

# Micro-core processing – A time and efficient protocol

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*Background* - Due to their small size, micro cores taken with a Trephor (Rossi *et al.*, 2006) of 2 mm diameter have to be glued on wooden holders before they can be clamped to the microtome for thin sectioning. However, during the hardening of the glue, the sample is drying and thus shrinking (Bodig and Jayne, 1993). This can cause cracks in the sample, which will make the samples unusable for further preparation. Alternatively, samples can be embedded in paraffin or PEG (Rossi *et al.*, 2006; Čufar *et al.*, 2008). Although embedding of samples provides excellent results, it is a very time-consuming procedure. Therefore, we developed a simple protocol for efficient processing of micro cores.

You need

- Wooden micro-core holder
  - Pattex Repair Gel®
  - Forceps
  - Sliding microtome (e.g. WSL, Switzerland)
  - Brush
  - Jar with 50% ethanol
  - Object holder
  - Stain (depending on your purpose, for example an alloy 150 mg astrablue, 40 mg safranin and 2 ml acetic acid in 100 ml distilled water; van der Werf *et al.* 2007)
  - Pipettes (for the different liquids)
  - Waste jar
  - Distilled water
  - Glycerin
  - Cover slips
  - Tissue
1. After sampling, the micro core has to be stored in 50% ethanol in order to prevent the sample from drying out and from being infested with fungi. In this solution, the micro core can be stored in the fridge at 5°C for a long time.
  2. For processing, let the sample dry on a tissue for circa half a minute. This enables the glue to better attach to the wood. In the meantime, put some Pattex Repair Gel® on the holder<sup>1</sup>.
  3. Use the forceps to mount the sample on the holder with the glue. Take care that you mount the sample with the fibres perpendicular to the holder in order to attain good thin sections.

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<sup>1</sup> Pattex Repair Gel® proved to be most suitable for mounting micro-cores on wooden holders due to its advantageous characteristics (extra strong, waterproof, gap filling and precise grip). This glue was tested against several other types of glue (like Pattex Uni Rapid Superglue®, Bison Kombi Epoxy Glue® and Ponal Wood Glue®) and led by far to the best results.

4. Push the sample with the forceps into the notch of the holder and cover the sample completely with glue. This will prevent a fast drying of the sample and thus the formation of cracks.
5. Let the glue harden for 24 hours.
6. Clamp the sample with the wooden holder into the microtome. Take care that you clamp the sample in such a way that you can cut towards the pith. This will prevent the bark from breaking-off during cutting.
7. Brush the knife and the sample with water or 50% ethanol (ethanol hardens the sample surface and thus prevents the thin section from tearing).
8. Let the sample approach the knife and cut several times through the glue until you reach the micro core. Depending on the species, chose a thickness between 5 and 15  $\mu\text{m}$ . Brush the sample with water or 50% ethanol before every cut. Place the brush on the thin section to prevent the cut thin section from curling. Sometimes the glue around the thin section stays attached to the glue around the sample. Therefore, you regularly have to remove superfluous glue around the sample with an additional knife.
9. Use the brush to put the thin section on an object holder and use water to keep it wet.
10. Wash the thin section with distilled water in order to remove any impurity. You can hold the thin section on the object holder with the pipette and pump the water through. Let the superfluous water flow in the waste jar.
11. Use another pipette to stain the thin section with the i.e., mixture of safranin and astrablue on the object holder. Use sufficient stain and let the thin section “float” in the drop for c. 10 minutes.
12. Let the superfluous stain flow in the waste jar and wash your thin sections with distilled water (see 10.)
13. Use a tissue to dry the object holder around the thin section .
14. Give a few (often 1 is enough) droplets of glycerine on the thin section and cover it with a cover slip. Take care that you do not include any air bubbles.
15. Clean the object holder from superfluous glycerin with a tissue.

### References

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