Sectioning

1) Leave the frozen specimen block in the cryostat to acclimatize for a while (10-20min) after fixing it to the cryomicrotome stage.

2) Trim the specimen until a cut surface of the specimen appears.

3) Place the Cryofilm on the cut surface.

4) Then tightly adhere the Cryofilm to the surface with a fitting tool (Fig. 2).

5) Cut the specimen slowly at a constant speed (Fig. 3).
Note: Be sure not to move the frozen section out of the cryostat until this step is completed.

6) Take the section from the cryostat and thaw it (Fig. 4).
Note1: If you want to carry out immunostaining with non-fixed sections, use the freeze-dried sections.
Note 2: For the freeze-drying, keep the frozen sections in the cryostat at –20C for approx. 2 hours.

7) After 10 to 70 seconds, turn the specimen side down and immerse the section in 100% ethanol. The time depends on the specimen.
Note: In order to remove any bubbles in the tissues; place the section in 100% ethanol, then move it to a low-pressure chamber

8) Then, move the section into the fixative.

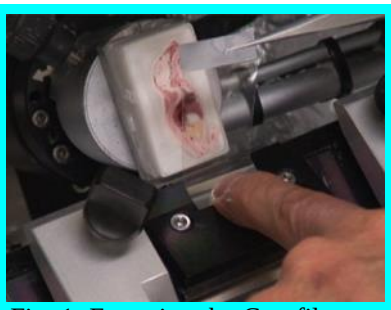


Fig. 1. Fastening the Cryofilm to the surface

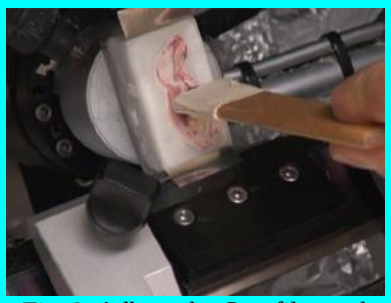


Fig. 2. Adhere the Cryofilm to the surface.


Fig. 3. Sectioning.


Fig. 4. Thawing the section.

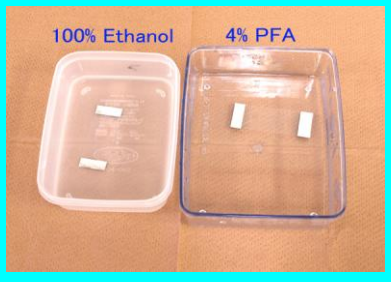
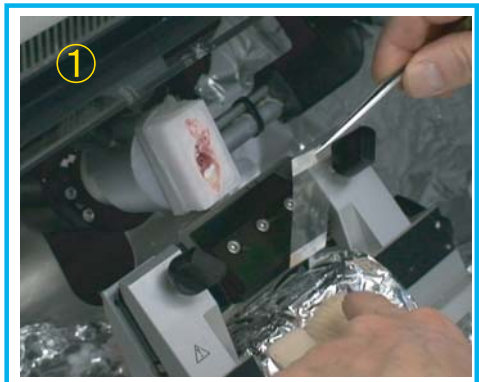
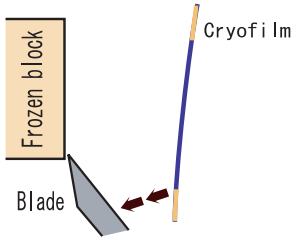


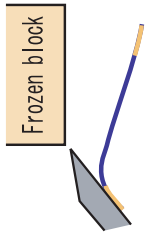
Fig. 5. Fixing the section.

Procedures for applying the Cryofilm to the surface of specimen block

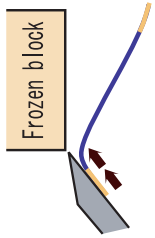




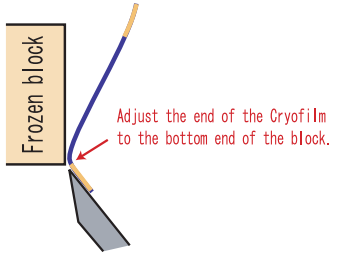
Move the Cryofilm toward the blade holder.



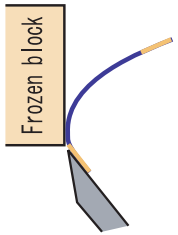
Keep the end of the Cryofilm on the holder.



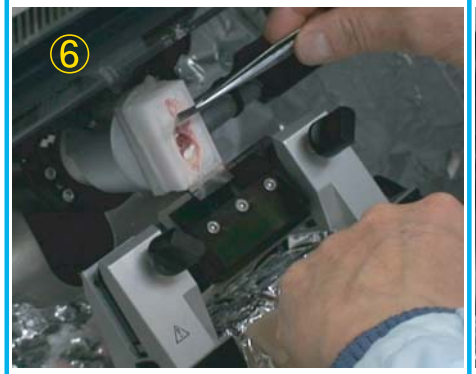
Move the Cryofilm to the frozen block.

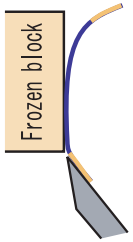


Fix the end of the Cryofilm to the bottom end of the block.



Bend the Cryofilm.





Mount the Cryofilm to the block surface carefully.