

**Institute for Genomic Biology
Core Facilities
Histology Laboratory**

Leica Rotary Microtome RM2255 Training Manual

July 2007



Institute for Genomic Biology

Leica RM225 Microtome training manual

Introduction:

Histology is the study of tissue sectioned as a thin slice, using a microtome. It can be described as microscopic anatomy.

A **microtome** is a mechanical instrument used to cut biological specimens into very thin segments for microscopic examination. Most microtomes use a steel blade and are used to prepare sections of animal or plant tissues for histology. The most common applications of microtomes are:

- Traditional histological technique: tissues are hardened by replacing water with paraffin. The tissue is then cut in the microtome at thicknesses varying from 2 to 25 μm (**micrometers**) thick. From there the tissue can be mounted on a microscope slide, stained and examined using a light microscope.
- Cryosection: water-rich tissues are hardened by freezing and cut frozen on a Cryostat; sections are stained and examined with a light microscope. This technique is much faster than traditional histology (5 minutes vs 16 hours) and are used in operations to achieve a quick diagnosis. Cryosections can also be used in immunohistochemistry as freezing tissue does not alter or mask its chemical composition as much as preserving it with a fixative.

A **micrometer**; (symbol μm) is an **SI** unit of [length](#) equal to one [millionth](#) of a [metre](#), or equivalently, one thousandth of a [millimetre](#). It is also commonly known as a **micron**. It can be written in [scientific notation](#) as 1×10^{-6} m, meaning 1 / 1,000,000 m.

Micrometer: 1/1000 of a millimeter or 1/25,000 of an inch. Also known as micron.

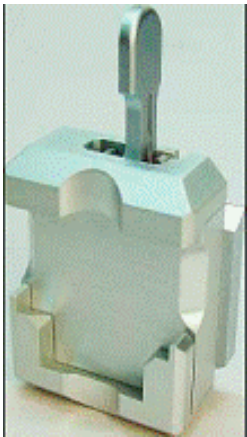
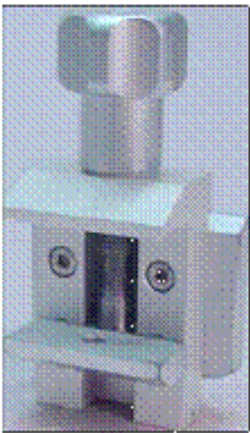
Block: After the tissues are processed, the tissues are embedded in a mold of paraffin. The block of paraffin can then be mounted on the microtome to be cut.



I. COMPONENT OVERVIEW

1.1. Switching on the instrument

When turning on the instrument with the main switch (back, bottom right), do not press any of the buttons of the control panel or the foot switch (optional accessory) at the same time! Turn the instrument on with the mains switch at the rear right side. This is followed by a beep. The instrument initializes.



1.2. To Insert/Remove Cassettes:

Universal Clamp - used to hold cassettes. Can be transferred out for various sizes of blocks.

1.3 Clamping Lever- Cassettes can be inserted and removed by: 1) pulling lever to the left. 2) Insert a cassette horizontally or vertically and 3) Pull lever to the right to secure the cassette in position.



2. SAFETY FEATURES

2.1. Emergency Stop Switch-Push in for immediate stop.



2.2. Handwheel brake lever- (under handwheel) used to activate the handwheel brake. To unlock handwheel lever should be pushed back completely towards the wall.

2.3. Handwheel Locking Mechanism- located on the top of handwheel. Use lock to guard against unwanted movement and when inactive. Slide lever right to lock, move left to unlock.

3.1 Repositioning the knife holder base

The one-piece knife holder base can be moved forwards and backwards on the microtome base plate. This vertical displacement allows bringing the knife holder into the optimal cutting position in relation to the specimen.

To reposition the knife holder base 1) release, rotate the clamping lever on the right side of the microtome base plate counterclockwise 2) reposition the knife holder together with the knife holder base forward or backward as appropriate 3) Secure the clamping mechanism by rotating the lever clockwise.

3. KNIFE



H. Clamping Lever for Knife:

3.2 Clamping Lever for Knife:

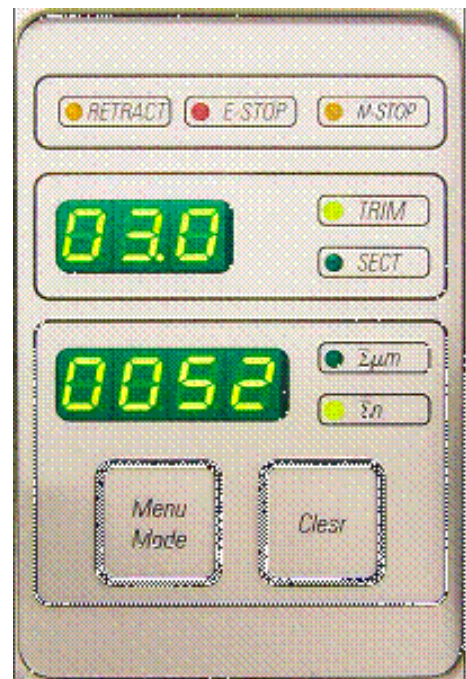


The lateral movement feature of the knife holder base enables the use of the entire length of the blade or knife, eliminating the need for readjusting the knife holder. To release the clamp and move the knife 1) rotate the lever on the left side of the knife holder forwards 2) move the knife holder sideways and 3) rotate the lever back to clamp.

3.3. Knife Guard-Used to guard knife from unwanted cutting.

Always put on top of knife when changing knife, block or when inactive. To insert or remove blade 1) fold knife guard forward (on top of knife) 2) pull right clamping lever forward and down 3) use ejector to push blade out, put guard down 4) insert new blade from right side. Making sure blade is clamped parallel to upper edge of pressure plate 5) push

right clamping lever backward and up



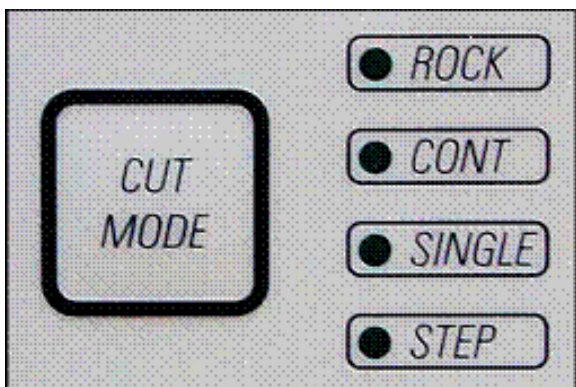
4. INSTRUMENT CONTROL PANEL::

4.1 The three-digit LED display indicates the last value set for section or trimming thickness. This is indicated on the control panel as well.

4.2 When red **E-STOP** field on control panel is illuminated either the emergency-stop function has been activated by either pushing in the E-STOP button or footswitch depressed completely.

4.3 When yellow **M-STOP** field is illuminated either the mechanical handwheel is locked or the handwheel brake is activated. The microtome can not be used as long as this is lit.

5. SEPARATE CONTROL PANEL:



5.1 Sectioning Modes:

5.1a Rock- Turn the handwheel a short distance forwards and backwards for cutting, without completing a full handwheel rotation.

5.1b Single: A single slice of paraffin embedded tissue from the microtome. The routine section is usually cut at 4-6 micrometers (microns).

5.1c Step: A section of tissue from paraffin-embedded blocks is cut by the microtome and a slide is prepared than another section is taken after a specified distance into the tissue (e.g. 200 micrometers from the original section)

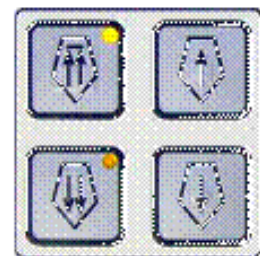
5.1d Continuous: Sections from the paraffin embedded tissue are taken consecutively for slides to be prepared. The paraffin forms a ribbon after being cut on the microtome. The consecutive sections are taken from the ribbon.



5.2 +/- Buttons for setting the section thickness/trimming section thickness



5.3 Speed control knob- determines speed of sectioning.



5.4 Course feed arrows:

1) ↑↑- Rapid movement of specimen away from the knife.

3

2) ↑- Slow movement of specimen away from knife.

4

3) ↓↓ - Rapid movement of specimen towards the knife.

4) ↓ - Slow movement of specimen towards the knife.



5.5 Trim/Sect Button:

Button for switching between sectioning mode and trimming mode. Able to set different thicknesses for each one.

5.6 Run/Stop + Enable buttons

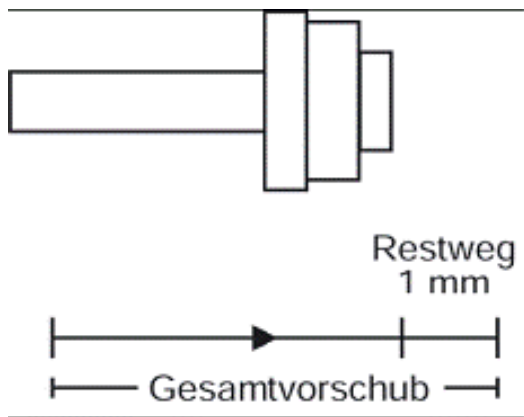


- To start motorized sectioning, press the **RUN/STOP** and **ENABLE**

buttons simultaneously after selecting the desired operating mode.

- While the sectioning motor is running, the yellow LED in the **RUN/STOP** key lights up.
- To stop motorized sectioning, press **RUN/STOP** or **ENABLE**.

Object Head



5.7. Indication of remaining horizontal feed

The visible and audible remaining feed indication feature informs the user during trimming and sectioning when a remaining feed of approximately 1 mm is available before

Remaining
Horizontal
Feed 1mm

the front limit is reached.

The yellow LED in the COARSE FEED button lights up from the beginning of the remaining feed. In addition, you

HOME

will hear a beep for approx. 2 seconds.

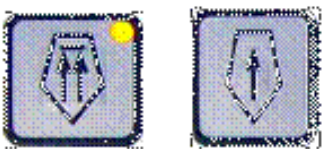
Total horizontal feed

The sectioning process is interrupted and the object head stops

in the upper end position.

From this point on, a remaining feed of approx. 1 mm is available.

In the remaining feed area, no more object feeding to the knife is possible using the coarse feed button. When the front end position is reached, the sectioning process stops automatically, and the yellow LED in the COARSE FEED button lights up.



You can continue to work on the specimen by pressing the corresponding coarse feed button until the Object Head has fully receded into the rear end position (**HOME**).