Tecnai on-line help manual -- Column description

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1 Apertures

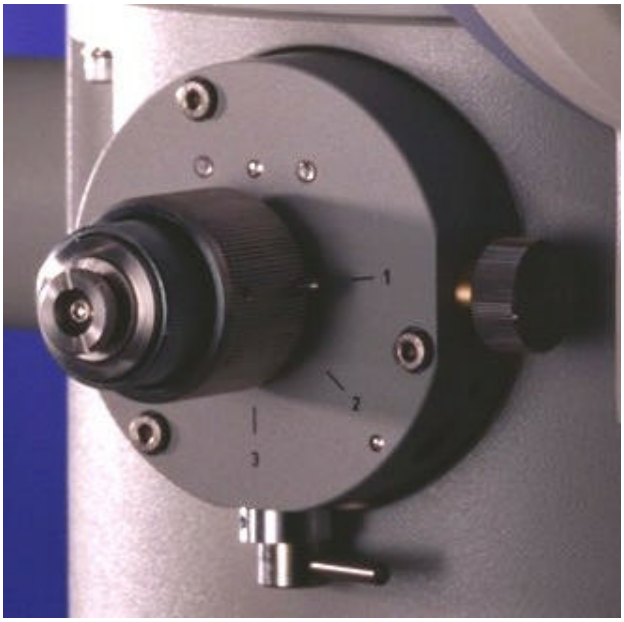
The microscope is equipped with three aperture controls, from top to bottom:

- Condenser aperture
- Objective aperture
- Selected-area aperture

Most aperture mechanisms are four-stop mechanisms, but the objective aperture may have a seven-stop mechanism. The type of mechanism can be seen from the numbers indicated on the outside of the aperture. Four-stop mechanisms consist of an aperture holder rod with four separate apertures in them. The seven-stop objective aperture has a blade with two parallel rows of four apertures.

Note: Aperture mechanisms, like all other mechanical controls on the microscope should move easily and not require excessive force. If the aperture doesn't move easily, do not try to force it as this may damage the mechanism. The sideways movement of the aperture is equipped with end stops to keep the aperture close to a centered position. The end stops are located in holes going in horizontally in the lower half of the grey, circular block with the aperture numbers, one on the left and another on the right. Do not try to force the aperture beyond these end stops. if the aperture cannot be centered properly, use a small (metric) Allen tool to adjust the position of the end stops.

A side-ways retractable, four-stop aperture mechanism.



1.1 Operation

1.1.1 Sideways retraction

Apertures so equipped can be retracted sideways out of the beam by a lever mounted at the underside of the aperture mechanism. Rotate the lever to the right to remove the aperture out of the beam, turn it to the left to insert it in the beam. Aperture mechanisms without the lever either cannot be removed from the beam at all (condenser apertures) or require retraction with the aperture selection ring (retraction along the aperture holder axis).

1.1.2 Aperture selection

Turn the large ribbed ring (closest to the column) clock-wise or anti-clockwise to select a different aperture. The small pin on the side of the ring points to the aperture number. On the objective aperture, position 7 has the aperture blade retracted completely from the gap between the objective-lens pole pieces. Position 5 is not usable (it is on the edge of the aperture blade). Positions 4 to 1 are the real aperture positions.

1.1.3 Aperture centering

The aperture selected can be centered using the axial (smaller ring inside aperture selector ring) and perpendicular (knob on side of ring with aperture numbers) movements.

1.2 Aperture sizes mounted as default

Aperture	1	2	3	4	5	6	7
Condenser 2	30 um	50 um	100 um	200 um‡			
Objective (TWIN)	10 um	20 um	40 um	100 um			
Objective (S-TWIN)	20 um	40 um	60 um	100 um	-	-	-
	10 um	30 um	50 um	70 um	-	-	-
Objective (U-TWIN)	20 um*	60um*	100 um*	750um			
Selected Area	10 um	40 um	200 um	800 um			

‡ 200um is maximum allowed size for the largest condenser aperture. X-ray safety under all operating conditions cannot be guaranteed if larger apertures are mounted.

* Thin-foil Au apertures.

2 CompuStage

The CompuStage is a motor-driven goniometer that provides computer-controlled movement of the specimen on five axes (X, Y, Z, α, β). The CompuStage consists of the following elements:

- Hardware (the goniometer), including motor drives and position-measuring system.
- Control electronics, including a dedicated microprocessor.
- CompuStage server software which provides the link between the user interface (software and hardware controls like the track ball for X-Y motion) and the CompuStage microprocessor.
- Hardware controls, consisting of a track ball for X-Y motion and pressure-sensitive up-down switches for Z, α and β.
- User interface software with translates operator input into goniometer actions.

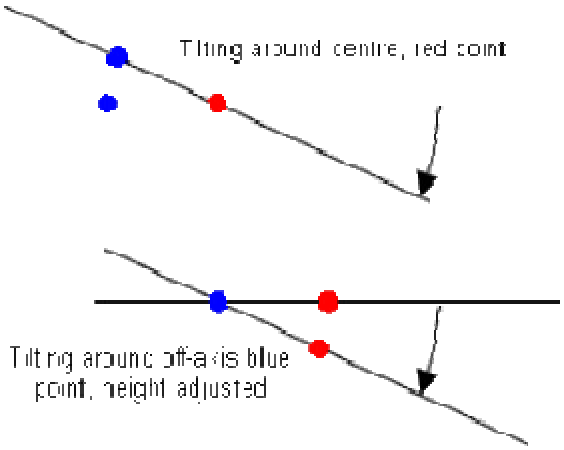
The definition of the physical position of the stage axes in the microscope column is covered in a separate section.

2.1 Specimen holders

The CompuStage can be equipped with a variety of holders, which are inserted or removed via an airlock. Specimen holders are inserted into ultra-high vacuum and must be kept clean. Special handling instructions for specimen holders should be adhered to.

2.2 Eucentric height

The α tilt of the CompuStage is constructed in such a way that it is possible to tilt around it without having large apparent movements of the point of interest on the specimen. This is called eucentric tilting and is achieved by bringing the point of interest to the same height (with the Z axis) as the α tilt axis itself: the eucentric height. The eucentric height is important because it not only provides an easy way of tilting without having to correct specimen position continuously, but it also defines the reference point inside the microscope for all alignments, magnification, camera lengths, and so on. In general one should work at the eucentric height (the only reason for deviating could be that at very high β tilts and specimen positions away from the center, the range of the Z axis may not be sufficient to bring the specimen to the eucentric height).



2.2.1 To set the specimen to the eucentric height

Method 1 Eucentric Focus Preset
Once the microscope has been aligned (as it normally shou ld be), the eucentric focus preset (obtained by pressing the Eucentric Focus button) sets the objective-lens setting to the correct value for a specimen that would be exactly at the eucentric height. If the specimen is not at the eucentric height, it will appear out of focus. If you now bring the specimen into focus by changing the Z height, not the focus, it will go to the eucentric height. It may help to switch on the wobbler, since the apparent displacement between the two wobbler images makes it easy to see whether the height is changed in the right direction (the displacement between the images becomes smaller).

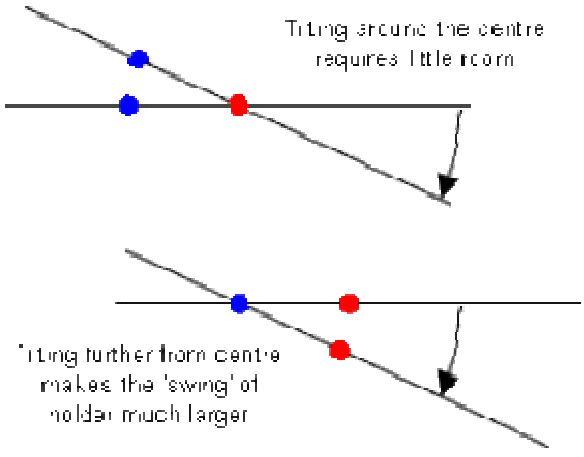
Method 2 The Alpha Wobbler
Since the image displacement on tilting is minimised at the eucentric height, the eucentric height can be set by minimising the displacement while the stage is tilting. For this purpose the CompuStage has the Alpha Wobbler function. When this function is activated, the CompuStage is tilted continuously between two preset tilts (typically -15 and +15°). Change the Z height to make the displacement smaller. When the displacement is minimised the specimen is at the eucentric height.

2.3 Safety features

For safety the CompuStage is equipped with a number of special features like the MaxiTilt system which allows maximum use of the available space between the pole piece for tilting while guarding against damage of holder, pole pieces and other objects in the pole -piece gap like the objective aperture holder. Another feature is the sEntry system which guards against insertion of holder that are incompatible (too big) for the objective -lens pole-piece configuration of the microscope.

2.3.1 MaxiTilt system

Because tilting takes place around the eucentric point, the motion of the specimen holder may require a lot of space (see picture below).



In the restricted space available between the pole pieces of some objective lens types, there may not be enough room to tilt very far. But the pictures above demonstrate that the available tilt range is very much dependent on the position of the stage (mostly the Y and Z axes). The MaxiTilt system of the CompuStage provides a flexible way of keeping the tilt within a safe range, while at the same time maximising the tilt available. The MaxiTilt senses what the maximum tilt is for the current stage position. If this range is exceeded (a situation called a pole hit), the CompuStage will move a little bit back on the axis that was changed last. If there is no axis identifiable, the B or A tilt will move back. The microscope will display an information message that a pole hit has been detected.

2.3.2 sEntry system

The CompuStage is equipped with a SafeEntry (or sEntry) system that prevents holders from being inserted into microscopes where the objective-lens pole-piece configuration is not compatible with the particular holder (like thick holders into objective lenses with a gap that is too narrow for the holder to fit).

The sEntry system consists of a key (a pin varying in shape and diameter) on the holder defining the holder dimensions and a lock on the CompuStage defining the pole-piece configuration. The length of the sEntry key is such that if an incompatible holder is inserted, the key (blocked by the lock) prevents the holder from going between the pole pieces.

Although older specimen holders designed fo the manual goniometer will fit in the CompuStage (provided their O-ring is exchanged for a - thicker - CompuStage O-ring), these holders are not equipped with a sEntry key and may therefore be unsafe with objective pole-piece configurations with narrow gaps such as the U-TWIN.

2.4 Red CompuStage light

The red CompuStage light has a more generic function than previously used on manual goniometers. It simply means that **no airlock actions** (insertion or removal) should be executed. This will happen of course while the airlock is being pumped when a holder is inserted into the microscope. It can also mean that it is unsafe to extract the holder under the current conditions (when the β tilt is more than 5 degrees or during movement the red light will also be on). In that case, the unsafe situation must be rectified before the holder can be extracted (reset β tilt to zero or wait until movement is finished).

2.5 Homing

Before the CompuStage is ready for use (when the microscope has been switched off altogether or after the CompuStage has been disabled), it must be homed, a procedure in which it finds the zero positions. During homing the CompuStage will move each of the four fixed axes (X, Y, Z and α; the β tilt axis is homed separately whenever a double-tilt holder is inserted) to one extreme. Because of the high tilt applied, this procedure must be executed without a specimen holder. In order to make sure that there is no specimen holder present, the microscope has to ask the operator to identify the specimen holder (which in this case should be 'No specimen holder', otherwise the homing cannot proceed).
If no user interface is active, there is no way for communicating with the operator. Consequently, the homing procedure after a start-up will only proceed once the use r interface has started. Even if the user interface does not show a message to select a specimen holder after a restart of the microscope, but the CompuStage does not move (easily checked by moving the right-hand track ball a bit), it is very likely that the CompuStage has not been homed. Select the Stage Configuration Control Panel and check the Goniometer enabled check box. The homing procedure will start.

2.6 Parking position

Occasionally it is necessary to get the specimen and/or specimen holder out of the way (for example, when no electron beam is visible initially or in some alignment steps if the specimen blocks the beam). Although it is possible to retract the holder fully and turn it slightly (the initial part of removing the specimen holder), it is be tter not to do this, because it can lead to a potential leak. The preferred method to 'remove' the specimen holder is to retract it about one or two centimeters and stick something elongated (a pen with a diameter of a centimeter or so will do nicely) between the inside cap of the holder and the CompuStage. The 'elongated' item will prevent the holder from moving back in and the distance is sufficient to remove any obstruction from the field of view.

Note 1: Always retract the holder slowly to allow the O-ring to keep the seal.

Note 2: Manual-goniometer specimen holders with the sapphire at the end have a longer tip than CompuStage holders and must be retracted at least three centimeters before they are clear from the field of view.

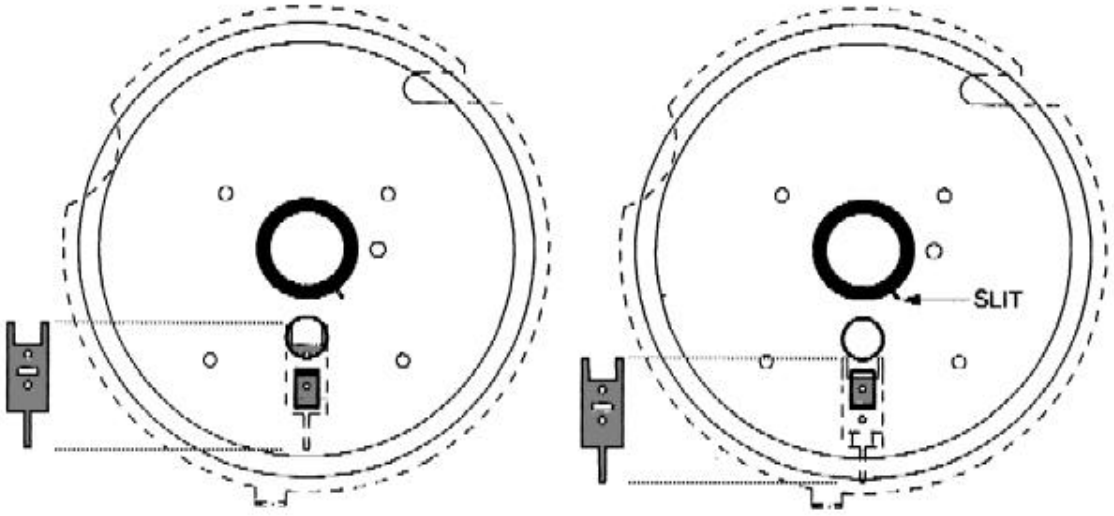
2.7 Specimen-holder handling

2.7.1 Specimen-holder selection

There are two ways for informing the microscope which specimen holder is being used:

- Not automatic: Each time a holder is inserted, the holder must be selected from the list that pops up in the message area of the Tecnai user interface.
- Default: Only for single-tilt holders, the last-used setting is selected automatically.

The CompuStage is equipped with the sEntry system. Associated with this system is an up-down switch on the outer panel of the CompuStage. The top part of the switch depends on the type of objective lens (with smaller lens gaps, the hole in the top is made smaller so only specific types of holders will fit).



The shield of the CompuStage showing the position of the holder-selection switch (gray). The switch itself is controlled by a notch in the lower rectangle. The actual shape of the top of the switch varies and on some microscope will block the sEntry key of the specimen holder from being inserted.

- In the **up position** (left-hand picture above), the **Default holder selection** is enabled. In this case the microscope will automatically select a single-tilt holder. In case there are more single-tilt holders present on the system (e.g. normal single - tilt and cryo holder), the holder selected is the last one chosen. If there are more single-tilt holders present, then for the first insertion, move the switch down, insert and identify the holder you are using, then move the switch up again. Thereafter the microscope will automatically select the same holder again.
- In the **down position**, the holder used must be identified by the user. This must always be done for double-tilt holders (the reason this is necessary has to do with the b tilt cable which must be connected before the holder can be inserted into the microscope), so do not use the switch up position with double-tilt holders.
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2.7.2 Handling instructions

Specimen holders are a bridge between the air pressure outside the column and the ultra-high vacuum inside. Their cleanliness is an important factor in keeping contamination down (specimen holders are the second-most important source of the contamination - specimens themselves are the primary source

nowadays). Caution should therefore applied to handling specimen holders. The following instructions should be adhered to:

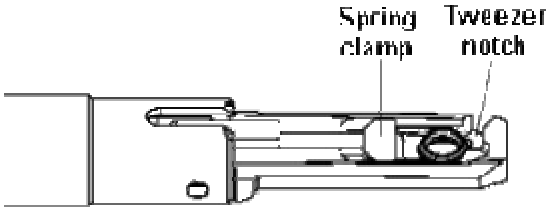
- Always use clean nylon or similar gloves when handling specimen-holder parts that enter the vacuum (that is, between the tip of the holder and the sealing O-ring).
- Clean the tip of the holder only with special cleaning fluids or with a fresh piece of window-cleaning (chamois) leather.
- Specimen, spacing washers and clamping devices should be manipulated only using pointed tweezers or the tools provided. Tools like the hex-ring tool or the needle for levering the single-tilt holder clamp should never be touched by hand on the wrong side (in the case of the hex-ring tool the use of gloves is advised because it is easy to pick it up at the wrong end). Clean the washers, tools and tweezers on a regular basis.
- The O-ring on the specimen-holder rod should be checked for possible dirt or excessive quantities of grease although it should not be completely dry. A very light coating of Fomblin grease (supplied with the microscope) is advised. Take care not apply grease to the conical part of the holder (the part between the thicker and thinner sections of the rod). The conical part is the area where the holder is 'seated' in the CompuStage. Any grease there will very likely result in drift in excess of specimen.
- When a specimen holder is not in use, in sert it in the protective holder cover supplied or reinsert it into the microscope. The latter keeps the holder thermally equilibrated with the microscope and CompuStage, thereby reducing drift after holder insertion (especially if there is a considerable temperature difference between the room and the microscope column).

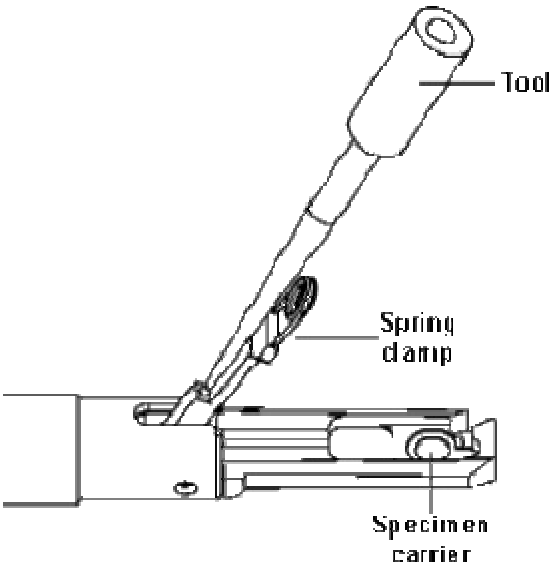
2.7.3 Inserting a specimen into the single-tilt holder

(Instructions for mounting specimens in other holders are covered in the manual accompanying these holders.)

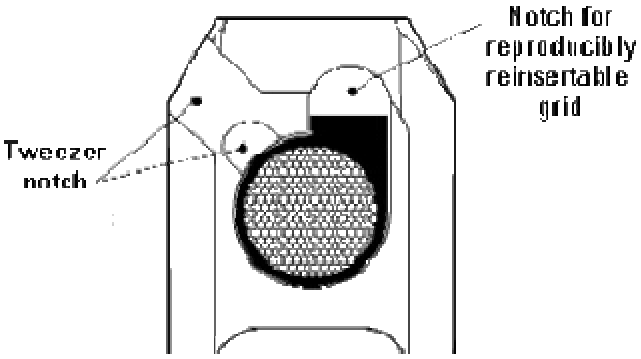
Note: For easy removal of specimens from the holder, a notch is provided on the holder (see the images below). One tip of a pair of tweezers can be inserted in this notch underneath the specimen (once the clamp has been lifted), making it easy to take the specimen out of the holder.

- If necessary, remove the cap at the end of the tube of the specimen-holder cover.
- Check that the tip of the holder and the clamping device are clean and dry.
- Keep one hand against the cap of the holder, making sure it cannot move out of the cover tube.
- Fit the tool (stored in one of the holes in the supports of the cover tube) into the hole in front of the clamp. Then lift the clamp to its fullest extent.





- Place the specimen in the (roughly) circular recess of the specimen-holder tip. If the specimen is on a re-insertable grid, place the 'ear' of the grid in the 'ear' recess.



- Carefully lower the clamp with the tool onto the specimen. Make sure the specimen remains correctly in position.

Caution: The specimen-securing clamp must be lowered carefully, otherwise the specimen and/or clamp can be damaged.

- Retract the holder slightly in the cover and turn it upside down. Tap the cap at the end a few times. Turn the holder back and check that the specimen has not moved (movement is a sign that it isn't clamped properly).

Note: Never mount magnetic specimens (disks) in the single-tilt holder. The clamp is normally not strong enough to prevent the specimen from flying out due to the objective-lens magnetic field and sticking to the objective-lens pole pieces.

2.7.4 Inserting a specimen holder into the microscope

Caution: The following instructions apply to all specimen holders and must be followed completely or damage to airlock, specimen holder or specimen stage may result.

For microscopes equipped with a turbo-molecular pump: The turbo-molecular pump (which should not remain running under normal microscopy because of the vibrations it causes) takes several minutes (2-3) to reach operational speed. To speed up specimen exchange, it is advised to switch the pump on (use the toolbar button) before extracting the holder. By the time the specimen has been exchanged the turbo-molecular pump will be near or at its operation speed and pumping on the airlock will begin (almost) immediately. Switch the pump off again after the holder has been inserted fully into the microscope.
If the turbo-molecular pump is running on when the specimen holder is inserted into the airlock, the pump will first spin up and only after a few minutes start pumping on the airlock. In this situation (holder triggers the pump), the pump will be switched off automatically after pumping on the airlock has finished.

Note 1: Read the specimen-holder handling instructions before proceeding.

Note 2: The specimen airlock of the CompuStage and the specimen holder consist of fine, high-quality mechanics. If considerable force is needed for any manual actions on the holder or CompuStage, it is a

sign of something being wrong. It should never be necessary to exert strong force and doing so may well result in damage to specimen holder or CompuStage.

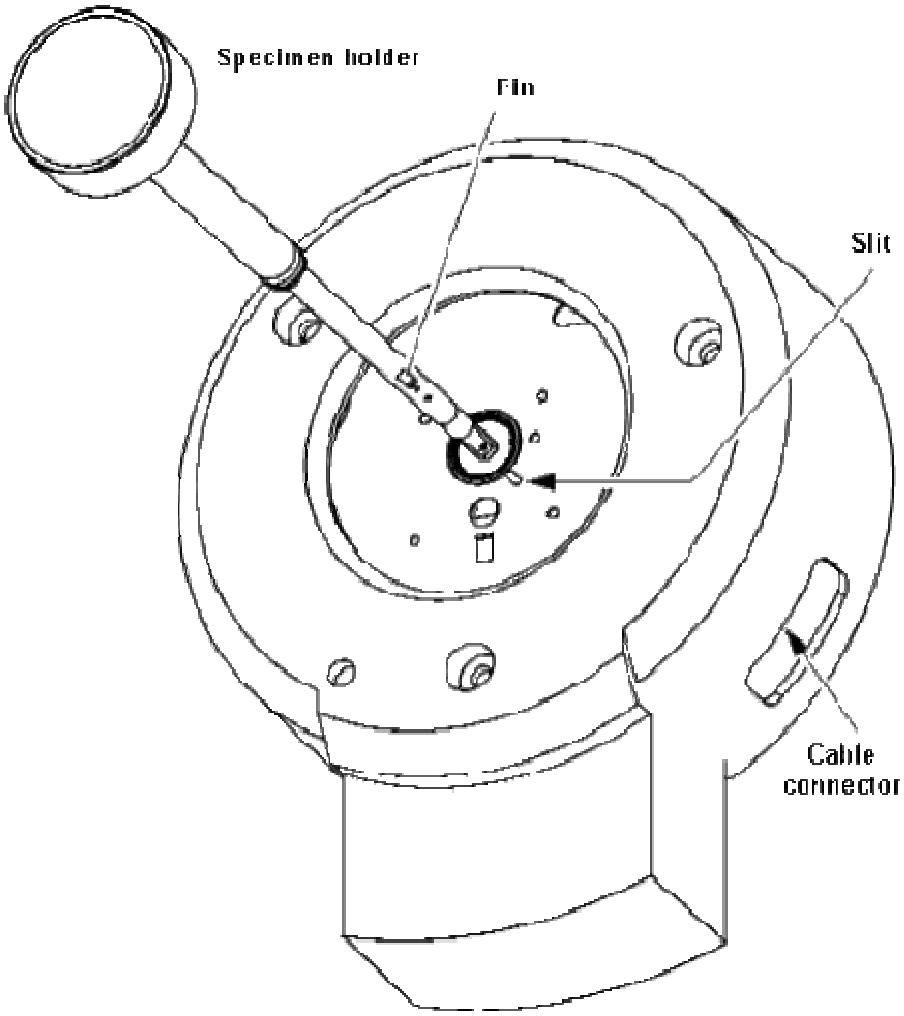
Note 3: It is not necessary to switch off high tension or filament during specimen-holder insertion, since the gun is separated from the column by the gun valve (V7) which is closed during specimen insertion (even if the user doesn't close the column valves, the microscope will automatically do so when it detects that a specimen holder has been inserted; it is good practice, however, to close the column valves before extracting the specimen holder from the microscope so they will still be closed when a holder is inserted again). It is advised, however, to keep the column valves closed after insertion of the holder (typically for a few minutes) while the specimen-area vacuum recovers - if only to reduce contamination of the specimen. Using the cold trap is advised to trap water vapour quickly (ion-getter pumps have difficulty in pumping water).

Note 4: Always carry out the complete insertion procedure. If the specimen is left in a retracted position, vacuum leakage can occur with consequent contamination (or, if left for a long period, loss of vacuum in the airlock and a crash of the column if the specimen holder is then inserted). If you do not want to insert the specimen holder after all (or if the airlock isn't pumped properly, for example because of a leaking O-ring), leave the airlock cycle to finish pumping (the red LED on the CompuStage goes off) before removing the holder from the airlock. Do not extract the holder while the airlock is being pumped.

The specimen holder is introduced through a pre-pumped airlock which ensures that air, introduced with the holder, is pumped away before the airlock is opened to the microscope column.

Procedure

- Hold the specimen holder with the airlock trigger pin parallel to the small slit in the CompuStage front plate (at roughly four o'clock). Carefully insert the end of the specimen holder into the airlock cylinder and slide the holder in until a stop is reached. At this point the prepumping of the airlock will start as indicated by the red CompuStage light which will be illuminated. If the light does not come on, the trigger has not been positioned correctly. Slowly turn the holder slightly to the left and the right until it will go in a bit further (the airlock trigger pin now falls properly into its groove).
- The Tecnai user interface will display a message asking for identification of the specimen holder. Select the type of holder from the list and press the Enter button.



Note: Unless a particular type of holder is specifically listed (in which case its has control parameters separate from the 'generic' holder types), it falls in one of two categories: single tilt or double tilt. Single tilt applies to any holder that doesn't have a computer-controlled b tilt (including manual-goniometer double-tilt holders!). For double-tilt holders a few different types may exist (typically only the Philips double-tilt holder is listed unless the control parameters for the other double-holders have been installed).

- If the holder is a double-tilt holder, insert the b tilt cable (as also instructed by the microscope) and press the Enter button.

Caution: As long as the red CompuStage light is illuminated, it is unsafe to insert the specimen holder further into the microscope. It is also good practice to observe the pressure in the backing line (which is connected to the airlock during airlock pumping) to make sure the airlock is being pumped properly on a microscope with the standard vacuum system (airlock is pumped by the rotary pump PVP). On systems equipped with a turbo-molecular pump, listen to the sound of the pump. If the turbo doesn't run more smoothly with ongoing pumping, the airlock is leaking.

- When the red CompuStage light has been switched off, rotate the specimen about 120 degrees counter-clockwise as far as it will, then allow it slide in further into the microscope.

Caution: Maintain a firm grip on the holder while it is sliding in (it is being sucked in by the column vacuum) to safeguard against any possible damage to CompuStage or holder.

- Once the holder is fully in, carefully tap a few times with a finger against the cap of the holder to help it settle into position and thereby improve stability.

2.7.5 Removing a specimen holder from the microscope

Caution: The following instructions apply to all specimen holders and must be followed completely or damage to airlock, specimen holder or specimen stage may result.

For microscopes equipped with a turbo-molecular pump: The turbo-molecular pump (which should not remain running under normal microscopy because of the vibrations it causes) takes several minutes (2-3) to reach operational speed. To speed up specimen exchange, it is advised to switch the pump on (use the toolbar button) before extracting the holder. By the time the specimen has been exchanged the turbo-molecular pump will be near or at its operation speed and pumping on the airlock will begin (almost) immediately. Switch the pump off again after the holder has been inserted fully into the microscope.
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Note 4: Always carry out the complete removal procedure. If the specimen is left in a retracted position, vacuum leakage can occur with consequent contamination (or, if left for a long period, loss of vacuum in the airlock and a crash of the column if the specimen holder is then inserted).

Procedure
- Close the column valves.
- Make sure the specimen holder is in a safe position (if necessary reset a and b to zero, or set all axes to 0). The red CompuStage light should be off.
- Pull the holder as far out of the CompuStage as it will go, then rotate it clockwise as far as it will go (about 120 degrees).
- Carefully extract the holder from the airlock (you have to pull against the vacuum remaining in the airlock).
- For double-tilt holders disconnect the cable plug.

2.8 Stage movement

The specimen stage movement is controlled by a track ball (normally the right-hand one, but the assignment can be changed by the user) and/or by Multifunction knob. The movement has two modes of operation:
- The track ball (discontinuous movement) mode.
- The 'joy stick' (continuous movement) mode.

In the track ball mode (the default) the stage will move whenever the track ball has been moved, in the direction and with a displacement related to the direction and displacement of the track ball. As soon as the track ball stops moving, the stage will stop moving as well. For normal operation at moderate to high magnifications this is the preferred mode. However, for searching at low magnifications the track ball mode requires that the user keeps moving the track ball continuously.
In the 'joy stick' mode the stage will move in the direction indicated by the movement of the track ball and with a speed related to the displacement of the track ball, and the stage will keep moving in the indicated direction without requiring further input through the track ball. The direction and speed of movement can be influenced by further control of the track ball. To stop the stage movement in the 'joy stick' mode, press one of the track ball buttons.
Switching between the two modes is achieved by pressing the two track ball buttons simultaneously.

2.8.1 Speed control

The speed of movement of the stage is related to three parameters :
- The speed value setting (1 to 9) as defined by pressing the left- (speed down) and right-hand (speed up) track ball buttons.
- The current magnification.
- The displacement of the track ball.

Note 1: One exception is at the lowermost speed setting. In this case the CompuStage will make its smallest steps, independent of magnification. At low magnifications this may mean that the stage doesn't seem to move at all. If the latter is the case, click once on the right-hand track-ball button to switch the speed one step up.

Note 2: At very low magnifications the speed control may appear to function no longer (speed up doesn't increase the speed of the CompuStage). This means that the CompuStage has reached its maximum speed and can go no faster.

2.8.2 Specimen stage movement by Multifunction knob

The specimen stage movement can also be assigned to the Multifunction knobs. In this case the Multifunction knobs duplicate the track ball directions (that is, Multifunction X = track ball X). The knobs are thus not connected directly to a stage axis (technically this is not possible within the software

architecture). For most microscopes this means that the X axis (the direction of the a tilt axis) is approximately connected to the Multifunction Y knob.

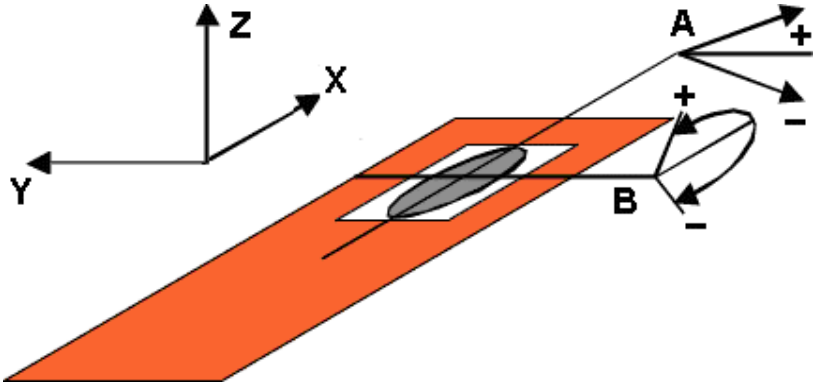
2.9 Stage Axes

The CompuStage is a goniometer with five axe s. Three of these are orthogonal translation movements X, Y and Z. The other two are mutually perpendicular rotation movements, α and β. The α tilt is parallel to the X axis and β tilt parallel to the Y axis.

The orientation in space of these axes can only be described at a tilt at 0° (since the other axes are mounted on top of the α tilt, they will change their orientation with it). At a tilt 0° the X and Y axes are horizontal and the Z axis vertical. The X axis runs along the rod of the specimen holder. If you look down on the microscope and define the front of the microscope as south, the X axis thus runs NW-SE, with SE in the + direction and the Y axis NE-SW with NE as the + direction. Up is the + direction of the Z axis. The X axis describes a truly linear motion, the Y and Z are in fact circular motions but with such a wide radius that the motion remains close to linear. The Y and Z motions cause the specimen holder rod to pivot around the conical face where it narrows down, just beyond the O-ring.

Because of this pivoting motion, the end of the holder on the outside of the CompuStage moves in the opposite direction!

Be low is a schematic 3-D view of the specimen holder tip and the orientation of the stage axes.



Note: The notation differs from that on the earlier CompuStage of the CM microscopes where the sign of the X and Y axes is reversed.

3 An introduction to vacuum

Electron microscopes cannot operate in air for a number of reasons. The penetration of electrons through air is typically no more than 1 meter, so after coming on meter from the gun, the whole beam would be lost to collisions of the electrons with the air molecules. It is also not possible to generate the high charge difference between the anode and cathode in the gun because air is not a perfect insulator. Finally, the beam on the specimen while in air would trap all sorts of rubbish (air is full of hydrocarbon molecules) on the specimen, crack them (removing hydrogen, oxygen, etc.) and thus leave a thick carbon contamination layer on the specimen. Each electron microscope therefore has a vacuum system. the degree of sophistication of the vacuum system depends on the requirements. Simple imaging of biological thin sections is much less demanding than cryo applications or small-probe analysis in materials science, and a thermionic gun can operate under much worse vacuum than a Field Emission Gun.

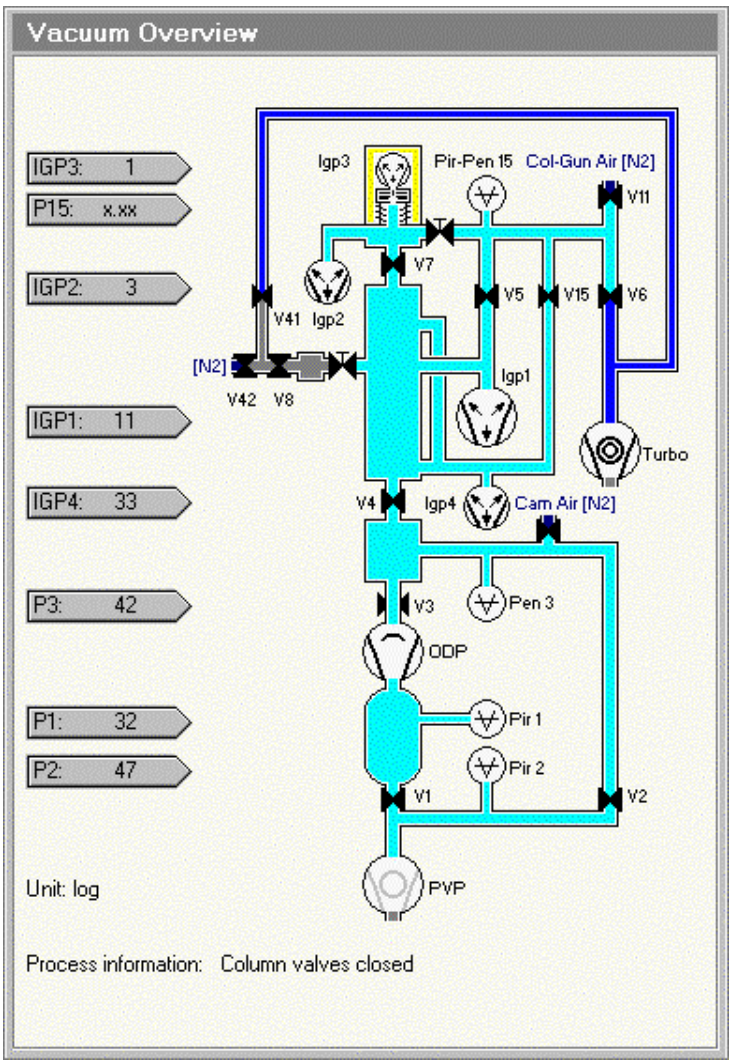
The most basic vacuum system consists of a vessel connected to a pump that removes the air. The vacuum system of an electron microscope is considerably more complicated, containing a number of vessels, pumps, valves (to separate different vessels) and gauges (to measure vacuum pressures). From the bottom up we can distinguish four vessels in the vacuum system:

- The buffer tank
- The projection chamber
- The column (specimen area)
- The electron gun area

These are not pumped by single pump, because there is no pump available that handle the full range in vacuum from air pressure (as present after a vessel has been vented) to ultra-high vacuum (in specimen are or gun). The microscope can in essence be divided in two parts, separated by a very small apertu re (200 micrometers), the differential pumping aperture, located between the projection chamber and the column.

The lower part basically consists of the projection chamber where we observe the image and where plate camera and TV cameras are located. This is pumped by an oil-diffusion pump. Behind the oil-diffusion pump is a rotary pump (the oil-diffusion pump cannot go from vacuum to air, it needs some other pump to back it up). Since the rotary (or pre-vacuum) pump is noisy, it is not running continuously but only when needed. In order to have continuous backing of the diffusion, there is a buffer tank in between them. The buffer tank is slowly filled by the oil-diffusion pump. When its pressure is becoming high, it is emptied by the rotary pump.

The upper part consists of the specimen and gun areas which are pumped by one or more ion-getter pumps. These pumps use no oil and are therefore clean. They also achieve higher vacuum than the oil-diffusion pump. The number of ion-getter pumps may range from one to four. Initial pumping of the column and gun on many systems is done by the rotary and oil-diffusion pumps, except for systems equipped with a turbo-molecular pump. In the latter case the oil-diffusion and rotary pumps never pump on the column and gun areas.

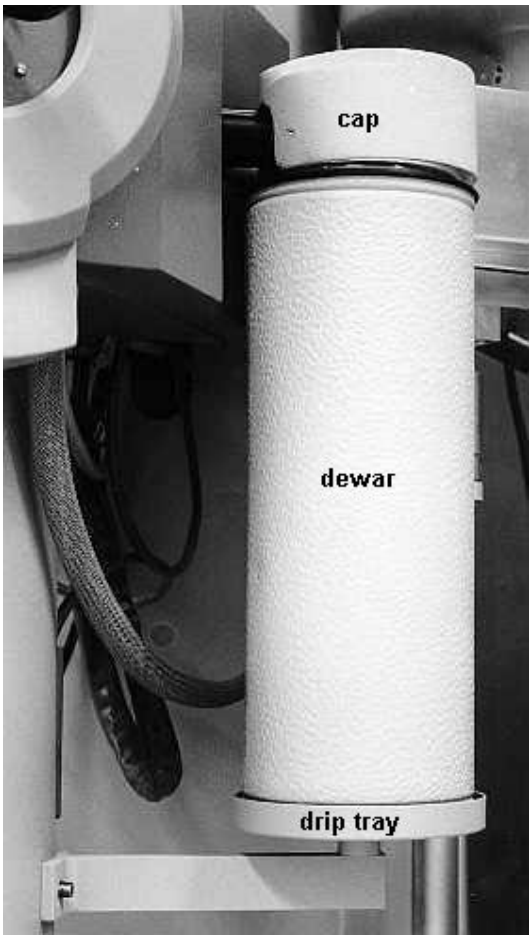


The diagram above shows the vacuum system of the Tecnai F20. At the bottom it starts with the rotary pump (PVP) which is not running here (otherwise its symbol would be black). Above PVP is the buffer tank (cyan color), separated by a valve (V1) from the PVP. Another valve (V2) is also closed. When open this would give the PVP access to the projection chamber (the area above V3, the valve that separates it from the oil-diffusion pump ODP). Pir (Pir 1 and Pir 2) and Pen (3) stand for Pirani and Penning which are vacuum-pressure measuring gauges. Valve 4 (closed) separates the projection chamber from the upper part of the column. Pressures are also measured on the basis of the current running in the IGPs. The large cyan area represents the specimen area (as well as some connecting pipes), pumped by a main ion-getter pump (IGP1) and the liner-tube ion-getter pump (IGP4) (liner tubes are thin pipes that connect the projection chamber to the specimen area and the specimen area to the gun). At the same height, to the right, is the turbo-molecular pump (Turbo) that is used to prepump the specimen and gun areas and also pumps on the specimen-holder airlock (on the CompuStage; through the line that is drawn over the top, towards valves 42 and 8). The gun is pumped by two ion-getter pumps (IGP2 and IGP3).

4 Cold Trap

The Cold Trap (also called Liquid-nitrogen cooling device or Cryo Trap) consists of a piece of metal around the specimen environment that is cooled to liquid-nitrogen temperature. The cooling is done by the liquid nitrogen in the dewar on the right-hand side of the column. Gases in the vacuum (predominantly water vapour) condense on the cold surface inside the microscope and thereby the partial pressures of these gases is reduced. Use of the cold trap is particularly effective when a holder is introduced into the microscope, because the water vapour coming in with the holder (either from the residual gases in the airlock or adsorbed on the holder surface) is trapped quickly.

To use the cooling device, first remove the dewar vessel (lift it up slightly, then tilt away at the bottom). Fill it with liquid nitrogen and replace it onto the support. **Make sure not to spill liquid nitrogen onto the viewing windows of the projection chamber as this may cause the glass to crack.**



Effective use of the cold trap depends on three factors: the level of the liquid nitrogen in the dewar, the time the cold trap has been in use (effectively the amount of ice already deposited), and the adjustment of the dewar to ensure that no vibrations from bubbling liquid nitrogen are transmitted to the microscope column.

Liquid-nitrogen level
If the level of the liquid nitrogen is below the half-full level of the dewar, the temperature of the cold trap inside the microscope may increase and some gases may start to evaporate again from the cold surface. In general keep the dewar between full and 3/4 full except when the cold trap has been cold for a long time already (when the rate of liquid-nitrogen boiling will be much reduced).

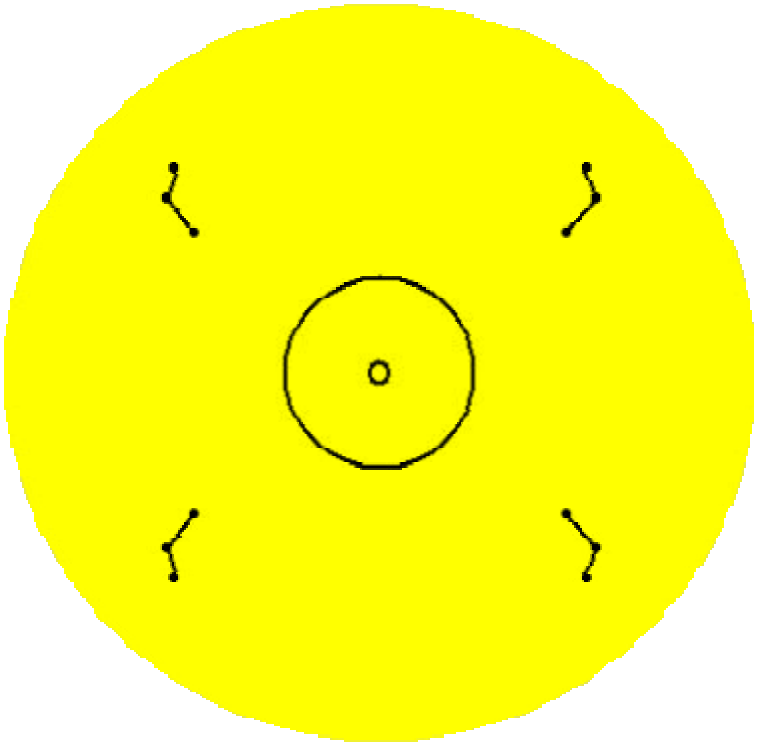
Cold trap effectiveness over time
As more and more ice is building up on the cold trap, the effectiveness of the removal of gases is reduced. Especially in environments where a lot of water is introduced with specimens (e.g. cryo-transfer work), the cold trap should be warmed up at least once a day. Rapid warm up can be done either by filling the dewar with hot water (simply throw out the remaining liquid nitrogen and fill the dewar under a hot tap; the dewar doesn't need to be warmed up first, it can stand the temperature change) or remove the dewar and blow with a hair dryer against the copper braids that hang inside the dewar.

Dewar adjustment
The dewar should not come into direct contact with the copper braids (wire bundles), insulating cap or the conductor (the rod running from braids towards the microscope). At the same time the amount of space between the dewar and cap must be minimized to prevent cold air from flowing out easily. If this situation cannot be obtained, then the height of the dewar on the drip tray support can be adjusted by removing the screw in the center of the drip tray and adding or removing washers between the dewar support table and the bottom of the drip tray or between the latter and the support rod below the drip tray.

5 Viewing screens

The Tecnai microscope is equipped with two (fluorescent) viewing screens, a main screen and a focusing screen.

5.1 Main screen



The main viewing screen has a diameter of 160 millimeters (the size of the phosphor, not including the outer rim) except on 300 kV instruments where it is 140 millimeters. The screen is equipped with markings that identify the various sizes of film as well as two circles that aid in identifying the screen center. The small circle in the center has a diameter of 5 millimeters while the larger circle has a diameter of 40 millimeters. The film size indicators take the shape of 'elbows'. Each elbow has three 'dot' markers. The dot markers on the ends closest to the screen center mark the sheet-film negative size 6.5x9 cm, while the dots on the 'elbow' itself (at the corner) marks the 3 1/4x4 inch size. The markers furthest outside are for a film type that is no longer used.

The main screen is moved up and down using a motor drive mechanism. The motor drive is under software control. One, dedicated button for moving the screen up and down is present on the left-hand side of the projection chamber (about five centimeters behind the lever for the small screen). Additionally it is possible to define any of the user buttons to have the screen lift function.

5.2 Focusing screen

The small focusing screen is moved in and out by hand with a lever mechanism on the lower left-hand side of the projection chamber. The focusing screen is used together with the binoculars.

6 Plate camera

The plate camera is located behind and inside the projection chamber of the microscope. The plate camera consists of a mechanism and a removable cassette.

6.1 Plate camera mechanism

The mechanism consists of a pneumatically driven rod that moves forward from behind the cassette. The rod pushes the central section of the cassette out towards the front. This central section is a transport tray, holding a single sheet film holder. The transport tray slides over guides in the bottom of the projection chamber. When it reaches the front, a pin located in the projection chamber pushes in a hole in the front of the tray. This moves two supports in the tray back, allowing the sheet-film holder to fall down inside the tray (until then it was carried at the top of the tray). The exposure is then made. The rod moves back, drawing the transport tray with it. When the tray is withdrawn fully, the sheet-film holder drops down into the receiving part of the cassette.

Note: When an exposure is made, the negative lies open in the projection chamber and can be struck by **light** coming from the room through the front or side windows of the projection chamber. In order to prevent fogging or outright exposure by light, switch off any room lights before the exposure and keep the covers on the projection chamber windows.

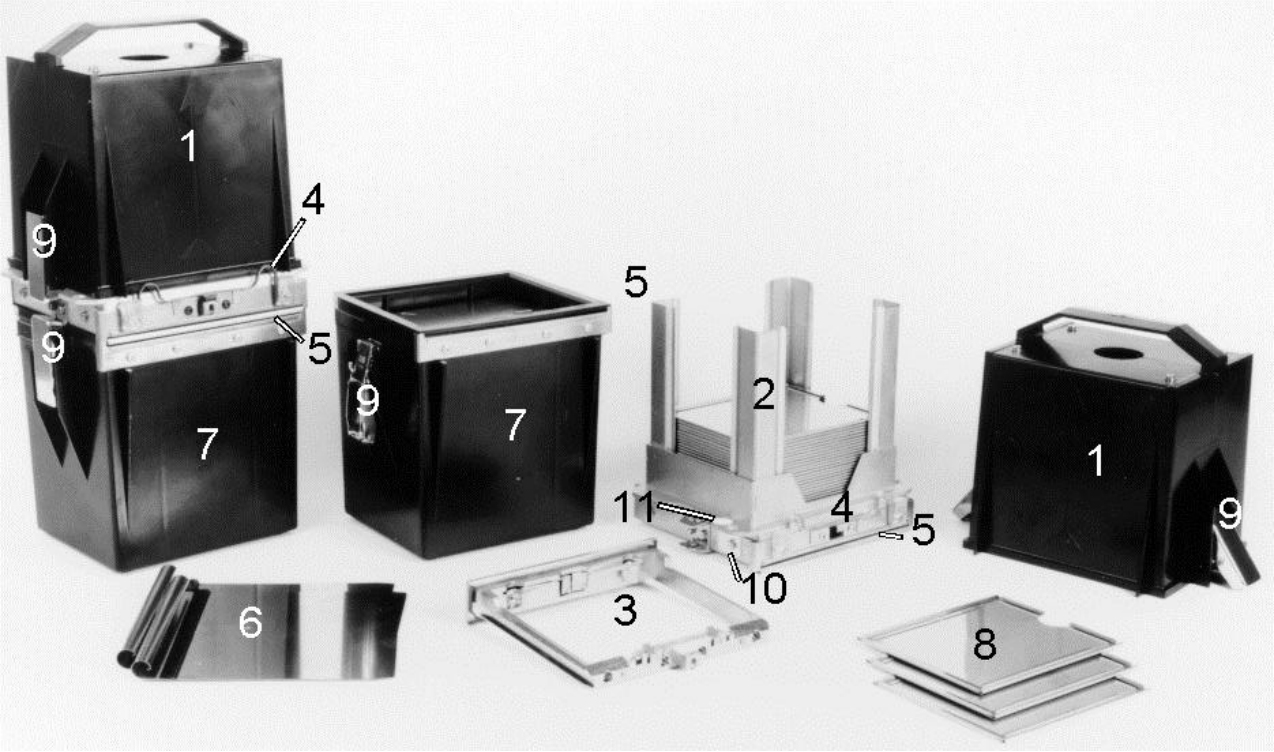
6.2 Plate-camera cassette

The cassette for sheet film can hold a maximum of 56 sheet-film holders (item 8 in the picture below). This cassette replaces the one holding 36 glass plates or sheet-film inserts.

Note for owners of older Philips microscopes: The plate-camera cassettes look similar to older designs for the CM-series and EM400 series (EM400, EM410, EM420, EM430) microscopes but are different. The older versions could hold only 36 plates. If you have such older cassettes, you should make sure the 56- and 36-plate cassettes should not be mixed as this will give rise to plate jam and other problems (this applies to both the cassette top section which has a different width of slit and to the bottom section which has a different spring). The 56-plate have markings to distinguish them from the older cassettes (a marker plate with the number 56 in relief - item 10 in the picture below - and a fluorescing sticker - item 11).

The 56-plate cassette is compatible with older microscopes (EM400, EM410, EM420, EM430, all CM microscopes).

6.2.1 Cassette parts description



The complete cassette consists of:

- a top magazine (1) carrying the unexposed film material between plate-stack guides (2).
- a transport tra y (3) belonging in the center of the cassette.
- a bottom magazine (7) to receive the exposed film material.

Blanking plates (6) can be used to split the cassette in room-light conditions so that a fresh receiving magazine can be put underneath the magazine with unexposed film material. Slide both blanking plates fully into the slits (5) provided above and below the transport tray (3), with the top blanking plate having its curved grip facing upward and the lower one with its grip facing down. Release the clips (9) from the **LOWER, RECEIVING MAGAZINE**, remove it and replace it with a fresh receiving magazine. **Do not take the cover off the top magazine as this will expose the unexposed film material to light!**

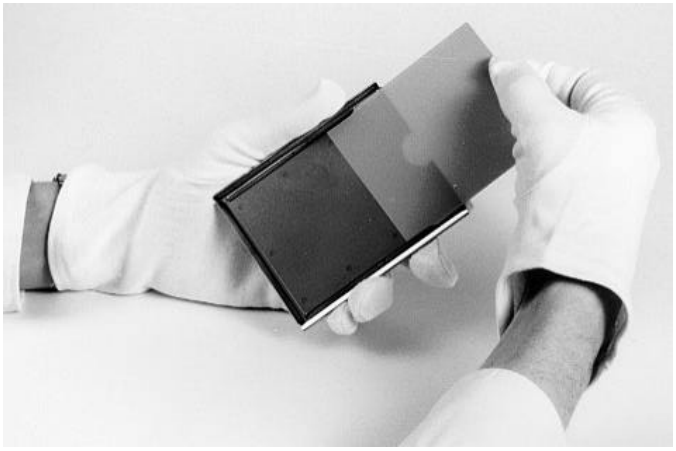
To prevent the transport tray from dropping out during transportation, it is fixed in position by a securing spring (4).

6.3 Inserting sheet film in a holder (dark room only!)

The sheet-film holder is a metal plate with upturned edges that will hold a sheet film into position. The top end of the sheet-film holder has a semi-circular cut-out (the finger slot).

To load a sheet film in the holder:

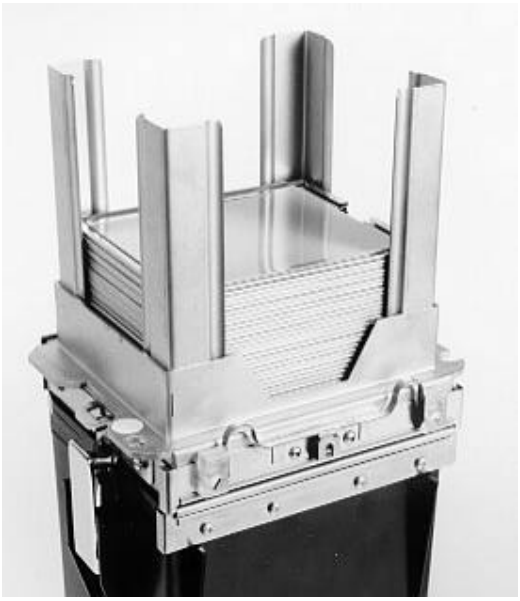
- Take the sheet-film holder in one hand with the finger slot at the top.
- Take hold of the top of a sheet film with the other hand and with the emulsion side upward (the small notch in the sheet film should be on the top right-hand side).
- Slide the sheet film downward underneath the securing rims until the top of the film fits below the rim inside the holder.



Inserting a sheet film into a sheet-film holder.

6.4 Loading the sheet-film magazine (dark room only!)

- Remove the upper magazine cover by releasing the clips (move the top end outward, then release the bottom part from the grip) and pulling it off.
- Stack the required number of sheet-film holders between the guides, with the emulsion (sheet film) upward. Do not exceed 56 plates per magazine.
- Replace the upper magazine cover and fix the securing clips. Note that the cover fits on in only one way (otherwise the clips are misaligned).



The plate stack between the guides of the magazine for unexposed sheet film.

6.5 Loading a cassette into the microscope

- Select the tab containing a Vacuum control panel.
- If nitrogen gas is available, check that the valve on the bottle or line is open.
- Press the Camera Air button and confirm (press the button with the 'V' sign).
- Wait until the projection chamber has reached air pressure.
- Lift the plate-camera cover (behind the column).
- Remove the magazine inside (if present) and insert blanking plates. Replace the magazine with the new magazine (make sure the blanking plates have been removed and that the magazine settles properly in place). The magazine fits in in only one way, with the guiding pins of the camera housing through the holes alongside the magazine.
- Check the O-ring of the plate-camera cover for cleanliness and proper position.
- Replace the cover and make it fits in properly.
- Press Camera Air off.
- If necessary, close the nitrogen gas valve.
- If necessary, adjust the plate-camera stock value.

Note: To prevent fogging of the unexposed film material, keep the blanking plates inserted into the cassette during transportation, and load and unload cassettes under somewhat dimmed room-light conditions.

6.6 Removing a cassette from the microscope

- Select the tab containing a Vacuum control panel.
- If nitrogen gas is available, check that the valve on the bottle or line is open.
- Press the Camera Air button and confirm (press the button with the 'V' sign).
- Wait until the projection chamber has reached air pressure.
- Lift the plate-camera cover (behind the column).
- Remove the magazine inside. Insert blanking plates to prevent fogging of the topmost exposures.
- Check the O-ring of the plate-camera cover for cleanliness and proper position.
- Replace the cover and make it fits in properly.
- Press Camera Air off.
- If necessary, close the nitrogen gas valve.
- If necessary, adjust the plate-camera stock value to zero.

Note: To prevent fogging of the unexposed film material, keep the blanking plates inserted into the cassette during transportation, and load and unload cassettes under somewhat dimmed room-light conditions.

6.7 Removing exposed film from the receiving magazine (dark room only!)

- Remove the lower magazine from the cassette by releasing the clips (move the bottom end outward, then release the top part from the grip). The top magazine for unexposed plates can now be lifted off and put aside.
- If a blanking plate is used, pull it out.
- Take the exposed plates out of the receiving magazine. It may help to tilt the magazine a little. The exposed plates rest on a spring-supported platform that will automatically rise when plates are removed.

6.8 Removing sheet film from a holder (dark room only!)

- Take the sheet-film holder in one hand with the finger slot at the top (the semi-circular cut-out). Press with a finger against the sheet film from the back through the finger slot so that the film comes up.
- Take hold of the top edge of the sheet film with the other hand and retract it upward out of the holder.

Pressing the sheet film from the back through the finger slot.

Take hold of the top edge and withdraw the film from the holder.

6.9 Troubleshooting

6.9.1 No exposure is being made

When the Exposure button has been pressed and still no exposure is being made:

- Check that the exposure time in automatic mode has a possible value (not 0.0 seconds) and that the LED of the Exposure button is on.
- Check that the screen moves up when the exposure has been started. If the screen does not move up, the exposure cycle has (probably) tried to move it up and failed within the given time. Move the screen up by pressing the Screen lift control and press the Exposure button.
- Check that the plate stock is higher than zero.

6.9.2 Plate-camera jam

The plate-camera mechanism may jam when it moves a plate in or out. In some case the jam is easy to resolve, in other cases it may be more difficult. Below is a description of the procedures to use to clear the plate jam.

Warning: do not attempt this when you are uncertain of what you are doing. If you are not the microscope supervisor, please warn the supervisor and let him/her handle this. If you are the supervisor and doubt whether you can handle this, call service!

There can be several reasons for plate jams. The procedures for clearing them differ.
1. A sheet-film holder does not come out properly of the upper magazine.
2. A sheet-film holder does not drop down inside the transport tray.

Transport tray moves back properly into cassette
The most critical aspect in solving a plate jam is whether the transport tray moves back properly into the cassette or is stuck inside the microscope. If the tray moves back, then close the column valves, vent the plate camera/projection chamber, and remove the cassette from the microscope. Go into the dark room, remove exposed and unexposed film material, and then try to establish why the camera jams while using empty sheet-film holders. When removing the film material, pay attention, so you can reload the empty sheet-film holders into the cassette exactly as they were.

Possible reasons for plate jams :
- A bent sheet-film holder.
- Old (36) and new (56) cassettes have been mixed. The slit through which the sheet-film holder comes out is wider for the old-type of cassette. Loading new sheet-film holders in this cassette will certainly jam, since two holders will try to come out together. The other way around, the old plate holder will not pass through the slit because it is too narrow.

If you have found the reason for the jam, load a cassette with empty sheet-film holders into the microscope. Switch to manual exposure time (you do not need to have the plate camera/projection chamber at vacuum) and 'shoot' a whole magazine to make sure the plate jam does not occur again. If necessary you can look through the window on the left-hand side of the projection chamber and observe the process (on 300 kV machines lift out the cover to see inside - when replacing it, make sure the O-ring is clean and properly seated).

Transport tray does not move back into cassette
This case is more difficult to solve. You cannot remove the cassette because the rod that moves the transport tray sticks through the tray.

Warning: the procedure described below can be dangerous if not handled properly. When the transport tray is moving, the forces on it are such that you damage your hands when they are in the way.

- Close the column valves, switch off the HT and vent the plate camera/projection chamber.
- Remove the window or cover plate (300kV instruments) on the left-hand side of the projection chamber.
- Switch the plate camera to manual exposure with the longest possible exposure time, and switch to HOLD (to prevent it switching back to automatic).
- Press Exposure. The rod with the transport tray should now come forward again.
- If possible, try to remove the jamming plate(s) by lifting them out.

- When done, press exposure again. The rod and transport tray should now move back properly into the cassette.
- If the rod and transport tray are back properly, remove the cassette from the microscope and in the dark room, remove the film material and then reload with empty sheet-film holders, put it back into the microscope and check. If everything works properly, the re-insert the window/cover plate, making sure the O-ring is clean and in its correct place. Replace the test cassette with a full one and pump the plate camera/projection chamber.

If the rod and tray did not move back properly, repeat the exposure (make sure it is still on manual exposure) and try and see if it is clear.

If it still doesn't work or if it fails again, call service.

Always remove the particular sheet-film holder(s) and inspect for damage. If damaged, discard (do not attempt to repair; that will probably only lead to repeated jams).