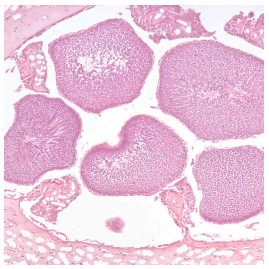
Apr 10, 2024

Histological processing of octocoral tissue



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We use this protocol and it's working

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Disclaimer

We have put great effort to include all relevant information and warnings, however the reader should make their own evaluation as to the appropriateness of any experimental technique for their objectives. Histological processing requires handling of toxic and corrosive substances, therefore the reader should take all essential precautionary measures. This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein

Abstract

In recent years, there has been a significant focus on coral habitats, encompassing both shallow and deep ecosystems. Historically, most studies concentrated on scleractinian corals, however contemporary research acknowledges the ecological importance of octocoral species, and the need to improve our understanding of their biology. Histology, which describes the internal morphology of animal tissues, is an essential tool for a wide range of biological studies, including reproductive biology, life history, taxonomy and phylogeny. Processing octocoral tissue histologically can be a complex task, given the unique skeletal structure of these organisms. Octocorals have internal skeletal structures called sclerites, which are composed of calcium carbonate and are embedded into the coral tissue. Decalcification of these structures creates air pockets in the tissue that hault further histological processing. Moreover, many octocorals possess a skeletal axis that is predominantly composed of organic matter, but may contain varying degrees of calcium carbonate. Including the axis in histological analysis may require experimentation to adjust the processing times. Although several studies report processing times for histological analysis, it is difficult to find detailed information on how to process octocoral tissue. Here, we present a detailed protocol that separates octocoral polyps from the skeletal axis, and utilizes vacuum to address issues with decalcification. The use of single polyps, or small polyp groups, decreases the processing time, and allows for manual processing without the use of automated systems. Vacuum removes trapped air from decalcification and increases the quality of the histological sections. We also include an optional step for specimens that were directly preserved in ethanol without prior fixation. Lastly, we demonstrate simple ways to section paraffin blocks that do not require extensive experience in histological sectioning.

Guidelines

- Decalcification causes entrapment of air in the coral tissue, therefore steps 10-15 have to be performed in a vacuum hood. If a vacuum hood/machine is not available, use a vacuum pump attached to a desiccator.
- The protocol is for small pieces of tissue or individual polyps without the coral axis. It will still work with larger specimens, or with the axis included, but the times in processing will have to be adjusted (increased)
- We include a step of *Rehydration and post-fixation*, that can be used if your specimens were not fixed in a fixative that is commonly used in histology (e.g. formalin, Bouin), but were preserved directly in alcohol. If you fixed your specimens, you do not need to use this step.

Materials

Reagents

- 10% seawater formalin
- Distilled water
- Formic acid 10%
- Ethanol solutions (15%, 30%, 45%, 70%, 80%, 90%,95%,100%)
- Xylene
- Paraffin
- Hydrochloric acid (1%)
- Harris haematoxylin
- Eosin
- DPX

Equipment

- Oven
- Vacuum hood or similar
- Heating and stirring plate
- Refrigerator and freezer
- Microtome
- Forceps and scalpel
- Histological molds
- Holders and vials for histological staining

- Water bath (or other way to keep water at ~50 ºC)

Consumables

- Eppendorf vials
- Glass and/or plastic pipettes
- Beakers
- Histological slides
- Coverslips

Troubleshooting

Safety warnings

- Formic acid and hydrochloric acid are corrosive and toxic, use a lab hood, gloves and glasses and take care when handling them
- Xylene is toxic, use a lab hood and gloves during processing and especially during staining
- Formalin is toxic, use a lab hood and gloves
- DPX is toxic, use a lab hood and gloves

Before start

- Read timings and plan the protocol beforehand. It includes several long steps (<6h) and if these steps are not planed beforehand, the protocol might have to continue through the night

Fixation

1 Use 10% seawater formalin to fix the samples

1d

2 After 24-48 hours transfer specimens in 70% ethanol to preserve. This step might be omitted (the protocol will work with specimens preserved in formalin) but leaving specimens for too long in formalin will make the tissue harder, and may complicate sectioning.

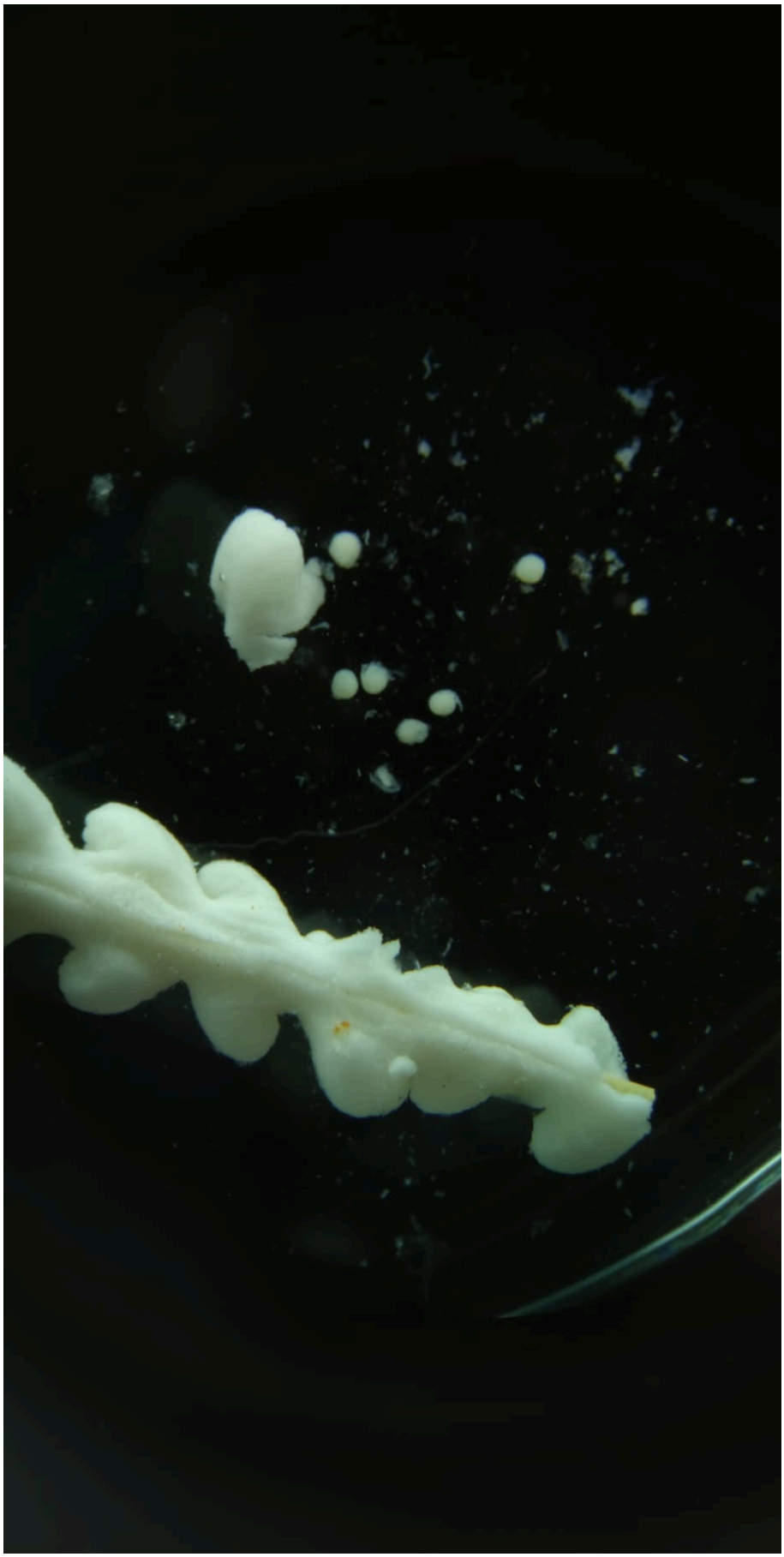
Dissection

3 Using a scalpel and forceps, make an incision parallel to the coral axis. Carefully separate the tissue from the axis by using the forceps, and remove the axis completely.

4 Dissect polyps or small groups of polyps by using the scalpel. If working with corals from the family Primnoidae, do not separate individual polyps from each whorl, i.e. you can work with entire whorls.

Note

There is a thin tissue layer separating the polyp and the coral axis. Make sure you don't cut through the base of the polyp, otherwise its content including gametes, may be lost.



Example of dissection that destroyed the base of the polyp, exposing and spilling coral gametes. (species: *Viminella flagellum*)

Rehydration and post-fixation

5 For samples that were fixed in alcohol, these should be rehydrated before starting decalcification *

Note

This section (*Rehydration and post-fixation*) is only needed if the specimens were fixed and preserved in ethanol, i.e. if there was no fixation in formalin, Bouin solution, or other fixatives typically used in histology. The goal of the section is to reverse the dehydrating effect of ethanol that may have caused shrinkage of tissue and fixing it in formalin before proceeding with the rest of the protocol.
Attention: If you have fixed your specimens in formalin or other fixatives, and then transferred them to ethanol you do not need to perform this section. Jump directly to decalcification.

5.1 Transfer tissue to Ethanol 70% for 15 min 15m

5.2 Transfer tissue to Ethanol 45% for 30 min 30m

5.3 Transfer tissue to Ethanol 30% for 45 min 45m

5.4 Transfer tissue to Ethanol 15% for 1h 1h

5.5 Transfer tissue to distilled water for 2h 2h

6 Transfer tissue to 10% formalin for 12h

12h

Decalcification

7 Place tissue in eppendorfs (or other plastic/glass vials), use stickers to label

8 Rinse fixative very well with distilled water (see steps 9.1-9.2)

8.1 Using a pipette, ideally with a thin tip, remove as much fixative as possible. Fill vial with distilled water. Leave for 2-3 hours. Repeat at least 3 times

9h

8.2 Leave specimens overnight in distilled water

12h

8.3 In the morning, change the water one more time and leave for another 30-60 mins

1h

9 Remove water completely, add formic acid solution (10%). Do not close the lid! Solution will start bubbling. Leave samples in formic acid until no traces of calcified material is left (typically 20-45 mins, will depend on the species)

45m

10 Transfer decalcified samples in clean vials with distilled water. Rinse 2,3 times, leave at least 30 mins between each rinse 1h 30m

Processing

11 Leave paraffin in 46-50 ºC overnight to melt 12h

12 Dehydration of the tissue in ethanol series in a vaccum chamber

Note

Ideally processing should be done in a vacuum chamber, to remove all the bubbles that were created during decalcification. Without the vacuum chamber, bubbles will not let the solutions to reach the tissue and the procedure will fail. A desiccator attached to a vacuum pump can work too.

12.1 Transfer tissue into Ethanol 70% for 30 mins 100 mbar 30m

12.2 Transfer tissue into Ethanol 80% for 30 mins (or just change the solution in the same vial) 100 mbar 30m

12.3 Transfer tissue into Ethanol 90% for 15 mins 100 mbar 15m

12.4 Transfer tissue into Ethanol 95% for 15 mins 100 mbar 15m

12.5 Transfer tissue into Ethanol 100% for 20 mins x 3 times 100 mbar 1h

12.6 Transfer tissue in Xylene for 20 mins 100 mbar 20m

Samples will turn transparent, any white remnants are sclerites that did not get decalcified properly, discard these samples. This step needs to be done in clean vials, do not just remove alcohol and put xylene.

13 Transfer tissue into paraffin and leave in oven for 1 hour 50 °C oven 1h

Embedding

14 Leave paraffin, molds and forceps in an oven at 50 ºC for at least 5-6h 50 °C oven 6h

15 Place paraffin on a magnetic stirring hot plate at 50 ºC, keep the stirrer on at low speed to keep the paraffin temperature homogenous 50 °C stirring hot plate

16 Pour a small amount of warm paraffin into the mold

17 Pick up sample with warm forceps and position it into the mold

18 Let it cool down slightly and top up with paraffin Room temperature

> Note
>
> During embedding, histological cassettes are often used to aid with securing the block during sectioning. We do not use them here (see sectioning for how we position and secure the block), but feel free to use them.

19 Make sure there are no bubbles

20 Move mold to a cold area (fridge) and subsequently place in refrigerator until completely solid

21 Using the edge of a knife, remove carefully the block from the mold.

Sectioning

22 Prepare microtome

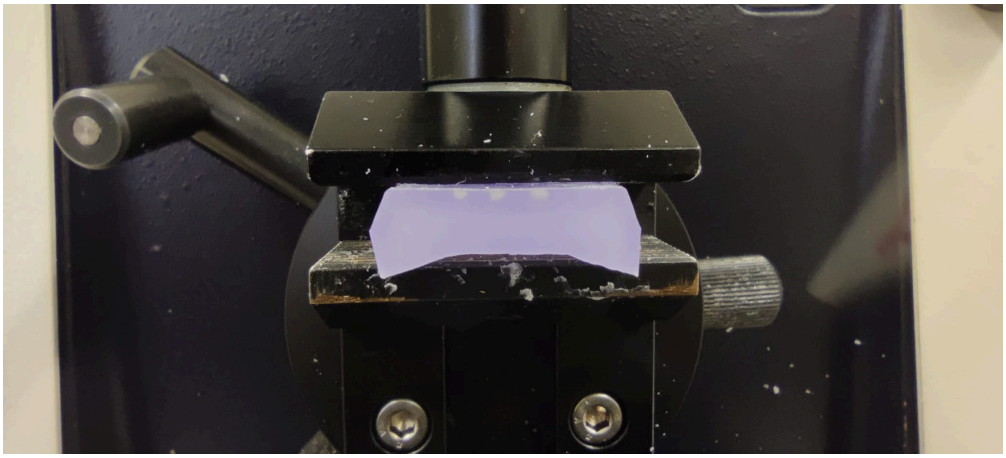
23 Prepare cold water bath and hot water bath ~50 ºC

24 Name slides with pencil

25 Trim the paraffin block until the area you want to section is clean

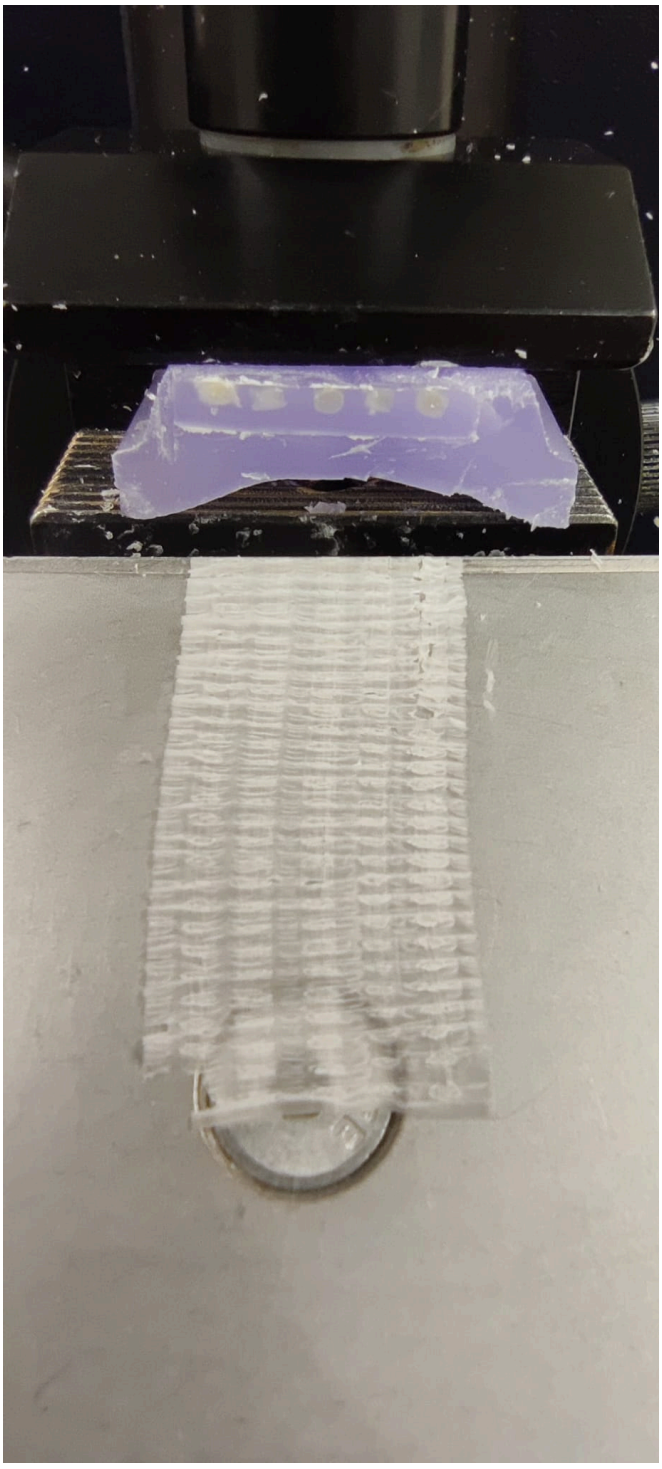
Note

As shown in the images below, we use an unconventional way to section our blocks. Instead of using cassettes and taking frontal sections of each block, we rotate blocks and take transverse sections. This reduces the required time to to trim the blocks, and does not require histological cassettes.



Paraffin block with samples placed on the microtome to be sectioned.

26 Perform cross sections (5-10 µm), creating a ribbon



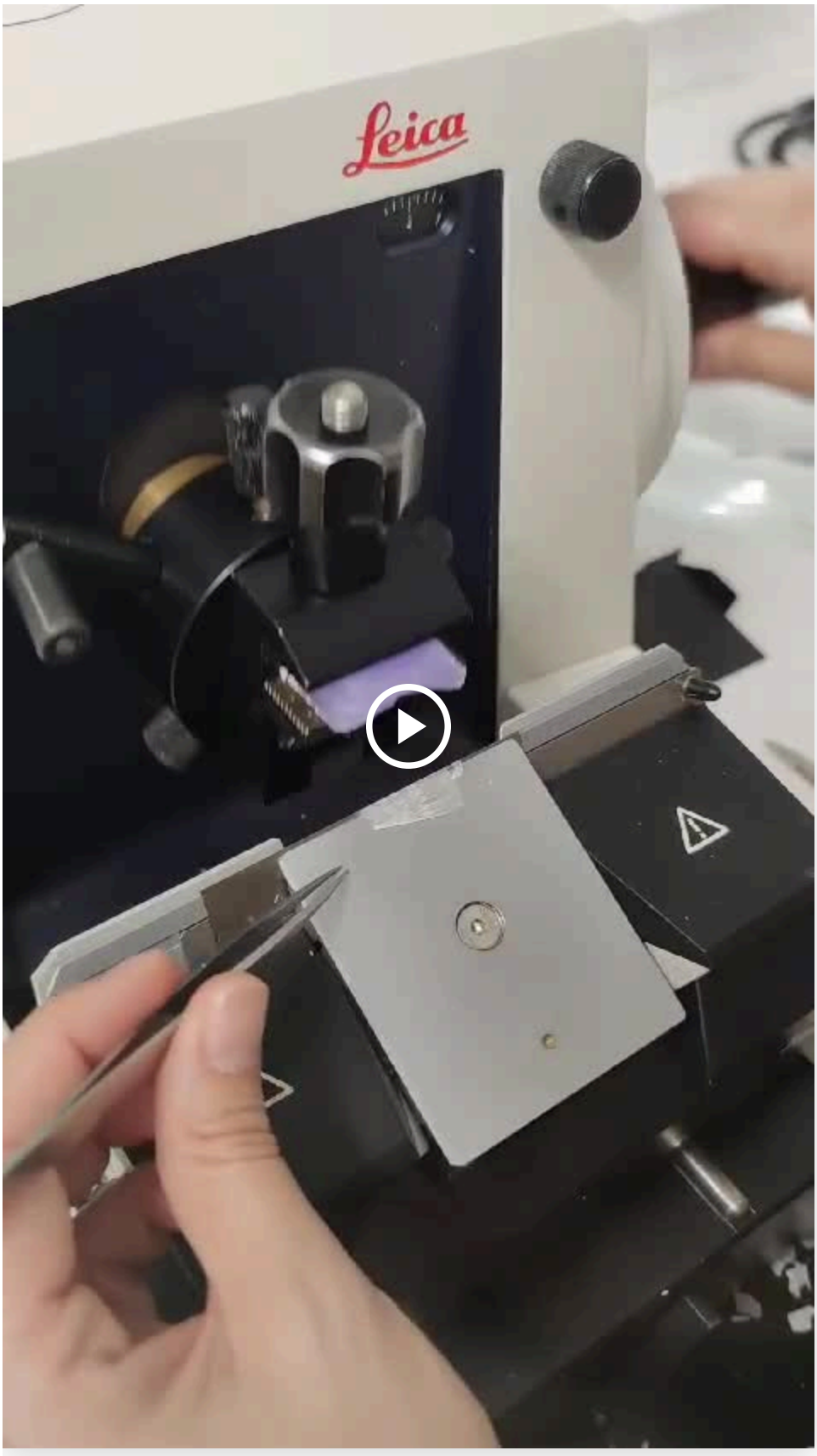
Paraffin ribbon after block sectioning on the microtome.

27 Detach ribbon from microtome with scalpel, pick it carefully with forceps

28 Place ribbon in cold water bath

29 Pick ribbon with histological slide

30 Place histological slide in warm bath to stretch the ribbon, be careful not to lose the ribbon in the water bath and do not leave for long

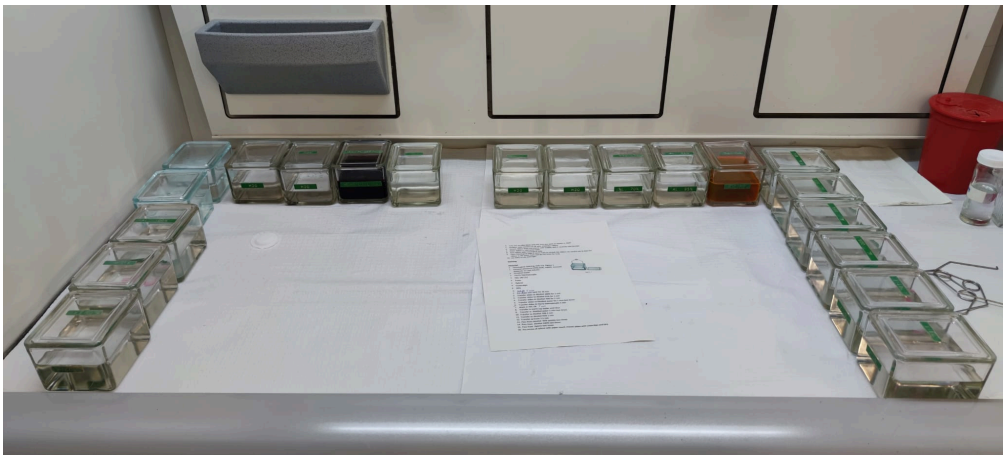

Leica

31 Let slides to dry overnight

Staining

37m 20s

32 Prepare the staining table. Place a label with the content on the staining vials. It is also usefull to write the time of each bath on the label.



Staining set up.

33 Place slides into an oven for 5 mins. This will help to remove the paraffin 50 °C oven 5m

34 Place slides into Xylene for 20 min 20m

35 Transfer slides to Ethanol 100% for 1 min 1m

36 Transfer slides to Ethanol 95% for 1 min 1m

37 Transfer slides to Ethanol 70% for 1 min 1m

38 Transfer slides to Distilled water for 1 min-two times 2m

39 Transfer slides to Harris haematoxylin 5 min — 5m

40 Wash in HCL 1% — 10s

41 Transfer in warm tap water until blue — 10s

42 Transfer in Distilled water 1 min-two times — 2m

43 Transfer to Ethanol 70% 1 min — 1m

44 Transfer to Ethanol 95% 1 min — 1m

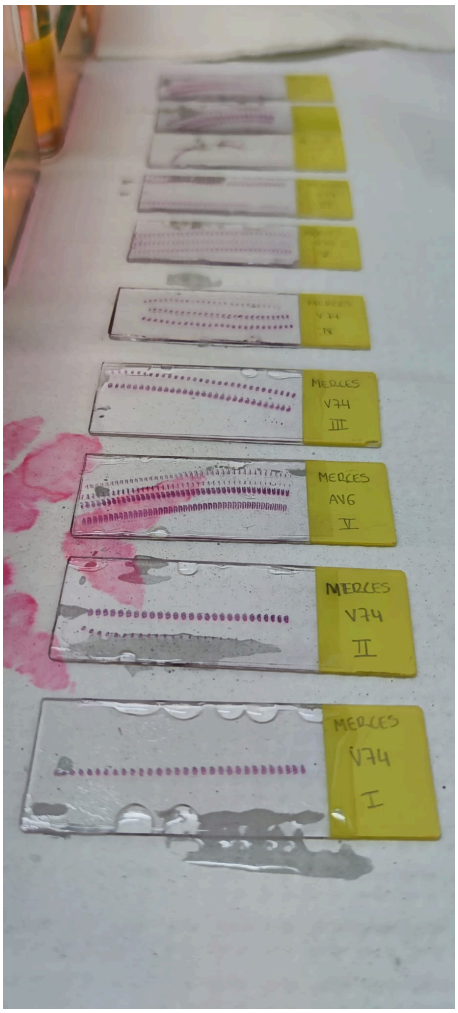
45 Transfer to Eosin 2.5 min — 2m 30s

46 Pass from Ethanol 95% quickly two times — 10s

47 Pass from Ethanol 100% two times — 10s

48 Pass from Xylene two times — 10s

49 Dry excess of xylene with paper towel, mount slides with coverslips and DPX



Final histological preparations.

50 Put slight pressure on slides with the forceps and guide bubbles to the margins of the coverslip. Make sure there are no bubbles left on the DPX, especially on tissue. This will ensure good visualization and long-term preservation of the section.

Protocol references

Price K, Peters E (2018) Histological techniques for corals. Draft2Digital