Embedding

1) Put the specimen on the strainer, and immerse it in the coolant (Fig. 1).
Coolants: Hexane-dry ice (approx. –75℃)
Hexane cooled with a cooling apparatus (–94℃)
Pentane in liquid nitrogen (approx. -130)
Isopentane in liquid nitrogen (approx. -160℃)
Note: Prepare more than 500ml of hexane at least when the hexane-dry ice is used as a coolant.

2) Put the proper quantity of embedding medium in the stainless steel container, then partly freeze the embedding medium (Fig. 2).
Note: Use the cooled embedding medium kept in a refregerator.

3) Place the frozen specimen in the embedding medium.
Note: Place the sectioning side face down.

4) Submerge the container into the coolant (Fig. 3A)
Note: In order to avoid melting the specimen, carry out steps 3) and 4) rapidly.

5) After 5-15 seconds (the time depends on the container size), raise the embedding medium so that the top is exposed from the coolant and freeze it completely (Fig. 3B).
Note: If the embedding medium is frozen completely in the coolant, cracks will occur in the frozen specimen block.

6) After freezing, place the frozen specimen block on the extractor (a wooden stage) and remove it with a wooden hammer (Fig. 4).

7) Slightly melt the specimen holder side of the frozen block and attach it to the holder (Fig. 5).

8) Fix the holder to the Cryomicrotome specimen stage.

Fig. 1. Freezing the specimen.

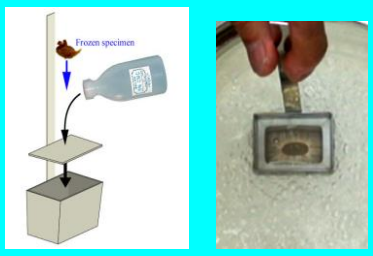

Fig. 2. Embedding

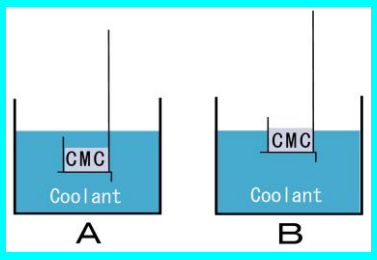

Fig. 3. Freeze-embedding.
(A): Early freezing
(B): Complete freezing.

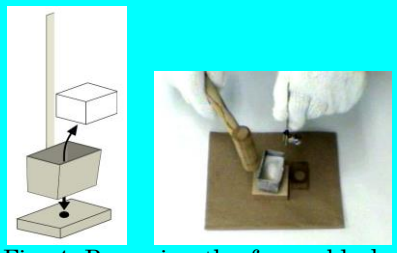

Fig. 4. Removing the frozen block from the container.

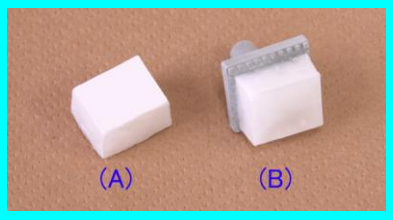

Fig. 5.
(A): Frozen specimen block
(B): The block fixed to the specimen holder.