

Transmission Electron Microscope Accessory

Side entry Cryosystem

Operating Manual
G34 - 683 e

LEO

Remark:

The accessory which is described in the following fits the LEO 912 OMEGA

The accessory is valid for LEO 910 without the OMEGA filter operation.

The accessory is valid for LEO 906 without the OMEGA filter operation and Koehler illumination.

The use of the Oxford Cryotransfer System is described in this manual.

There are hints regarding the Gatan Cryotransfer System.



Important !

First read the complete manual, since many steps can be practiced before a cryotransfer is done.

The entire operation (transfer and microscopy) should first be simulated and practiced with a holey grid at room temperature.

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1 Description

1.1 General

Every cryotransfer holder is generally delivered with the corresponding workstation. The workstations are only used to load the specimen into the holder in order to transfer it into the microscope. The specimen preparation has to be done in a separate preparation chamber.

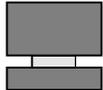
The LEO cryo workstation covers both application steps. The specimens can be prepared in the chamber and loaded into the holder for the transfer into the electron microscope.

Thus the ice contamination is minimized, and besides the consumption of liquid nitrogen is lowered.

Originally the LEO workstation was designed for the top entry system. With the special kit the workstation can be modified for the side entry system.

For the operation of the cryosystem nitrogen gas is necessary. The purity should be 4.6 at least.

1.2 Parts of delivery

- Cryoadaptor 
- Teflon plug 
- LN₂ container 
- Magnetic switch 
- Adjustable support block 

The order number of the complete cryoadaptor is 340719 - 9008.

In the Gatan system the LN₂ container is replaced by a supporting device for the tip of the cryoholder.

1.3 Modification

To modify the system to the side entry version remove the two screws and take off the adaptor from the workstation.

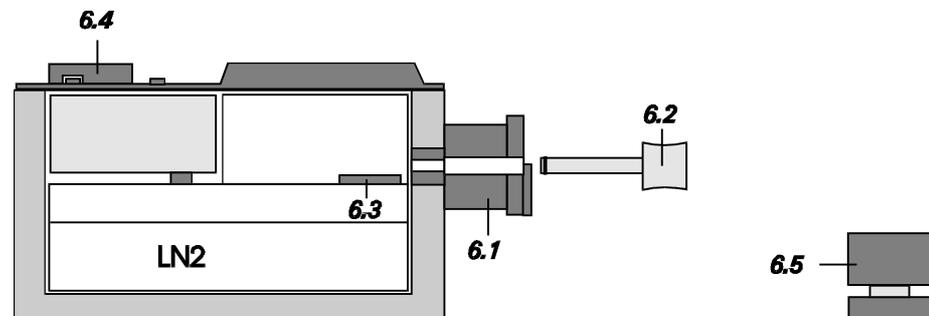


Fig. 1: Cryoworkstation

The new adaptor (6.1) is fixed at the workstation with the two screws in the same way (Fig. 1). Because of the better overview the metal fork is not displayed in figure 1.

1.4 Operating principle

The function principle of the workstation is described in the manual "Cryo workstation". This chapter deals with the handling of the side entry cryosystem only.

The additional magnetic switch (6.4) allows to switch on the heating even when the lid is closed. In this condition the cryoholder is locked in or out. The heating guarantees that the opened adaptor is sufficiently purged by nitrogen gas during the transfers. As soon as the adaptor is closed by the plug (6.2), the heating can be switched off.

The adaptor also accepts the bellows housing to purge the rod with nitrogen gas.

The workstation is turned by 90° for transfers as shown in figure 2.

1.5 Maintenance

The cryosystem is generally maintenance-free. But before use it should be checked in regard of cleanliness. The cleaning is done according to the manual of the manufacturer. All parts which are being cooled have to be dry. A hair dryer is the proper equipment for this job.

2 Operation

2.1 General

There are special manuals available for the operation of the cryoworkstation and the cryotransferholder.

2.2 Cooling of the EM dewars

After the start of the EM the dewars can be filled with liquid nitrogen. **Note the safety rules for liquid nitrogen !!**

The upright dewar has a socket which can be connected to a temperature display (option). After about 1 h cooling time or after reaching -170°C the inserted cryoholder is ready to be cooled with liquid nitrogen.

There is no temperature display for the dewar in the pumping tube. Therefore take care that the dewar does not warm up during operation. In principle all dewars should be filled up in periodical intervals.

When the cryoholder is at -160°C , it can be transferred to the cooled workstation.



Remark:

If the cryoworkstation shall be used to freeze samples, the cryogen has to be liquified before the cryoholder is transferred to the workstation.

2.3 Transfer of a cryofixed specimen

2.3.1 Removing the cryoholder from the EM

- Tilt the goniometer to $+60^{\circ}$
- Close the shutter on the cryoholder
- Connect the gas line for the nitrogen flush (only Oxford)
- Regulate the gas flow so that the nitrogen flush is audible
- Retract the cryoholder until it stops and turn it 90° counterclockwise until click stop
- Push the holder somewhat into the airlock and turn the holder further 90° counterclockwise.



Liquid nitrogen might be spilled, while the cryoholder is turned up !!

- Push button N2-Flush in the VAC menu.
- The airlock is flushed by nitrogen gas via the prevacuum tube.

- Retract the cryoholder until the metal pin on the rod is visible; then turn the holder into normal position (dewar faces up).
- Refill some liquid nitrogen into the dewar and leave the cryoholder in this position to continue with 2.3.2.

2.3.2 Inserting the cryoholder into the cryoworkstation

- Take out the plastic plug (6.2) of the workstation and switch on the heating with the moveable magnetic switch (switch in midposition on the lid).
- Remove the cryoholder from the goniometer keeping the bellows fully extended over the holder. Take care as the nitrogen dewar becomes upturned.
- Engage the bellows into the workstation interface (6.1) and put the dewar on the adjustable support block (6.5).

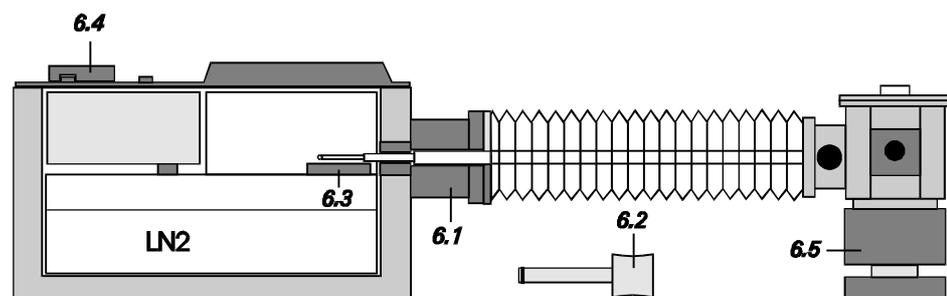


Fig. 2: Cryoholder in the cryoworkstation

- Push the dewar tight onto the block (6.5) while moving the holder into the workstation (adjust the height of the block if necessary).
- Refill dewar with liquid nitrogen and pour liquid nitrogen over the tip as well.
- Disconnect gas line to the bellows to stop the nitrogen flush.

2.3.3 Loading a specimen

- Open the shutter on the cryoholder and loosen the screwed insert with the cooled insertion tool.
- Take out the screwed insert with cold forceps and keep it cold in the liquid nitrogen container below the tip.
- Remove the grid from specimen cup.
- Place the new grid in specimen cup, put the screwed insert on the grid with cold forceps and turn it tight with the cold insertion tool.
- Close the shutter immediately and pour liquid nitrogen over the tip.
- Close the workstation with the lid and switch on the heating by the magnetic switch (magnetic switch in midposition on the lid).

2.3.4

Inserting the cryoholder into the EM

- Activate the button N2 FLUSH in the VAC menu.
- Tilt the goniometer to 60°, if the tilt was reset to 0° after taking out the rod.
- Connect the nitrogen gas line to the bellows.
- Pour again liquid nitrogen over the tip.
- Retract the holder into the bellows, while holding the flange at the workstation interface.
- Remove the cryoholder from the workstation keeping the bellows fully extended.
- Engage the bellows into the goniometer interface.
- Carefully move in the holder until it stops.
- Turn the holder by about 10° counterclockwise and push it all the way in.

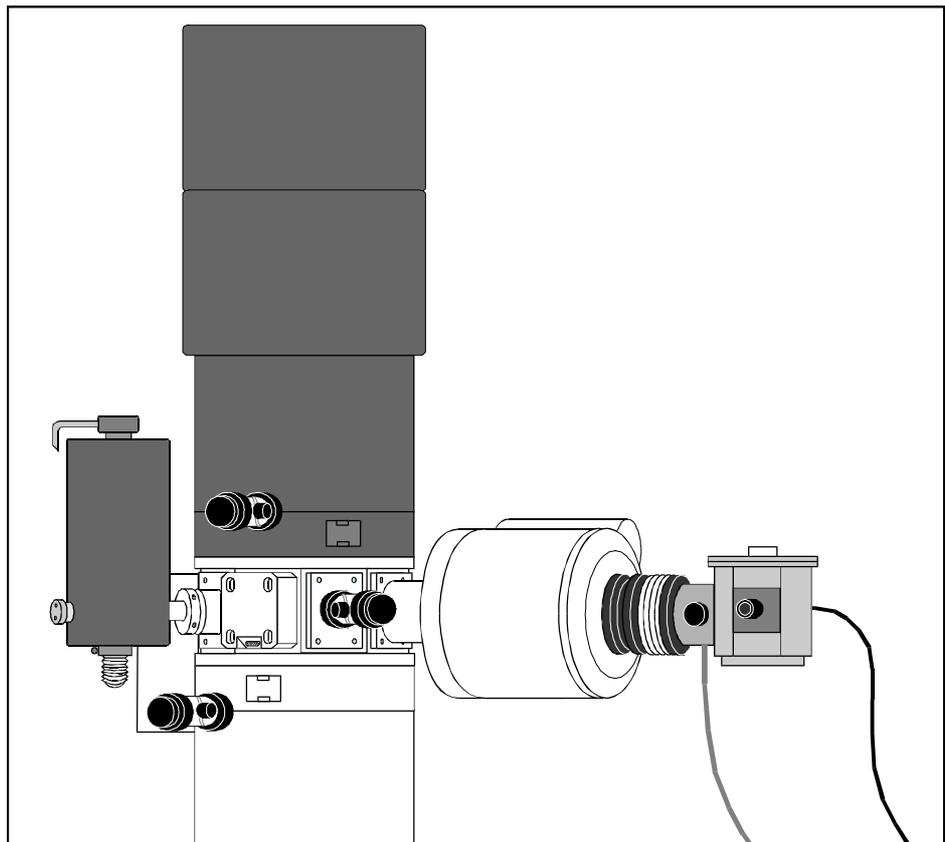


Fig. 3: Cryoholder in the goniometer



Take care as the dewar is almost upturned nitrogen might be spilled depending on the level (Follow the safety rules !)

- Prepump the airlock as usual and let slide in the holder slowly into the microscope vacuum.
- Disconnect the gas line from the bellows.
- Reset the goniometer tilt to zero.
- Refill the dewar with liquid nitrogen.
- Allow the the cryoholder to stabilise in the high vacuum for 20 - 30 min.

2.4 Microscopy

Before getting started all dewars should be refilled with liquid nitrogen as there are stable conditions during the microscopy work.

2.4.1 Microscope setup

The EM setting and the base adjustment should be done before a specimen is transferred. Thus only the fine tuning is left for the cryo specimen.

Every excessive radiation of the specimen has to be avoided.

2.4.2 Storage of specimen positions in the LowMag range

- Switch on high voltage and filament.
- Select LM mode in the main menu.
- Illumination angle as small as possible.
- Retract all apertures.
- Open the shutter on the cryoholder.
- Look for thin specimen positions and store it in the GON menu.
- Find one specimen position which can be used for the fine tuning of the EM (if possible far away from the stored positions; i. e. use the carbon film)

2.4.3 Fine tuning of the EM

- Switch to Mag mode in the main menu.
- Switch on AIS mode in the main menu.
- Adapt the brightness.
- Centre objective lens aperture.
- Check the AIS alignment.
- Check the illumination tilt.
- Compensate objective lens astigmatism.
- Check the spectrometer alignment.
- Introduce spectrometer entrance aperture and slit aperture, and centre it.

2.4.4 Presetting the Low Dose system

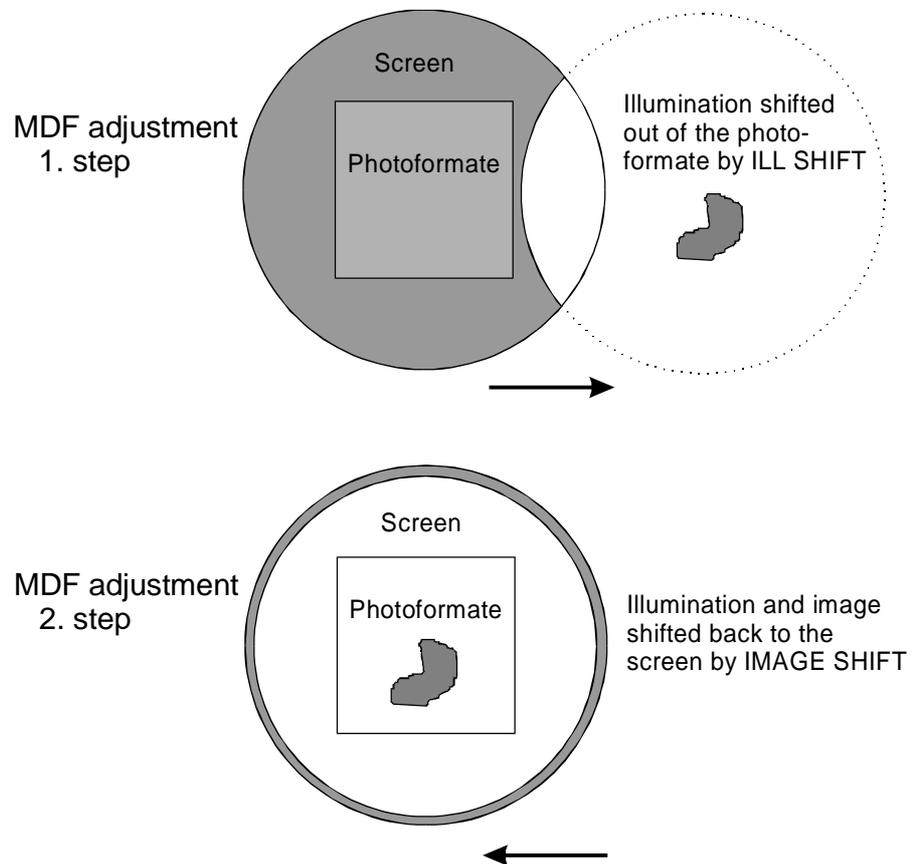
(see also chap. 3 p. 47 - 50)

- Select magnification for the photograph.
- Activate MDF menu in the PARAMETER menu.
- Push RESET MDF OFFS. to set all values to zero.
- Activate ADJ. MDF OFFS.
- Shift the illumination field out of the photo formate with encoders III SHIFT.



Remark:

The option PARALLEL / FREE allows deflection in all directions or parallel to the tilt axis.



- Shift the off axis area to the screen centre with encoder IMAGE SHIFT.



Remark:

The off axis specimen area, which is now visible on the screen is used for focusing. If the MDF system is switched off, the area to be photographed is imaged on the screen.

2.4.5

Creating macros for cryo application

(see also chap. 3 page 54 - 57)

To obtain acceptable results in cryo microscopy the number of pictures is much higher than in standard microscopy. Also the number of steps to expose a negative is increased, and it requires more attention.

Therefore it is recommended to automatize the procedure after a good specimen area was found.

That can be realized by the MACRO option. Each microscope of the series LEO 906, LEO 910 und LEO 912 can be easily upgraded by this option.

- Select the MACRO option in the PARAMETER menu.
- Toggle the function SELECT FILE for the desired macro, and push LOAD FILE (at the beginning there is only MACRO.MCR).

It is useful to attach the fixed functions to the same function key within the application.

The search mode e. g. is always on F1. In this configuration the specimen is imaged between 4000x and 8000x with low intensity. Here the specimen detail is selected to be photographed at higher magnification.

The focus mode e. g. is always on F2. Here an offaxis specimen detail is imaged, where the focusing can be done at the desired magnification.

The beam blanker function e. g. should be on F7 and F8 in all macros.

The cryo macros mainly distinguish in magnification and intensity setting. Of course one macro can also contain different magnifications, but the MDF setting is only optimized for one magnification.

A proper name for a cryo macro is e. g. cryo25.mcr. 25 stands for the magnification 25.000x.

Creating a cryo macro for M = 25.000x

- Push button SELECT Fx FUNC. until line F1 is activated
- Select edit mode with button EDIT Fx FUNC.
- Put in the following sequence of commands:
R1T024T001T005T022T038T003S10104S10007T017T025R0

The commands R1 and R0 open and close the REMOTE function.

This sequence of commands contains the setting for the search mode.

The commands in detail:

Beam Blanker	= ON	T024	ILL Mode	= TEM	T003
Image Mode	= ON	T001	ILL Pointer (0.04mrad)	= 4	S10104
MAG Mode	= MAG	T005	MAG Pointer (4.000x)	= 7	S10006
AIS Mode	= ON	T022	MDF Mode	= OFF	T017
MIS Mode	= OFF	T038	Beam Blanker	= OFF	T025

Put in the commands for the focus mode on F2.

- Select line F2 with SELECT Fx FUNC.
- Activate edit mode with EDIT Fx FUNC.
- Put in the following commands:
R1T024T016S10015S10115T025R0

The commands in detail:

Beam Blanker	= ON	T024
MDF Mode	= ON	T016
MAG Pointer (25.000x)	= 15	S10015
ILL Pointer (0.4 mrad)	= 15	S10115
Beam Blanker	= OFF	T025

It is provided that this command line is used after the search mode (F1) was activated. If not, add the commands T001, T003, T005 und T022.

Follow the same procedure to put in the function BEAM BLANKER ON to F8 and BEAM BLANKER OFF to F7.

Cryo macros for different magnifications only require a change of magnification and brightness in the F2 line.

The storage of a modified macro is done by the function SAVE AS. Then the macro can be stored under a new name, and the original one is not overwritten.

2.4.6. Dose calculation for the exposure

The dose for the exposure is one important parameter in cryo microscopy.

The automatic exposure time mode is not based on the dose measurement. It guarantees a defined density on the negative independent of the dose. It measures the intensity beyond the specimen. Therefore the exposure time mode should be changed to fixed mode.

Knowing the illumination angle (Köhler illumination) the dose value can be reproduced or changed in well defined steps.

Starting point for the dose calculation is the current measurement on the camera detector (only LEO 912):

- Switch on HV and filament.
- Set the emission current to 5 - 10 μA .
- Retract specimen holder and all apertures below the specimen.
- Set magnification to 25.000x.
- Fully illuminated fluorescent screen.
- Set illumination angle to 0.5 mrad.
- Lift large fluorescent screen.
- Activate CAM SET in the CAM menü.
- Push button SELECT EXP: METER until camera collector is selected.

- Toggle button I / δf / θ until the current is displayed on the negative data field.
- Note the current and all corresponding data as you can see in the table below.

The dose rate is calculated according the following formular:

$$d_s = I \times M^2 \times 1400 \left[e^- / nm^2 s \right]$$

Example:

High voltage	120 kV
Emission current	7 μ A
Filament heatin	3,5 A
Magnification	25.000x
Illumination angle	0.5 mrad
Current (camera collector)	51 E-11 A
Dose rate	446 [e⁻/nm²s]

If the High voltage, the emission current, and the filament heating is kept constant, the dose rate can easily be calculated for each illumination angle, since there is a square relation for the different illumination angles. The magnification is not relevant to the result.

To get the total dose the exposure time has to be considered. Therefore the time has to be added to the above formula:

$$D_s = I \times M^2 \times T \times 1400 \left[e^- / nm^2 s \right]$$

If the image is taken with the above setting and a fix exposure time of 2 s the total dose results in

$$892 [e^- / nm^2].$$

The following table contains all factors to calculate the dose rate at different illumination angles.

Example:

The calibration measurement was done at 0.5 mrad. That leads to a dose rate of 446 [e⁻/nm²s].

But the picture was taken at 0.32 mrad. Go to 0.5 mrad in the left column, follow the line to the 0.32 mrad column, and get the factor.

Calculation of the dose rate: 446 [e⁻/nm²s] x 0.41 =

$$183 [e^- / nm^2 s]$$

For the total dose calculation the exposure time has to be considered as mentioned before.

Calculation table for the dose rate

mrad	0.1	0.13	0.16	0.2	0.25	0.32	0.4	0.5	0.63	0.8	1.0
0.1		1.69	2.56	4.0	6.25	10.2	16.0	25.0	40.0	64.0	100.0
0.13	0.59		1.51	2.37	3.69	6.05	9.49	14.75	23.5	37.8	60.0
0.16	0.39	0.66		1.56	2.43	4.0	6.25	9.8	15.5	25.0	39.0
0.2	0.25	0.42	0.64		1.56	2.56	4.0	6.25	10	16.0	25.0
0.25	0.16	0.27	0.41	0.64		1.64	2.56	4.0	6.35	10.2	16.0
0.32	0.1	0.17	0.25	0.4	0.61		1.56	2.43	3.9	6.25	9.8
0.4	0.06	0.11	0.16	0.25	0.4	0.64		1.56	2.5	4.0	6.25
0.5	0.04	0.068	0.1	0.16	0.25	0.41	0.64		1.59	2.56	4.0
0.63	0.026	0.044	0.063	0.1	0.16	0.26	0.4	0.64		1.61	2.53
0.8	0.017	0.026	0.04	0.063	0.01	0.16	0.25	0.4	0.62		1.56
1.0	0.01	0.017	0.026	0.04	0.063	0.1	0.16	0.25	0.4	0.64	

2.4.8

Searching good specimen areas in the Mag range

For microscopy all apertures and the specimen holder have to be inserted.

Good areas are thin ice layers in which the specimen is embedded. Thick ice layers exposed to the same dose result in a poor density on the negative.

But thin ice layers can only be used, if they still contain the specimen emulsion or suspension. That you can check in a mash which is not stored for photography. Then you go to a higher magnification and look at the thin ice layers either on the viewing screen or on the TV monitor to get an impression of the particle distribution. For this kind of work the beam damage is not relevant. On the contrary it is helpful, since the specimen distribution is much better visible due to the excessive irradiation.

By this information you are able to select good specimen areas at 4.000x (F1).

- Open the shutter on the cryoholder.
- Use a not stored position for the fine tuning of the EM.
- Push function key F1 after loading the cryo macro (M=4.000x).
- Recall the first stored specimen position in the GON menu.
- Move the interesting specimen position to the centre of the screen.

2.4.9

Focusing and photography

- Push F2 to activate the photo mode of the loaded macro.
- Focus the specimen on the off axis detail.
- Push the exposure button on the left panel.

After the exposure the focus position is shown again on the viewing screen. You can change the settings for a further exposure (focus, brightness, specimen tilt).

The focusing conditions are comparable to those in conventional microscopy. The mag. depending defocus mode is not recommended in cryomicroscopy since here the defocus value is well defined and independent of the magnification.

2.5 Finishing the cryo work

Allow the anticontaminator to warm up. To shorten the time you may empty the dewar. During the warm up the IGP can be switched off (Software Version V.3.1) to evacuate the column by the turbo pump.

The cryoholder can stay in the vacuum. If that is not possible, evacuate the tip of the holder by an external vacuum system. Thus any condensation onto the holder is avoided.

3 Deviations from the LEO 906 operation

The operation of the LEO 906 is described in the brief manual.

For cryo applications there are differences in operation compared to LEO 910 and LEO 912 OMEGA.

- The cool trap is in the pumping tube behind the column.
- The goniometer tilt is done manually.
- The size of the illuminated specimen area is not defined by field apertures but by the setting of C2. In TEM mode the area size is selected automatically.
- The dose measurement is different.
- The illumination angle is not displayed. Therefore there is no macro command which could be implemented in a macro.