RMC Products

by Boeckeler

CR-X
Instruction Manual

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CR-X

Cryosectioning System
Instruction Manual

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Chapter

One

Introduction to Your CR-X Cryosectioning System

1.1 Introduction

All of us at Boekeler Instruments would like to thank you for purchasing your **CR-X Cryosectioning System**. To become familiar with your new instrument, please carefully read this manual. If the instrument has not yet been unpacked and set up, please refer to Chapter Two for unpacking and installation procedures.

This instruction manual assumes that you are already an experienced cryoultramicrotomist. If you are not, we suggest that you refer to one of the many excellent books on the subject listed in Appendix Three. Personal instruction from an experienced cryoultramicrotomist would be most helpful in learning techniques. Your representative will also be able to help instruct you in the use of your new instrument. In addition, the staff of the Boeckeler Applications Laboratory is available to help you with your specific application needs.

For additional information, ordering information, or applications help, call 520-745-0001 or Fax 520-745-0004 in the continental U.S. or email us at info@rmcproducts.com. In other countries, contact your local distributor service center. Please visit our website at www.rmcproducts.com.

1.2 System Description

This section provides an overview of the CR-X, its features and capabilities, and general descriptions of the various parts of the system. Refer to Figure 1.1 on page 1.2.

We suggest that you read this portion of the manual in order to familiarize yourself with the CR-X System before you proceed with installation and operation of the instrument. Specific details of installation and operation are covered in later Chapters of this manual.

General Features and Capabilities

The CR-X is a self-contained, microprocessor-controlled cryosectioning system, which has been designed to be compatible with the entire line of RMC ultramicrotomes in addition to other brands of and many other modern ultramicrotomes. The cryosectioning system is easily installed on the ultramicrotome by removing the upper knife stage, installing the cryochamber on the lower stage, and attaching the specimen arm extension into the end of the cutting arm of the ultramicrotome.

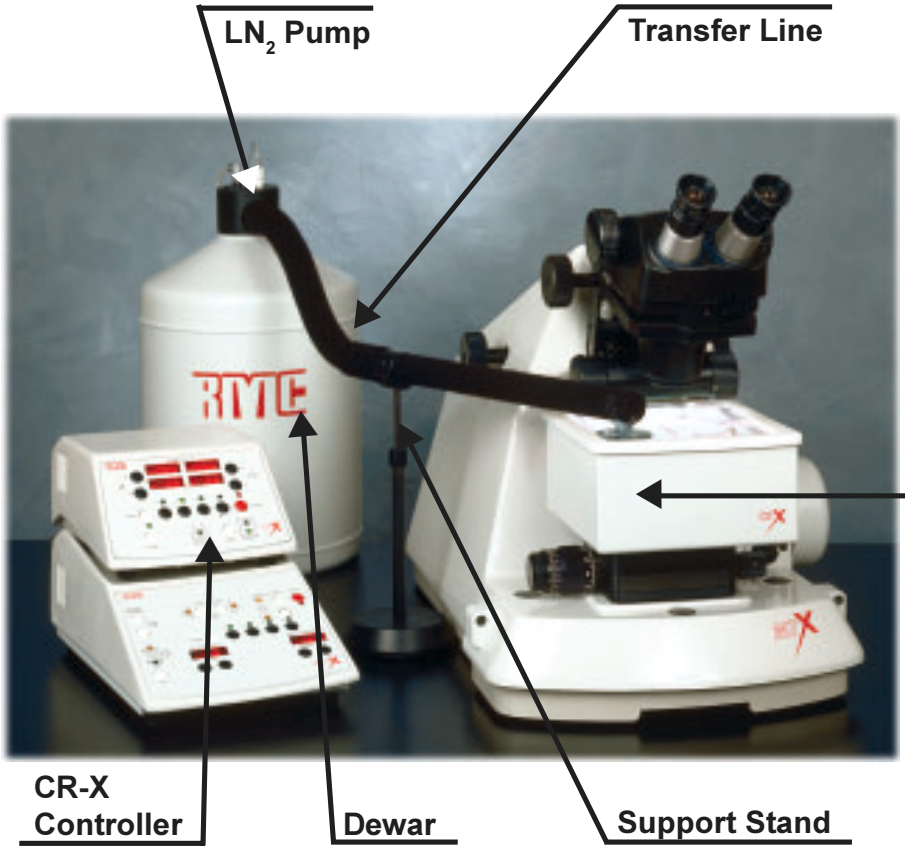


Figure 1.1 CR-X on the RMC MT-X Ultramicrotome

Liquid Nitrogen (LN_2) is used to cool the specimen and the knife. It is delivered to the cryochamber by a unique LN_2 pump and low-consumption transfer line. Level sensors in the cryochamber automatically regulate the level of LN_2 by turning a liquid nitrogen pump on and off. Thermal conductors immersed in the liquid nitrogen cool the specimen and knife. Temperature sensors and heaters permit independent regulation of the specimen and knife temperatures.

Controller

The controller for the CR-X accurately regulates temperatures and LN_2 delivery. The temperatures of the specimen and the knife are shown on the controller's LED (Lighting Emitting Diode) displays. **Refer to Figure 1.2 on page 1.3.** The controller will also indicate when the LN_2 level in the Dewar is low. The controller provides the means for the operator to select temperatures for the specimen or knife and to store, recall, or modify these temperatures. Additionally one can turn the LN_2 pump on and off, select either a high or low liquid nitrogen level in the trough, and initiate the warming cycle for the cryochamber.

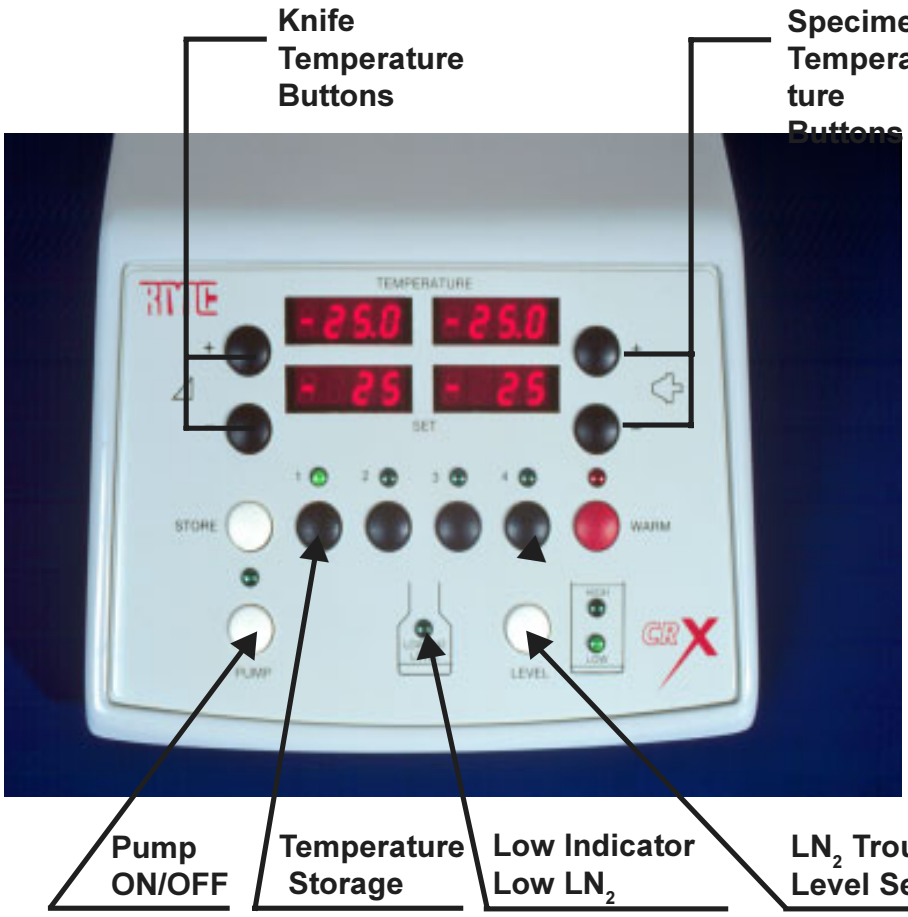


Figure 1.2
Controller (Overview)

Detailed instructions for the controller are found in **Chapter Three** of this manual.

The main controller has four temperature storage memory locations; each location permits the storage of separate specimen and knife temperatures - any of the stored temperatures may be instantly recalled for use. The knife and the specimen may be set to any temperature from +35° C to -185°C with a temperature stability of ±0.1°C, (for temperature lower than -185°C, allow LN_2 to fill directly into chamber).

There is also an automatic “warm” cycle which evaporates any remaining liquid nitrogen and warms and dries out the components in the cryochamber. This feature is especially useful for regenerating the system after it has been used.

Dewar and Pump

A 9 liter Dewar serves as the liquid nitrogen reservoir. Under normal operating conditions, liquid consumption is 0.75 liters per hour. One full Dewar of liquid nitrogen will therefore be sufficient to operate the CR-X for a complete working day.

The submersible portion of the LN_2 pump also contains the actual LN_2 displacement pump and the LN_2 low level sensor. When the LN_2 Dewar needs to be refilled, the “low level” display will be activated on the controller front panel.

Cryochamber and Specimen Arm

The cryochamber, shown in Figure 1.3, maintains both the knives and specimen at the desired temperature. Holders are provided for glass, diamond and triangular tungsten carbide knives.

The specimen arm extension enters the cryochamber via a thru-the-wall baffle seal. The specimen arm in turn attaches to the end of the microtome arm. The cryochamber slides onto the dovetail guides of the lower stage of the ultramicrotome.

Figure 1.2 Cryochamber

Electrical connections from the specimen arm extension and a baffle heater are made on the side of the cryochamber. These connections, along with the necessary cables from the cryochamber, are routed to the controller.

The knob located on the right side of the cryochamber on the shaft is used to rotate the knife stage to orient the knife to the specimen.

The knife stage is easily seen in the center of the cryochamber. Knife holders (with knives) are placed in the curved recess of the upper knife stage. Also inside the cryochamber, note the u-shaped metal trough, which is located along the inside walls of the chamber. This trough is for liquid nitrogen, which is delivered by the liquid nitrogen transfer line. A metal heat sink from the knife stage extends into this trough: it acts to cool the knife stage. In a similar fashion, two heat sinks on the specimen arm extend into the trough on each side.

A small heater located in the back wall of the liquid nitrogen trough gently vaporizes liquid nitrogen. This heater is under microprocessor control. The controlled boiling of liquid nitrogen causes a continual outflow of dry nitrogen gas from the cryochamber, thereby preventing atmospheric moisture from entering the chamber and collecting as frost on the cold surfaces. A small toggle switch located on the back of controller, labeled "LN_2 boil-off", switches this boiling to its maximum level when the atmospheric humidity is especially high and troublesome.

A perforated metal cover fits over the liquid nitrogen trough. When the system is installed the liquid nitrogen delivery tube is suspended directly above the perforated metal cover. The delivery tube should be installed so that it does not touch the cryochamber walls.

Liquid Nitrogen Dewar and Cryogen Transfer System

The liquid nitrogen delivery system has three components: the LN_2 pump, the transfer line, and the power supply. The LN_2 pump submerged in the Dewar of liquid nitrogen which pumps and transfers LN_2 through an insulated transfer line, **Refer to Figure 1.1 on page 1.2,** and the power supply unit, which also controls the bellows pump. The bellows pump provides the air pulses which power the LN_2 pump. The power supply is normally kept on the floor.

Chapter

Two

Unpacking and Installation of the CR-X

Unpacking and installing of the CR-X is best left to a trained RMC representative. However, if necessary, you may unpack and install the instrument yourself by following these instructions carefully.

2.1 Unpacking Instructions Carefully unpack each individual package. Do not bend the long black transfer line. Lay it flat on a table until you are ready to install it. Carefully inspect all packing materials to be certain that no parts are discarded accidently. Be sure to identify and inspect all parts for loss or shipping damage. **Refer to the Figures in Chapter One and Appendix Four** in the back of this manual.

2.2 Electrical Set-Up Requirements The CR-X cryosectioning system is equipped with a universal power supply adapter, which is located at one end of the power supply unit. It can be adjusted to make the instrument compatible with any of the following power sources:

100V, 50/60 Hz Single Phase	**220V, 50/60 Hz Single Phase**
120V, 50/60 Hz Single Phase	**240V, 50/60 Hz Single Phase**

The power voltage is selected on the mini printed circuit board located inside the adapter next to the power cord connection and main power switch. The position of the white pin indicates the voltage selected.

Warning: Do not plug in the main power cord until the correct voltage selection change-over has been made. Failure to make this adjustment before supplying power to the instrument can result in severe damage to the instrument.

2.2.1 Voltage Selection & Fuses Adjust the voltage selector as follows:

1. Remove the fuse housing on the back of the power supply by prying it off at the left-hand side with a small screwdriver. **Refer to Figures: 2.1, 2.2, 2.3, & 2.4 on pages 2.2 and 2.3.**

2. Remove the P.C. board using either a hook fashioned from a paper clip or a pair of needle nose pliers. **Refer to Figure 2.2 on the next page.**

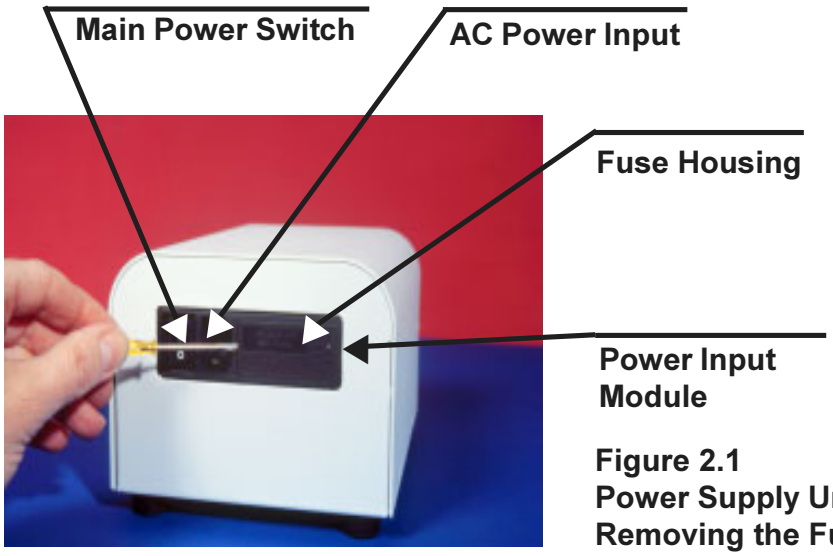


Figure 2.1
Power Supply Unit Showing
Removing the Fuse Housing

Figure 2.2
Removing the Voltage Selector Board

Select the appropriate voltage by rotating the board so that the desired voltage (printed on the board) and the nearby arrow are oriented on the left side of the board and are pointing into the slot which accepts the board. Then rotate the central white plastic part on the board so that when the board is inserted into its slot the pin points outward and projects through the appropriate hole on the voltage selector cover. Insert the board back into its slot and firmly seat the board in the slot.

3. Check the fuse. Use a one amp fuse for 100 or 120 volts or a 0.5 amp fuse for a 220/240 volt operation, **Refer to Figure 2.3 on page 2.3.** Always use 250 V slow blow fuses. The one amp fuses are the larger supplied. To change to the smaller 0.5 AMP fuses: first remove the screw, which secures the fuse holder to the cover, **Refer to Figure 2.4 on page 2.3;** turn the fuse holder over so its opposite side is visible; reattach the holder to the voltage selector cover, using the screw this will expose the fuse holder; put the appropriate fuses into place; and then snap the fuse housing back into place. Verify that the white plastic pin is projecting through the correct hole to indicate the local voltage.

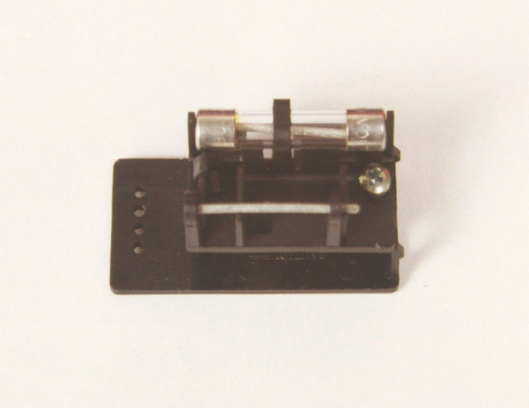


Figure 2.3 Fuseholder
Configured for 1.0 amp U.S. Fuses

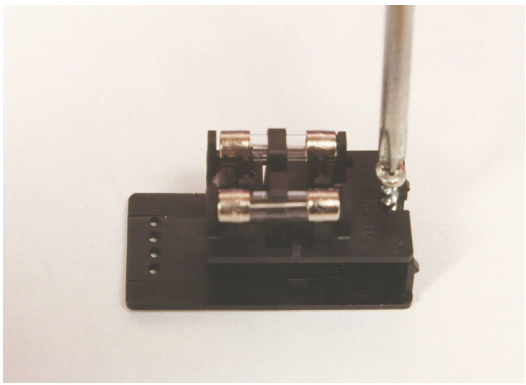


Figure 2.4 Fuseholder
Configured for 0.5 amp European Fuses

2.3 Installation Procedures The following instructions explain how to install the CR-X onto the RMC model MT-X, or MT-XL ultramicrotomes. Installation on a Reichart or Leica is very similar. **Refer to Appendix 7, page 10.** These instructions are written with the assumption that you have already set up the ultramicrotome, familiarized yourself with its operation, and have used the instrument for room temperature sectioning. Please refer to your **Ultramicrotome Operator's Manual. Figure 2.6 on page 2.5** illustrates the CRX cryosectioning chamber and specimen arm, showing the location of components referenced in the following procedure.

CAUTION: To avoid possible damage to the microtome, the motor should always be off whenever the handwheel is turned by hand.

2.3.1 Preparing the Ultramicrotome Remove the upper knife stage from the microtome. To do so, release the stage locking lever located on the right side of the lower stage. Slide the upper stage off. Remove the specimen orientation head (arc segment holder) by loosening the locking screw. The microscope arm can be swung away 90° to the right or left, taking care not to overstretch the light cord. Reset the advance mechanism of the microtome so it is at the beginning of its travel.

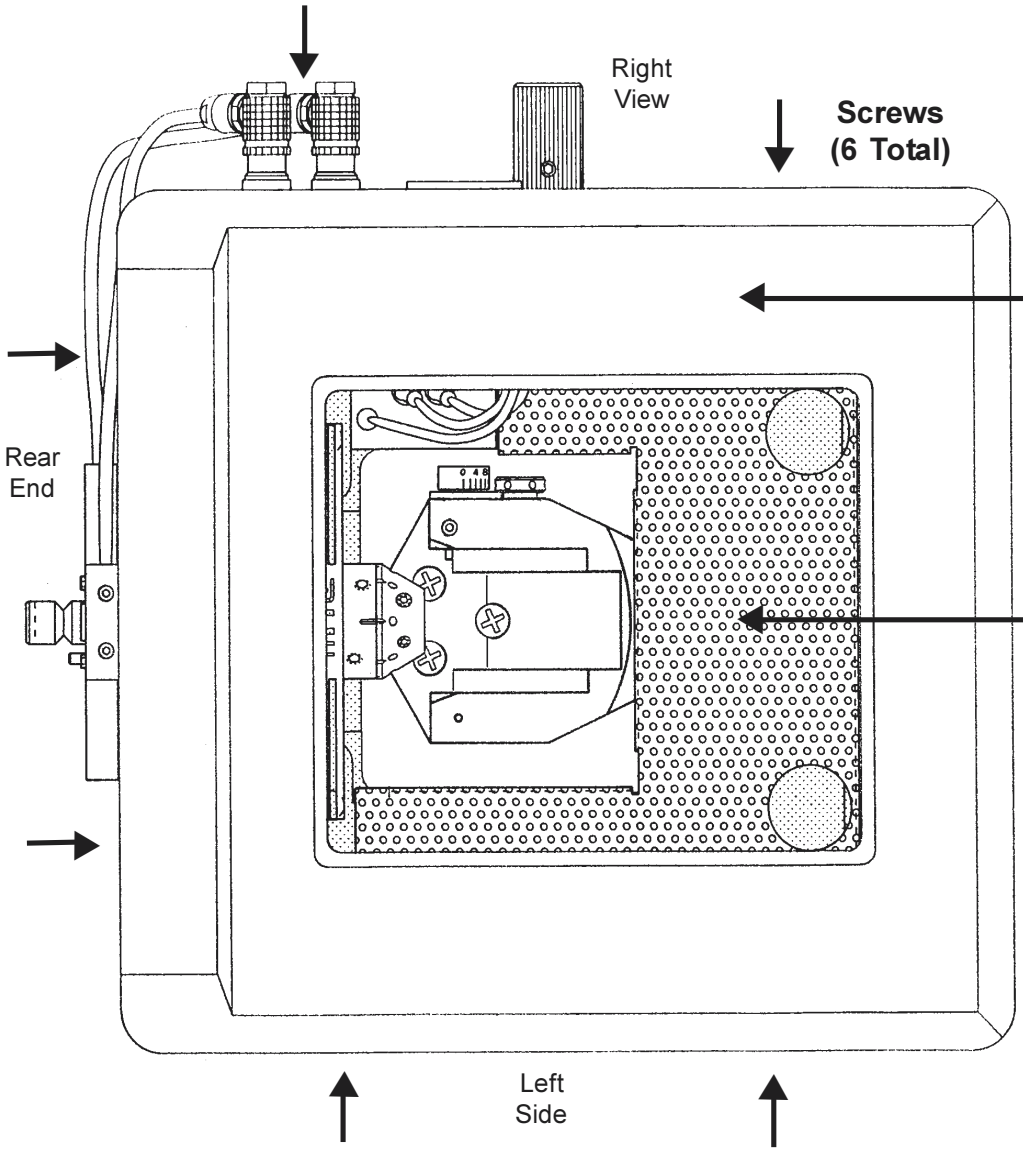


Figure 2.5 Cryo-Chamber with Cover (Top View)

Key to Figure 2.5 Cryo-Chamber with Cover (Top View) location of mounting screws are shown by the short arrows.

2.3.2 Specimen Holder Installation For better access to the cryo-chamber for installation, remove the chamber cover. **Refer to Figure 2.5.** There are six set screws that hold this cover on. Use the hexagonal wrench with the white band ($^{1}/_{16}$ inch) supplied in your accessory kit, to loosening the six mounting screws. Next remove the **perforated metal trough cover (7), Figure 2.6 on page 2.5.** Carefully insert the **specimen arm (11)** through the hole in the **baffle seal (14)** from inside the cryochamber.

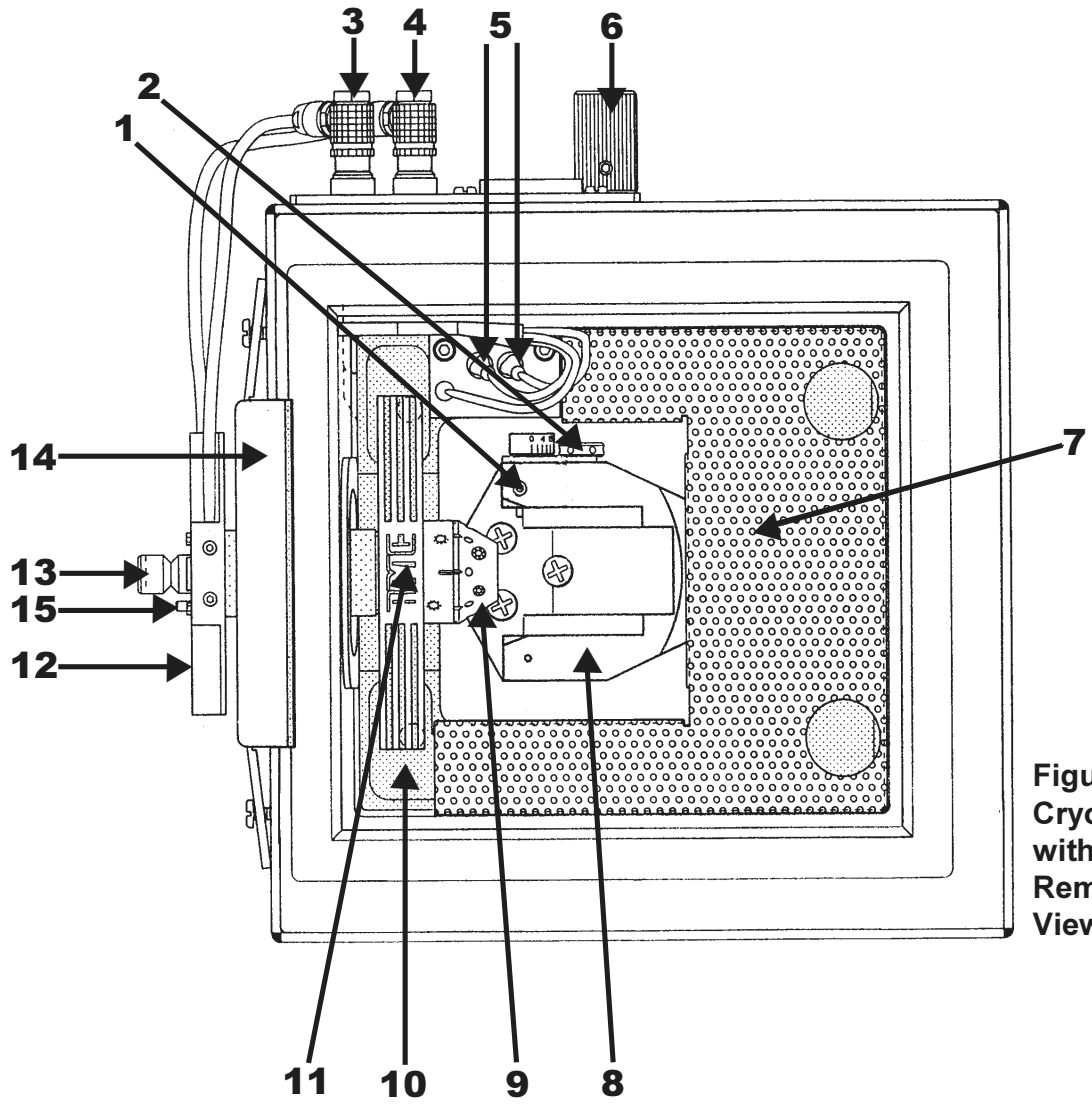


Figure 2.6 Cryo-Chamber with Cover Removed (Top View)

Key to Figure 2.6 Cryo-Chamber with Cover Removed (Top View)

1. Knife Angle Adjustment Screen
2. Knife Holder Clamping Wheel
3. Baffle Heater Connector Cables
4. Specimen Arm Connector
5. Level Sensors
6. Knife Stage Rotation
7. Trough Cover
8. Knife Stage
9. Specimen Mount
10. LN_2 Trough
11. Specimen Arm
12. Baffle Heater Plate
13. Specimen Arm Mounting Stud
14. Baffle Seal
15. Alignment Pin

Next, replace the **perforated metal trough cover (7)** and finally the painted cryochamber cover. Slide the **baffle heater plate (12)** over the end of the specimen arm and tighten it into place with the two set screws. Next, plug in the **specimen arm connector (4)** and **baffle heater connector cables (3)** into their respective sockets on the right-hand side of the cryo-chamber.

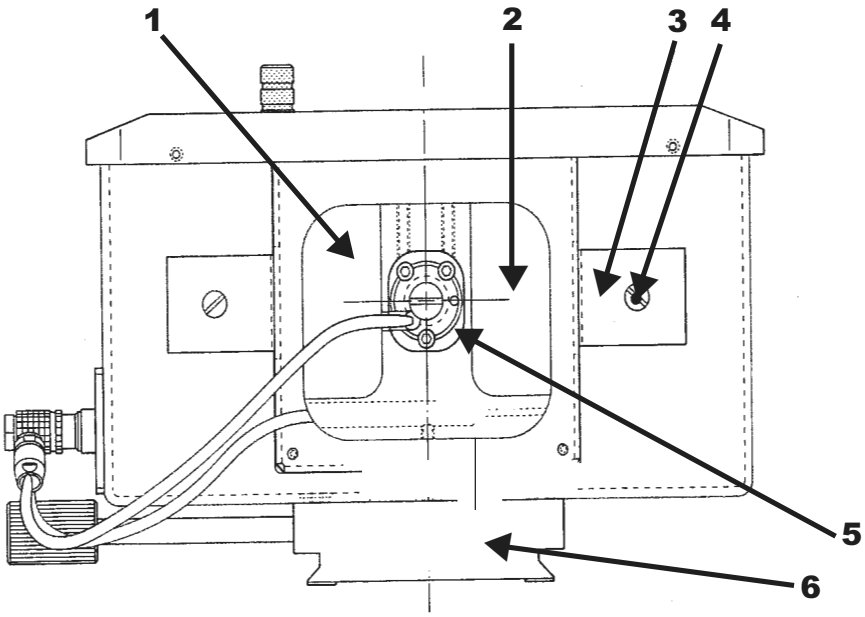


Figure 2.7 Cryo-Chamber (Rear View)

Key to Figure 2.7 Cryo-Chamber with Cover Removed (Top View)

1. **Baffle Heater Plate**
2. Baffle Seal
3. Baffle Seal Clips
4. Screws for Clips
5. Specimen Arm
6. Dovetail Mounting Plate

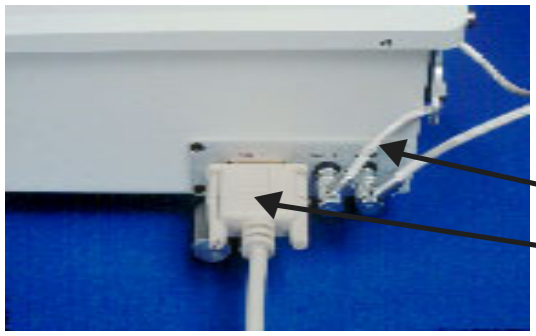


Figure 2.8 Cryo-Chamber (Side View)

2.3.3 Cryochamber Installation Slide the **dovetail mounting plate (6)** and the cryochamber onto the lower knife stage. At the same time, gently guide the **specimen arm mounting stud** into the end of the ultramicrotome **cutting arm** as far as it will go. Align the specimen arm so that the **alignment pin (15) Figure 2.6 on page 2.5**, fits into the corresponding hole on the cutting arm. Tighten the specimen holder using the locking screw on the cutting arm. The position of the cryochamber is very important. It must be positioned such that the **specimen mount, (9) Figure 2.6,** is neither too far nor too close to the knives. Rotate the handwheet slowly and carefully to be sure the back of the specimen arm does not hit the back inside wall of the cryochamber. Also, be sure that the round chrome-plated heat sinks extending beneath the specimen arm do not contact the walls of the **LN_2 trough (10) Figure 2.6.** Now lock the cyrochamber into place using the locking screw on the right side of the ultramicrotome lower stage.

2.3.4 Power Supply and Control Unit Location Locate the power supply unit on the floor or on a bench top separate from the one the ultramicrotome is on. This will prevent the vibrations of the power supply unit from interfering with specimen sectioning. Locate the controller at any convenient position on the microtomy table or on top of the MT-X or MT-XL control unit.

2.3.5 Preparation of the Transfer Line Before using the CR-X for the first time, you must attach the support stand to the liquid nitrogen transfer line. **Refer to Figure 2.9 below.**

Remove the two screws securing the upper portion of the clamp on the transfer line support stand. **Refer to Figure 2.10 on page 2.8.**

Place the support stand on the rigid part of the transfer line, next to connection with the flexible part. This is about 33cm (13 inches) from the delivery tube end of the line. Then place the transfer line into the lower portion of the support stand's clamping device. Place the top portion of the clamp over the transfer line, and secure the halves of the clamp around the transfer line by reinserting the two screws. **Refer to Figure 2.10 on page 2.8.** Before tightening the screws, be sure the delivery tube is pointing straight down.

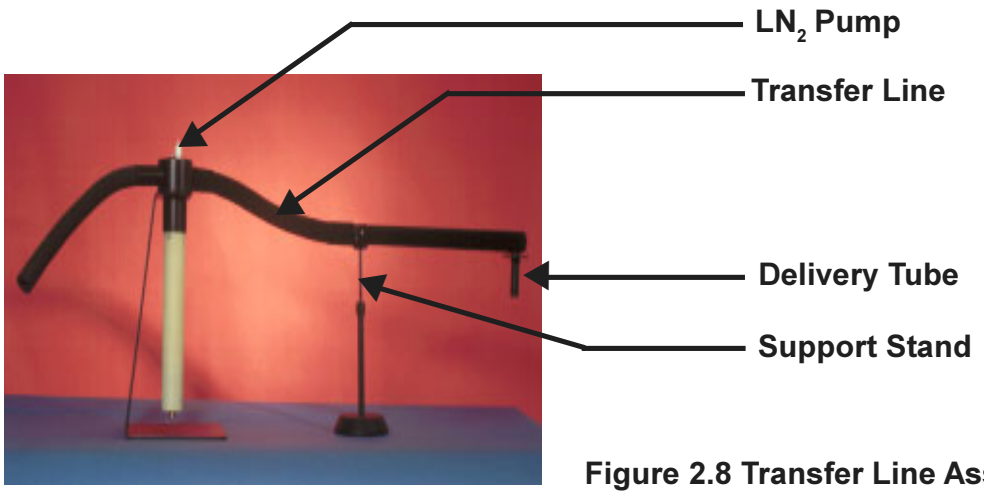


Figure 2.8 Transfer Line Assembly Overview

Figure 2.9 Attaching Support Stand to Transfer Line

2.3.6 Preparation of the LN_2 Pump

Place the transfer line stand to the left of the microtome (or the right side if preferred). Be careful not to bend the transfer line more than 45°, this can do permanent damage to the line.

Loosen the knurled ring on the transfer line support stand, insert the long black rod on the transfer line into the transfer line stand, tighten as needed.

Note that the transfer line and the LN_2 pump head are a single unit. **DO NOT** attempt to separate the transfer line from the LN_2 pump.

Connect the clear plastic tubing between the top of the LN_2 pump, **Figure 2.11, page 2.9** and the tubing fitting on the power supply unit and the power supply. Place the LN_2 pump in the support stand.

Place the Dewar to the left or to the right of the ultramicrotome, whichever is most convenient, so that the fill line can be lowered into the cryochamber. The height of the transfer line can be adjusted by raising or lowering the transfer line stand. Secure the transfer line in place by tightening the knurled knob.

Lower the delivery tube on the transfer line into the cryochamber so that LN_2 will flow into the one of the holes in the trough cover. Install the exhaust hose onto the cold nitrogen gas vent on the side of the LN_2 pump. This tube vents the cold nitrogen gas, which boils off as LN_2 flows through the transfer line.

2.3.7 Electrical Connections Verify the power switches on the back of the controller and on the pump/transformer box are both in the OFF (“O”) position.

WARNING: To avoid potential electrical shock, the power cord to the controller should be disconnected and the power switch should be off while connecting the cable to the controller.

Plug the white cable into the four-hole socket on the top of the displacement pump; use the right angle end of the cable. Plug the other end (straight connector) of this cable into the socket on the back of the controller, **Figure 2.12 (3) on page 2.10.**

Plug the male 15 pin D connector cable into the back of the controller and the female connector on this cable into the side of the cryochamber.

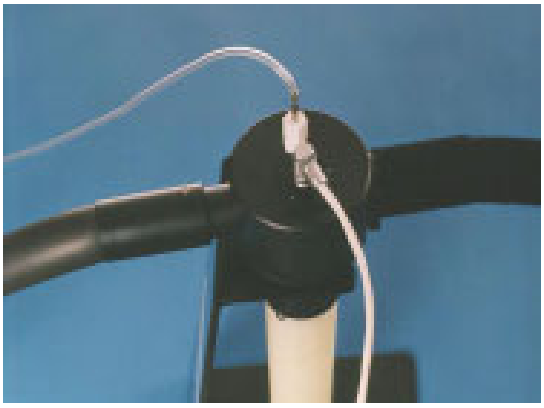


Figure 2.10 Top of the LN_2 Pump

Verify that the electrical connector from the arm is plugged into its receptacle on the side of the cryochamber (labeled **“ARM”**), and that the electrical connector from the baffle is plugged into its receptacle on the side of the cryochamber (labeled **“BAFFLE”**). To plug these in, match the red dot on the connector to the red dot on the receptacle.

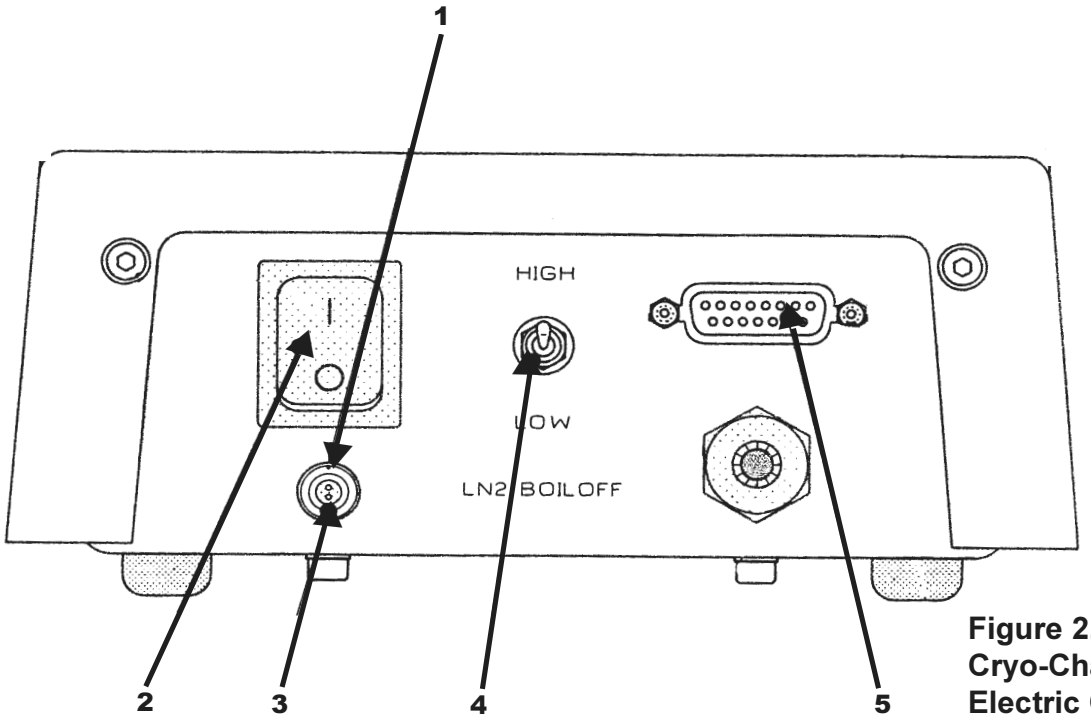


Figure 2.12 Cryo-Chamber Electric Controller (Rear View)

Key to Figure 2.12 Cryo-Chamber Electric Controller (Rear View)

1. Red Dot on Connector *(Item 3)*
2. Power Switch
3. Dewar Level Cable Connector
4. LN_2 Boil-Off
5. Cryo-Chamber Connector

Connect the AC cable between the power supply unit and the controller. Be sure that the voltage selector board has been set to the proper voltage. **Refer to Section 2.2.1 - Voltage Selection and Fuses on page 2.1.** Connect the power cord to the back of the power supply. Make sure the main power switch to the CR-X is in the OFF ("O") position.Then plug the power cord into the wall outlet.

> **NOTE: If a wall outlet having a 3-wire ground is not available, a three prong adapter with the green wire connected to a verified ground should be used to establish proper grounding of the instrument.**

The power to the controller may now be turned on.

2.4 Final Connections and Set-Up

2.4.1 Connect the clear plastic tubing to the fittings (metal tubes) on the power supply and the top of the LN_2 Pump. **Refer to Figure 2.11 on page 2.9.**

2.4.2 Fill the LN_2 Pump Dewar, and place the cap on the Dewar. The Dewar holds nine liters of LN_2 . **Do not completely fill the Dewar. Leave approximately (3 inches - 7.5 cm) of empty space at the top to allow for the submersion of the LN_2 Pump.** The first filling will induce a significant amount of "nucleate boiling." After the boiling has subsided and the interior of the Dewar has cooled, more LN_2 may be added if necessary.

2.4.3 Verify the "U"-shaped protective mesh trough cover is installed in the cryochamber, over the LN_2 trough. **Refer to Figure 2.6 (7) on page 2.5.**

2.4.4 Install the disk-shaped plastic vapor block ring over the end of the LN_2 delivery tube, and then install the O-ring over the delivery tube to hold the vapor block ring in place, **Figure 2.14 below.**

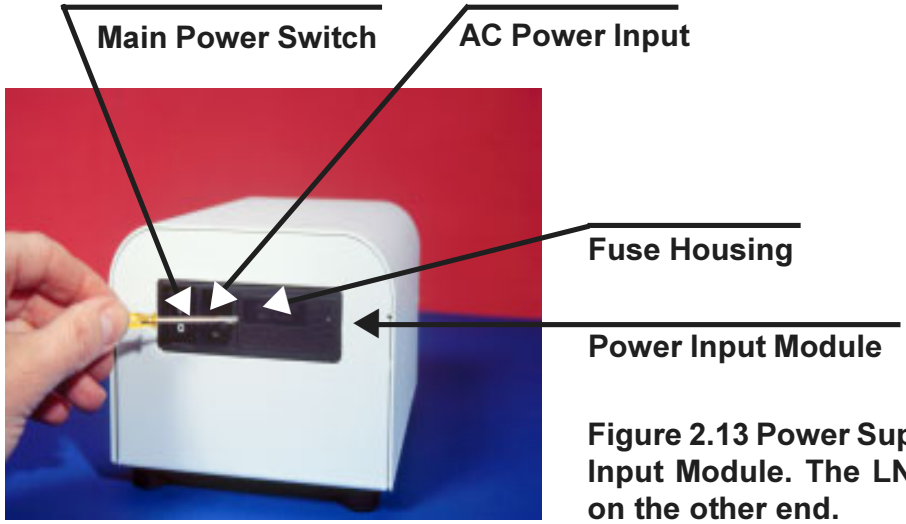


Figure 2.13 Power Supply Unit Showing Power Input Module. The LN_2 pump tubing outlet is on the other end.

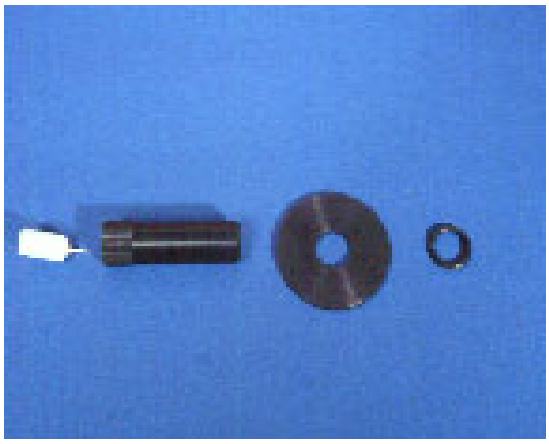


Figure 2.14 Delivery Tube (Exploded View)

The parts appear from left to right:
Float Valve, Delivery Tube,
Vapor Block Ring and O-Ring

2.4.5 Position the liquid nitrogen LN_2 pump to the left or alternatively the right of the cryochamber. Adjust the height of the support stand (if nessary) so the end of the delivery tube is approximately 2.5 cm (one inch) above the mesh screen in the cryochamber.

2.4.6 Place the LN_2 pump Dewar near the LN_2 pump.

2.4.7 Place the clear plastic cover on the cryochamber. Orient the cover so the cutout in the cover is in the lower left-hand corner and the vapor block ring rests on top of the cover.

2.4.8 Grasp the head of the LN_2 pump with one hand, and the support stand with the other hand. Lift the entire LN_2 pump as a unit, keeping the transfer line parallel to the bench top. Gradually lower the LN_2 pump into the LN_2 pump Dewar, at the same time returning the LN_2 delivery tube to its original position in the cryochamber. Now move the transfer line support stand so that the delivery tube is over the circular hole on the trough cover. **Refer to Figure 2.5 on page 2.4.**

CAUTION: Rapid nucleate boiling will occur after about one minute. Lower the pump in the Dewar slowly so that this fast boiling will not force the LN_2 out of the vent tube.

Make small adjustments to the position of the liquid nitrogen Dewar as needed. During operation, after the transfer line has cooled and contracted, it will be necessary to make small adjustments to the position of both the LN_2 Dewar and transfer line support stand, so that the delivery tube does not touch the cryochamber cover.

Chapter

Three

Basic Operation of Your CR-X Controller

WARNING: Observe safety procedures for handling liquid nitrogen as outline in Appendix 1 before removing the CR-X from the microtome.

3.1 Controller

Figure 3.1 - CR-X-Controller (Front View)

3.1.1 Start up Turn on the power switch located on the power supply. At this point the normal displays will be as follows:

1. Top Displays: both will indicate the actual temperatures of the knife (left) and specimen (right).

2. Bottom Displays: both will read **OFF**

3. Low LN_2 Light: **FLASHING** green when at room temperature or when LN_2 level is low.

4. Turn on the power switch located on the back of the controller.

5. Press one of the four black temperature memory buttons labeled 1, 2, 3, or 4 to select a set temperature for the knife and the specimen. Select Low Level for LN_2 level unless going colder than -90°C.

NOTE: The factory preset values, Table 3.1 o n the next page, have the same temperatures for the knife and specimen in each memory storage location. These are only suggested temperature ranges. You can change all of the temperatures and store your new selections in any of the four memory storage locations.

MEMORY LOCATION	KNIFE TEMP, °C	SPECIMEN TEMP, °C
1	- 20	- 20
2	- 60	- 60
3	-120	-120
4	-140	-140

Table 3.1 Factory Pre-Set Temperature Chart

3.1.2 Temperature Control The control unit has four windows with red LEDs for the temperature displays. The bottom two windows display the temperature set by the user, and the top two windows display the actual temperature. The two windows on the left indicate actual and set temperatures for the knife; the two windows on the right indicate actual and set temperatures for the specimen.

1. Knife temperature control To set the temperature for the knife, use the two black buttons on the left side of the LED displays beside the knife icon to move either up or down toward the desired setting.

2. Specimen temperature control To set the temperature for the specimen, use the two black buttons on the right side of the LED displays beside the specimen icon to move either up or down toward the desired setting.

3.1.3 Memory Storage Buttons The CR-X can store up to four temperature settings. This enables the user to set temperatures, store them into the memory, and then recall the temperature settings at any time by pressing the button. To store a set of temperatures: first press one of the memory storage buttons - these buttons are black and are numbered 1 through 4 - when a button is pressed, the corresponding green light above the button will illuminate; Set the temperature desired; and then press the white **STORE** button. The temperatures selected will be stored under the corresponding button until the numbers are reset.

3.1.4 Pump ON/OFF To begin pumping LN_2 into the cryochamber, press the white pump button. LN_2 will begin flowing from the transfer line in less than one minute.

NOTE: LN_2 should begin flowing within one minute. The LN_2 trough should fill in less than ten minutes. The level of LN_2 in the trough will be controlled automatically. The chamber will be ready for sectioning in 20 to 60 minutes, depending upon the selected temperatures. Refer to Chapter 4 Diagnostics Maintenance, if no LN_2 flows.

3.1.5 Low LN_2 Level Indicator When the green light of the low LN_2 level symbol illuminates, the level of the LN_2 in the Dewar is low.

3.1.6 Setting the Required LN_2 Level The level of LN_2 in the cryochamber trough can be selected using the level button and choosing either **HIGH** or **LOW**. The corresponding green indicator light will illuminate when a button setting is chosen.

> **NOTE: If a temperature of -90°C or lower is being used, use a "HIGH" LN_2 level. If a temperature warmer than -90°C is being used, use the "LOW" LN_2 level. These settings ensure maximum stability and economy of LN_2 consumption.**
>
> **NOTE: For a more rapid cool down from ambient temperature, use a "HIGH" LN_2 level. After the knife and specimen have reached their set temperatures, the level can be changed to "LOW", if work is being done above -90°C,**

Two sensors in the chamber trough automatically control the LN_2 system. The LN_2 levels is by physically raising or lowering the sensors.

3.1.7 Warm Up The **WARM** switch is selected at the end of a sectioning session. It turns off the trough heater, LN_2 pump, and programs the specimen and knife set point temperatures to +35°C. This helps the cryochamber dry out before the next use. The warm function may be left on overnight, but this is not usually necessary.

3.2 System Preparation

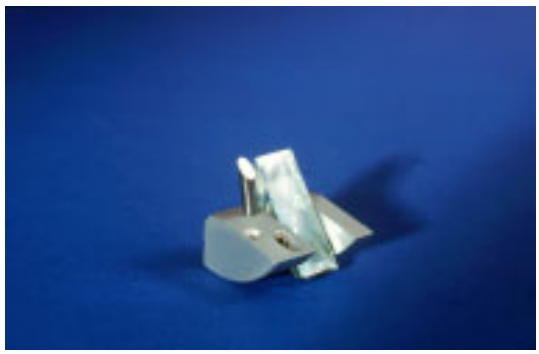
3.2.1 Remove the plastic chamber cover and remove the transfer line delvery for more access room.

3.2.2 Select the appropriate size specimen pin adapter to be used, and insert it into the specimen holder. **Refer to Figure 3.2 page 3.4**. Lock the pin adapter in place by tightening the adapter screw using the $^1/_{16}$" white band hex wrench.

NOTE: The flat specimen clamp can be mounted directly into the specimen arm adapter and tightened using the white band hex wrench ($^{1}/_{16}$ inch). Be certain that the knife stage is far enough back so the specimen will clear the knife edge when it is later placed in the specimen holder. Use the hex wrench with the blue band ($^{5}/_{64}$ inch) to tighten the specimen in the flat specimen holder.

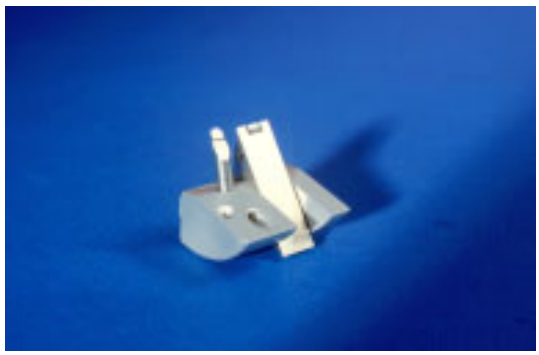
3.2.3 Place a glass or diamond knife into the appropriate holder and tighten the locking screw, **Refer to Figure 3.2 below,** with the blue-white band hex wrench ($^{3}/_{32}$ inch) ball driver. Do not over-tighten.

NOTE: To use an 8 or 9mm glass knife, use either the thick glass knife holder, Figure 3.2 a, with the 3mm wide triangular metal spacer provided in the accessory box or the diamond knife holder, Figure 3.2b.



a.

Thick Glass Knife Holder. Shown with a 12mm Glass Knife and a Tungsten Carbide Trimming Tool.



b.

Diamond Knife Holder. Shown with a Dry-Cryo Diamond Knife and a Diamond Trimming Tool.

c.

Double Glass Knife Holder Shown with a 6mm Glass Knives and a Tungsten Carbide Trimming Tool.

Figure 3.2 Knife Holders

3.2.4 Screw the Knife retrieval tool into the hole on the top of the knife holder and use this tool as a handle to help place the knife holder assembly into the stage in the cryochamber. Be sure that the curved surface of the knife holder makes good contact with the curved surface of the knife stage. The front of the knife holder should make contact with the pin which sets the knife clearance angle.

NOTE: The threads of the retrieval tool should be dry before re-use.

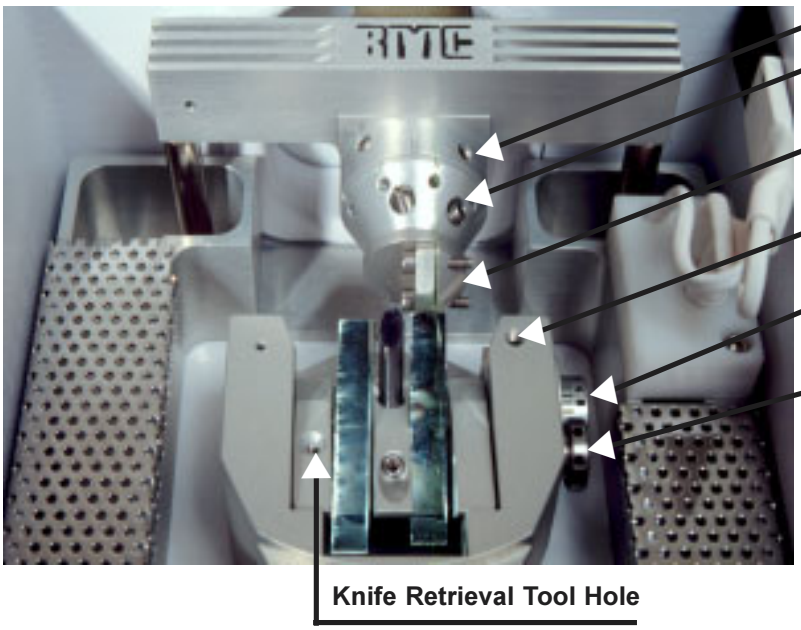


Figure 3.3 Cryo-Chamber (Inside View)

3.2.5 Adjust the knife clearance angle using the white band hex wrench ($^{1}/_{16}$") and the knife angle adjusting screw **Refer to Figure 3.3.**

3.2.6 Clamp the knife holder assembly into place by tightening the knife holder locking screw, **Figure 3.3** using the white band hex wrench ($^{1}/_{16}$").

3.2.7 You may pre-cool a spare knife holder and knife, in the front right or left-hand corner of the cryochamber.

3.2.8 Cover the cryochamber opening with either the large single cover or the two-piece splits covers.

3.2.9 Lower the LN_2 delivery tube through the corner hole in the plastic chamber cover. Make sure that the tube does not touch the cover of the cryochamber. When LN_2 is pumped through the transfer line, it will cool and contract. Before final knife approach and cyro-sectioning, reposition the delivery tube by moving the transfer line support stand so that the delivery tube does not touch the cryochamber walls or covers.

3.2.10 Attach the optional grid holding device. **(Catalog 74091)**, to the knife holder such that the grids are adjacent to the back edges of the knives.

3.3. CR-X LN_2 Withdrawal System

3.3.1 Fill the LN_2 Dewar to about 5cm below the neck opening. Do not overfill into the neck tube. Allow the Dewar to stabilize, which is indicated by the cessation of violent boiling. This normally only takes a few minutes.

3.3.2 Turn on power switch at power supply and the switch on the controller.

3.3.3 Set temperatures on controller, **Refer to Figure 3.1 on page 3.1,** and select the LN_2 level on front of the controller according to the examples on the temperature chart, **Table 3.2 on page 3.7.**

3.3.4 Turn on pump and allow it to run for approximately 5 minutes before placing it in the Dewar. This is to remove any residual moisture.

3.3.5 Slowly lower the LN_2 pump into the Dewar with the pump turned off.

WARNING: If the warm pump is inserted rapidly in the Dewar, eruptive boiling will occur, emitting liquid nitrogen from the neck of the Dewar. Wear protective clothing and a face shield. Take precautions to avoid LN_2 droplets from becoming trapped between skin and clothing. This can result in serve "burn" damage. Refer to page 1 of Appendix One - Safety Precautions Using Liquid Nitrogen.

After the boiling has stopped, position the transfer line and delivery tube over the LN_2 trough in the cryochamber. Then turn on the pump switch on the controller.

3.3.6 LN_2 should start to flow into the cryochamber within 30 seconds. If the pump does not start delivering LN_2 from the delivery tube within 2 minutes, then "prime" the pump by slipping off the plastic tubing from the pump head for approximately 20 seconds. This allows the pump chamber/valve assembly to fill with LN_2 and clear any trapped gas. Reattach the plastic line and the pump should deliver LN_2 within a few minutes.

NOTE: If the pump fails to deliver liquid nitrogen, refer to Chapter 4 Diagnostics & Maintenance.

3.3.7 The LN_2 trough in the cryochamber should fill in less than five minutes and then the level will be controlled automatically. The chamber will be ready for sectioning between 10 and 45 minutes, depending upon the temperature selected.

SET TEMP.	KNIFE MINUTES	SPECIMEN MINUTES	HIGH/LOW LEVEL SETTING
- 20°C	7	6	Low
- 50°C	10	8	Low
- 80°C	15	13	Low
-100°C	16	14	Low
-120°C	18	18	High
-140°C	22	24	High
-160°C	28	28	High

Table 3.2 Typical Cool Down Times

3.4 Setting Baffle Heater Power Level

Set the power level to the Baffle Heater can be set as follows:

1. Turn OFF the power completely at the Power Supply. Note that you may leave the second power switch on the back of the Controller in either the ON or OFF position.

2. Depress the PUMP button on the Controller.

3. Turn the power back ON, while holding the PUMP button depressed.

4. Look at the Lower Left Display on the front panel of the Controller. It should show a number from 1 to 100, which represents the percent of maximum power being delivered to the Baffle Heater. Use the (+) or (-) buttons for the Knife (Triangle Icon) to select a power level from 1 to 100%. NOTE: If you are installing new Software for the first time, it will show a 0 or 255 until you begin to select a new value.

Suggested Settings:

Low Room Humidity	**30%**
High Room Humidity	**60%**

5. To store the selected power level, depress the LEVEL button. This stores the power level and reverts the Controller to normal operation. OFF will be displayed in the two lower displays if the Controller power switch has been left in the OFF position, or 35 will be displayed and the controller will be in WARM if the Controller switch was left in ON.

6. Operate the unit normally. If frost accumulates on the back of the Specimen Arm, increase the power level to the Baffle Heater by 10% by repeating steps 1 thru 5 above.

Don't forget that on the CR-X model for RMC ultramicrotomes, the baffle heater is incorporated in the baffle shield, whose position can be adjusted on the cutting arm. For greater de-frosting effect, move it closer to the baffle, however be certain that it will not touch the baffle housing after knife approach and during sectioning.

On the CR-X model for the Leica Ultracuts, two heaters are located on the back of the baffle shield.

Avoid using more power to the baffle heater than necessary, as the excess heat might effect sectioning.

3.5. Care After Use: Warming Up the System

3.5.1 When sectioning is completed and specimens removed, press the WARM button on the controller. This switches off the LN_2 pump and resets the knife and specimen holder heaters to 35°C, evaporating moisture from these areas. The system can be safely left on WARM for overnight drying.

3.5.2 At the end of the day, wait five to ten minutes after switching off the pump to allow the line to become pliable, then remove the withdrawal device from the Dewar and replace it onto the support stand. It is very important to leave the plastic tubing attached to the LN_2 pump during warm-up, so that moisture does not condense inside.

It is also important to keep the LN_2 pump upright. If it is laid down in a horizontal position, the valves and tubes could be contaminated by condensation.

3.6 Specimen Preparation and Transfer

3.6.1 The specimen to be sectioned must be affixed to the appropriate specimen pin using a suitable mounting medium and then quickly frozen. The method of freezing will vary with technique and should be determined beforehand. Alternatively, many samples such as polymers may be clamped in the flat specimen clamp as shown in **Figure 3.3 on page 3.5.**

3.6.2 The specimen pins must be kept under LN_2 in a small transfer cup (a plastic 50 ml beaker which has been cut to a height of 1.5 cm is ideal). Plastic bottle caps may also work well. This transfer cup is kept in a small LN_2 Dewar flask until the cryo system temperatures are stable.

3.6.3 When the knife and specimen temperatures have stabilized, remove the covers from the cryochamber and place the transfer cup in the chamber on top of the knife holder, or on the LN_2 trough cover.

3.6.4 Stop the microtome cutting arm at the upper part of its cycle and use precooled forceps to quickly insert the specimen pin into the specimen pin adapter.

3.6.5 Put the transfer cup back into the small liquid nitrogen Dewar flask.

3.6.6 Lock the specimen pin in place by tightening the specimen pin locking screw using the one white band hex wrench ($^1/_{16}$ inch). The screw should be snug, but not too tight; if the screw is too tight it could possibly damage the specimen pin.

3.6.7 If specimen must be trimmed, it may be done at this point. The specimens may be conveniently rotated for trimming by loosening the specimen mount locking screw, **Figure 3.3 on page 3.5**, and rotating the specimen mount. The white band hex wrench ($^{1}/_{16}$ inch) can be inserted in any of the 8 holes. Engraving marks every 45 degrees also permit precise rotations for trimming. The specimen pin adapter itself may also be easily rotated by first loosening its locking screw and rotating it by pushing the tooth-like grooves with the wrench.

3.7 Flat Specimen Mounting

The flat specimen holder is supplied in the accessory kit and is used for mounting specimens frozen by ultrarapid freezing (for example with a MF-7000 Metal Block Freezer or a MF-7200 Propane Jet Freezer) or for various materials science specimens. It is shown on the specimen mount in **Figure 3.3 on page 3.5.**

3.7.1 Place the flat specimen holder clamping screw facing up, into the recess of the clamp holder. Then, place the clamp holder (with flat specimen holder) into the black mounting dish**.**

3.7.2 Place the mounting dish into a styrofoam insulating tray, then fill the container with LN_2 to completely cover the clamp holder, flat specimen holder, and mounting dish. Allow the mounting dish, clamp holder, and flat specimen holder to reach LN_2 temperature.

3.7.3 For better visibility and accessibility allow the LN_2 to evaporate from the inside of the mounting dish. Be sure to maintain a level of LN_2 around the outside of the mounting dish to keep the working area cold.

> **NOTE: Do not expose the frozen sample to warm air. Immerse the sample in LN_2 after ultrarapid freezing before transferring it to the clamp holder.**

3.7.4 Once the LN_2 has evaporated from the inside of the mounting dish, transfer the frozen sample onto the flat surface of the clamp holder.

3.7.5 Using the flat surface of the clamp holder as a work surface, use precooled forceps and a scalpel to carefully break the sample. Shape the sample into a trapezoid having a block face of approximately 1 mm to 2 mm in length.

3.7.6 Use the blue band hex wrench ($^{5}/_{64}$ inch) hex ball driver to loosen the flat specimen holder clamping screw.

3.7.7 Use precooled forceps and gently slide the sample (to be cryosectioned) into the jaws of the flat specimen holder. Be sure to align the block face of the sample so that is parallel with the jaws of the flat specimen holder.

CAUTION: When securing the sample in the flat specimen holder, be sure not to over-tighten the clamping screw, as this could fracture the sample.

3.7.8 Tighten the clamping screw on the flat specimen holder with the wrench.

3.7.9 Check that the sample is securely mounted by gently tapping it with the tip of the forceps. If the sample is loose, realign the sample and retighten the clamping screw.

3.7.10 Partially fill a shallow container, such as a small disposable plastic beaker, with LN_2. Place the flat specimen holder (with sample) into the container and transfer it to the cryochamber. When the knife and specimen have reached sectioning temperature, insert the flat specimen holder into the specimen mount and tighten it in place with the corresponding set screw.

3.8 Sectioning Procedures

The sectioning procedures that follow are general. Detailed, final procedures will vary depending on the needs of the individual project. Various modifications may be necessary to achieve specific results.

3.8.1 Thin Sectioning

(50nm to 200nm, temperature range of -80°C to -140°C). For sucrose, cryoprotected biologicals.

1. View the specimen as follows:
   Tilt the microscope and light forward until the light housing just clears the top of the cryochamber, then move the microscope forward until the specimen is visible.

CAUTION: The drive motor should be off whenever you use the handwheel.

2. Approach specimen using the coarse control on the ultramicrotome knife stage. At the same time, if you are using an MT-X, MTX-XL, MT-7 or MT6000-XL Ultramicrotome you may use the handwheel to rock the specimen up and down until you can easily see the distance between the knife and the specimen. Normally, the specimen will have some small ice crystals on it from being stored in the LN_2; continue to approach the specimen in this manner until the ice crystals contact the knife-edge.

3. Switch the microtome to automatic and continue to approach the specimen with the thickness control set at 500nm or less. When the first section comes off, reset the thickness control to the desired ultrathin thickness (i.e. 50-100nm).

4. Set the sectioning speed between 0.5 and 2.5 mm/sec and begin to section (Optimal speed settings will vary depending on many things, such as type of tissue, condition of knife, humidity, section thickness, etc.). Many microtomists prefer cryosectioning via manually turning the handwheel. This gives the ability to immediately slow down if curling is seen or speed up if the sections are fine.

3.8 Removing the CR-X from the Microtome

The CR-X can easily removed from the ultramicrotome to resume normal room-temperature operation:

1. Press WARM on the controller front panel, this will initiate the automatic shut down procedure and warm the knife and specimen holders to 35°C. Wait 5 minutes before trying to move the transfer line, it must become pliable.

2. Carefully raise the LN_2 pump and fill line assembly together without excessively or bending the fill line. The outer insulating layer of the fill line is fabricated from foam rubber, which freezes solid during cryogenic operation. Bending or stressing the line when frozen may result in damage and require replacement. Place the pump in its storage stand in a vertical position.

3. Remove the cryochamber from the microtome by reversing the assembly procedure.

NOTE: Normal temperature ultramicrotomy can be resumed as soon as the cryochamber has been removed.

Chapter

Four Troubleshooting & Maintenance Guide

4.1 Sectioning

The following refers to cryosectioning of biological specimens infilitrated with sucrose and/or cyroprotectants, but the advice also applies to cryosectioning of many polymers.

Section thickness for a given specimen will vary with conditions and may not be the same as the thickness selected on the ultramicrotome. Observe the appearance and behavior of the sections as they are being cut, then adjust the thickness setting on the ultramicrotome accordingly. Cryosections 70 to 90nm thick should appear translucent, with yellow to green interference colors.

A **Problem: Sections appear snowy.**

Possible Causes:

1. Section thickness setting too large.
2. Specimen insufficiently infiltrated with cryoprotectant specimen frozen too slowly.

B **Problem: Sections fracture at the knife edge.**

Possible Causes:

1. Sections are too thick
2. Specimen and knife temperatures too cold
3. Section thickness setting too large

C **Problem: Sections not cutting with each stroke, or are cut with uneven thicknesses.**

Possible Causes:

1. Knife angle is not optimal for the specimen
2. Knife is dull
3. Specimen locking screw is loose
4. Specimen pin adapter is loose in the specimen holder
5. Knife holder is loose
6. Microtome cutting arm locking screw is not tight
7. Cryochamber is not properly clamped to the microtome stage
8. Specimen arm is touching the chamber during cutting
9. Specimen was inadequately cryoprotected and frozen
10. Specimen arm heat sinks are touching the LN_2 trough during the cutting stroke.

4.2 LN_2 Delivery Malfunction

4.2.1 LN_2 will not pump when pump button is depressed.

1. First determine that there is adequate LN_2 in the Dewar (the Dewar low level LED is not on) and that the **air line tubing** from the power supply unit to the fitting on the top of the LN_2 pump is in place.

2. Determine that the bellows pump in the power supply unit is running. It makes a distinctive noise which can be heard in a quiet room.

> **NOTE: The bellows pump will not operate if the controller cable is not plugged into the cryochamber.**

3. Determine that the bellows pump is functioning:

a.) Remove the plastic tubing from the fitting on the LN_2 pump and place it against a sensitive area of your skin, such as the back of your hand. You should be able to feel the slight pulses or puffs of air, followed by brief periods of suction.

b.) If no air movements can be detected, **first unplug the power supply unit,** then remove the eight covers screws.

c.) Determine that the outlet tubing is connected to the bellows.

d.) Remove and inspect the bellows for leaks. Replace if necessary **(Catalog #314790).**

4. The LN_2 pump and transfer line are obstructed. If all the above appear to be in place and are functioning properly, it is possible that moisture has entered into the system and has become frozen. The LN_2 pump and transfer line will have to be dried out before resuming operation.

Refer to 4.2.2 LN_2 Delivery Pump Dry Procedure on following page.

4.2.2 LN_2 Delivery Pump Drying Procedure Normally it is quite adequate to leave the pump to air dry in the vertical position in its stand. However, to completely eliminate the possibility of moisture contamination, the following procedure is recommended:

1. Gently warm the LN_2 pump with a hair dryer. Note: Do not use a high-power commercial hair dryer or overheat the pump. Components being dried should never become too hot to touch.

2. Unscrew the delivery tube. Note: It should first be returned to room temperature for ease of removal. Use a hair dryer to speed warming. Do not use excessive force or tools to gain mechanical advantage. You should be able to unscrew the delivery tube by hand.

3. Remove the float valve from the delivery tube, thoroughly dry both the delivery tube orfice and the float and set aside.

4. Start the pump running and place a 100 ml beaker with approximately 50 ml of 100% anhydrous ETOH underneath the LN_2 pump. The ETOH will be pumped through the screen phase separator and should be collected with a second beaker.

5. Continue pumping all the ETOH.

6. Remove the beakers and leave pump running for 45-60 minutes while continuing to gently warm the LN_2 pump with the hair dyer. Keep pumping until no alcohol odor can be detected at the phase separator.

7. Visually inspect the plastic tubing for moisture droplets, if moisture is detected, repeat steps 4 through 6.

4.2.3 LN_2 The transfer line is freezing excessively and LN_2 is coming out the exhaust hose. This can occur is the float value sticks in the down, shut position.

1. Tap your finger under the end of the delivery tube to see if the float valve pin can move upwards. Take care to avoid frost-bite. It may be frozen shut if moisture remained from a previous use.

2. Remove the LN_2 pump from the Dewar and allow it and the transfer line to return to room temperature.

3. Remove the delivery tube and be certain that the metal screen phase separator is not extending so far below the transfer line that it would prevent the float valve from rising and opening to deliver LN_2 into the cryochamber.

4. If the problem is intermittant, no other cause for the float sticking in the down position can be found, the cause may be frost contamination in your LN_2 Dewar. Discard the LN_2 and replace it with fresh LN_2 directly form the supply vessel is possible.

4.3 Lubricating of the bellows pump in the power supply unit

Lubricate the shaft with any machine or motor oil as shown. The black plastic cam and bellows piston should be lubricated with a light automotive grease.

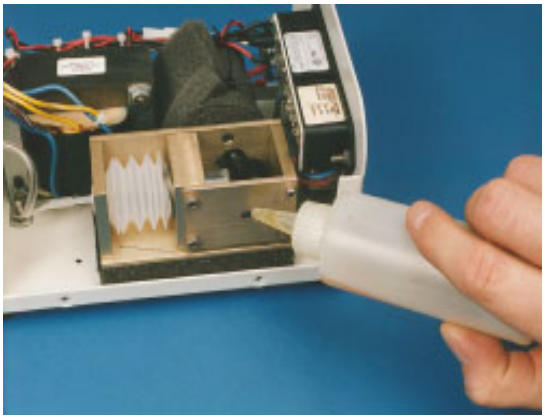


Figure 4.1 - Lubricating the Bellow Pump in the Power Supply Unit

4.4 Adjusting LN_2 Transfer Line

If the screen phase separator at the delivery tube end of the transfer line protudes too far, it will prevent the float valve from rising to let the LN_2 flow. In that case, pull the tubing back at the LN_2 pump end (arrow) and replace the pump shield.

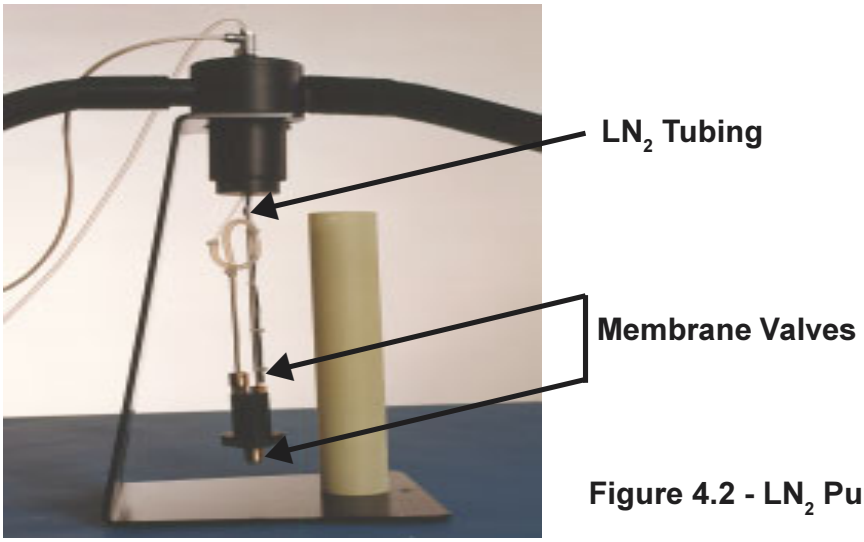


Figure 4.2 - LN_2 Pump with Shield Removed

4.5 Valve Replacement

The membrane values are located within brass valve fittings. **Refer to Figure 4.2.** They ordinarily last for years, but may eventually need replacement. Carefully unscrew the brass valve fittings with an adjustable wrench (top value) or by inserting the white band hex wrench ($^1/_{16}$") supplied into the holes (bottom valve) for the leverage. Inspect the membrane valves. If they are torn, replace them. **(Catalog #74321)**

4.6 Cleaning

CAUTION: Always remove the knife from the cryochamber before cleaning in order to prevent serious cuts to your hands and fingers.

CAUTION: Do not clean any of the parts until the cryochamber and the liquid nitrogen transfer line have come to room temperature.

Turn off the power at the main switch on the power supply unit and disconnect the power cord. The cryochamber is best cleaned using a moist cloth or tissue. A very mild solution of standard dishwashing soap or a mild window glass cleaner may be used. Do not use strong ionic detergents such as Alconox^{R} which can damage the black anodized finish on the metal parts such as the knife stage. When cleaning, be sure not to disturb the level sensor and trough heater wiring, which is located inside the cryochamber on the right side. Avoid allowing any liquids to enter or remain on any of the electrical connectors.

4.7 Resetting the CR-X Controller

This procedure downloads the software to the microprocessor and resets the Controller. This may be useful to restore normal functioning of the Controller if a malfunction has occurred.

1. Turn OFF the main power switch on the Power Supply Unit (figure 2.1)
2. Make sure that the power switch on the back of the CR-X Controller, arrow number 2, Figure 2.12, is in the ON position.
3. Depress and hold the + and – Knife Temperature Buttons, Figure 1.2.
4. Turn ON the main power switch on the Power Supply Unit (figure 2.1)

The Set Temperature display will read -25 for both the Knife and Specimen (Figure 1.2) and the four Temperature Storage buttons will go back to the default values stored in the software.

Appendix

One

Safety Precautions in Using Liquid Nitrogen

The following is taken from Union Carbide Corporation, Linde Division, publication F-3499E. Nitrogen is an inert, colorless, odorless, and tasteless gas that makes up about 78 percent of the earth's atmosphere.

WARNING: **Nitrogen can cause asphyxiation and death in confined, poorly ventilated areas. Nitrogen as a liquid or a cold gas can cause severe frostbite to the eyes or skin. Do not touch frosted pipes or valves. If accidental exposure to liquid nitrogen or cold nitrogen gas occurs, warm the areas affected by frostbite with water that is near body temperature, and consult a physician at once. Use a pressure-reducing regulator when withdrawing gaseous nitrogen from cylinder or other pressurized source.**

KEEP EQUIPMENT AREA WELL VENTILATED. Nitrogen is nontoxic, but it can cause asphyxiation without any warning in a confined area that does not have adequate ventilation. Any atmosphere, which does not contain enough oxygen for breathing (at least 18%), can cause dizziness, unconsciousness, or even death. When there is doubt about the adequacy of ventilation, do not enter the room. Ventilate the room thoroughly and if possible, use an oxygen analyzer with a 0 to 25% scale to check for oxygen.

Nitrogen cannot be detected by the human senses and will be inhaled. If adequate ventilation is not provided, the gas may displace normal air without warning that a life-depriving atmosphere is developing. Store containers outdoors or in other well-ventilated areas. Never enter any tank, pit, or other confined area where this gas may be present until purged with air and tested for a breathable atmosphere, using a gas analyzer.

LIQUID NITROGEN IS EXTREMELY COLD (about -195°C or -320°F). **COVER EYES AND SKIN.** Accidental contact of liquid or cold gas with the eyes or skin may cause severe frostbite. Handle liquid so that it will not splash or spill. Protect your eyes with safety goggles or a face shield, and cover he skin to prevent contact with the liquid or cold gas, or with cold system components. Protective gloves that can be quickly and easily removed and long sleeves are recommended for arm protection. Wear cuffless trousers outside boots or over high-top shoes to shed spilled liquid.

CAUTION: Never use containers, equipment, or replacement parts other than those specifically designed for use with liquid nitrogen.

Appendix

One

Safety Precautions in Using Liquid Nitrogen *continued*

IF NECESSARY TO DISPOSE OF WASTE GAS OR LIQUID USE CAUTION. Gaseous nitrogen should be released only in an open outdoor area. Liquid nitrogen should be dumped into an outdoor pit filled with clean, grease-free and oil-free gravel, where it will evaporate rapidly and safely.

Appendix

Two

Specifications

Temperature:

- Range: +35°C to -185°C
- Settability: 1°C
- Stability: ± 0.1°C
- Display: Backlit red LCD

LN_2 Delivery System:

- Compact 9 liter Dewar
- 0.8 liters/hour at sectioning temperature at -90°C
- Efficient Non-Contact Transfer Line
- Non-pressurized, positive displacement type LN_2 pump

Knives:

- 3 knife holders provided: a dual 6mm glass knife holder, thick glass knife holder and a diamond knife holder. Each accept a trimming tool.
- Glass Sizes Accommodated: 6mm, 8 through 12mm. 45° triangular knives 25mm x 25mm
- Diamond Sizes Accommodated: RMC and all other popular brands
- External Knife Rotation Control Knob
- Lateral movement of 25mm
- Knife Clearance Angle Adjustment - adjustable at any time from 0° to 12°

Control Unit:

- Compact Design; can be positioned on tabletop or on top of MT-*X* or MT-*XL* controllers
- Microprocessor control with 4 user-adjustable programs for knife and specimen temperatures
- LED Displays of knife and specimen set temperatures and actual temperature readings
- Warm cycle quickly brings cryochamber back to room temperature

Cryochamber:

- Quickly installs on ultramicrotome stage
- Large open design for easy access to specimen and knives
- Transparent cryochamber cover with large sample access window

Electrical Data:

- 100/110/120/220/240 VAC, 50/60 Hz Power input

Dimensional Data

- Cryochamber: 12cm X 21cm X 21cm HXWXL
- Controller: 10.5 X 19 X 24cm HXWXL
- Power Supply Unit: 11.5 X 14.5 X 23CM HXWXL
- LN_2 pump and transfer line: 84 cm long X 40cm high X 7.5 cm maximum width
- Dewar: 25.0 cm diameter X 39.0 cm high
- Gross shipping weight: 33kgs
- Shipping dimensions: 94 X 51 X 41cm

Appendix

Three

Appendix

Four

CR-X Shipping List

1	Controller	74929	1
2	Cable, dewar level	74203	1
3	15 PIN Cable, cryochamber to controller	284106	1
4	Power Cord	67080	1
5	Clear tubing for Pump	60453	6 feet
6	Cryochamber Assembly	74950	1
7	Arm Assy for RMC	74082	1
8	LN_2 Pump and Transfer Line Assy	74310	1
9	Fill Line Stand	74065	1
10	Nitrogen Vent Hose (black)	74322	1
11	LN_2 Dewar	60454	1
12	Power Supply Unit	74951	1
13	Cryochamber Cover, Single Piece	74410	1
14	Cryochamber Cover, 2 pieces	74220	2
15	Mounting Dish	71181	1
16	Instruction Manual	74355	1
17	Pump Stand	74208	1
18	Dewar Cap	74257	1
19	Hand Rest	74254	1
20	Accessory Box, Including:	71005	1
	Plastic box	68882	1
	Holder, two 6mm glass knives & trimming tool	74414	1
	Holder, diamond knife & trimming tool	74417	1
	Holder, 12mm glass knives & trimming tool	74420	1
	Pin Holder, 3mm	71091	1
	Pin Holder, 2mm	71092	1
	2mm Specimen Pins, pkg. 10	70861	1
	3mm Specimen Pins, pkg. 10	70862	1
	6.5mm Specimen Pins, pkg. 10	70863	1
	Specimen Pin Support Disc	71291	1
	Flat Specimen Clamp	71292	1
	White Band Hex Wrench ($^1/_{16}$ inch)	74290	1
	Blue Band Hex Wrench ($^5/_{64}$ inch)	74291	1
	Blue-White Hex Wrench ($^3/_{32}$ inch)	74292	1

CRX-Shipping List *continued*

Item	Description	Part	Quantity
	Accessory Box Contents Continued		
	Fuse, 1 Amp, slow blow	68003	2
	Fuse, 0.5 Amp, slow blow	314908	4
	Spacer, for 6mm glass	71130	1
	Spacer, for 9mm glass	71170	1
	Spacer, cryotrimmer 0.5mm	74245	1
	Spacer cryotrimmer 1mm	74246	1
	Knife Retrieval Tool	71220	1
	Needle Valve Float	74037	1

Appendix

Five CRX Accessories

Part #	Description
50285	Tungsten Carbide Cryo-Trimming Tool. Requires P/N 74332 Cryo-Trimming Tool Holder (below) for CR-2000 and CR-21 cryosectioning systems.
50286	Diamond Cryo-Trimming Tool
70861	Specimen Pins, 2mm, package of 10 aluminum with slotted on one end
70862	Specimen Pins, 3mm, package of 10 aluminum with slotted
50285	Tungsten Carbide Cryotrimming Tool
50286	Diamond Cryotrimming Tool 70861
70861	Specimen Pins, 2mm, package of 10 aluminum, slotted on one end
70862	Specimen Pins, 3mm, package of 10 aluminum, slotted on one end
70863	Specimen Pins, 6.5mm, package of 10 aluminum with cross-slotted on one end
71091	Pin adapter for 3mm pins (Part nos. 70862 and 70863)
71092	Pin adapter for 2mm pins
71291	Specimen Pin Mounting Disc-holds specimen pins for mounting specimens under stereomicroscope
71101	Torme flat specimen vise
71180	Flat specimen clamp holder, for holding specimens into P/N 71101 above
71292	Flat specimen clamp for specimens 4mm x 8mm x 6mm
74414	Glass knife holder, for 2-6mm wide knives and trimming tool
74417	Diamond knife holder, also holds a trimming tool
74693	Breath shield, mounts on stereo microscope of MT-X, MT-XL between eyepieces
74420	12mm glass knife holder, also holds a trimming tool
71171	Breath shield, mounts on stereo microscopy, between eyepieces
74189	Grid Holding Device
74410	Cryochamber cover, clear, one piece
74449	Cryochamber cover, clear, two pieces
74254	Hand rest, for use while cryosectioning
74245	CryoTrimming Spacer, spaces glass knives 0.5mm apart for trimming the specimen between two glass knives
74262	Flat specimen clamp for specimens 4mm x 5mm x 6mm
74247	Dewar cap
71282	Cryosectioning kit including: loops, microscalpels, forceps, specimen pin holders, and all accessories required for the Tokuyasu technique

CR-X Accessories *continued*

Section Floater Ionization depolarization device for electrostatic control during cryosectioning

74800	Section floater for CR-X, 115 VAC
74801	Section floater for CR-X, 220-240 VAC

Appendix

Six

Models Available

Catalog Number	Description
74350	For use on the MT-7000 Ultra, MT-X, and MT-XL ultramicrotomes.
74352	For use on MT-7, MT6000XL, and MT6000 ultramicrotomes manufactured by RMC and previously DuPont.
74353	For use in Reichert Ultracut E and Leica Models S and T.

Appendix

Seven

Installation on the Reichert and Leica Ultramicrotome

Installation of the CR-X for Reichert and Leica Ultracuts **(Catalog# 74353)** is very similar to the models for RMC Ultramicrotomes. However, the heater for the specimen arm shown in **Figure 2.6, (12) on page 2.5**, is replaced by strip heaters bonded to the back of the **baffle seal (14)**, whose cable also plugs into the connector labeled **baffle,** on the right side of the cryochamber, **Figure 2.8 on page 2.6.**

The cryochamber **mounting plate (6)** shown for RMC Models, **Figure 2.7 on page 2.6,** is different for the Reichert and Leica Models. The sides are straight-walled rather than dovetailed. It slides onto the lower knife stage of the ultramicrotome and locks into place using the clamp on right side of the lower knife stage.