Enhancing Microscope Specimen Images

A guide for digitally editing images for online identification guides

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This guide was developed specifically for LinEpig, an online reference for spiders in the linyphiid subfamily Erigoninae. However, similar practices may prove useful in imaging other small arthropods.

Imaging systems. In recent years, the Field Museum and other institutions have increasingly moved to more-sophisticated imaging systems, such as the Leica Application Suite (leica-microsystems.com), which create a stack of focal slices and then auto-composite them into a montage, using software to determine the depth and focus of different parts of the image.

Compositing tools allow you to produce a much better image, with more of it in focus. In some cases, one system is used to generate the image stack and a separate application – such as Helicon Focus (heliconsoft.com) or earlier tools like CombineZ (combinezm.en.lo4d.com) – to create the montage. Either way, the software can sometimes make poor "guesses" that generate artifacts or miss elements of detail. Missing or obscured detail can generally be repaired in the compositing interface, by selecting and applying a "patch" from the slice where it was in focus.

Digital editing tools, such as Photoshop, are used to sharpen the features of an image and improve its overall clarity to help users see structures. Compositing artifacts that lie outside of the actual subject (such as a "fractal halo" effect) are typically easier to fix in Photoshop than in the compositor. We also use Photoshop to sharpen the image, adjust the lighting, correct the color and/or clean up distracting elements as needed.The earlier images in LinEpig were single-shot jpegs produced with an eyepiece-mounted camera attachment; some images contributed by other researchers continue to be of this sort. These photos can often also benefit from digital editing to enhance weak focus or correct over- or under-exposure; such adjustments are relatively simple.

Types of images. Images used to identify arthropod specimens to species generally focus on the genitalia (in spiders, the palps and epigyna) and may also include pictures of the whole animal, or "habitus," or of particular characteristic features. These subject categories call for somewhat different cleanup techniques, as they often have been produced using different lighting setups, magnification, and focal points. A habitus image may need fewer color and value adjustments than one focusing on genitalia. Genital images must clearly show the structure, which generally means more-nuanced color and value adjustments than for the habitus.

Contents:

# I.   General Rules of Thumb

- **ALWAYS make a copy of the image** you are editing. Do not transform the only version of the image you have available. It helps to organize copies and originals into separate folders so you never accidentally edit an original.
- **Only edit out artifacts that can be seen at 100% magnification**. Zooming in with the magnifying glass (press Ctrl+ to zoom in and Ctrl- to zoom out) will reveal artifacts galore. The magnification percent will be displayed beside the bottom scrollbar and will read 100% when the image's resolution matches the monitor's.
- **Only edit out artifacts if you know what lies below**. It is better to leave an artifact than reconstruct a structure incorrectly. With palps, a different view (ectal, mesal, ventral) can help distinguish the lines of an obstructed structure.
- **It is okay to delete a taxonomically unimportant part of the image** (a single leg, for example) marred too severely by artifacts.

General Procedure

1. Crop the image close to the subject, minimizing background. Note that the scale bars for habitus images *must* be in before cropping (this is done in Leica editing).
2. To ensure accuracy, **duplicate the Background layer** and keep the copy selected so you can toggle your changes on and off.

Uncheck the box with the eye icon to see the original image:
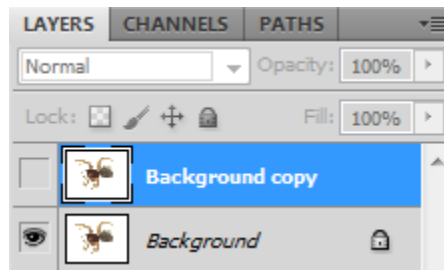
3. Adjust **Levels** (Ctrl+L), **Hue/Saturation** (Ctrl+U), and/or **Brightness/Contrast** (Image>Adjustments).

   The Levels window uses a histogram to visualize the tonal range of an image. The sliders (from left to right) represent the image's black point, midtones, and white point.
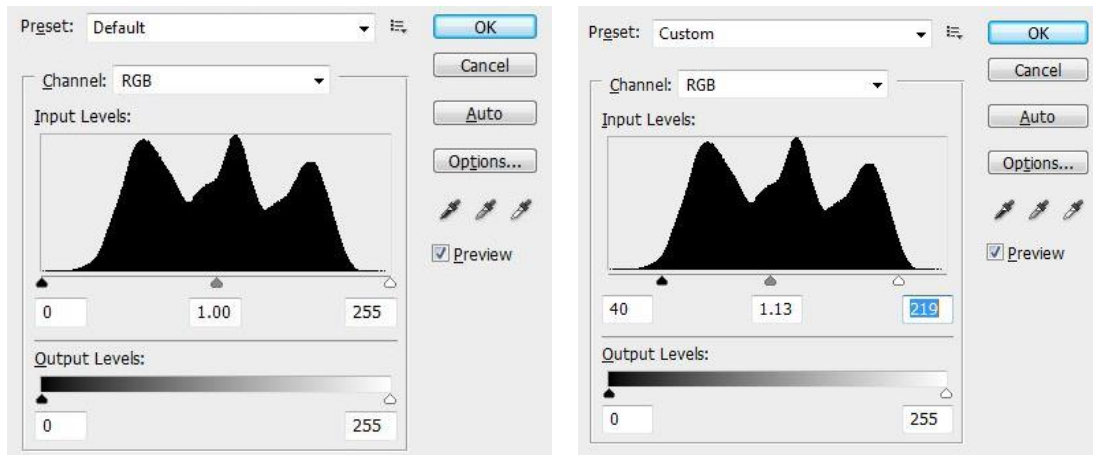
Adjust Levels (Ctrl+L) until internal structures are apparent by dragging the black point slider rightwards and the white point leftwards. Mess around with the midpoint and see what looks best. Do not black out or white out any details on the specimen.

Hue/Saturation (Ctrl+U) has a dropdown with the different color channels. Use this window to remedy images inappropriately dominated by a certain color, for example: cyan. Go to the cyan channel and desaturate, lighten, or darken the cyan values until the image is legible – do not overdo it and black/white-out any details of the specimen.
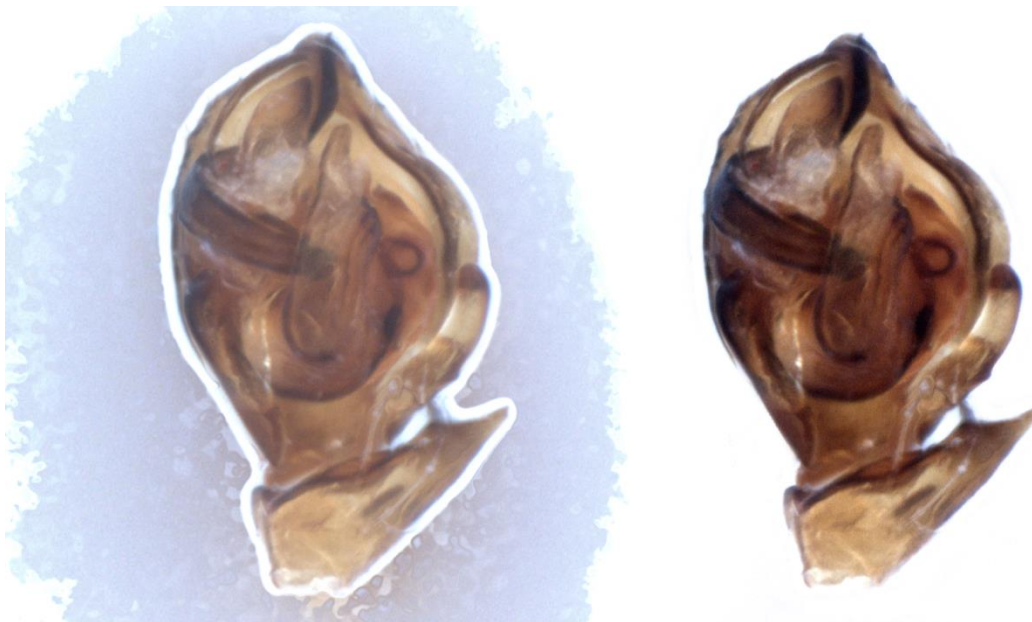
The image above underwent lightening of the cyan channel in the Hue/Saturation window and adjustments to the black and white points in the Levels window.

When you are satisfied with the result, **merge the two layers by right clicking the top layer and selecting "merge down" and then duplicate the layer left over**. This makes comparison between your edits and the original easier without the distracting color difference.

II. Habitus Workflow

1. **White-out the Background**
   - Make a new layer and use the polygon lasso (click and hold lasso icon for options) around the spider so that its setae – the stiff bristle-like hairs– are included in the selection. Presence or absence of hairs and bristles can be important to identification.
   - Invert the selection (Ctrl+Shift+I), hold Shift and click to lasso any interior areas missed in the first selection.
   - Use the eye dropper (Alt+left click while using Brush tool) to pick a color from the inner halo surrounding the spider – usually white or a light neutral color. Paint bucket (G) the selected area to cover up background artifacts. (Note: G defaults to the gradient tool's spot in the tool box. Press and hold the gradient tool icon to find the paint bucket option.)
   - Deselect (Ctrl+D). Zoom in until you can comfortably use the brush tool to color over the remaining artifacts and in between the hairs.

The black line in the right figure represents the selection made by the polygonal lasso tool.

Note: ***Do not use the magic select tool*** *because A) you will have to select varying values separately, and B) the magic wand often ignores setae, leaving you with the job of manually erasing each individual bristle from your white-out layer. Selecting around the hairs may feel tedious but it is nowhere near as laborious as making a poor selection and having to clean up around them stroke by stroke with the brush tool or eraser.*

Now you may the **edit artifacts and imperfections as you encounter them**.

III. Palp Workflow

Open all views of the same palp (ectal, mesal, ventral, etc.) to easily compare structures from different angles and differentiate them from dirt and artifacts.

1. **Color and Value Balance**

Before and after color and value editing.

Palp images seem to be more susceptible to color domination. Open up the Hue/Saturation (Ctrl+U) window first.

- Check and see if you can eliminate artifacts from various color channels by moving the Lightness and Saturation sliders, making sure to revert unproblematic channels to their original state.
- Adjust Levels (Ctrl+L) until internal structures are apparent, as described in *General Rules of Thumb*.
- Make your top layer invisible and compare your edits to the original image. It should be clearer. Are your edits detrimental to the image's legibility? Hit Ctrl+Z (or Ctrl+Alt+Z if Photoshop rather than Elements) and try again.

2. **Background**
   Ideally, the adjustments made in Step 1 will have helped with any unwanted background artifacts. If the background is still problematic…
   - Crop the image around the palp so you don't end up cleaning extra areas.
     - ➢ **If the background has many artifacts:** make a new layer and try selecting around the specimen using the lasso tool, inverting the selection (Shift+Ctrl+I) and filling it in with the paint bucket tool (G). Deselect (Ctrl+D) and use the

brush tool to remove artifacts between hairs. *Do not use the magic wand tool, as it ignores setae and creates a more tedious workflow in the long run.*

> ➢ **If the artifacts in the background are negligible**: use the brush tool, hold alt and click to sample a color, and cover as needed. Then use the brush tool to define individual bristles.

3. **Edit artifacts as encountered.** The most frequent offenders in Palp images are droplet artifacts, though every once in a while you'll get something obstructing a structure. ONLY ATTEMPT TO FIX THIS IF YOU ARE SURE OF THE SHAPE OF THE STRUCTURE. Duplicate the work layer before attempting such a feat.

IV. Epigynum Workflow

Spider epigyna are often dissected for imaging and identification, but because those of Erigoninae are relatively flat, the epigyna in LinEpig are generally photographed *in situ*. This means there is little to help the compositing software differentiate the structures from the background.

To compensate for this, place light sources on either side of the imaging arena when photographing. Side lighting creates harsher shadows, which helps the camera read depth more precisely. Diffuse the harsh light by placing a plain-paper loop around the specimen, separating it from the. This technique yields more clarity than a dome light and/or an acetate diffuser. Bringing the image slices into Helicon for compositing will improve the quality of the montage over the native Leica compositor.

Left: dome light, Leica
Center: side lights with paper diffuser, Leica
Right: side lights with paper diffuser, Helicon

1. **Adjust levels (L)** as descibed in *I. General Rules of Thumb* to give structures maximum definition.
2. Use the spot healing brush tool (J) to **remove any distracting droplet artifacts**.
3. Use the clone stamp tool (S) to **remove obstructions when nothing important lies underneath**.

V. Artifact Index

Fractal Halos

One dramatic type of artifact that is common with subjects placed against a background, such as habitus and palp images, are a kind of fractal halo surrounding the subject. **They can be easily fixed with the polygonal lasso (L), the paint bucket (G), and brush tool (B).** See *II. Habitus* workflow for more detailed instructions.

Hard pixelated edges

Abrupt changes formed by imperfect compilation create hard, pixelated edges. **These can be softened** (anti-aliased) **with the blur tool.**
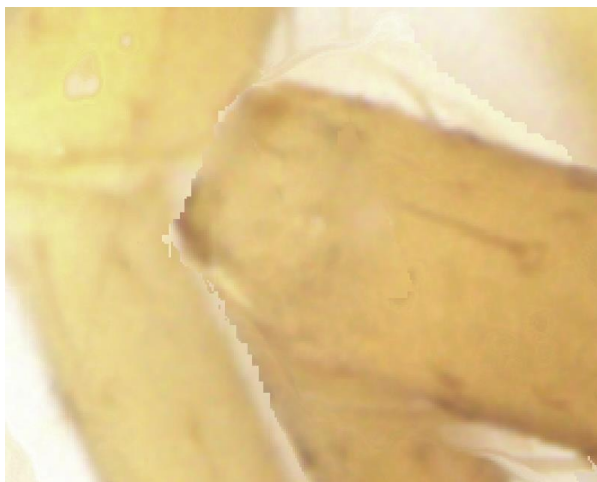
Droplets

A common type of artifact looks like droplets of liquid pressed between transparent pieces of plastic. This is the most common artifact you will encounter using the Leica compositing software. On close inspection, these droplets are made up of concentric shapes with many colors sampled from the nearby area. Some droplet artifacts are very close to the colors of the ground they sit on; it is up to you to omit them or leave them depending on how distracting they are. **Higher contrast droplet artifacts should be removed with the spot healing brush tool (J) OR by sampling either side with the clone stamp tool (S).**


Many arthropods have bumps on their bodies. While bumps may seem difficult to distinguish from droplet artifacts, droplets can be easily identified on very close inspection. Zoom in until you can begin to see the pixels in the image. Droplets and other artificial blobs will have a higher resolution than morphological bumps. Morphological bumps will always have the grain quality of the rest of the image, while artificial droplets appear to sit on the surface of an image. Their shape conforms to the pixel grid in which they exist. In the figure below, red arrows point to artifacts while the green arrow points to a morphological bump that should be left alone.
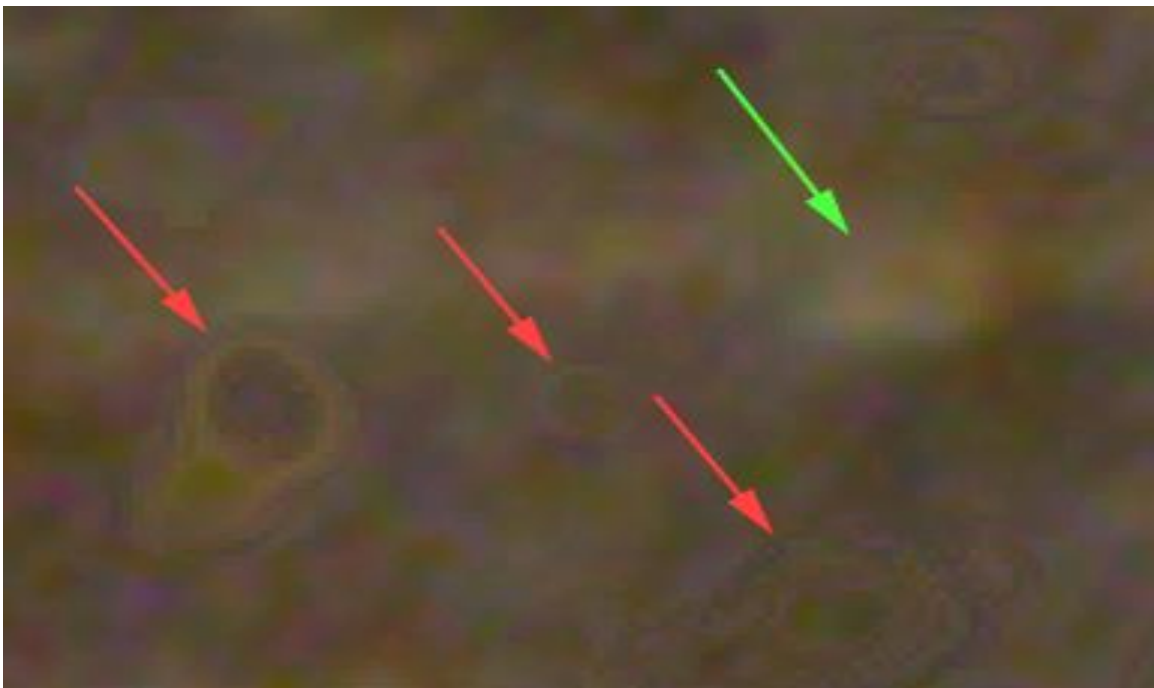
Wet Interference

In areas where one object overlaps another, a wet-looking border may interfere with the representation of form. This effect is especially common when the upper object has delicate, protruding elements, such as hairs. The object on top will appear to have a watery barrier between itself and the object below it. While this should have been corrected in composite editing, it can still be fixed in Photoshop.
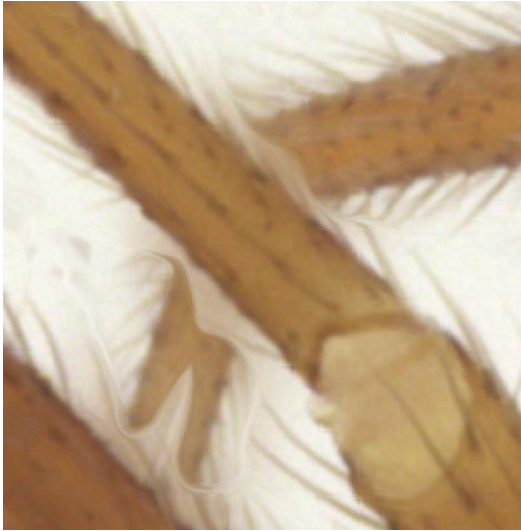
To fix wet interference, **remove the barrier between the objects with the clone stamp tool, sample the limb just before the interference, and continue the structure to close the gap**. Allow a small line to form between the overlapping parts to ensure their visual separation.

If the form of the object below the wet interference is unnecessary, consider omitting it instead of reconstructing it.

If you are uncertain about the form of the structure obscured by the wet interference, do not try to reconstruct it by guessing. Clean up the area around it but leave the structure alone.

Apparent wetness can interfere with the representation of hairs in an image. If a hair looks as if it's been warped or smudged, it's likely due to wet interference. First reconstruct the setae, assume they're stiff and relatively straight. Then remove the remaining border of concentrated color surrounding the problem area.
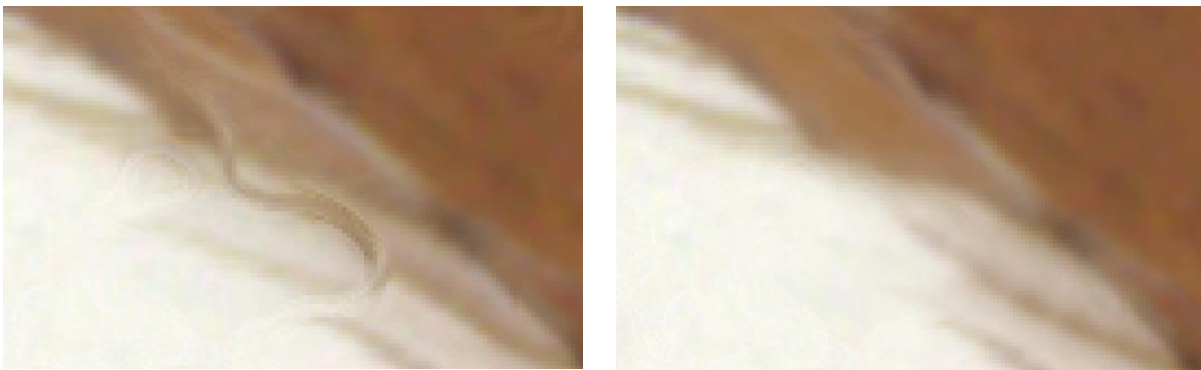
VI. Tool Index

The following is a list of the Photoshop tools and their keyboard shortcuts that are referenced in this guide. If the keyboard shortcut does not activate the proper tool, click and hold the tool selected on the tool box for a menu of tools stacked beneath it. Hover over the option for a description and release the mouse button to select it.

Use the left and right [bracket] keys to change the cursor size when applicable.

Crop tool (C)

Cropping allows you to manually change the size and aspect ratio of an image, and is a very effective way of focusing a viewer's attention. Drag a box around the portion of the image you wish to keep. Grab the center of an edge to change one dimension, grab the corner to change both dimensions. Unless you uncheck Delete Cropped Pixels, the data you crop will be deleted, so be sure you're keeping all the information you need before pressing Enter to apply the crop.

Blur Tool

The blur tool softens color transitions in an image. Keep the cursor the same size as the narrowest point of the hard edge you wish to soften. Use the blur tool to soften pixelated, and/or hard edges.

Polygonal Lasso (L)

Selecting parts of an image limits the effects of your influence to a specified area. The polygonal lasso tool allows you to make a selection without having to hold a left click throughout. Left click to begin a selection, follow the perimeter of the object you're selecting, left clicking to plot vertices. Hit the backspace key to undo the last vertex. Click the first point to complete the selection. The perimeter should look like marching ants when the selection is completed. When you are done editing the selection, hit Ctrl+D to deselect so you can continue to edit the image as a whole. This tool is useful for whiting out backgrounds.

Paint Bucket (G)

The paint bucket tool fills a selected area with color. If you paint bucket your selection and are unhappy with its hard edge, go to Select>Modify>Feather. Feathering works like the blur tool but instead of editing preexisting hard edges, you're preemptively softening the transition between your selection and the original image. This tool is useful for whiting out backgrounds.

Brush tool (B)

The brush tool changes the color of pixels within your cursor. This is most useful in cleaning around a newly paint-bucketed background and separating hairs. The color can be sampled from the image using the Eyedropper tool (Alt+left click while using the brush or paint bucket tools), and used to cover up distracting artifacts in the background. Do not use the brush tool on the specimen itself, as it lays down

flat color and obliterates textural details. Use the clone stamp tool (S) to reconstruct parts of the specimen to ensure tonal accuracy.

Clone Stamp tool (S)

The clone stamp tool allows you to duplicate a sampled area elsewhere on an image. Sample, or choose a reference point, by holding Alt and left clicking. Then move your cursor to the problem area and cover it with the sampled data by left clicking and dragging as you would the brush tool. When using the clone stamp tool (S), do not sample the same data over and over again. This can create a pattern and in extreme cases it can leave the ground streaky and blurry. Be aware of the sample diameter so that you do not include the unwanted element in the sample and end up simply moving it around or repeating it.

Left: Unedited artifact
Middle: Repetition caused by stamping too close to the sample source.
Right: Streak formed by clicking too often and too close to the sample source.

Spot Healing Brush tool (J)

The spot healing brush tool does not require manual sampling; it simply averages the ground around it. With droplet artifacts surrounded by unblemished ground, use the spot healing brush tool (J) before bothering with the clone stamp tool. If the spot healing brush tool produces an undesired effect, undo (Ctrl+Z), and try the clone stamp tool (S).

Additional Resources

How to Use Adobe Photoshop Tools
https://www.wikihow.com/Use-Adobe-Photoshop-Tools

Retouch and repair photos
https://helpx.adobe.com/photoshop/using/retouching-repairing-images.html

Photoshop Elements User Guide
https://helpx.adobe.com/photoshop-elements/user-guide.html

Leica Application Suite manual
http://users.stlcc.edu/Departments/fvbio/Microscope_LeicaDMI_LAS_Help_Manual.pdf

Helicon Focus manual
https://www.heliconsoft.com/focus/help/english/HeliconFocus.pdf

iDigBio Digitization Resources
https://www.idigbio.org/wiki/index.php/Digitization_Resources