extensive due to its broad scope. The most important chapters in the Metashape Pro manual are Chapter 3: General workflow; Chapter 4: Referencing (e.g., camera/photos alignment); and Chapter 5: Measurements (distance, area, and volume). There is also a searchable help function in both the software and in the user manuals (where one can colour mark the text etc.). One can also find useful information in Knowledge base (https://agisoft.freshdesk.com/support/solutions). One should follow the Agisoft homepage and other 3D and photography internet sites in order to improve modelling and photography skills and to update the software.

Part III is intended as a simple and practical introduction to Metashape and in particular its pro edition. Once one feels comfortable using this guide, one can start exploring the Metashape manual in detail and experiment with options and different processing parameters that are not mentioned here. To help beginners to get started, we only provide the information that is most relevant to the 3D modelling of small animals and we only describe how we ourselves created our first models and the mistakes we made. We detail, step by step, the basic modelling process for the final 3D model, and how to send and present it.

We begin with a short description of the basic command structure and the general workflow. In Metashape there are often alternative options for opening panes and commands. We try to adhere to one option only to make things simpler. When they first appear, some of the features may not be easily understood, but they will all be explained in detail later on when we actually use them. The words “camera” and “photo” are used, more or less, interchangeable in this text!

THE BASIC COMMAND STRUCTURE AND THE WORKFLOW

At the top pane, one will find:



Left-click on any of these and drop-down options will appear which are self-explanatory. There is a toolbar under the top pane (**View** > *Toolbar*) with a number of icons with an explanatory text which appears when the mouse arrow is pointed at them. The icons light up when they can be activated and some have drop-down commands. We will refer to the relevant command options in detail, as and when they are needed.

We begin with the first dialogue box (**fig. 13)** choices: ***Tools*** > *Preferences* > *General*. Choose: English, Point Cloud, Dark (or Classic or Light*)*, Anaglyph, Parallax to 1.0, Decimal degree,

Coordinate system units > **Tick** Check for updates and Write log to file > OK. **Do not tick** *Use derived units* if other units than meter are used.

In the *Advanced* option > choose; ***Keep key points*** and ***Keep depth maps*** > *OK*

Note that the Metashape manual 1.8.4 describes all the parameters available in the different Metashape Preferences dialogue windows in Appendix C. Metashape Preferences.

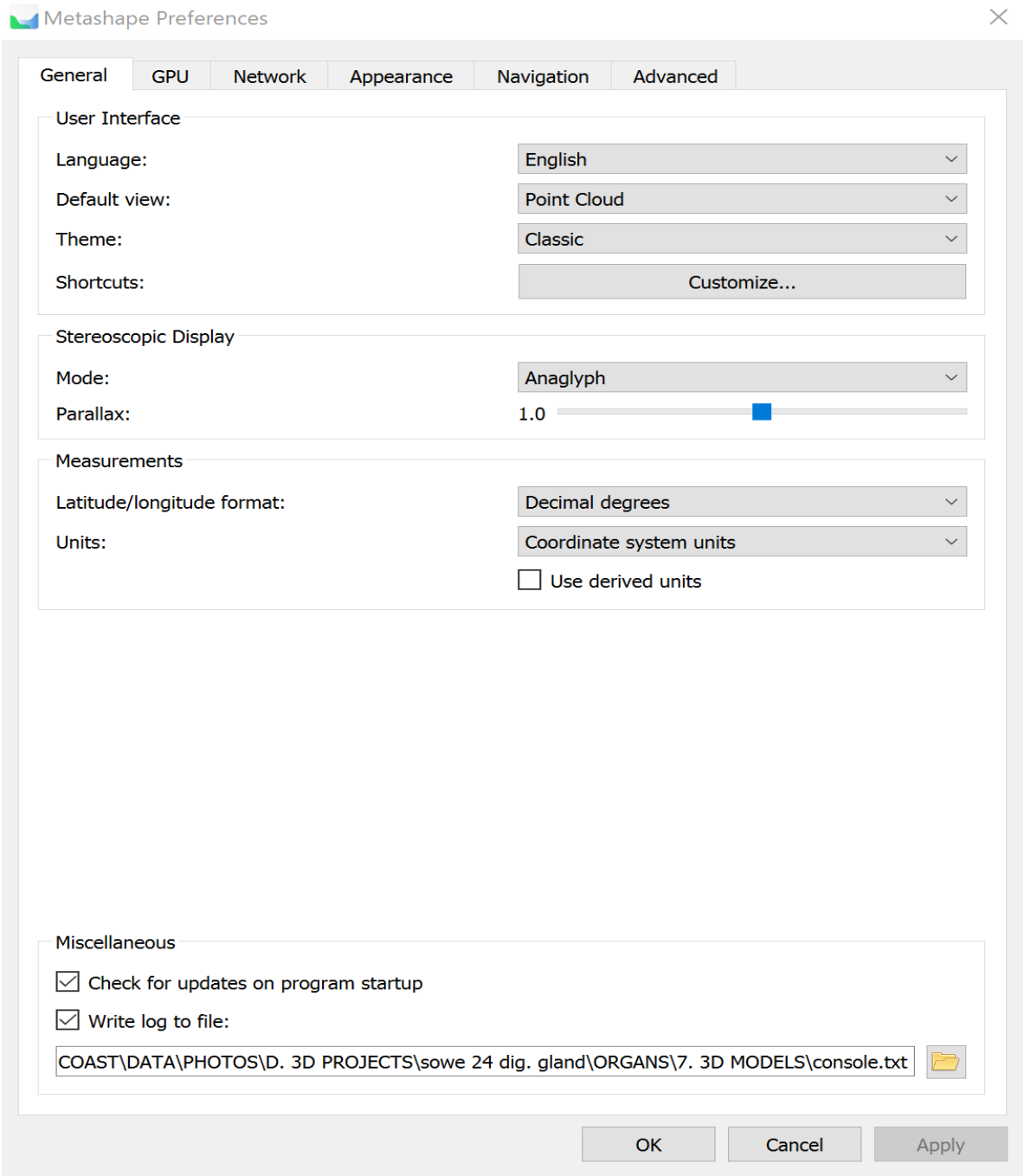


Fig. 13. Metashape preferences including check for updates and the log file. Note: **Do not tick** *Use derived units* if other units than meter are used.

Under **View >** select the three main panes below:

1. **Workspace**: shows the *Chunk* which is the place where the cameras/photos are imported into the project and the total number of markers and tie points that are used to connect the photos are shown. Under C*ameras*, the individual photos are shown with their file names (we use running numbers) and the **NC** is displayed if the camera is not calibrated (not vital for microscope photos) and **NA** if not aligned. Below *Camera*s is found detailed model information about Markers, Scale Bars, Depth Maps, etc. (left-click to see the information). **Chunk**s can be d**uplicated** which is very useful since all camera/photos, markers, scale bars, Alignment, Mesh, Texture etc., can then be used in a separated model (but saved in the same file). This is practical when one would like to test different modelling options without starting from scratch.

2**. Reference:** shows different "**quality**" information regarding, e.g., *Error (pix) and Projections* of Cameras and Markers as well as *Distance*, *Accuracy* and *Error of Scale Bars*. Move the columns of interest to the left for easy access.

3. **Photos:** shows individual photos and their properties. **View** > *Photos* > ***Change View (***a small square icon to the far right on the Photos toolbar with drop-down options). One can here choose to see the thumbnail photos in different sizes and by left double-clicking them brings them to the screen (this can also be done from *Details*). *Detail*s will show, e.g., *Aligned*, *Quality* and *Size* of each photo. Move the important columns to the left for quick access.

Note: if one *right-clicks on* a camera/photo in any of the above three panes a number of more frequently used options will appear. One can shift quickly between *Reference*, *Workspace* and *Photos* by clicking on their shortcuts in the bottom left corner of the screen. When opening Metashape the last used projects will appear at the bottom of the **File** drop-down list (above *Exit*). This function saves time.

Workflow

See **Appendix I** for all workflow settings.

The 3D modelling workflow consists of 4-6 main steps depending on the chosen end result.

1. *Add Photos* or *Add Folder*. Adding the photo folder is the quickest way to add multiple photos.

2. *Align Photos.* Common points (feature points) are located in the photos and matches are made across photos into tie points. The result of this process consists of a **tie point cloud** containing triangulated positions of key points matched across the images. In other words: Tie point: where same feature has been recognized in multiple images. Key point: a distinct feature in **one** single image.

3. *Bild Dense Cloud.* This is not dealt with here as we build a Mesh from Depth maps and since *Dense Cloud* doesn't affect the texture for 3D model.

4. *Build Mesh.* Generates a 3D model (from Depth maps).

5. *Build Texture.* Texturing the 3D-mesh model gives it the final photographic appearance.

6. *Build Tiled Model.* Tiled models in TLS format can be viewed in Agisoft Viewer (which is a free download software). In this guide tiled models are based on depth map data. They can only

be built from the PSX format and can provide a very high resolution. They can be saved as ***PSX** (which is a project format) and as ***TLS**. "Hierarchical tiles" format is a good solution for city scale modelling since it allows for responsive visualization of large area 3D models in high resolution. However, it can be used for models of any size.

Saving models

The 3D project in progress is always saved in folder 6. 3D MODELS and different final versions in folder 7. FINAL 3D MODELS AND 2D PHOTOS. Save the project as a Metashape project file (*PSX) after each modelling stage and everything that was created in the project is displayed in the Workspace pane. If one creates a new copy of the project after each change one may may run out of memory. We strongly recommend making **backups** of the **photos** and just save the of the project during the modelling. However, for the backups of final projects one should include the entire folder system, using the **File** > *Save as* command **(not** Copy). Note that the drive letter (C:, D:, etc.) may change during backup to other sources.

The differences between the Metashape formats PSX and PSZ (not used here, but see **Appendix III**)) are well described in the Metashape manual. The Metashape Project file (*.PSX) basically stores the processing links between the *PSX file , the *.files structured archive and the photos. This format enables responsive loading of large data (dense point clouds, meshes, etc.), thus avoiding delays on reopening photos.

The various formats that can be used for, e.g., exporting, copying, sending and displaying textured models to others, are given in **Appendix III**. When copying and sending a textured **PSX** model one must include three items: the PSX model, the photos folder and the *files folder. Since Project files use relative paths to reference photos, one must always include **the relevant folder structure** when saving the project file to another location. If not, Metashape will fail to run any operation requiring source images, although the project file including the reconstructed model may be loaded up correctly.

One can ***disable*** *Store absolute image paths option in the Metashape Preferences dialog window:* **Tools** > *Preferences* > *Advanced. Store absolute image paths.* We recommend *enabling* this parameter if project is **not** moved to another folder or computer.

One problem encountered when backing up a 3D model to an external hard drive was that we could open the 3D model but not the photos correctly in the **Photo** pane (because Metashape could not find the them).

If this occurs:

a) Ensure that the photos have been copied to the correct folder (5. Z-STACKED PHOTOS) in the external memory.

b) The path of the images may be wrong and thus need to be changed: *Right-click* on the **Photo** pane > *Change Paths* > *All cameras* and find your folder 5. Z-STACKED PHOTOS > left-click on the photo that appears > *Open*. The path is now changed and the photos should appear correctly in the **Photo** pane. Right-click on a photo > *Ope*n, or double-left-click it to see the photo on the screen.

c) If the above does not work. *Right-click* on a single photo in the Photo pane or Workspace pane > *Check Paths* > *All cameras.* One may get the message: *Some images are either missing or resized*. One reason for this might be that some photos have a deviant size: **Photos** > *Change*

View (icon on the Photos toolbar) and check in the size column for deviations from the normal x/y size. One should remove such photos after making new ones. Large differences in x/y pixel numbers (> 5 pixels) among z-stacked photos only occurred when we tested a stereomicroscope with two light beams (see **Part II, Microscope photography).**

d) Check by choosing a photo in the **Photos** pane >*right-click* > *Estimate Image Quality* > *All cameras*. One can now be able to see the quality estimates in the *Quality* column and the photos can be shown correctly on the screen.

Note. When the same set of photos is used for different 3D purposes, it should be copied into the **relevant project** photo folders before starting a 3D project to avoid the path problem. We also once experienced that when photos in the **Photos** pane were opened, the **colours were inaccurate**. If that happens; the *Show Depth Maps* button on the Toolbar (**fig. 14**) should not be enabled.

Fig. 14. *Show Depth Maps* button.

ADD PHOTOS

Here we will add all photos (including the five "scale " photos) to the 3D model), check their quality and take new photos to replace any poor ones. **Moisten** the specimen f**requently** and keep the camera ready for complementary photography.

1. Start a **new project: File** > *New* **>** *Saves as* (include the ID code in the project filename) in folder D. 3D PROJECTS.
2. Click on *Workflow* > *Add folder* > select folder 5. Z-STACKED PHOTOS > Choose *Single cameras* > *Open.* The camera/photos can now be seen in the Workspace pane.

Save +backups (using *Save as*)

Camera calibration

After adding the photos, Metashape must be provided with some important camera data. The **NC** (not calibrated) found in **Workspace** pane > *Chunk* > *Cameras* indicates that the EXIF data available is **not** sufficient to estimate the camera focal length and a manual calibration is therefore required. Focal length is the distance from the sensor to the rear nodal point of the lens. Click on ***Tool****s* > *Camera calibration* and in the dialogue box: in Camera type choose *Frame* and enter 0.00422 **mm** in both Pixel size boxes (for Nikon D500 the pixel size is 4.22 µm). Contact the camera manufacturer for pixel size information. Enter 85 mm in the *Focal length* box if you use an 85 mm macro lens. Ignore other options. **NC** will be **removed** from the Workspace pane **after aligning (**see **below)**. When using **compound microscope** and **stereomicroscope cameras,** Agisoft recommends not specifying the pixel size and focal length in the *Camera calibration dialogue* box since they may not correspond to the resulting image (the focal length changes with

zooming and change of objectives). This means that the NC will not be removed from the **Workspace** pane when using microscopes.

Reference settings

Here (**fig. 15)** we use **Local coordinate system** and specify information about units and accuracy. **View** > *Reference* > Click on the *wrench icon* button to the far right at the top of the reference pane and in the *Reference Settings* dialogue box choose **mm** coordinate system; **tick** the Camera and Reference boxes; set camera, marker and scale accuracy **to 0.001 mm** (delete all old numbers first). Leave other settings as they are. **Important:** click on the *wrench icon* button **again** to **check** the input values. Obviously, the accuracy settings should be as realistic as possible and a 0.001 mm setting requires that the glass slide micrometer scale photo allows for it and that the markers are set at pixel level.

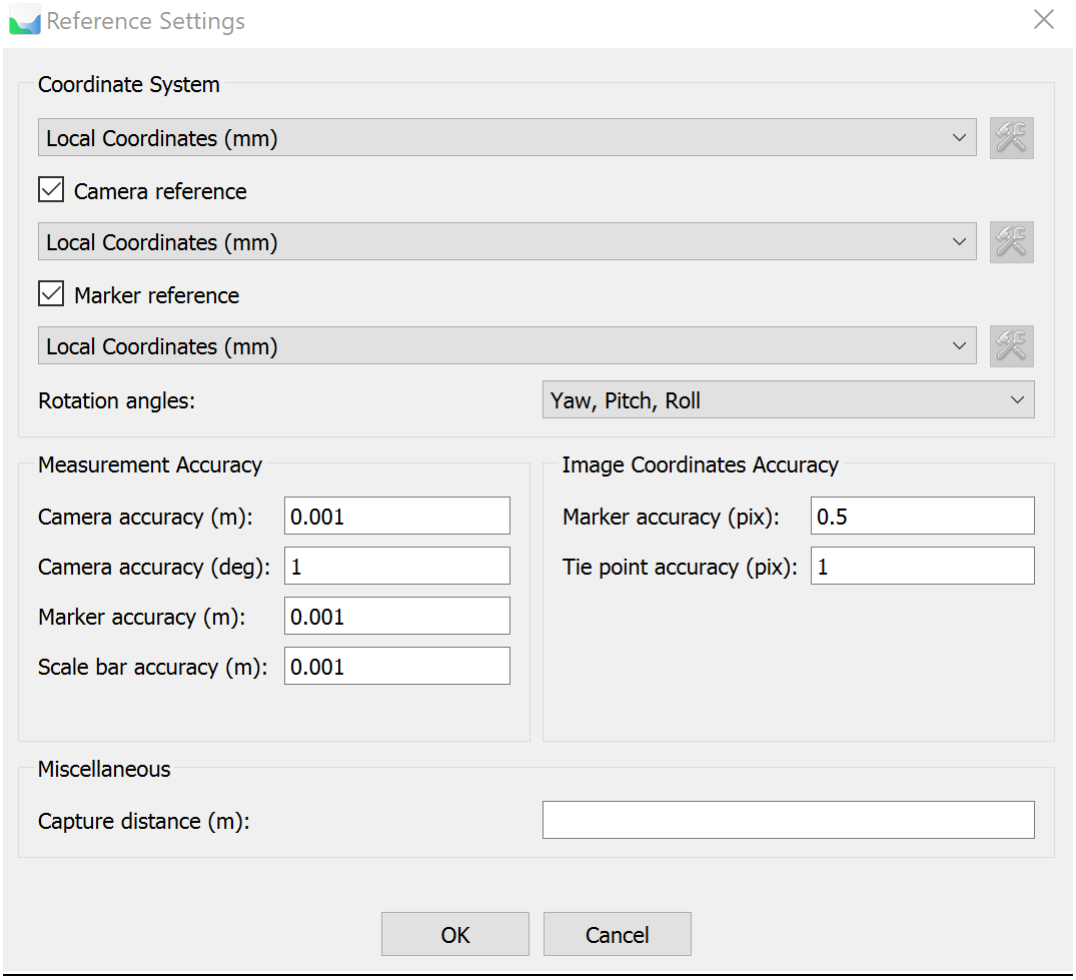


Fig. 15. Setting the Local coordinate system and Measurement accuracy.

Poor quality photos

1. In the **Photos** pane > *Change View* (far right icon on the **Photos** tool bar) > *Details* > under *Label* > *right-click* a camera/photo > *Estimate image quality* > *All cameras* > OK.

2. Move the *Quality* column to the far left for easy access. Press the *Quality* heading to see the quality in increasing order (low quality at the top).

3. **Disable** photos of a quality **< 0.5** that appear in the **Photos** pane > *Details* > *right-click on* them > *Disable Cameras*. One may occasionally have to accept lower quality photos if it´s impossible to achieve better ones. **Do not forget** that photos with very different colours, due to, e.g., that an organ has dried and become darker during the photo session, should not be used since they may affect aligning and texture. The dry organ can also be smaller. It is therefore extremely important to **moisten** the organs **frequently** (every 5-10 minute). Once an organ has dried it is almost impossible to get it back to its natural appearance.

4. Make new z-stacked photos to replace the poor ones. Open the first disabled photo in Metashape and open **Remote Helicon** or **NIS** (if a microscope photo is to be taken) so that the two images can be compared. Make sure to obtain approximately the same frame view from the Nikon D500 or microscope camera and try to take a better photo. Give the new photo a new file name (**same running number + new)**, and save it in folder 5. Z-STACKED PHOTOS. Add the photo to the model: **Workflow** > *Add Photos.* Check whether the quality has been improved: **Photos** pane bar > *Details* > *right-click on* the photo > *Estimate image quality* > *Selected cameras* > OK. Remove the old poor photo: *right-click* on it > *Remove Cameras*. Take the remaining complementary photos. They should all be of an acceptable quality before proceeding.

Save

Note: **Do not yet remove** your specimen and camera and **moisten** the specimen **frequently**!

Masking photos

Before we start aligning the photos, we **manually** mask all **unwanted areas**. Masked areas will be **excluded** during feature point detection in order to improve **alignment** and **quality** of the **tie points**. This is **important** when using a turntable since the specimen is not static as regards the background. Masking can be used prior to; Aligning photos and when creating Mesh, Texture and tiled Models. Here we only mask photos in order to improve alignment (see **Chapter 6. Editing** in the Metashape manual for further information**).** Masks are available in both the Professional and Standard versions.

Here we have a dark background, a rotating turntable and a camera whose position on its stand changes during the photo session. The background may therefore not always be completely dark. Tie points associated with the background and the metal bar will have a negative effect on the alignment process. Masks can to be created manually or automatically for each image (where possible). Here we show the manual method and mask all the photos where the metal bar is visible (**fig. 16**). One can also mask the entire background in all the photos but if the object is complex only a part of the background can be masked.

1. Open a photo showing the metal bar from the *Photo* pane.

2. Left-click on **Photo** > Select one of the selection tools from the drop-down list, e.g., *Intelligent Scissors.*

3. Select the metal bar area.

4. Right-click the photo > *Add Selection* (or its icon on the toolbar) and a solid line is indicating the selection made.

5. Select a photo > Click on the *Show mask* icon (white quadrant with a black circle) on the toolbar and the selection appears/disappears.

6. Repeat for all remaining photos containing the metal bar.

Save

The photos are now ready to be aligned.

Fig. 16. A mask has been manually placed around the metal bar. Note that the arms could not be stretched out (see **fig. 9**).

ALIGN PHOTOS

Alignment is a critical and time-consuming part of the 3D modelling. Remember that **many** of the **alignment problems,** that we will deal with below, will not occur if **optimal photos** with **good overlap** and an **optimal background** are available. During alignment Metashape finds identifiable feature points (up to the maximum set **Key point limit** in the *Align Photos* dialogue box) and matches them across photos (up to the maximum set **Tie point limit**) as shown in **fig. 17.** If there are more than 150 photos one may choose not to use *Generic preselection* since it will take some time. However, it is really the photography that takes time.

Workflow > *Align Photos*: See **fig. 17** and **Appendix I** for **all** workflow settings.

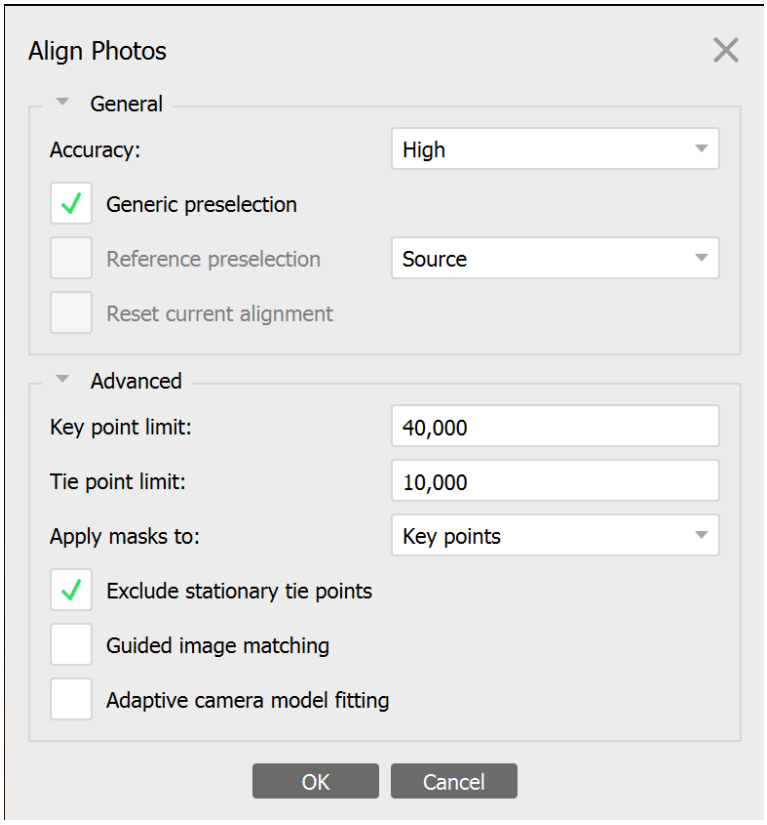


Fig. 17. The *Align Photos* dialogue box.

Check the effect of Masking by comparing the alignment of unmasked and masked photos (**fig. 18**). One can also compare the **number** of aligned photos and the **quality** of the **tie points**. If one does not want to use masks during alignment one can select *Apply mask to*: *None* in the Alignment dialogue box.

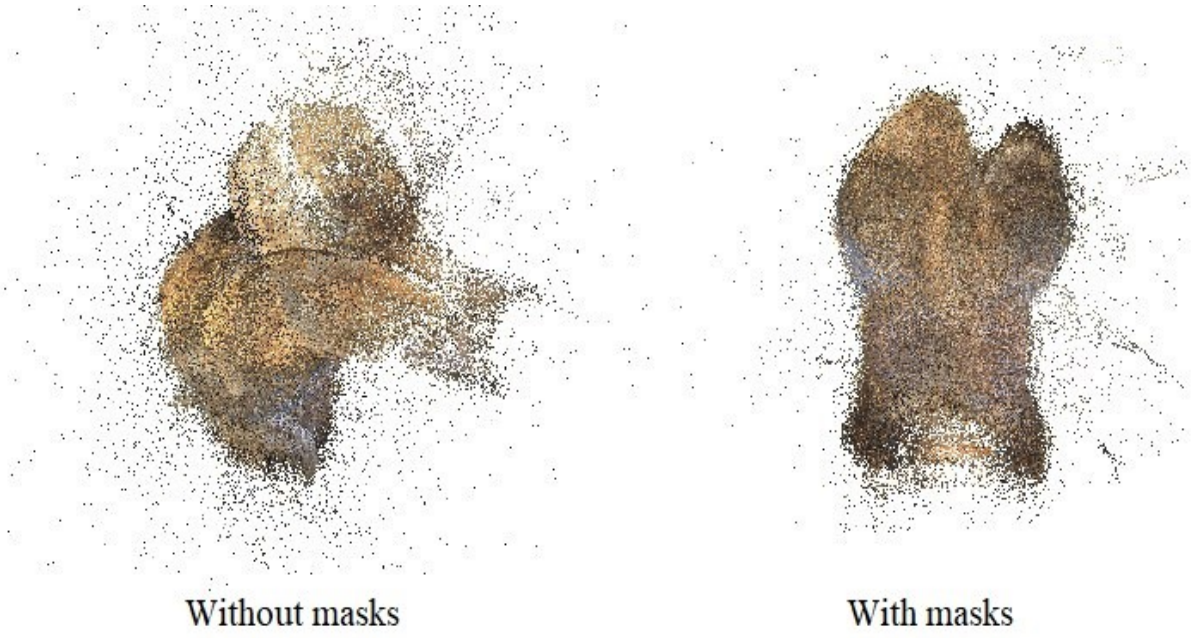


Fig. 18. These alignments with point clouds are based on stereomicroscope photos of a squid digestive gland (fig. **28**). The background texture during the z-stacking photography was not optimal. The background of all photos was therefore masked.

Save

If the model is **very small** or **cannot be seen** in the Model view should make sure that *Model,* and ***not Ortho,*** is chosen on the Tool bar. Left-click on the *Show Cameras* icon> *Show Thumbnails* in order to locate the model. One can also try: **Model** > *Transform Object* > *Move Object:* the object is where the red, green and blue lines meet. *Right-click* were the lines meet > *Center View.* Scroll the mouse courser forward or click the **Zoom In** button until the model can be seen. One often has to use "*Center View*" a few times during zooming.

We now have our first model (in *Model* view) consisting of a tie point cloud but this would appear **incomplete** if we didn´t have optimal quality photos, overlap and background. This is rarely the case and several actions with Metashape would be needed to improve the situation**.** We have already checked photo **quality,** but this must be **repeated** for all **new** photos. Check that the bounding box fits around the specimen: **Model** > *Transform Region* > *Resize Region* or ***Reset Region*** and Metashape will automatically configure the bounding box. Use also the other options in *Transform objects and Region* to *resize, move*, *rotate,* etc.

A dialogue box will appear with the message "**Some photos failed to align"**. To **align** such photos: **View** > *Photos* > *Details* > Click on *Aligned* so that the **unaligned** photos appear at the top of the column > *Select the unaligned photos* > *Align selected Photos* and some of them, it not all, will be aligned.

Save

Below we will deal with areas of the specimen that have **not** been **photographed,** the **use of Markers** and photos that are **still unaligned** or have **few projections**. **Note** the **number** of **aligned photos** (*Chunk* > *cameras* (number of aligned/total number of photos)) in order to follow the success of the following actions.

Areas that have not been photographed

Open **View** > Workspace, Photos, and Reference.

1. Centre the model cloud by double-left clicking on it. The thumbnail photos can also be viewed (click the *camera icon* in the toolbar, *Show Thumbnails*) together with their file names (**Model** > *Show/Hide Items* > *Show Labels).* The rotation of the turntable between photos should be about 10-15 degrees which corresponds to 24 -36 photos.

2. Downsize the model (scrolling the mouse) and rotate it (**Model** > *Transform Object* > *Rotate object*) and the camera positions then appear from different angles (**fig. 19**).

3. Examine the model and its surrounding thumbnail photos and identify areas that need more photos. Click on a photo in the **Photo** pane and a red rectangle then appears around the camera position (if it is aligned). Take notes of where cameras are absent and where the nearest ones are available.

4. **Take all new photos** that are needed to cover a particular area but do not move the camera or the specimen before the area has been covered in full. It is easier and much quicker to take new photos when needed if the camera is already in position for photography. **Do not forget to moisten the specimen.**

5. **Save** the new photos in folder 5. Z-STACKED PHOTOS (file name: use t**he nearest aligned photo number** + **missing** + **a running number)**.

6. **Workflow >** *Add Photos* > *select* the new photos in folder 5. Z-STACKED PHOTOS.

7. **Align again: Photos** > s*elect* the new photos > **Workflow** > *Align Photos.*

8. Did the missing photos align? Check their **photo quality** (as **above**, **Poor quality photos**).

At this stage, it can be difficult sometimes to judge whether any more photos are needed. In such cases we just proceed to the next step.

Save

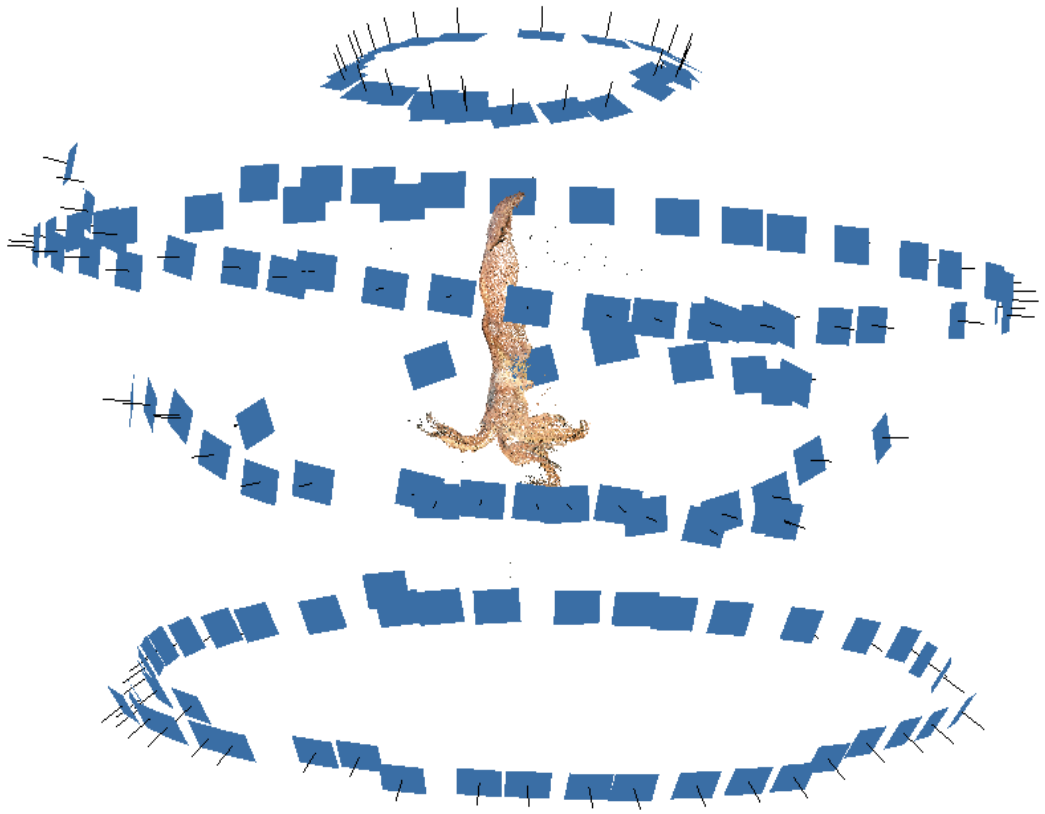


Fig. 19. Camera positions in four circles around a squid specimen. In this case, photos are needed from below the squid.

Markers

It often happens that some of the photos are still unaligned, mostly due to poor photo quality and/or poor overlap (both of which we tried to correct by taking new photos). One can then either disable or remove the photos (if they are not needed) or try to align them. However, in some situations, e.g., when taking photos in nature or for photo technical reasons, one may not be able to take better or more overlapping photos. One would then need to boost Metashape´s alignment by placing markers to small pixel clusters (easily identifiable feature points) in the photos, thus enabling connections (tie points) to be made between them. Aligning photos using markers is particularly useful when one needs to couple chains of photos together and close holes in the model. Markers are only available in the Pro version. **Note** that markers cannot be of use **in all cases**, which emphasizes the importance of **taking good photos with at least 80 % overlap from the start.**

Markers are indicated by flags:
Green flag = a marker that is manually placed on the photo after checking its position.
Blue flag = an accepted marker point with matching tie points/pixel clusters. The blue markers are automatically placed on the images by Metashape.
Grey flag = an uncertain position which, **if** corrected, becomes green. In many situations they are just left as they are and of no use.
A red/white line = a helpline to manually place a marker in another aligned photo. Note that the helpline may not always cross the optimal marker position.

We will now deal with three alignment issues: 1. Unaligned photos; 2. Photos with few < 150 connections and 3. Partly aligned photos that are placed in different **Components (see below)** and we will use Markers to align the cameras/photos.

Opening photos for marker placement

The principle here is to **Place** markers in **four easily identifiable and identical points** in **four** (if possible) nearby **aligned** photos and **all** the nearby **unaligned** photos. This marking procedure is the same for the three alignment issues. If there are separate **areas** of the specimen having these problems one will have to **repeat** the procedures described below **for each area**.

Opening the photos*: right-click* the first aligned photo in the **Photo** pane > *Open in New Tab* and the photo appears on the main window and its file name is shown on the window pane above the photo. Repeat this for the **remaining aligned and unaligned** photos and all photos will appear in the same Tab group. *Right-click* on the first aligned photo > *Move to Other Tab Group* and we will have two Tab groups as shown in **fig. 20**. All chosen photos are now available for markers to be placed. Click the letter X next to the file name to remove any unwanted photos from any Tab group.

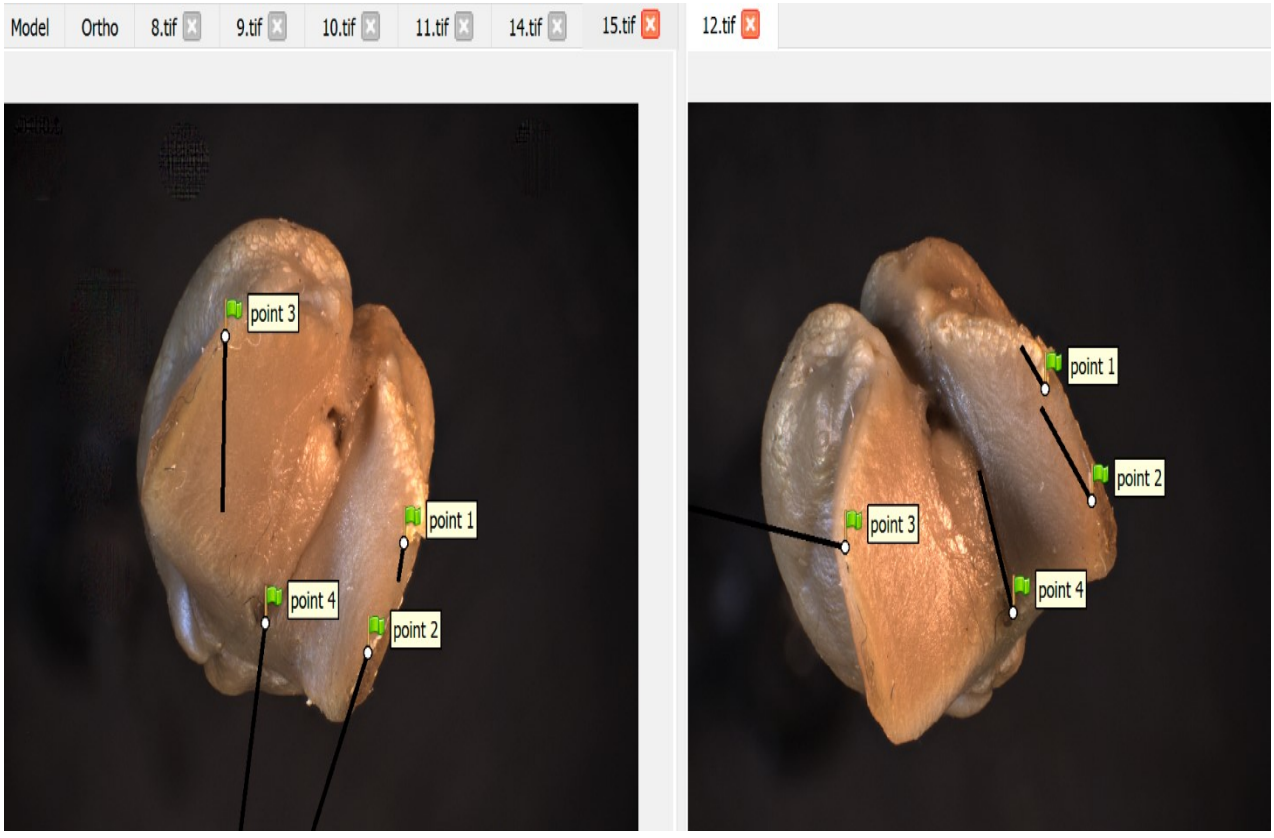


Fig. 20. The digestive gland of the bob tail squid *Sepietta oweniana.* The photos 8-15 and 12 are in two different Tab groups and photos 15 and 12 are shown side by side with four identical markers.

Applying markers for aligning photos

We will now open the first aligned photo and P**lace** four markers in it (**fig. 20**) and then clarify their positions on the **remaining three** aligned and **all** nearby unaligned photos. When the **Place** marker option is used on **at least three aligned** photos, those markers will be **automatically placed** on all remaining photos where possible but their **positions** must be **checked**.

1. **View >** *Toolbar* > left-click on ***Show Markers*** (the **large flag** icon to the right) and the marker flags will be seen on the photos when markers are applied. If the **Show Markers** option is not enabled the Marker dialogue box will not appear.
2. **Zoom** the first and the second aligned photos to **high magnification** (pixel level), and find a **good** marker point that is clearly visible in **all** the selected photos.
3. **Place markers**. *Right-click* on a good point in the first aligned photo > *Place Marker* > *New Marker.* A **green** flag named Point 1 appears **(**marker points are automatically given a running number). If not visible, click on the large flag icon on the toolbar. A grey flag and a red-white help line will now appear in the second aligned photo. The position of the grey flag must be corrected. Left-click on it so that the flag dot turns red and move it to the supposedly correct position where the flag will turn green (manually placed marker).
4. Marker Point 1 will appear in the *Referenc*e pane under *Marker*. Place **three more** markers (that do not lay on the same line) in the first two aligned photos.
5. Select the remaining nearby aligned and unaligned photos, one by one, from the first Tab group and Place markers 1-4 in them using the *Place Marker submenu* (point 1 - 4).
6. **Photo** pane > S*elect* (Ctrl and left-click) all the unaligned selected cameras/photos > *Right-click* > **Align Selected Cameras** (**it is important not to use the reset alignment option).** NA (not aligned) should now disappear for the unaligned cameras in the *Workspace* pane and the thumbnail photos in the *Photos* pane should be ticked in the upper right corner. An **Error (pix)** estimate and the number of **Projections** can now be seen in the *Reference* pane > *Marker.*

NOTE: Right-click a Marker in *Reference* pane > *Show Info* and all photos with that markers will be shown. This is a very practical option when placing and checking markers.

Applying markers to photos with few projections

It is important that an aligned camera/photo has >**100 projections** (projections of valid tie points) **after the Error corrections** (see **below**) in order to optimize the model. The goal is therefore to make sure that all photos have well over **100 projections before** the Error corrections are made. The reasons for low projection numbers are bad photo quality, **insufficient overla**p and inefficient use of the images as shown in **fig. 21**. The **primary action,** however, should be to increase overlap by taking **additional photos**!

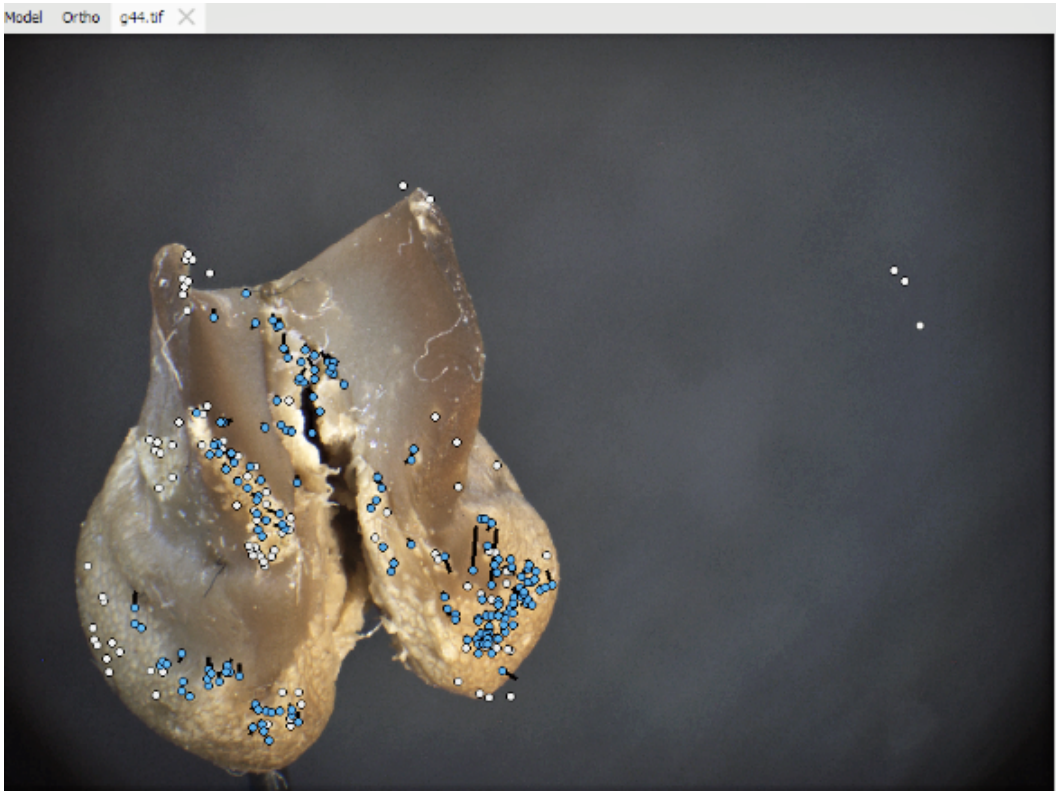


Fig. **21**. A photo of a digestive gland with inefficient use of the image.

A small number of projections can lead to poor alignment quality (orientations of the image will not be correct or distorted) and the result of the alignment of the images will affect the quality of the texture. Therefore, we need to identify such images and increase their numbers of projections by adding markers if no more photos can be taken. If there are only one or two nearby photos available, one should place many markers on all of them.

To identify photos with few **projections: Reference** > sort the *Projections* table in increasing order. One can also see projection details for each photo: **Photos** > *View Matches*. Select a photo at the top bar and its valid matches to other photos then appear.

1. **Open** the first photo with the lowest number of projections seen in the **Reference** P*rojection* table. Then open **all** nearby photos and **Place** many markers in all photos. Check the *View Matches* to see matches to nearby photos.

2. **Align** the selected photos and check the change in number of projections in the *Projection* table. Add more markers if the number of projections is not well over **100** (if possible).

3. Repeat for the remaining photos where the number of projections is not well over **100.**

Applying markers for aligning Components

Subsets of cameras/photos can, after the alignment process, be split into **different Components** that are displayed in the chunk's content of the *Workspace* pane (**fig. 22**). Such subsets of photos have **not** been aligned with **each other**. Only Component 1 is shown when **all** photos are aligned **together.**

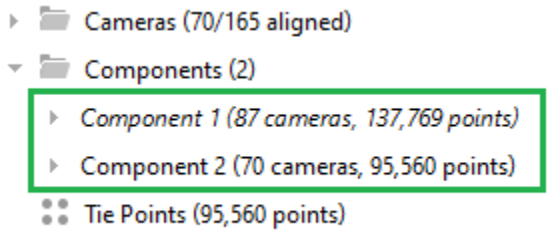


Fig. 22 Chunk content with Component information**.**

Parts are groups of photos that **are aligned** with each other **only if** they appear in the **same Component** (**fig. 23**). Cameras that are put into different Parts, during the Metashape alignment procedure, are used for alignment analysis which we do not deal with here.

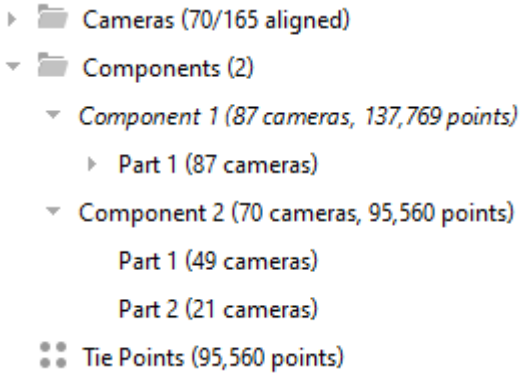


Fig. 23. Chunk content showing Parts within two Components.
The marking process for components is almost identical to that for aligning photos.

1. **Double-click** on the **first Component** and make it **active** and **open four nearby photos** and move the **first** photo to a **tab group of its own** on the right side of the screen.
2. **Place four** markers in the **first** photo: *Place Marker* > *New Marker* in the first photo and then use the *Place Marker submenu* to copy the markers **into the other three photos**. A red-white help line will appear. Remove these photos (click on X) but keep the first one on the right side of the screen.
3. **Double-click** on the **next Component** and make it **active** and open **four** nearby photos on the left side of the screen.
4. Continue the marker placement operation for the four photos using the **existing marker** selection (point 1-4) from the *Place Marker submenu.*
5. After all Components / cameras been aligned: *Workspace* > *Components* > ***Merge Components***.

Save

ERROR CORRECTIONS AND MAKING SCALE BARS

All photos, including the scale bar photos, are now aligned but the 3D model must be improved. We will optimize the model by **removing/reducing** any **errors** in the Point cloud and by creating **scale bars** (which add supportive reference data to the 3D project so that distance, area and volume can be estimated).

We used the following parameters:
The goal of the following optimization and filtering procedures is to reduce each camera (photo) **Error (pix)** value to <1.0 whilst preserving a high **Projection value > 100** (both given in the *Reference pane > Cameras).* Keep the ***Reference*** **pane open** during the entire correction and scale bar procedure and monitor the changes.

Error (pix) is the mean square reprojection error for the marker calculated over all photos where it is visible. **Projections** is the total number of projections of valid tie points in a photo. **Tie points** are the numbers of acceptable connected points between a pair of photos.

Before starting the error correction procedure, one should:

1. Make sure that any previously made Marker boxes in *Reference* are **unticked until** just before the **final filtering** method, *Reprojection Error*, is started.
2. In *Reference* > *cameras*: sort the individual photo **Errors (pix)** in descending order. Error and Projection values will change during the correction process described **below**, and their values should be monitored.
3. *Right-click* on the model > *Center View* (or double left-click on it).
4. Check that the bounding box fits around the specimen. **Model** > *Transform Region* > *Resize Region* > *left-click* the specimen and turn it around.

Optimization and Filtering

When observing the **Sparse cloud** (the four dots icon on the top pane) of the 3D model, one can see that several dots are obviously wrong (e.g., they are outside the specimen) and need to be removed.

Four optimisation/filtering methods can now be utilized:

1. Reconstruction uncertainty (filtering)
2. Projection accuracy (filtering)
3. Bundle alignment (making scale bars)
4. Projection error (filtering)

Within each of the above filtering methods 1, 2, and 4, **three steps** are **repeated** a number of times until the target levels are achieved (while reducing Error (pix) and maintaining as many Projections as possible):

1. **Detection** of unwanted points

2. **Deletion** of filtered red points

3. **Optimize cameras**

Reconstruction uncertainty

This procedure helps to remove **outlier points** of little value. Double Left-click or Right-click the 3D model cloud, centre and enlarge it and then go to the top pane on the screen: select **Model >***Transform Object* > *Rotate*, so that all irrelevant points are visible during the filtration. Keep the **Marker boxes** in the reference pane **unticked**.

a) Choose **Model** > *Gradual Selection* > **Reconstruction uncertainty**. Note the current value (on the left under the slide bar). **The target level is 20-40**. Start with **70** by moving the bar to the right > OK and filtered points will then turn red. Do not reduce more than **50 %** in each step (if >50 % use **Edit** > *Undo* and use a higher value).

b) **Delete** red points: **Edit >** Delete selection.

c) **Tools** > *Optimize Cameras* and monitor the *Projection* value (>100) and *Error* (pix) (should be reduced). Accept general (green) settings and always choose Estimate tie point covariance **>** OK.

Repeat these steps (usually 4-5 times) until a **level of 20- 40** is reached.

Projection accuracy

This procedure removes projection errors by filtering **points of large size**. Points are **clusters of pixels** and consequently smaller clusters reduce the variance of the point position estimations. Keep the **Marker boxes** in the reference pane **unticked**.

a) Choose **Model** > *Gradual Selection* > *Projection accuracy.* **The target level is 2-3.** Start at **40**. Sometimes there will be a high reduction of the key points and the target level cannot be reached.

b**) Delete** red points: **Edit** > D*elete Selection*.

c) **Tools** > *Optimize Cameras*. Accept general settings only and choose *Estimate tie point covariance* > OK).

Repeat these steps (usually 4-5 times) reducing the level until a **level of 2-3 is** reached.

Save

Making scale bars

The bundle of aligned photos is now ready for real-life constraints such as scales and we attempt to increase the model accuracy by creating **at least three scale bars, using markers,** which make it possible to estimate **lengths, area and volume**. A scale bar is essentially a known distance in the model. Adding more scale bars would eventually reach a "**no significant value"** point. One can **monitor** the refinement of Scale Bars ***Total Error*** in the *Reference* pane **as each scale bar is** added.

We create scale bars in three simple steps:

1. In **NIS,** we create and **burn** a **distance line** together with **length data** into **copies** of at least **three** of our five z-stacked "**scale** " photos taken at 90 degrees (see **Part II**, **Microscope photography)** that were saved in folder 5. Z-STACKED PHOTOS and aligned together with all other z-stacked photos. We save these **burned copies** in folder *2D SCALE PHOTOS.* If we need more than three scale bars, we can use the remaining two "scale" photos. The burned copies with a distance line and length data must **never** be deleted since they enable a check of scale bar accuracy. The burned copies will also be saved in their own camera group in Metashape and **disabled** (see **below**).

2. In **Metashape,** we open the first pair of photos: the **burned copy** with a distance line and length data and the aligned **original "scale"** photo, side by side. We **Place markers** in the aligned scale photo at points that correspond **exactly** (at pixel level) to the two endpoints of the burned **distance line**. We then Place the same markers on **three more aligned photos**. This process is repeated for at least two more "scale" photos.

3. In **Reference** > *Scale Bars* pane we add the distance that was burned into the copy.

Creating a distance line in NIS:

1. Open NIS and choose the correct **short cut** calibration on the NIS access bar (e.g., 40wild1.0x or Nikon D500 85 mm 0,35). Check that a correct distance can be measured on a glass slide micrometer (see **fig. 11**) or a ruler (**fig. 12).**

2. Open the **first scale** photo from folder 5. Z-STACKED PHOTOS

3. **Zoom** in on **two easily identified microstructures** (they must be some distance apart and in an absolutely flat area of the photo). Make sure that the two identified microstructures can be found in at least **three nearby photos**. Measure the distance between them. **Burn** the distance line into the photo together with the estimated distance in µm in large font size (**fig. 24)** and save the photo (file name: running number + burned) using "**Save as**", in folder 3**.** *2D SCALE PHOTOS*. Repeat for **at least two more** scale photos.

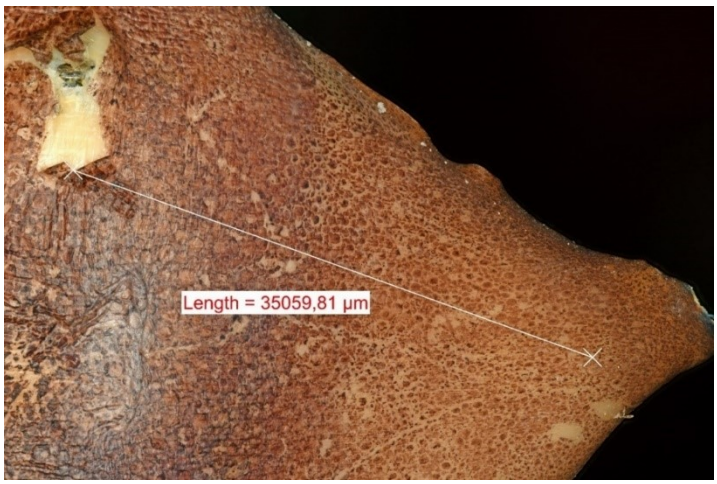


Fig. 24. A "burned " photo with a distance line and its estimated length.

Creating a scale bar in Metashape:

1. **Open Metashape** and **add** the three **burned** photos: *Workflow* > *Add photo* > select (Ctrl + left-click) the **burned** photos in folder 3. *2D SCALE PHOTOS* > *Open*. *Select* the three photos in the **Photos** pane > *right-click* on them and choose **Disable Cameras**. A **red dot** appears in the disabled cameras and photos.

2. Make a special camera group for the **disabled burned** photos so that they can be found easily: *Workspace* > *Select* the three burned photos, *right-click* on them and choose *Move Cameras* > *New Camera Group*.

3. *Right-click* on the new camera group "Group 1" with the burned photos > *Rename* > and name it "**Burned**".

4. **Check** that the previously selected *Reference Settings* are correct: **View** > *Reference* > *Click* on *the wrench icon* button to the far right at the top of the *Reference* pane and in the *Reference Settings* dialogue box, choose **mm** coordinate system; tick the Camera and Reference boxes; set camera, marker and scale accuracy to **0.001 mm.** Leave the other settings as they are; > OK. Click on the *wrench icon* button **again** to check the input values.

5. Open the **first pair** of photos in different tab groups, the **burned one with a distance line and the original scale photo,** and place them side by side in different Tab Groups on the screen: **Workspace >** *Chunk* **>** *Cameras* > *right-click* on the photo >*Open in New Tab* > *Move to Other Tab Group.*

6. Zoom in the **burned** photo to **pixel** level where the **burned distance line starts** and the corresponding area in the **scale** photo and place the first marker on it that corresponds to the start point of the burned distance line: *Right-click* > ***Place marker*** > *New Marker* and do the same for the end point of the distance line (**fig. 25**). Be **very precise** when doing this!

7**.** Both markers are now appearing with a green flag and different Point numbers in the *Reference* ***Markers*** pane. Give **new names** to the pair of markers in the *Reference Markers* pane: *Right-click* on the **first** marker > ***Rename*** and change the point name to, e.g., **55scale1a** (running number of the photo, Scale1 is the first scale and a is the first marker) and do the same for the **second** marker and named it 55scale1b (repeat this naming procedure for the two remaining pairs of scale photos). It is important that one knows on which photo a scale bar is based if problems occur with the length and volume estimates and rescaling is required.

8. **IMPORTANT**. **Clarify the marker positions** on the image by adjusting the position of **each marker on at least three nearby aligned images**. Left-click the grey flag dots in those images and they will become red and adjust their positions. Their flags will turn green after adjustment. This will create more projections and provide a better precision for the scale bar.

9. Select the first pair of markers (*Ctrl and left-click*) in *Reference* ***Marker****s* pane (both marker areas become darker) > *right-click* > ***Create Scale*** *Bar.* The two markers then appear as a scale bar, with a joint name, in the ***Scale Bars*** pane.

10. Click on the ***View Source*** *icon* (a small square icon) in the *Reference* pane heading and **m** should change to **mm** in the ***Scale Bars*** pane.

11. In the ***Scale*** **Bars** pane > **double** left-click **under** *Distance (mm*) and enter the scale distance (that was burned into the **burned** photo in **µm)** in **mm**. 1000 **µm** is one mm. Use a full stop, not a comma, in the scale distance. Note that the **scale box is** now **ticked** (**fig. 26**).

12. **Repeat** all above and create at least **two more scale bars** using remaining pairs of "burned" and "scale" photos.

13. Check that the **scale bar boxes** are **ticked.** The scale bars contribute to the adjustment procedure, however, if one believes that the **scale bars** have **poor accuracy**, they should not be included in the optimization process and their boxes should be **disabled**.

14. ***Update transform*** (the icon with two blue arrows) in the *Reference* pane heading (**fig. 26**) in order to **apply the changes** and **Error (mm)** will be shown.

15. ***Tools > Optimize Cameras*** (or click on the *Star* icon in the *Reference* pane heading) improves the model estimation. **Tick** the ***Estimate tie point covariance*** in the dialogue window and accept the green default choices > *OK*. **The Point cloud** will now be **reconstructed** and when finalized the **Ruler icon should light up!**

16. **Check** the distance estimate: Open all the **aligned** "**scale**" photos and click on the *Show Marker* (large flag icon on the top pane). Choose the ***Ruler*** icon at the toolbar pane and measure the distance between the first and second scale markers. Measure close to the markers but not on them. Check that the distance is the same as given in *Reference > Scale Bars distance (mm)* for each photo. Do not (yet) worry if these tests show some minor discrepancies. Later, when the 3D model is finished, we will check the accuracy of distance estimates made on the final 3D model against the scale photos. However, again, it is **extremely important** that both the burned distances and the scale markers are correct and based on easily **identifiable** points in **all photos** that are used for making scales. See **Make a Report** how to include tests of model accuracy.

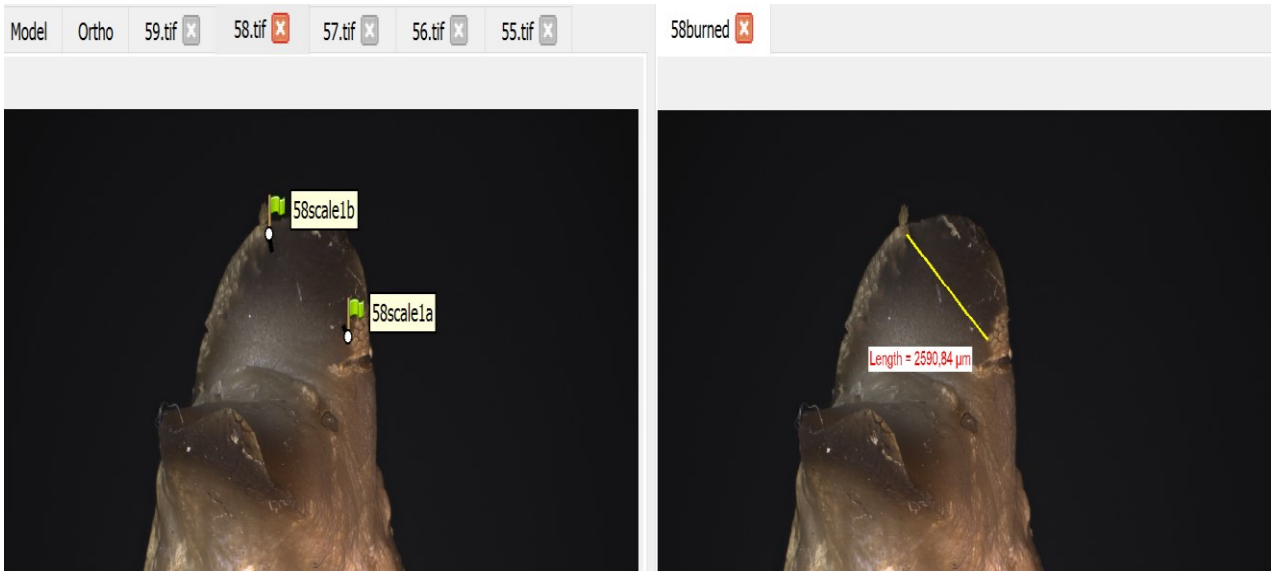


Fig. 25. Distance line for a scale bar. Four nearby not yet opened photos (59, 57, 56 and 55) and the 58 scale photo and the 58 burned one with its burned distance line and length data. The two markers, 58scale1a and 58scale1b, correspond to the start and end points of the burned distance line. The two markers will be placed on the nearby (minimum three) photos.

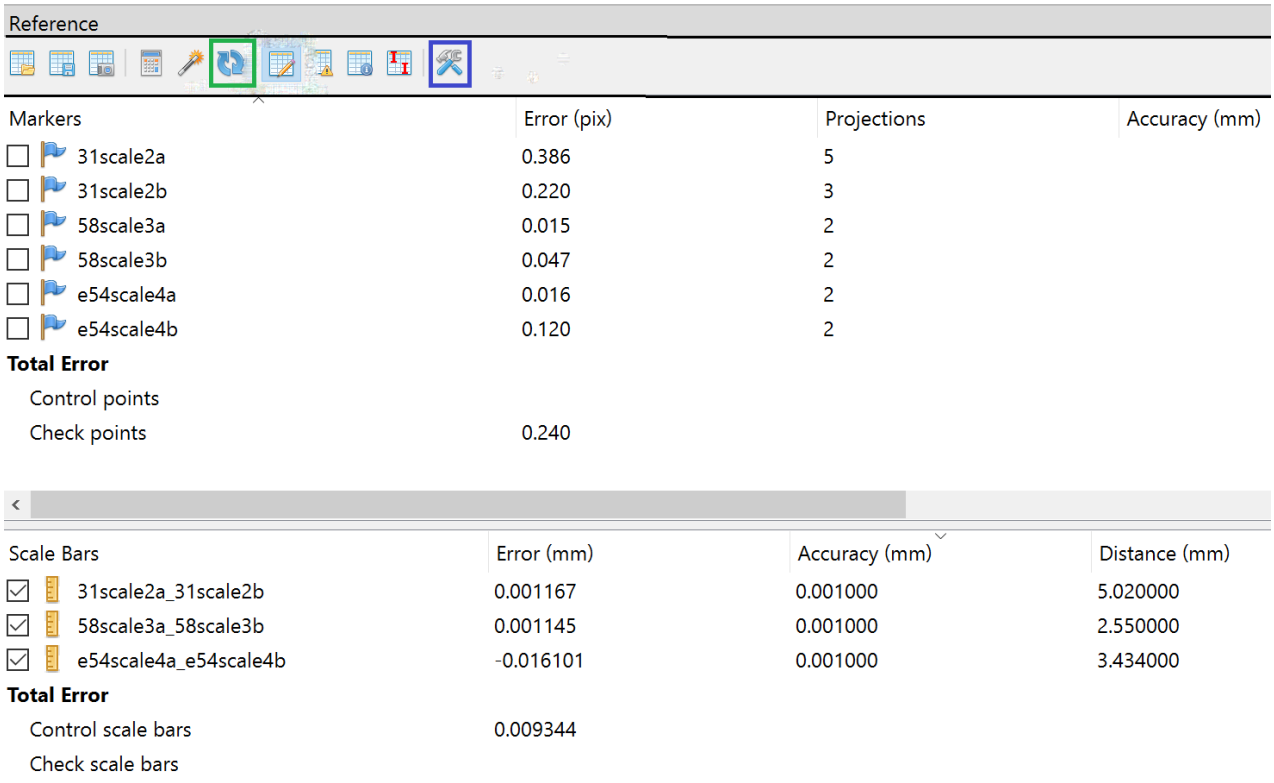


Fig. 26. Markers and three Scale Bars with distance given in mm. The green rectangle shows the U*pdate Transform* icon. Accuracy was estimated in the *Reference Settings* dialogue box (wrench icon inside the blue rectangle). The numbers of projections are few since the markers were not yet placed in nearby photos.

Note that it **is important** that the **assumed** accuracy in the *Reference Settings* dialogue box is realistic in relation to one's scale photos, scale markers and scale bars since the Error estimate and the length, area and volume estimates could otherwise be affected!

Note that after adding or changing any information in the *Scale bars* section of the *Reference* pane, on must **press the *Update*** button and *Optimize Cameras* in T**ools** apply the changes.

Save

Reprojection Error

The reprojection error in a photo is the distance between two **projected** 3D points **on the photo**, the **reconstructed** and the **original** one. The reprojection error demonstrates the accuracy of point positioning and is specified in pixels. During the reprojection error filtering process one sets the criterion to how many points in the Dense Cloud one wants to delete. One should change the viewing angle of the model so that most of the points are visible during this process in order to check which point are highlighted before deleting them.

Tick the **scale bar** boxes.

a) **Model** > *Gradual Selection* > *Reprojection error*. We often use the target level **0.3-0.4**. Start with **0.7.** If reduced by more than **10%:** press **Edit** >*Undo* and try a higher value. Note that this target level is not generally recommended since **higher levels might be preferred** depending on the camera used, etc. One should **not to delete to many points** since it may lead to a small number of tie points.

b) **Delete** red points. **Edit** > D*elete Selection*.

c) ***Tool >*** *Optimize cameras*. Accept general (green) settings and always choose *Estimate tie point covariance* > OK.

Repeat this until the preferred target level is achieved.

Save

MESH

1. **Model** > *Transform Region* > *Resize Region* and make sure that the bounding box fits around the object.
2. Choose **Workflow** > *Build Mesh*. See **Appendix I** for settings in the dialogue box.

If one gets the message **Empty surface** it means that that the **Bounding** box is misplaced during *Build Mesh* operation and doesn't contain any information.

Save

Editing the mesh

Background features and other unwanted features that could negatively affect the final model should be removed, as per some of the various methods described below. **Test** different methods on **duplicates** of the model in **different chunks**; Click the ***Workspace*** > *right-click on* the *Chunk* > *Duplicate*. In the dialogue box select *Depth Maps* > OK. Give each new **Chunk a name** that corresponds to its purpose. Remember to *right-click* on the *Chunk* > *set active* when a model is to be viewed (and modified).

Remove unwanted points and objects. One can remove either individual or groups of points manually. Select **Model** > *Free-Form Selection*) and delete the selected points: **Edit** > *Delete Selection*. Click on the *Arrow* button on the **Toolbar** to rotate the model so that all unwanted points can be detected.

Mask defocus areas. One may have blurred areas in the photos which may affect the sharpness of the final texture. One can then **automatically** apply masks to such areas (based on the depth information of each point) in order to exclude them prior to the texture blending.

1. Select ***Tools*** > *Mesh* > *Generate Masks.*

2. In the *Generate Masks* dialogue box (**fig. 27**) select suitable parameters. Click on the *OK* button when done.

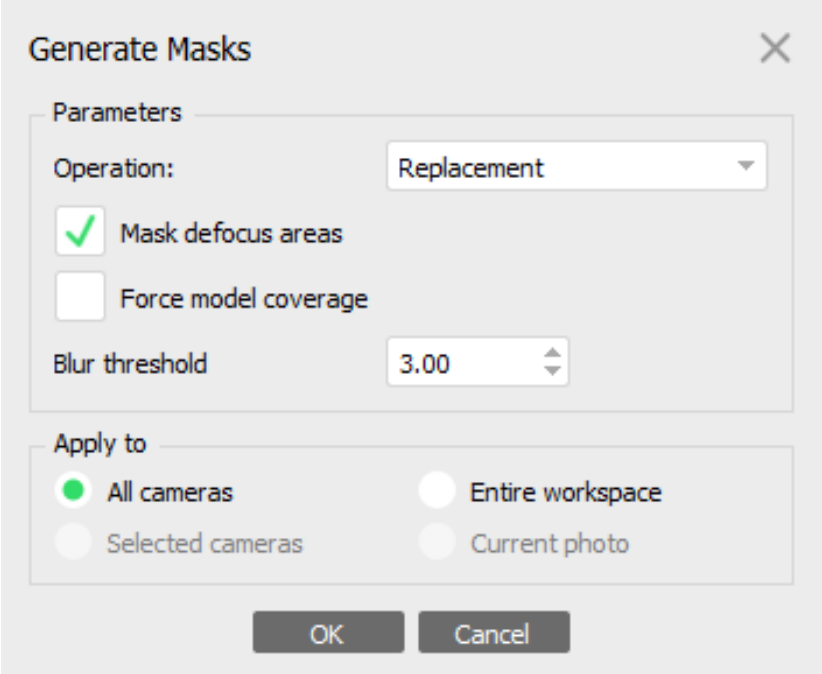


Fig. 27. In Generate Masks we can automatically apply masks to areas that are out of focus.

Colour calibration: (**Tools** > *Calibrate colours*). Use this option only if there are big light differences in photos. Obs**:** this can create bad colours and be time-consuming! Make the colour calibration in a **new Chunk**!

White Balance: **Tools** > *Calibrate Colours* > *Sparse Cloud* > *Calibrate white balance*. White balance should be done during the photography and used here only if there are considerable **light differences** in the photos. Note that the use of white balance here could cause error in the colours. Make the white balance in a **new Chunk**!

Save

Photoshop photos before texturing. Before starting texturing: make any necessary photoshop changes on the inferior photos.

1. Copy the original the z-stacked photos that need to be modified to folder 6. Z-STACKED FOLDERS FOR PHOTOSHOP and make any necessary adjustments on the inferior photos. **Do not change the frame** size by cropping nor the file name. Save and exchange the "photoshop" photos for the original ones in folder 5. Z-STACKED PHOTOS.

2. Repeat: **Editing the mesh model** as above if needed.

Save

TEXTURE

1. Click **Workflow** > *Build Texture*. See **Appendix I** for settings in the dialogue box.

Save

Editing the texture

In order to light up the model and adjust the contrast one can use: **Tools >** *Set Brightness*. Another option would be the *Remove lighting* tool which smooths out the dark and light areas of the model. Make the texture editing in a **new Chunk** and **rename** the new Chunk to **Lighting**. Select **Tools** > *Mesh* > *Remove lighting*. Use the *Colour/ Multi colour* mode if the model has different colours at first trying the given presets. Compare the two models by alternately setting the chunks to "active". **Check** the results **carefully** when using this option for scientific purposes.

One can also use the **Agisoft Texture de-lighter** (*dlz) software which is a free stand-alone tool designed to remove shadows from model **textures**, although in principle the photographs taken should reduce such problems to a minimum. This software requires user input only in the form of rough brush strokes marking lit and shadowed areas. It is optimized for **8-bit JPEG** compressed textures and does not need specific image formats with greater depth of colour. It can be downloaded for free **from Agisoft**. To get started: From Metashape > Export your model in ***OBJ** format to folder 7. FINAL 3D MODELS AND 2D PHOTOS and **import** it to T**exture de-lighter**: **File** > *Import Model***.** Click on the ***Blue*** **palette**, mark the shadowed areas and then click on the ***Yellow*** palette to mark the areas that are too light. Use the pointer to move the model around. Finish by clicking on the *Remove Cast Shadows* and *Remove Shading*. **Save** the model and, if needed, ***Export*** the model in any of the other available formats. **Check the results carefully** when using this software for **scientific** purposes.

Save as: Id code+de-lighter1.dlz in the folder 7. FINAL 3D MODELS AND 2D PHOTOS and in this format the model opens directly in **Texture de-lighter**.

MEASUREMENTS

The estimated scale lengths are now tested for accuracy in the 3D model. Open one of the "**burned scale**" photos with a burned distance line and length data and open the textured model. Rotate the model so that it appears identical to the burned photo. Zoom in them both and select the large *Ruler* icon at the top toolbar. *Left-click* in the model at the corresponding start and end points of the burned distance line in the photo. Are the distances identical? Right- and then left-click outside the model to remove a measurement. **Repeat** this process with the other burned "scale" photos. Make a **note** of the differences in length estimates between the model and "scale " photos (to be added to the **Report**, **see below**). Then estimate the area and volume (which should also be added to the report): **Tools >** *Mesh* > *Measure Area* and *Volume*. Are the estimates **realistic! Note** that if the 3D model is not closed, the volume option will not appear and area estimates would thus be incorrect!

With Metashape the volume of our rubber specimen (**fig. 7**) was estimated to be 1162 mm^3, which is comparable to the volume estimates (water displacement method) we made earlier. After

making 32 estimates we obtained a mean of 1245 mm^3 (minimum 1138 mm^3 and maximum 1453 mm^3) but a minor overestimate of the true volume was to be expected in view of the method used. We concluded that the Metashape volume estimate for small specimens works well, although of course the accuracy of any estimate is entirely dependent on that of the scale bars and the 3D model itself.

Save As: File name **IDcodefinal3D1** in folder 7. FINAL 3D MODELS AND 2D PHOTOS

Make backups ! Use **Save as. Check** the model can be opened using **backups**.

See **APPENDIX III** for a 3D model (**fig**. **28**) which can be viewed in the PDF version.

BUILD A TILED MODEL

The tiled Metashape models comprise more than 12 000 polygons (often >1 million). They are often used when 3D modelling large structures but can also be used for any other sizes. Tiled models (in the **TLS** format) are used, e.g., in the **Agisoft Viewer** which is a free of charge viewing software for textured models. See **Appendix III** for further **instructions**.

1. Open your textured model and check that the object is within the **bounding box.** If not, drag the corners and select **Model** > *Transform Region* > *Reset Region* or use the *Move, Resize* and *Rotate* options.

2. **Workflow** > *Build Tiled Model*

3. Choose in the **dialogue box**: Source data: *Depth maps or Mesh*; Quality: *High or Ultra-high*; Face count: *Medium*; Depth filtering: *Mild*; tick the *Enable Ghosting filter* box. If *Depth maps* are used one can choose among Quality options. *Ultra High* means that the **original z-stacked photos** are used, but it takes time, and one could instead use *Reuse depth map* (if *Medium Quality* is selected), to save time. Face count specifies the maximum number of polygons in the final mesh. We always chose the time demanding options (if we get a better result) since it is the photography that takes most of the time during 3D modelling.

Note. If small holes were repaired using the **Tools** > *Mes*h > *Close Holes* option, one **cannot use the depth maps for making a Tiled model.** This option applies only to the **Mesh** which then should be used for making a Tiled model.

4. **Export** the Tiled model in the Agisoft Tiled Model Format ***TLS: File >** Export > *Export Tiled Model.*

5. When saving a Tiled model in folder 7. FINAL 3D MODELS AND 2D PHOTOS 7 one will find an Export Tiled Model dialogue box with information regarding the coordinate system: check that the local coordinates are in **m (**not mm) **>** OK. **Agisoft Viewer cannot yet handle mm** in the **coordinate system** but distances can be given in m, cm or mm depending on the estimated distance.

Use "Save as": IDcode+tiled 1 with a ***TLS extension**

Due to the configuration of our computer operating system our Tiled models have an Adobe symbol in front of their file names. However, such files cannot be opened in Adobe photoshop.

6**. Save** the Tiled model with *PSX format (in the Metashape project).

7. View the ***TLS** model in Agisoft Viewer: **File > *Add layer*** (one cannot use *Open*) to import the model.

MAKE A REPORT

Whilst a 3D model report is extremely useful and important and should be mandatory for scientific 3D model documentation, it is also of value for non-scientific 3D models. The report should therefore, together with the 3D project`s data file, be kept in the same folder as the final 3D model. A report provides quick and exact information as to how a particular model was made. It has a title page with notations and the date, followed by the survey data, camera calibration, scale bars, digital elevation model, camera calibration data and parameters of the process. Make a **note** of the differences in length estimates between the model and the "scale " photos and the area and volume estimates. One can also include the 3D model in a PDF report (see Appendix III). Making a report in Metashape is simple:

1. **File** > *Export* > *Generate Reports:* In the *dialogue* box one can write the title and the 3D project file name plus other information of importance > *Save* as pdf in folder 7. FINAL 3D MODELS AND 2D PHOTOS.

Save as: ID code+ final 3D 1 report

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https://www.agisoft.com/. Includes, support, community, downloads and buy.

Knowledge base (https://agisoft.freshdesk.com/support/solutions).

https://www.facebook.com/groups/agisoft/. This is the biggest community for Agisoft Metashape, formerly known as Photoscan, outside the official Agisoft Forum. Please note: the Agisoft team don't administrate this community.

APPENDIX I: WORKFLOW SETTINGS

In order to save computer memory capacity, one should save all workflow work in **the same file** in folder 6. 3D MODELS using the project ID code. Do not forget to make **backups** to several external memories.

Below we show our most common workflow settings.

1. ADD PHOTOS/FOLDER

Save (+backups)

2. ALIGN PHOTOS
Accuracy

Highest or High

Generic preselection /Source

Reset current alignment (only when starting all over with new alignment)

Advanced

Key point limit: 40 000

Tie point limit: 4000-10 000

Exclude stationary tie points (removes background points)

Apply mask to: key points or tie points

After alignment: Check that the bounding box fits around the object. Use the *resize, move*, and *rotation* tools found in **Model** > *Transform Region* options. Delete all bad points outside the box**: Model >** *Free -Form Selection* **>** select the bad points area **> Edit >** *Delete Selection.*

Save (+backups)

3. DENSE CLOUD
Dense Clouds are **not** needed for our 3D models.
Quality
High or Ultra high

Depth filtering
Mild

USE: *Resume depth* maps if saved earlier as Dense cloud.

Calculate point colours, ALWAYS

Calculate point confidence: ALWAYS
This takes some time. When finished: **Model**/*Transform Region*/ *resize* and make sure that the bounding box fits around the object.

Save (+backups)

4. BUILD MESH
General
Source data: Depth maps, ALWAYS

Surface type: Arbitrary (3D)

Quality: Ultra high

Face count: high

Advanced
Interpolation: enabled (default)

Depth filtering: mild (no reuse of depth maps)

Calculate vertex colours: not needed for depth maps

Save (+backup)

5. BUILD TEXTURE
Before texturing:
Remove unwanted points and apply Masks.

Do photoshop changes (if needed) on the copies of z-stacked photos (***do not crop them***) and keep the same file codes as in the original z-stacked photos).

One can do a Colour calibration (*Tools > Calibrate colours*) *before* texturing but only if there are big light differences in photos. *Obs***:** this can create bad colours and be time-consuming!

One can also calibrate the white balance at this stage (in the same dialogue box). However, the white balance of photos should also be checked before adding them to Metashape.

Then build the texture model:

General
Texture type: Diffuse map, ALWAYS

Source data: Images, ALWAYS

Mapping mode: Generic, ALWAYS

Blending mode: mosaic, ALWAYS

Texture size/count USE SUGGESTED 4096-16000

Advanced
Hole filling, ALWAYS (**but not first time,** in order to detect missing photos).

Ghost filter, ALWAYS (some thin structures or moving specimens failed to be reconstructed and use this to avoid ghosting effects).

Save (+backup)

6. Build Tiled Model

General
Source data: **Dense Cloud**

Leave Pixel and Tile size as they are given

Face count: Medium (specifies the maximum number of polygons in the final mesh, should not be too low or too high).

Advanced
Ghost filter (if some thin structures or moving specimens failed to be reconstructed (to avoid ghosting effects).

Reuse depth maps > Ok

Save
Note that the tiled PSX model is **still open** in Metashape and it has a PSX* extension on the top file name bar.

Now we export the tiled model to get a TLS format:

File > *Export >Export Tiled Model*

"Save as" IDcode+tiled 2 TLS with an ***TLS extension** in folder 7. FINAL 3D MODELS AND 2D PHOTOS.

APPENDIX II: SOME COMMON BUTTONS

Be familiar with these functions before exploring others.

FILE

Open

Save

Save as

Export (one can export and save models and generate reports)

Import

Upload Data: to internet resources (4D Mapper, Cesium, PointBox, Pointscene and Sketchfab)

Last in the drop-down: One can see the last projects that have been opened: Open from here is quicker.

EDIT

Undo

Delete selection

VIEW

Capture view (save a photo)

Different panes: Workspace, Reference, Properties, Photos

Animation (video)

Toolbar

Full Screen (click it again to go back to normal, useful during **MAKING SCALE BARS** when one need to zoom in.

WORKFLOW

Add photos

Align photos

Build dense Cloud

Build Mesh

Build texture

Build Tiled Model

MODEL

Navigation (quicker to use the large arrow icon on the Toolbar)

Free form selection

Gradual Selection (for **ERROR CORRECTIONS)**

Ruler (quicker to use the Ruler icon on the Toolbar)

Transform objects (move, rotate)

Transform regions (move, rotate and resize). When using the *Resize* option both region and the object can be rotated simultaneously which is very useful during **ERROR CORRECTIONS**.

View mode (different views appear in the toolbar when available. This option is therefore an alternative to choosing among the large model icons on the Toolbar.

PHOTO (open a photo first)

Different selections tools. Click the Large arrow icon on the toolbar to go back to normal.

Show/Hide, e.g., Show Masks, Show markers and Show Points (very useful)

TOOLS

Markers

Mesh

Camera Calibration

Optimize Cameras

Set brightness (has its own Toolbar icon)

Preferences (language, etc, very important)

HELP

Content

Check for Updates

About Metashape

APPENDIX III: VIEWING AND SHARING 3D MODELS

3D models can be viewed and sent to others using different software, which requires appropriate model file formats. Metashape offers many different file formats (but some of them are of less use to the beginner). Metashape itself uses two formats, the ***PSX** and the ***PSZ** for saving a project. Such 3D models are not suited for e-mail due to their large size and instead one can use **Google Drive**, **Dropbox** or other cloud storage services.

PSX files (Metashape project file) is used when saving 3D models during the modelling process (see Part III **Saving models)** and for export in other formats**.** The *.PSX file stores the links to the processing results in the *.PSX file and the data itself in ***.files** (a structured archive in a yellow folder with the 3D model name). When copying and sending a PSX model one needs to **send three** things; the *.PSX file , the *.file folder and all the photos (folder).

PSZ (Metashape project archive) files are **ZIP compressed**. PSZ models are saved from the PSX model: **Edit** > ***Save as*** > *chose the latest psz format* > *Save.* When copying and sending a PSZ textured model, one only needs to send the PSZ file. Some data may be lost during saving in some formats, e.g., the Tiled format, but one will get a notice before saving starts. The PSZ models can only be opened in Metashape.

To export a 3D model:

1. Select Export Model... command from the **File** menu.
2. Browse the destination folder, choose the file type, and the file name. Click the *Save* button.
3. In the Export Model dialogue **specify the coordinate system** and indicate the applicabl export parameters to the selected file type.
4. Click the *OK* button to start the export.
5. The progress dialogue box will appear displaying the current processing status. To cancel processing click the Cancel button.

Metashape supports model export in the following formats:

- Wavefront OBJ (*.obj)
- 3DS file format (*.3ds)
- VRML models (*.wrl)
- COLLADA (*.dae)
- Stanford PLY (*.ply)
- X3D models (*.x3d)
- STL models (*.stl)
- Alembic (*.abc)
- Autodesk FBX (*.fbx)
- Autodesk DXF Polyline (*.dxf)
- Autodesk DXF 3DFace (*.dxf)
- Open Scene Graph (*.osgb)
- Binary glTF (*.glb)
- U3D models (*.u3d)

• Adobe PDF (*.pdf)
• Google Earth KMZ (*.kmz)

Agisoft Viewer
This stand-alone free software is practical since anybody can download it and use it to view your **tiled** 3D models in the **TLS** format (only one file to send). The options are relatively simple and self-explanatory. The Agisoft Viewer will show length in m, cm or mm **Note** that the tiled model must be set to **m** (not mm) **in Metashape** *Reference Settings* and choose in the Agisoft Viewer: ***Tools*** *> Preferences > dialogue window: General; Mode: Anaglyph; Units: Coordinate system units* and **tick** the *Use derived units* bo*x*. Check the distance estimates against the corresponding burned photos distances.

Open a tiled model:
1. Select *Add Layer* (**not** Open) from the *File* menu

2. Chose the *tiled TLS* model (with a photoshop icon in our case) > OK

The model will now be displayed in the *Viewer* workspace and on the *Layers* pane.

Save as ID code+viewer1vpx in the format Viewer Project *.**VPX** in folder 7. FINAL 3D MODELS AND 2D PHOTOS.

The VPX model can be **opened directly in Agisoft Viewer: File** > *Open.*

Making videos of 3D models (in both Agisoft Viewer and Metashape)
Below we show one of the quickest ways to make an mp4 video that can be viewed in, e.g., PowerPoint, PDF, Windows Media Player and as attached to an E-mail. Open the 3D model in Metashape. To the right in the **windows** screen there are four small icons: **camera**, **video recorder**, **screenrec gallery** and **screen settings** which are self-explanatory. Using the video recorder: Click it and mark the area around the model > after count down > move the object around > Stop > Save (by clicking the small ***Save video locally*** icon under the video) in folder 7. FINAL 3D MODELS AND 2D PHOTOS with the ID code + a name of choice. In the video, one can make measurements and draw in the volume/area estimate value box, make measurements, as well as use the pointer to pay attention to certain parts of the model. The result is saved automatically in the *Screenrec Gallery (*but only with a date/time file name, regardless of the file name in the 3D MODEL folder. From the gallery one has direct access to all videos and photos (as PNG) and the Preview option. It may be useful to play the video a 2x speed if many arrows and measurements are shown (which take some time to carry out during the recording).

We do not go into details regarding the use of 3D models in various other software since they are continuously being developed and occurs in many different editions.

Adobe Acrobat
In Acrobat, one can open and view a 3D PDF model created in Metashape. A 3D model (*U3D) can also be inserted into a PDF document using, e.g., **Adobe Acrobat Pro.**

PowerPoint
3D models and videos can be viewed.

Word
3D models and videos can be viewed.

3D viewers
A quick way to view models.

Excel
3D models and videos can be viewed.

Online
Uploading of generated data (Dense cloud, Textured model) to various online resources: 4DMapper, PointBox, Pointscene, Scetchfab: **File >** *Upload Data.*

Finally, we show a 3D model of the digestive gland of the bobtail squid *Sepietta oweniana* using our stereomicroscope camera (**fig. 28**). In the PDF version of this guide, this model can be zoomed and rotated. This is an incomplete model and we show it in order to **illustrate some of the problems** that can occur during the photography. The digestive gland has some holes due to poor overlap and to the fact that some of the photos could not be used since the organ was not moistened as frequently as it should have been, resulting in **discolouration** and **shrinking**. We could not model the octopus *Eledone cirrhosa* shown in **fig. 9**. The reason is that the specimen had been preserved in 4% formaldehyde prior to the mounting on the preservation tube and the arms could therefore **not** be stretched out in full in the preferred positions for photography, despite being in water for 24 hours. In other words, optimal objects and photography is the key to successful 3D modelling!

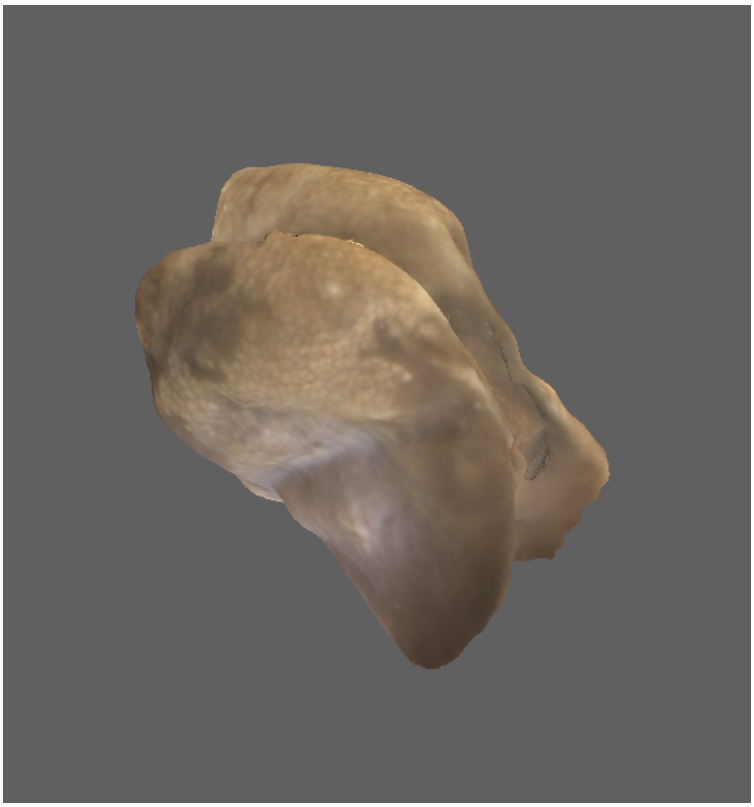

Fig 28. The digestive gland of a bobtail squid (*Sepietta oweniana*) to be viewed as a 3D model in the PDF version.

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Agisoft Metashape is a cutting-edge software solution, with its engine core driving photogrammetry to its ultimate limits, while the whole system is designed to deliver industry specific results relying on machine learning techniques for post-processing and analysis tasks.

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SI TECH is a Swedish company focusing on development, manufacturing and marketing of innovative safety solutions for protective suits such as; drysuits, rescue suits and similar protective garments. SI TECH is rooted in the diving industry and this is still the company's core market.

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