Protocol ♦ FFPE Sectioning

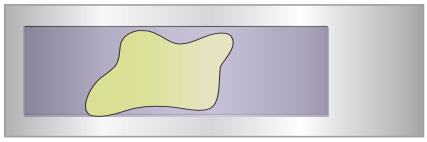
Paraffin embedded tissues are specimens that have been fixed in formalin to preserve their cellular structure and subsequently embedded in paraffin to stabilize it for long-term storage and easy sectioning. Fixation is mainly performed to preserve the morphology of the living tissue, thus, ideal for pathological analysis.

Degradation and difficulty in extraction of nucleic acids have been observed in FFPE tissue. Therefore, if the analysis of nucleic acids is your main interest, a different tissue preservation method may be preferred. To section and mount paraffin embedded tissue and to prepare slides suitable for subsequent Laser Microdissection, follow the guidelines below.

Materials:

- Paraffin molds
- Knifes, disposable blades
- Low melt paraffin
- Brushes, pins
- Water bath
- Heating plate
- MMI Membrane Slides (PN: 50102, 50103)
- Paraffinized tissue samples

Note: Before staining and microdissection, the slides must first be de-paraffinized.



Note: Section must be placed on the flat side of the MMI Membrane Slide.

Method:

Preparation:

1. Ensure paraffin block is at room temperature
2. Place block into holder of microtome
3. Use clean, sharp microtome knife or disposable blade
4. Cut section ~ 5 - 10 µm thick
5. Float paraffin ribbons on deionized H_2O (or RNase-free H_2O), heated to just below the melting point of the paraffin being used (usually 40 - 42 °C)
6. Mount sections on MMI MembraneSlide by slowly bringing the flat side of slide up from underneath the floating tissue section, so that the tissue section is centered in the membrane window
7. Place slide at an angle to allow water to drain and incubate sample at room temperature for 60 - 90 minutes
8. Slides can now be stored at room temperature for future use
9. Proceed with staining protocol

UV treatment of slide to sterilize and increase adherence (optional):

To help with adhesion of the sample to the slide one can incubate the slide under UV light for 15 - 30 minutes. A UV sterilization hood works best for this. The UV light will breakdown the membrane slightly and make it tacky, helping adhesion. Additionally this will sterilize the slide. Do not incubate for longer than 30 minutes or risk damaging the membrane.

Additional coatings (poly-L-Lysine, Agarose, or Gelatine) are recommended for tissues that are fatty, hard, fibrous, or contain carilage/bone

The most common method is coating the MMI Membrane Slide with 0.1 % poly-L-Lysine solution. Incubate the slides for 1 hour at room temperature or 30 minutes at 37 °C. Gelatine or agarose can be used as well by preparing a 0.01 % solution and incubating the slides using the above guidelines