Cryostat: frozen sectioning

EQUIPMENT

- Cryostat: Leica CM 3050S and CM 1950

MATERIAL

- Forceps
- Small and hard brushes, big brush
- Superfrost+ slides
- Disposable low profile microtome blades: Routine work "C35"
- Anti-roll plate 70mm

SAFETY

Wear gloves and lab coat.
Be careful not to be injured by the blade.

TO PREPARE

- It is important to choose the right cutting temperature for each tissue. Set the cryostat chamber temperature before anything else (Fig.4 and 5). For a summary see chart below (Fig.1).

- Since OCT blocks are stored at -80°C, they should equilibrate in the cryostat chamber for at least 15 minutes before cutting.

- In the meantime, place the anti-roll plate in the aluminum frame (Fig.2).

- Slide the C35 blade in the clamp of the knife holder (Fig.2) and tighten well.

PROCEDURE

- Move the block holder completely back using the arrow on the control panel (Fig.4/5).

- Check the angle of the knife holder (Fig.2), usually 0°.

- Stick your block on a specimen disc (Fig.3) with OCT.

- Insert the specimen disc into the specimen head, orientate and tighten the disc (Fig.3 and 5).

- Adjust the anti-roll plate (Fig.2) exactly at the edge of the knife. The sections will then easily slide between plate and knife.

- Move the knife holder until the block is placed at the edge of the blade (Fig.3).

- Set the thickness between 10 and 20 μm with the wheel (Fig.4 and 5) and trim the block until the full face of the tissue is sectioned.

- Set the advance feed to the desired thickness (usually 7-8μm) (Fig.4 and 5)

- Remove any debris associated with coarse cutting from the knife and plate with a small brush or alcohol (allow to dry before cutting).

- If necessary, move the blade to an unused area or install a new one in the clamp (Fig.2)

- Cut a section, remove the anti-roll plate and with a clean Superfrost^{+} slide collect the section. The slides are at room temperature before collection.

STORAGE

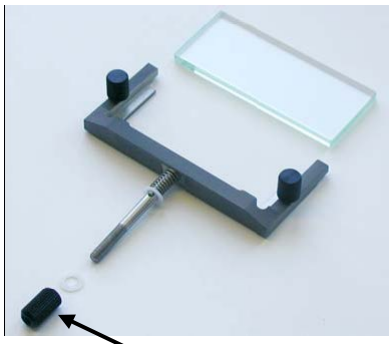
Slides are dried at room temperature during 30 minutes and stored at -20°C in a range box to avoid accumulating dust.

Fig. 1 Temperature chart (Leica)

Tissue	-10°C – -15°C	-15°C – -25°C	-25°C – -35°C
Adrenal	❄	❄	
Bone marrow		❄	
Brain		❄	
Bladder		❄	
Breast - fatty			❄
Breast - little fat		❄	
Cartilage	❄	❄	
Cervical		❄	
Fatty			❄
Heart and vascular		❄	
Intestinal		❄	
Kidney		❄	
Laryngeal		❄	
Lip		❄	
Liver		❄	
Lung			❄
Lymphoid		❄	
Muscular		❄	
Nose		❄	
Pancreatic		❄	
Prostate		❄	
Ovarian		❄	
Rectal		❄	
Skin with fat			❄
Skin without fat		❄	
Spleenal or bloody tissue		❄	
Testicular	❄	❄	
Thyroid		❄	
Tongue		❄	
Uterus curettage	❄		

The temperature values given above are based on long-term experience, however, these are only approximate values, as any tissue may require particular adjustments.

Fig. 2 : Knife holder



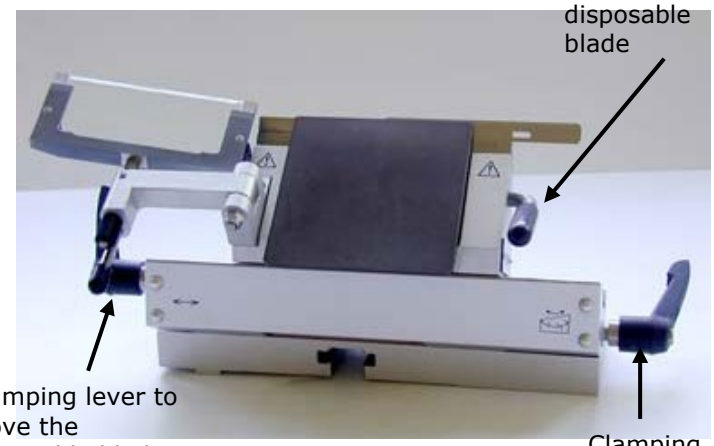
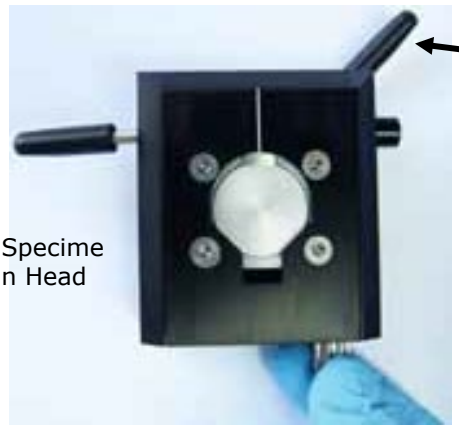


Fig. 3: Specimen holder



Specimen disc

Fig. 4: Control panels CM1950

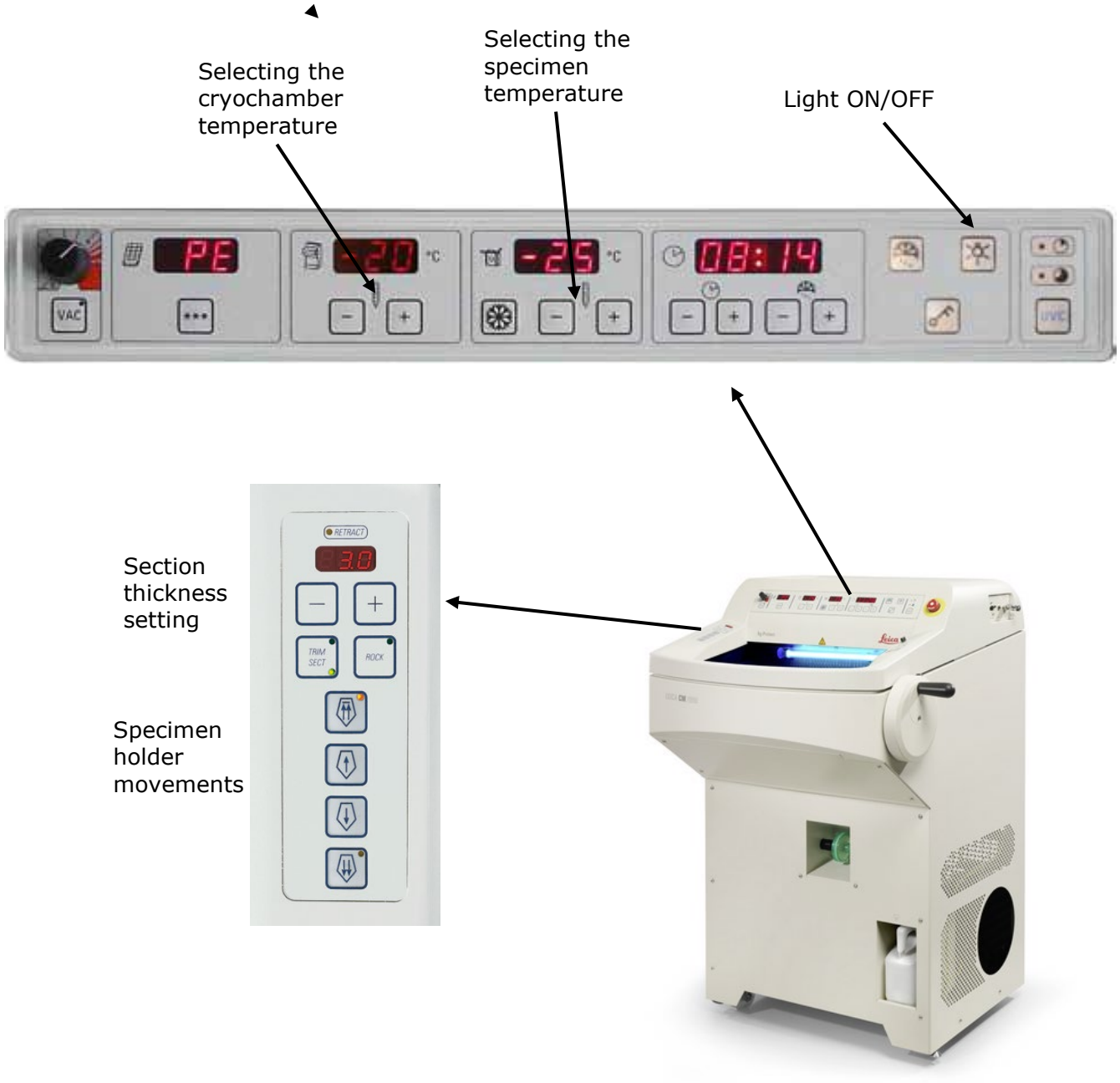
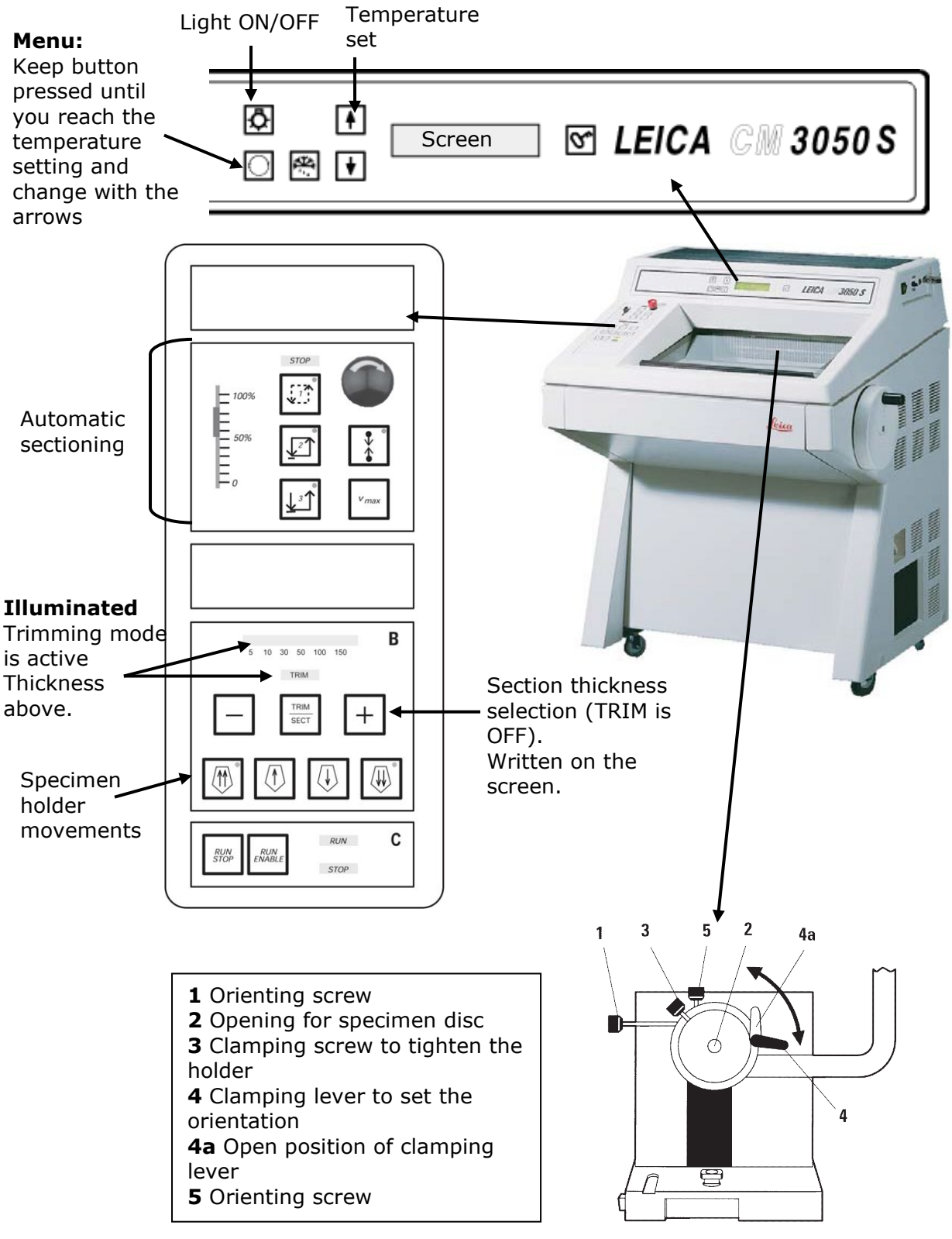


Fig. 5: CM3050S



1 Orienting screw
2 Opening for specimen disc
3 Clamping screw to tighten the holder
4 Clamping lever to set the orientation
4a Open position of clamping lever
5 Orienting screw