

Imaging of animal communities protocol v1.1

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IMAGING VIDEO: https://drive.google.com/file/d/1wa7FPBE-f3HOe3LUdNsRIKvXr2At_BxW/view?usp=sharing

Imaging has to be done within one month after the extraction to standardize coloration of animals. All animals from each sample are imaged together in 96% ethanol in one Petri dish, 5 photographs per sampling point: nematodes, enchytraeids, mesofauna, macrofauna and earthworms separately (25 photographs per site). All photographs are submitted to the **Central team**, which extracts abundance and individual body masses. This protocol has been developed based on our experience with the scanner EPSON Perfection V600 connected to a PC (the PC software has more settings than the one for Mac). Alternative: a scanner which allows for 3200dpi+ and has a CCD image sensor.

Differences between groups:

- Macrofauna and earthworms: plastic Petri dish ø 9 cm, 3200 dpi, white background
- Microarthropods: plastic Petri dish ø 9 cm, 3200 dpi, white background
- Nematoda and enchytraeids: plastic Petri dish ø 3.5 cm, 4800 dpi, black background (the white background panel is removed)

Key settings:

- Mode: **Professional**
- Export format: **JPEG**
- Document Type: **Reflective**
- Document Source: **Document Table**
- Auto Exposure Type: **Document**
- Image Type: **48-bit color**
- Resolution: **Nematoda and enchytraeids: 4800 dpi; Other: 3200 dpi**
- Unsharp Mask: **on**

Instructions:

1. Turn on the scanner and the computer
2. Shake the vial with animals and ethanol and pour all animals into a Petri dish
 - a. Ensure to transfer all animals by flushing the vial
 - b. All animals need to be completely covered by ethanol; do not put on the lid
 - c. Use transparent and thin Petri dish without scratches; avoid any bubbles
3. Gently shake the dish back and forth or use tweezers to distribute animals - try to avoid overlap among animals and other objects as much as possible
4. Put the dish into the scanner (in the center). Wait for ~40s so animals sediment.
5. Scanning process
 - a. Create scanning preview and choose scanning frame perfectly fitting the Petri dish outer contour; press "Zoom"
 - b. Press "Scan", choose picture format JPEG (*.jpg), select the correct folder.
 - c. Name the file according to the **sample code on the label**.
6. Wait until the scanning process is finished ****In case multiple scans are taken from the same sample, proceed with step 3.*
7. While the scanning process is running, prepare the next sample.
8. Check the image, put the next dish in the scanner, put the old sample back.