Simple Operation Manual for CT520 Cryostat Microtome

I. Startup



Fig. 1 Instrument overview



Fig. 2 Software main interface

As shown in Fig. 1, connect the power cord to the port at the back of the instrument (Fig. 1-3), move the circuit breaker switch up to turn the instrument on (Fig. 1-2). The display lights up, the compressors start simultaneously, and the freezing shelf, the specimen head, and the blade holder are refrigerated and under temperature control according to the respective set temperature (Fig. 1-1).

II. Interfaces

(i) Main interface

1. Sectioning module



Fig. 3 Sectioning module on the main interface

 \square Fig. 3-1 shows that the set section thickness is 5 μ m;

 \square Fig. 3-2 shows that the set trimming thickness is 25 μ m;

Fig. 3-3 shows the section/trimming thickness adjustment button. Click the "-" button to decrease section/trimming thickness according to step value. Clic the "+" button to increase section/trimming thickness according to step value. The dial in the middle displays the real-time section/trimming thickness;

□ Fig. 3-4 shows the sum of sectioning times and the sum of section thickness, where the sum of sectioning times is the cumulative number of times of sectioning and trimming, and the sum of section thickness is the cumulative total thickness of sectioning and trimming. Click the "Clear" button to reset the sum of sectioning times and the sum of sectioning thickness.

2. Freezing elements

The freezing elements can be used to adjust the temperature of the freeze shelf, the specimen head and the blade holder.

Freeze shelf: As shown in Fig. 4-2, the current temperature of the freeze shelf is displayed. The - and + buttons can be used to adjust the target temperature of the freeze shelf. When there is a difference between the target temperature and the current temperature, the instrument will refrigerate the freeze

shelf, and the corresponding refrigerating icon will be displayed. When the current temperature reaches the target temperature, the refrigerating icon will disappear, and only the current temperature will be displayed.

Specimen head: As shown Fig. 4-3, the current temperature of the specimen head is displayed. The - and + buttons can be used to adjust the target temperature of the specimen head. When there is a difference between the target temperature and the current temperature, the instrument will refrigerate the specimen head, and the corresponding refrigerating icon will be displayed. When the current temperature reaches the target temperature, the refrigerating icon will disappear, and only the current temperature will be displayed.

Blade holder: As shown in Fig. 4-4, the current temperature of the blade holder is displayed. The - and + buttons can be used to adjust the target temperature of the blade holder. When there is a difference between the target temperature and the current temperature, the instrument will refrigerate the blade holder, and the corresponding refrigerating icon will be displayed. When the current temperature reaches the target temperature, the refrigerating icon will disappear, and only the current temperature will be displayed.

Temperature presets: As shown in Fig. 4-1, Administrator account click the Settings button, and a popup will display the preset values of the components. The temperature of the freeze shelf, the specimen head and the blade holder can be set separately to meet the temperature requirements for different tissues.Presets can be selected directly on the interface.



Fig. 4 the Freezing elements on the Main Interface



Fig. 5 Set Temperature Presets

3. Specimen head position

The total feed and current position of the specimen head are indicated. The feed range of the specimen head is 30 mm. The specimen head position indicator moves with the operation of the forward and backward buttons on the control panel within the feed range. When the specimen head reaches the end of the feed, a buzzer will sound to alert the user. The specimen head distance bar turns from blue to yellow after the specimen head position is over 25 mm to alert that it's approaching the limit. Click "Set" button to record the current position of the specimen head (Fig. 6-3). A memory position is then recorded. The "Set" button will change to "Clear" when a memory position is set (Fig. 6-1). Click "Move" (Fig. 6-2) and the specimen head will return to the recorded memory position.

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4)					88 Menu
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10	15	60	Compo Freeze	shelf *	Current/target 0°C/-25°C +
-	5 _{um}	- +	Spec. h	ead * 🗕	0°C/-15°C +
	2.5 75		Blade h	older —	0°C/-20°C +
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Functions					
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Defrosting	UV disinfection	Rapid cooling	Illumina	tion 🔍 💳 🕻	•

Fig. 6 Specimen Head Position Indicator on the Main Interface

4. Functions



Fig. 7 Functions on the Main Interface

Defrosting: As shown in Fig. 7-1. Enable when too much vapor in the chamber. Click the defrosting toggle to start defrosting and vapor in the chamber and the microtome can be expelled. The defrosting icon on the interface will be highlighted.

UV disinfection: As shown in Fig. 7-2. Click the UV disinfection toggle to enable UV disinfection. The UV disinfection icon on the interface will be highlighted..

Rapid cooling: as shown in (Figure 5-12-3). To enable rapid cooling, the current freeze shelf temperature must be lower than -10 $^{\circ}$ C. After turning on, the Peltier element will refrigerate for the duration set in "Menu - Settings - Preferences" (the default duration is 10 min and can be adjusted within 5 - 30 min in "Preferences"). After the duration, rapid cooling will be turned off.

Illumination: As shown in Fig. 7-4. Click the illumination toggle to enable illuminating. The brightness can be adjusted on the main interface.

(ii) Control panel

		The screen is generally turned off. It displays the	
		contents in sectioning/trimming and freezing	
		elements when turned on;	
	TRIM SECT	Press this button to switch between	
		sectioning/trimming;	
	ON/OFF	Press this button to turn on/off the screen. The	
		screen displays the contents in sectioning/trimming	
		and freezing elements when turned on. The	
		temperature of the freeze shelf, the specimen head	
SECT ON/OFF		and the blade holder can be adjusted directly with	
)()		the control panel;	
		These buttons can be used to adjust thickness and	
		temperature;	
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	_₹	Specimen head fast backward movement till the	
		end;	
	\geq	Specimen head fast forward movement;	

III. Sectioning

(i) Freezing specimens

The freeze shelf is on the left side of the cryochamber with 20 stations, including 2 Peltier element for rapid freezing and 18 freezing stations.

- At room temperature, cut the specimen to size, place it on the specimen disc and cover it with freezing compound (OCT);
- Place the specimen disc on the freezing station to freeze at a low temperature. Place the disc on the Peltier element if rapid cooling is required;
- Place the heat extractor on the specimen to accelerate freezing and flatten the specimen if needed;

□ Place the specimen disc in the specimen head, clamp the disc properly, and start sectioning.

Note: Use the heat extractor carefully according to tissue type. It is recommend to use the heat extractor on soft tissues that are prone to distort ONLY after the tissue starts to freeze or not to use the heat extractor, or the tissue may be squeezed and distort.





- 1. Heat extractor
- 2. Freezing stations

3. Specimen disc

- 4. Peltier element
- (ii) Blade holder adjustment



Fig. 9 Blade holder

1. Blade ejector	2. Blade holder lateral shift lever	3. Anti-roll guide position
		adjustment knob
4. Blade clamp lever	5. Anti-roll guide screw	6. Blade holder base clamp lever
7. Pressure plate	8. Blade guard	

1. Front and rear position adjustment of the blade holder base

Rotate the "blade holder base clamp lever" counterclockwise so that the blade holder base can be

moved back and forth. Move the blade holder base to the desired position, and then rotate the lever to clamp. The blade holder base can be wholly removed away from the rail.

2. Lateral movement of the blade holder

Rotate the "blade holder lateral shift lever" on the left counterclockwise, and move the blade holder laterally to make use of the whole blade for sectioning. Rotate the lever clockwise to clamp the blade holder.



Always clamp the blade holder and the blade holder base after adjustment. Never touch the metal part of the blade holder when it is cold or frostbite may occur. Hold the rubber plug if moving the blade holder is required.

3. Adjust section angle

Use a #4 hex key to loosen (rotate counterclockwise) the "rotary tool apron locking screw", and then push the edge of the rotary tool apron by hand to make it rotate along the arc surface of the tool apron for angle adjustment. The adjustable angle range is $0 - 10^{\circ}$. After angle adjustment, fix the "rotary tool apron locking screw".

If the section quality is not satisfactory, try to change the angle by 1° until satisfactory. Do not change the angle for greater than 10°.



4. Adjust anti-roll plate

Fig. 10 Anti-roll guide structure

 1. Cutting edge
 2. Anti-roll plate edge
 3. Screw for parallelism between

 edge and anti-roll guide
 edge and anti-roll guide

Insert the blade and rotate the "blade clamp lever" to clamp;

- □ Tighten the two "anti-roll guide screw";
- Use a hex wrench to loosen the "screw for parallelism between the cutting edge and the anti-roll guide";
- Adjust the angle of the anti-roll guide so that the edge of the anti-roll guide is parallel to the cutting edge of the blade;
- I Tighten the "adjusting screw for parallelism between the cutting edge and the anti-roll guide".

Notes:

D Protect the part where the anti-roll guide is in contact with the tissue section from any damage;

I If the section quality is not satisfactory, check that there are no tissue debris, grease or foreign objects remaining on the cutting edge and anti-roll guide;

Avoid touching the edge of the anti-roll guide with hands or other high-temperature objects or it may cause tissue section adhesion.

5.Insert and replace a blade

- D Prepare the blade and fold the anti-roll guide to the left
- Insert the blade from the right side of the blade holder
- □ Fasten the blade clamp lever



Fig. 11 Insert a blade



The blade is extremely sharp! Handle it with care! Never try to catch a falling blade!

Make sure that the cutting edge has been covered by the safety guard when it's not in use!

(iii) Insert the specimen disc



	Fig. 12 Install specimen disc	;
1. Specimen holder clamp	2. Horizontal orientation	3. Specimen holder
lever	Screw	
4.Vertical orientation	5. Specimen head	
screw	orientation lever	

- Put the handwheel handle at the highest position, and push the handwheel locking lever into the bottom of the handwheel groove to lock the handwheel, so that the specimen head is locked at the highest position;
- Freeze the tissue on the specimen disc, lift the specimen head clamp lever, and insert the specimen disc to the specimen head, make sure the specimen disc is in proper contact with the specimen head.
 Press the specimen head clamp lever down to clamp the specimen disc to the specimen head.
- Rotate the "specimen head orientation lever" to loose the clamp of the specimen head, pull the "orientation screws" to adjust the angle of the specimen. Clamp the specimen head.

(iv) Trimming

Trimming is to prepare the specimen for sectioning. Following the instructions:

Insert the pre-cooled blade into the blade holder, clamp it, and adjust the anti-roll guide;

Check that the angle of the blade holder is $5^{\circ} \sim 8^{\circ}$. 5° is recommended (it is not recommended to adjust the angle as it has been adjusted to a proper angle);

- Adjust the relative position between the blade holder and the specimen to leave space for feed;
- □ Unfold the anti-roll guide;
- Unlock the handwheel;

Switch to trimming mode, press the fast forward key or the slow forward key to drive the specimen close to the blade. Spin the handwheel to start trimming. A recommended trimming thickness is $20 \sim 30 \ \mu m$;

I Trim the specimen to the desired surface, switch to section mode and start sectioning.



Fig. 13 Trimming

(v) Sectioning

One sectioning is completed when the handwheel is turned clockwise or counterclockwise for one round. The section thickness is the set section thickness value. The recommended section thickness is 5 -10 µm.

Notes:

- □ Clamp the specimen first and then insert the blade;
- □ Lock the handwheel properly;
- Use the protection of anti-roll guide and blade guard properly;

Never leave the blade in the blade holder after operation.

(vi) Sectioning reference

1. When using a Cryostat microtome, the key to ideal section is the appropriate sectioning speed and proper use of the anti-roll guide. The appropriate sectioning speed is mastered through practical experience and obtained by skillful operation of the handwheel. There are many parts to adjust the anti-roll guide. Some of them influence each other, so the anti-roll guide needs to be carefully adjusted and cannot be placed on the cutting edge casually.

2. When the living tissue is freezing, the water in it turns into ice and thus the tissue hardens. The hardness changes with its temperature. The lower the temperature is, the harder the tissue is. How to choose a proper temperature for a kind a tissue is explored through practice. The sectioning temperature of most tissues without fat and fixed with formalin is preferably between -13 °C and -23 °C.

- 3. To obtain quality sections, pay attention to the following aspects:
- (1) Select suitable temperature of the cryochamber;
- (2) Section properly;
- (3) Adjust anti-roll guide properly;
- (4) Sharp blade and suitable cutting angle.

4. Contrary to the general paraffin block section, for the frozen section, it is better to cut the short side of the tissue instead of the long side, that is, to make the line of contact between the tissue and the sectioning blade shorter





Fig. 14 Sectioning references

IV. Cleaning

After sectioning, dispose of the section waste in the waste tray, activate UV disinfection, and wipe the chamber, the blade holder and the freezing shelf with alcohol.