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NAMING IMAGES

Saving images

- Save the images on the '**nnm/dino/Sylvius Zeiss Microscope**' network disk, use a folder named with the project number. Use subsequent folders with names of the different collecting racks or taxonomic subgroups if desired.
- Use the **TIFF** image format.
- Use **underscores** for separation instead of spaces or dots.

Naming images

- First, the image name must include the **RMNH number**. Use underscores for separation instead of spaces or dots. Use the barcode scanner to scan the RMNH number if a datamatrix is present on the label, this helps to prevent typing errors.
- Second, the image name can include the **orientation** of the subject (if applicable).
  - For dorsal views use "**dor**".
  - For ventral views use "**ven**".
  - For lateral views use "**lat**".
  - For frontal views use "**front**".
- Third, the image can include an additional letter if there are multiple images of the same orientation.

- For plants, the collection number alone will suffice (e.g. L0873777.tif).

(A) RMNH_ARA_14509_dor.tif

(B) L0873777.tif

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NIKON SLR SETUP

Important! Make a reservation for the NIKON in the reservation book!

Getting started

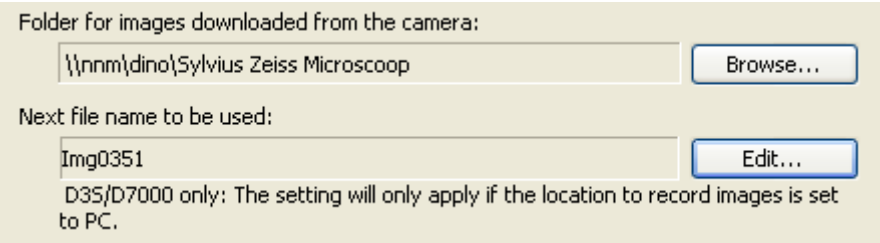
- Make sure that the window directly behind the SLR setup has been covered to avoid daylight coming into setup. If necessary, turn off as many other light sources as possible.
- Attach the camera to the support, with the lens facing down towards the subject area.
- Remove the lens cap and place the DR-6 right angle viewfinder onto the camera.
- Attach the USB connector cable to the camera (this cable is connected to the computer at any time).
- The ring light can be switched on with a button inside the light setup.

Background

Use a uniform background, preferably black, grey or white. The default grey background with white 1cm squares can be used as well, but these squares may prove difficult to remove from photos in a later stage. For small subjects such as Lepidoptera or Odonata, a special glass plate can be used in combination with supports and a grey background. This will eliminate shadows around the specimen. For more details, read the relevant group-specific instructions.

Acquiring and processing images

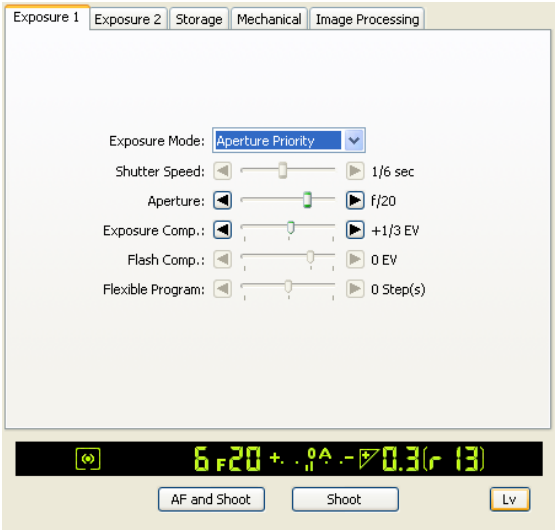
Switching on the camera will automatically start the software on the computer (if the USB connector cable is attached). First of all, the download directory will have to be appointed for the series of photos. In the menu, under "Tools", click "Download Options" to change directory and automatic filenames (figure A).



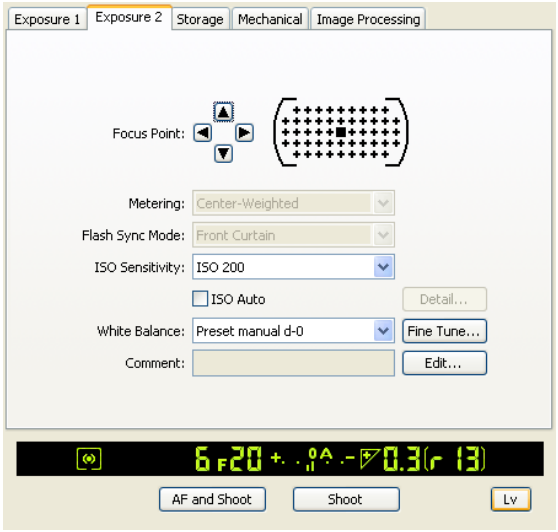
(A) part of the Download Options menu

In the "Camera Control Pro" screen, under the tab "Exposure 1", set the Exposure Mode to "Aperture Priority". You can now select an aperture value that fits best with the specimens (see group-specific instructions for more details on this) (figure B). Under the tab "Exposure 2", the focus point can be moved, so make sure that this square is placed on the specimen (figure C). The "Lv" button in the lower right corner of the "Camera

Control Pro" screen can be clicked to activate the live view, in which this focus point is also visualized (figure D). Keep in mind that the live view option will deplete the battery of the camera relatively fast!



(B) the Exposure 1 tab



(C) the Exposure 2 tab

Zooming in and out is done by moving the camera down or up with the winch on the camera support. For very small specimens, it is advised to bring the background and specimen closer to the camera, as the camera can only move down as far as the light setup allows.

(D) the Live View screen

Use the "AF and Shoot" button to auto-focus and shoot a photo. If a message pops up that the subject is not in focus, try moving the focus point, manually repositioning the specimen or zooming in/out. Images are automatically saved into the folder specified. **You will have to rename them manually to the standard format with RMNH numbers!**

Finishing up

- Place all the used equipment for the setup back into the appropriate drawers in the lab.
- Disconnect the camera and return it to the office (room 6.4.17), together with the DR-6 viewfinder.
- Recharge the battery if needed.

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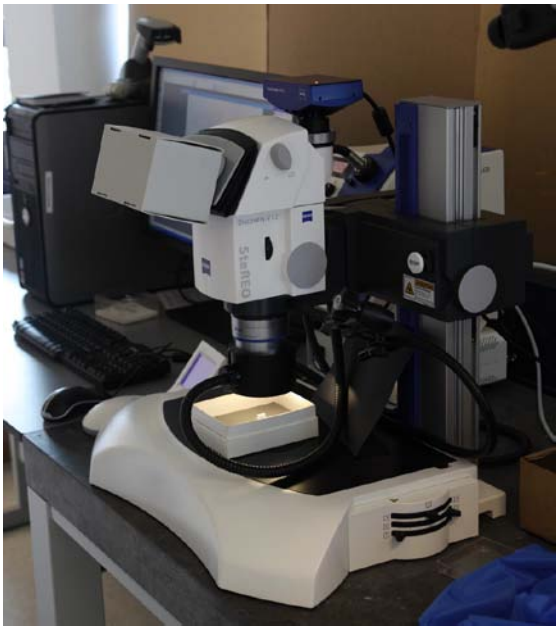
ZEISS STACKING-MICROSCOPE

Important! Make a reservation for the ZEISS microscope in the reservation book!

Getting started



- Make sure that the window directly behind the microscope setup has been covered to avoid daylight coming into setup. Also turn off as many other light sources as possible, especially when working with reflective objects.
- Switch on the microscope first, then start up the AXIOVISION software. Use the blue button on the EMS-2 power unit to turn the microscope on, and the switch(es) on the light source(s).
- Set-up the lighting as you will be using it. There is a ring-light available, a double swan-neck with optional focusing tips, a diffuser for diffuse lighting and table light for transmitted lighting.

Setting the white balance

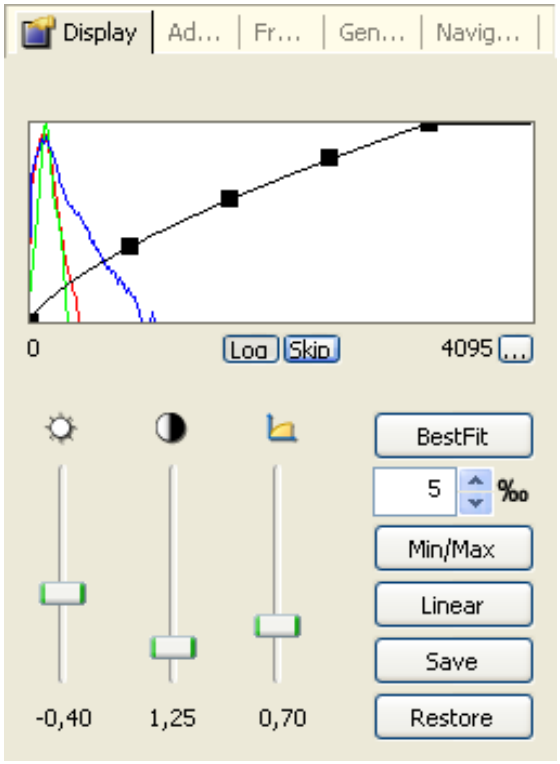
Before starting a series of photos, use a 70% grey card (KODAK) to set the white balance by placing it under the microscope and clicking the "Automatic" button under "White Balance" in the "Live Properties" window. Be sure to have all the lights set up as they will be used for photography, changing the setup and brightness of the lights will alter the white balance.

Acquiring and processing images

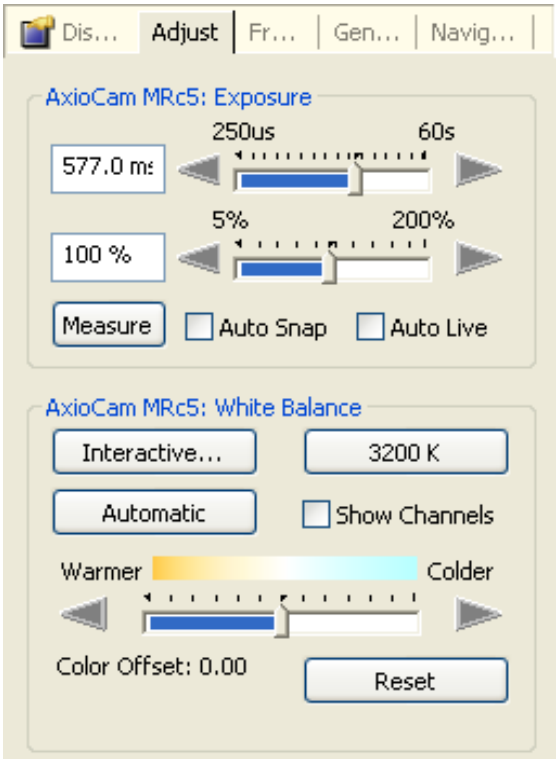
The microscope can be controlled using the attached joystick. The actions that can be performed with this joystick are; zoom in (right), zoom out (left), move focal plane downwards (down) and move focal plane upwards (up). For acquiring and processing of images, the AXIOVISION software has been outfitted with a "workflow" bar, with all the necessary tools displayed in order of use.

Live. This enables the live view (make sure the microscope is set to "camera"). Manually move the specimen to center the specimen in the frame and use the zoom function to zoom in or out until the specimen nearly fills the frame. Moving the focal plane up and down shifts the image sideways, so make sure there is enough space on the left and right side of the specimen to allow for this movement! The position of the focal plane is not important at this moment, but having part of the specimen in focus makes it easier to work with.

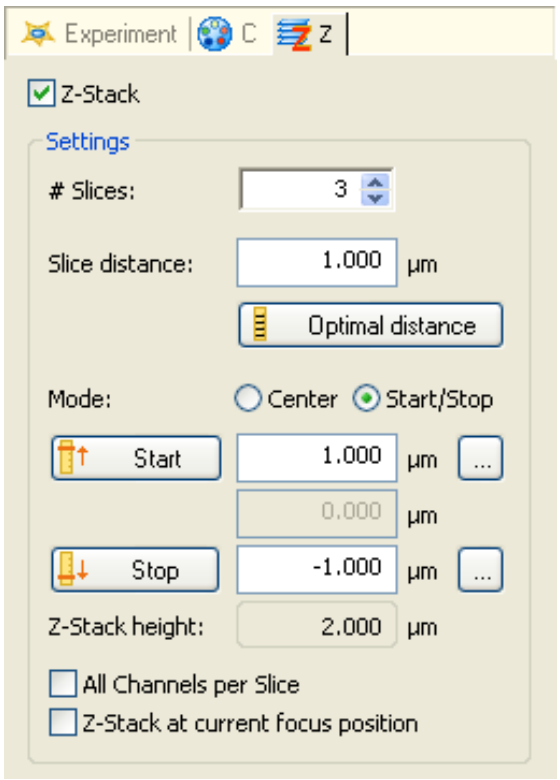
Live Properties. This window can be used for adjusting the settings of the camera. Under the tab "Display", the optimal brightness, contrast and gamma-value can be set for a series of photos. When the live image is optimal, click "Save" to store the values (figure A). Under the tab "Adjust" the exposure time and white balance can be changed. When the specimen is in the correct orientation and magnification, use the "Measure" button under "Exposure" to automatically adjust the exposure time (figure B).



(A) the Display tab of the Live Properties menu

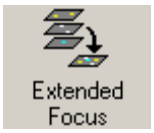


(B) the Adjust tab of the Live Properties menu



(C) the Z tab of the Multidimensional acquisition menu

Multidimensional Acquisition. This window enables multidimensional image acquisition. Select the tab "Z" (for Z-stack) and change the "Mode" to "start/stop". *If the "Z" tab is not available in the acquisition menu, close and restart the AxioVision software*. Now use the joystick or the onscreen "Z-stack navigation" to adjust the focal plane so that the topmost part of the specimen that needs to be included in the image is in-focus. When the focal plane is in the right position, click on "Start" to save this setting. Now move the focal plane towards the lowermost part of the specimen and click on "Stop". To calculate the amount of images in the stack, click "Optimal distance", which will automatically display the number of slices and the slice distance. Usually the number of slices is too high, so manually change the "Slice distance" by doubling it and click on the number of slices so that it automatically adjusts to the new settings. *Make sure the ocular (eyepiece) is covered to prevent any light from falling onto the specimen through the microscope*. Now press "Start" at the bottom of the window to acquire the images (figure C).



Extended Focus. This opens a window in which the stack of images from different focal depths can be converted into a single image. Set "Quality of alignment" to "High", make sure the correct stack is selected (the most recently viewed stack/image is selected by default) and click "OK". The new image is automatically added as a new tab in the main screen. The software automatically alters the brightness and contrast for the newly created image, which sometimes causes the stacked image to appear darker than the live image. Open the "Properties" menu for the image to restore the brightness and contrast to the original values that have been saved in the "Live Properties" menu before.

Scale Bar. This enables the addition of a scale bar into the image. The size of the scale bar may vary with the size of specimens, but it should be used consistently within a series of photos. Dorsal overviews can for instance use a 1mm or 2mm scale, close ups generally use 0.5mm scale bars. In the dorsal overview photo, the scale bar should preferably not overlap the specimen, unless the legs cannot be avoided. In addition to that, the scale bar should be placed in the lower-left corner, with its starting point equidistant from the side and bottom of the image.

Burn Image. This is an *optional* step to burn any annotations, such as the scale bar.

Save. This opens a new window in which the image can be saved. If the scale bar has not been burned into the image with the previous button in the "workflow", make sure to check the box "Burn in Annotations" in this screen. Select the correct folder and enter a name for the specimen (the barcode scanner can be used to scan the label that is with the specimen). Use the TIFF image format.

Finishing up

- Return processed specimens to their containers. Clean up and store all the used additional equipment.
- Exit AxioVision and wait for the software to shut down. Switch off the microscope by using the "Shut down" option on the joystick or the blue button on the EMS-2 power unit.
- The lights will automatically switch off, but be sure to flip the switches on their power units back in the "off" position. Place the nylon cover over the microscope.

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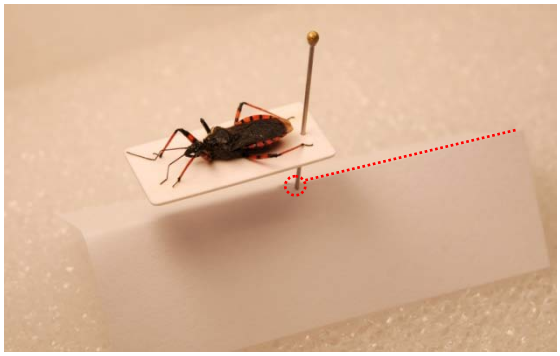
TIPS AND TRICKS

Hiding labels when working with pinned material (ZEISS)

- If pinned material contains labels that are visible on the image while using the ZEISS stacking microscope, instead of taking of all the labels, hide them by using a small piece of white (or other preferred color) paper.
- Take a (small) rectangular piece of paper and fold it in half. Cut along the fold until about halfway.
- Slide the cut around the pin, thereby positioning the paper in-between the specimen and the labels.
- With a small hole at the end of the cut you'll avoid creating a wedge if the paper is positioned around the pin. Make sure the hole is around the pin (figure A, red lines indicate cut).



(A) hiding the labels on a pinned specimen

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ODONATA

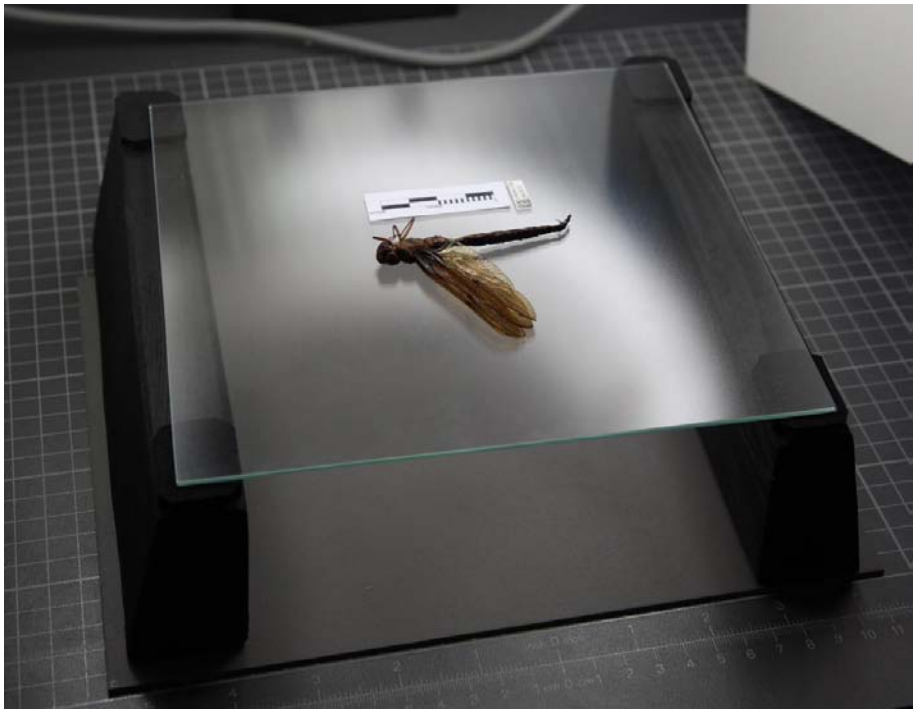
All specimens need to be photographed in landscape format, in which the specimen needs to fill the image as much as possible. Due to the method of preservation of dried specimens, in which the head is rotated 90°, the orientation of the specimen on the photo depends on the orientation of the head. The specimen should be photographed as a lateral view, in such way that the dorsal side of the head is visible (so the specimen can either be facing left or right).

Working with dried material

- The specimen is stored in a separate, smaller envelope within the sample envelope. The RMNH label is placed in the main sample envelope, not in the smaller one which contains the sample.
- Use insect tweezers to handle specimens, and pick them up by their wings.
- Do NOT blow away dust while the specimen is positioned under the NIKON setup, this may cause damage to the fragile specimens!

Working with alcohol material

- The specimens are stored in glass vials or eppendorf tubes.
- Use insect tweezers to take specimen out of the container and let them dry shortly before photography to avoid the formation of wet stains on the glass plate.



(A) overview of the NIKON setup

Pictures to be taken: NIKON

- A 70% grey card (KODAK) is used as a background. To get a uniform background, and to eliminate shadows around the specimen, a glass plate is used between the specimen and the background. Two supports are used to create a distance of approximately 5cm between background and glass plate.
- A 20 or 40mm scale (depending on the specimen size) should be placed at the left side of the image, with the RMNH label underneath it.
- Set the aperture on **f/13** using the onscreen menu ("Exposure 1" tab) when the camera is connected to the computer and switched on. The focus point can be moved up/down and left/right ("Exposure 2" tab), make sure it is located on the specimen (preferably on the wings or abdomen).
- One photo per specimen, with the dorsal side of its head visible (figures B and C).

(B) sample image using the NIKON setup

(C) alternate sample image using the NIKON setup

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LEPIDOPTERA

All specimens need to be photographed in the same orientation, a dorsal view in landscape format, in which the specimen needs to fill the image as much as possible (without leaving out the antennae).

Working with pinned material

- Remove all the labels from the pin (place them back after photography, using the same holes).
- Do NOT blow away dust, this may cause damage to the fragile specimens!
- Avoid movement near the specimen whilst taking photos (especially in the ZEISS setup).

Pictures to be taken: ZEISS (specimens <2cm)

- Only the ring light is used, without additional spotlights. The light source needs to be positioned at the front of the microscope (i.e. the tube that connects to the power source needs to be positioned towards the user).
- Make use of a box with foam and a grey background. The pin with the specimen needs to be stuck into a hole in the grey card (use the same hole every time to prevent holes from being visible in the background of the photos).
- A gamma-value of approximately **0.70** provides the most accurate colour representation.
- For every specimen a dorsal overview, with the anterior side of the specimen facing to the top (figure B). The pin does not need to be in focus.

Pictures to be taken: NIKON (specimens >2cm)

- A 70% grey card (KODAK) is used as a background. To get a uniform background, and to eliminate shadows around the specimen, a glass plate is used between the specimen and the background. Two supports are used to create a distance of approximately 5cm between background and glass plate.
- Small pieces of PRITT POSTER BUDDY are used to place the pin onto the glass plate. The size of these buddies should preferably not be visible on the final photo, so make them as small as possible.
- A special scale on two pins is available to use in the NIKON setup. The height of the scale can be adjusted to ensure it is in focus with the specimen. Next to the scale bar, there is room to place the corresponding RMNH label. This scale will also have to be placed onto the glass plate using buddies.
- The scale can either be placed on the right side of the image or on the bottom (what is best depends on the size and shape of the specimens), but the placement should be consistent throughout a series of photos (figures C and D).
- Set the aperture on **f/20** using the onscreen menu (“Exposure 1” tab) when the camera is connected to the computer and switched on. The focus point can be moved up/down and left/right (“Exposure 2” tab), make sure it is located on the specimen.
- For every specimen a dorsal overview, with the anterior side of the specimen facing to the top. The pin does not need to be in focus. Multiple photos may be taken by moving the focal point, if various important features of the specimen are not in focus in the overview.

(A) overview of the NIKON setup

(B) sample image using the ZEISS setup

(C) sample image using the NIKON setup

(D) alternative placing of the scalebar

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LEPIDOPTERA: NEPTICULIDAE

All specimens need to be photographed in the same orientation, a dorsal view in landscape format, in which the specimen needs to fill the image as much as possible (without leaving out the antennae).

Working with pinned material

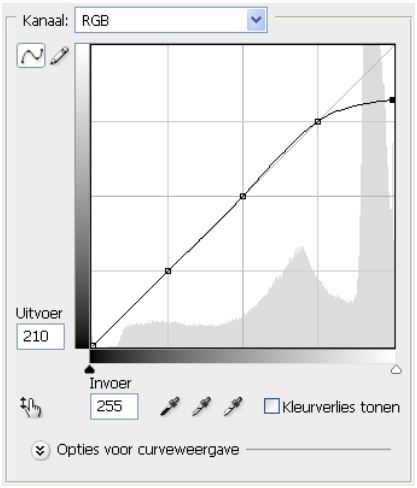
- Remove all the labels from the pin (place them back after photography, using the same holes).
- Do NOT blow away dust, this may cause damage to the fragile specimens!
- Avoid movement near the specimen whilst taking photos.

Pictures to be taken: ZEISS (specimens <2cm)

- The ring light (20%) is used in combination with the spotlights (65%), where the lighting is used to let the foam block (on which the specimen is pinned) blend in with the background as much as possible. To sort this effect, position the spotlights in such way that they illuminate the subject from above (not too sharp an angle).
- A white piece of foam is used as a background, without any additional boxes around it.
- A gamma-value of approximately **0.70** provides the most accurate colour representation.
- For every specimen a dorsal overview, with the anterior side of the specimen facing to the top.
- The background may be overexposed.

Editing the image in Photoshop

- "Afbeelding" > "Aanpassingen" > "Niveaus"
- "Afbeelding" > "Aanpassingen" > "Curven"
- "Afbeelding" > "Aanpassingen" > "Belichting"
- "Afbeelding" > "Afbeeldingsgrootte" > 1440px
- "Filter" > "Verscherpen" > "Scherper"
- "Afbeelding" > "Afbeeldingsgrootte" > 800px
- "Bestand" > "Opslaan als..." > JPG, kwaliteit 10



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COLEOPTERA

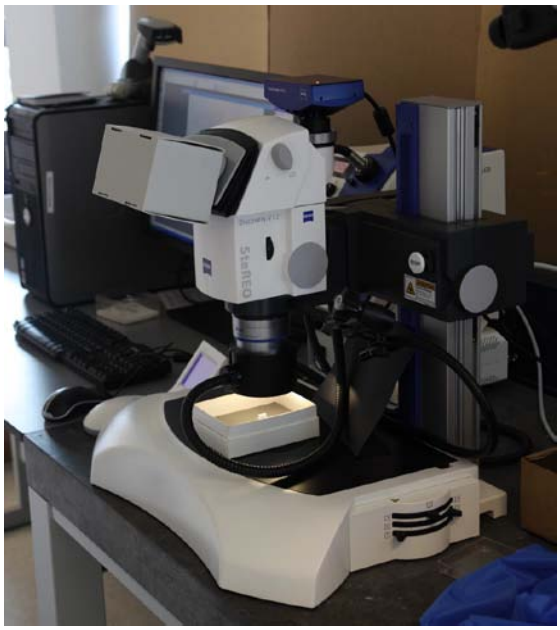
All specimens need to be photographed in the same orientation, a dorsal view in landscape format with the anterior side of the specimen facing left, in which the specimen needs to fill the image as much as possible.

Working with pinned material

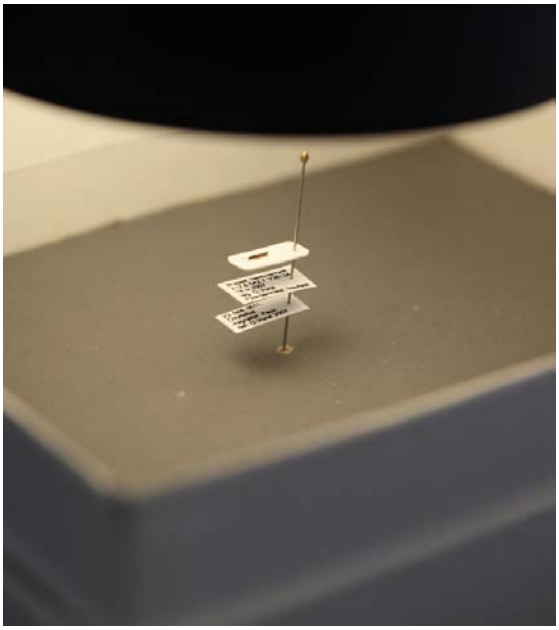
- Remove all the labels from the pin (place them back after photography, using the same holes).
- Do NOT blow away dust, this may cause damage to the fragile specimens!
- Avoid movement near the specimen whilst taking photos (especially in the ZEISS setup).

Working with glued material

- Specimens are glued to a white card that is attached to a pin in combination with its labels.
- Glued specimens have to be photographed on their card (figure B)



(A) overview of the ZEISS setup



(B) close-up of the specimen

Pictures to be taken: ZEISS (specimens <2cm)

- Only the ring light is used, without additional spotlights. The light source needs to be positioned at the front of the microscope (i.e. the tube that connects to the power source needs to be positioned towards the user).
- Make use of a box with foam and a grey background. The pin with the specimen needs to be stuck into a hole in the grey card (use the same hole every time to prevent holes from being visible in the background of the photos).
- A gamma-value of approximately **0.70** provides the most accurate colour representation.
- For every specimen a dorsal overview, with the anterior side of the specimen facing to the top (figures C and D). The pin is preferably out of view, and does not have to be in focus when included.

(C) pinned specimen under the ZEISS setup

(D) glued specimen under the ZEISS setup

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BRYOPHYTES

Specimens are highly heterogeneous in size and shape. Therefore, no specific orientation of the plants is used, but leaf shape and habit should be visible (e.g. a photo of one branch of circa 1cm). The specimen needs to fill the image as much as possible, without leaving out the apex. Ideally, the specimen leaves some margins surrounding it (white background), so that in stacking the image, no parts are left out of focus.

Working with herbarium material

- Use the main specimens in the envelope, not the included subsample with the collection number.
- Only the Zeiss setup is used, because leaves and habit of the plant are usually very clear under a binocular microscope. For larger plants, only apical parts should be photographed.
- Mixtures of species in the photo should be avoided. If the collection consists of several species, try to isolate or zoom in onto the relevant one.

Pictures to be taken: ZEISS

- Only the ring-light is used, without additional spotlights.
- Make use of a box with a white background (a piece of paper).
- Always check the white balance using the empty box with white background, for correct colour representation. See the general Zeiss protocol.
- All specimens, even relatively flat ones, are photographed using the "Z-stack" function in "Multidimensional Acquisition". Afterwards, we use "Extended Focus", "Scalebar" and "Save", as described in the general Zeiss protocol. The filename of the TIFF files is the collection number, for example L0873777.TIFF.

(A) sample image using the ZEISS setup

ARACHNIDA

All specimens need to be photographed in the same orientation, with different detail shots for male and female specimens (two photos per specimen). Male specimens can be recognized by the large bulbs at the end of its pedipalps (centre photo). Larger specimens have to be positioned in such way that the body (from chelicerae or eyes to posterior tip of abdomen and spinnerets) is in frame, the legs are of lesser importance. See figure D for anatomical features of an arachnid.

Working with alcohol material

- Specimens preserved in alcohol will have to be photographed submerged into alcohol. Prepare a glass petri dish with a layer of white sand and fill it with a layer of alcohol of the same percentage. Place the specimen in the petri dish in such way that it is submerged into the alcohol. Make use of the sand to stabilize the specimen by gently pressing it into the layer of sand, without covering the body of the specimen with grains of sand.
- Use a plastic tray in which the jar with samples can be placed, to avoid spilling alcohol over the workstation. Make sure all the different tools (various tweezers) are present. Also have a flask of alcohol at hand (for topping of the sample vials before closing).
- Think of a system to process the samples in an orderly fashion. For example, when there is a jar with specimens individually preserved in glass vials, take out all the vials at before starting and place them back into the jar once they have been photographed.
- A gamma-value of approximately **0.50** provides the most accurate colour representation.

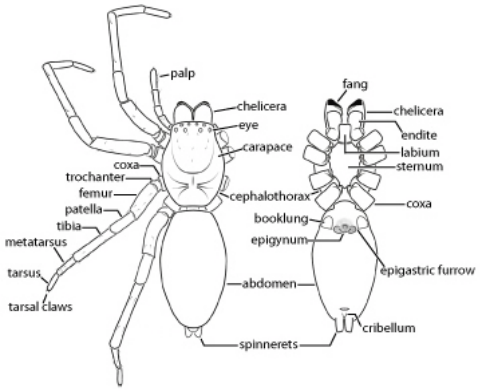
Pictures to be taken: ZEISS

- For every specimen a dorsal overview, with the anterior side of the specimen facing left (figure A).
- For male specimens a lateral view of the pedipalp, with the hairy and non-hairy part both visible, again with the anterior side of the specimen facing left (figure B).
- For female specimens a ventral view of the epigynum (entrance of the reproductive tract, genitalia), again with the anterior side of the specimen facing left (figure C).

(A) dorsal overview

(B) lateral view of the pedipalp (♂)

(C) ventral view of the epigynum (♀)



(D) anatomical features of arachnida

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PORIFERA

All specimens can to be photographed in an orientation that fits best. Porifera do not have “sides”, so the actual position under the camera is not as relevant as in other groups.

Working with alcohol material

- Specimens will be photographed using the NIKON setup, these specimens will have to be removed from the alcohol and dried to the extent that no puddles will form around the specimen. Due to the light colours of most alcohol-preserved sponges, a black background is used.

Pictures to be taken: NIKON

- For every specimen an overview, including a label.
- The label needs to be placed at the side of the image in such way that it can be cropped off, or an additional photo without label should be taken.
- Depending on the size of the specimen, use a 2, 4 or 10cm scale, which should preferably be positioned below the specimen on the photograph.
- Set the aperture on **f/20** or **f/30** using the onscreen menu (“Exposure 1” tab) when the camera is connected to the computer and switched on. The focus point can be moved up/down and left/right (“Exposure 2” tab), make sure it is located on the specimen.

(A) sample image using the NIKON setup