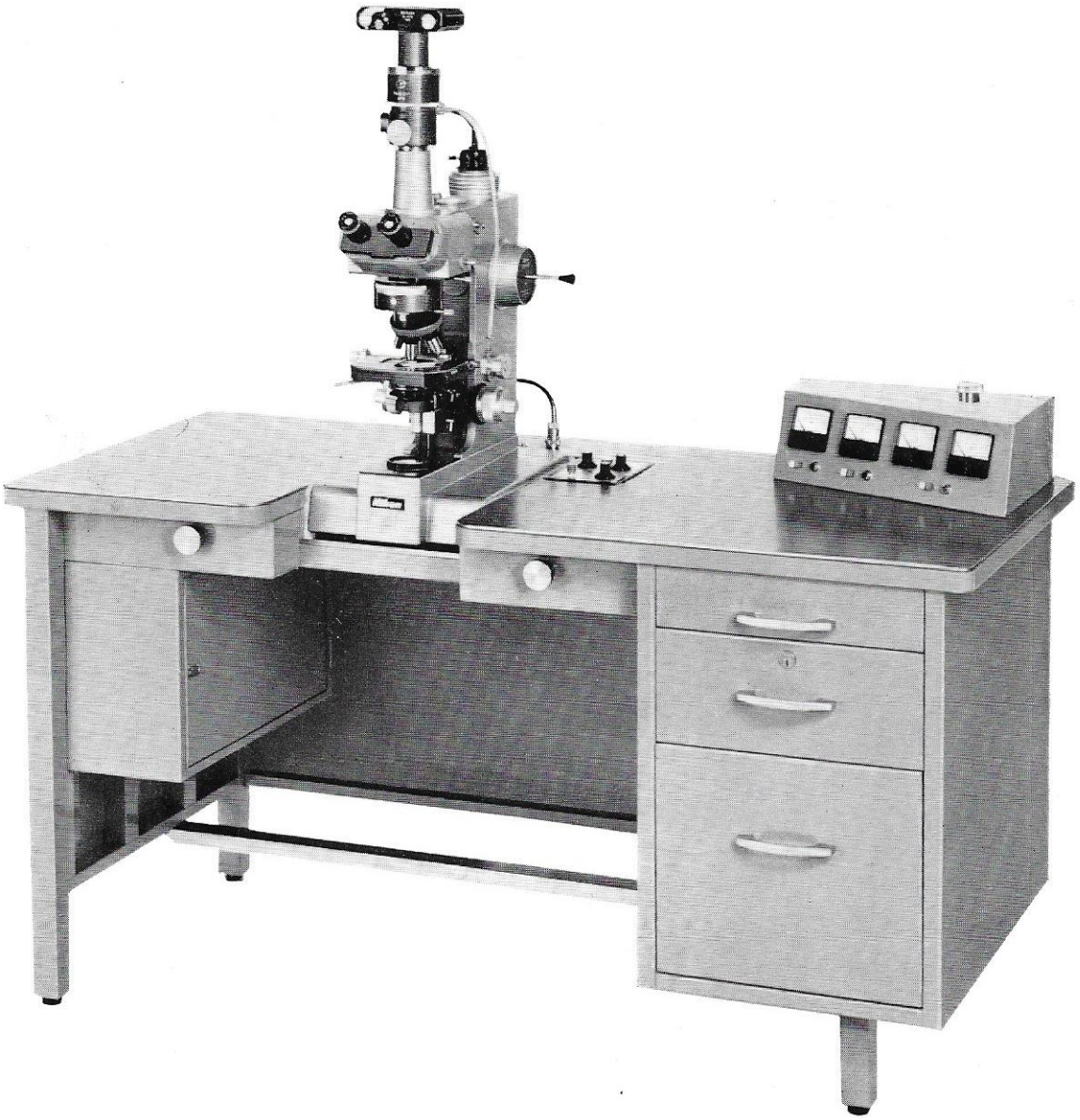
Nikon/APOPHOT

INSTRUCTIONS



NIPPON KOGAKU K.K.

APOPHOT

The APOPHOT microscope, being a high precision instrument, needs delicate handling in order to maintain its efficiency and accuracy as long as possible.

The instrument should be used in a room, dry and free from dust and vibration; if possible, of constant temperature and moisture. Be careful not to touch the lens surfaces to avoid leaving finger marks. For removing dust, use a soft camel hair brush. For removing grease, wipe then off carefully using soft, washed-out cotton cloth soaked with xylol.

Never use alcohol or ether for cleaning the objective lenses.

If any malfunctioning is discovered in use, check it immediately. When not in use, the microscope should be covered with the vinyl cover supplied as an accessory.

Store in a dry and dust-free place.

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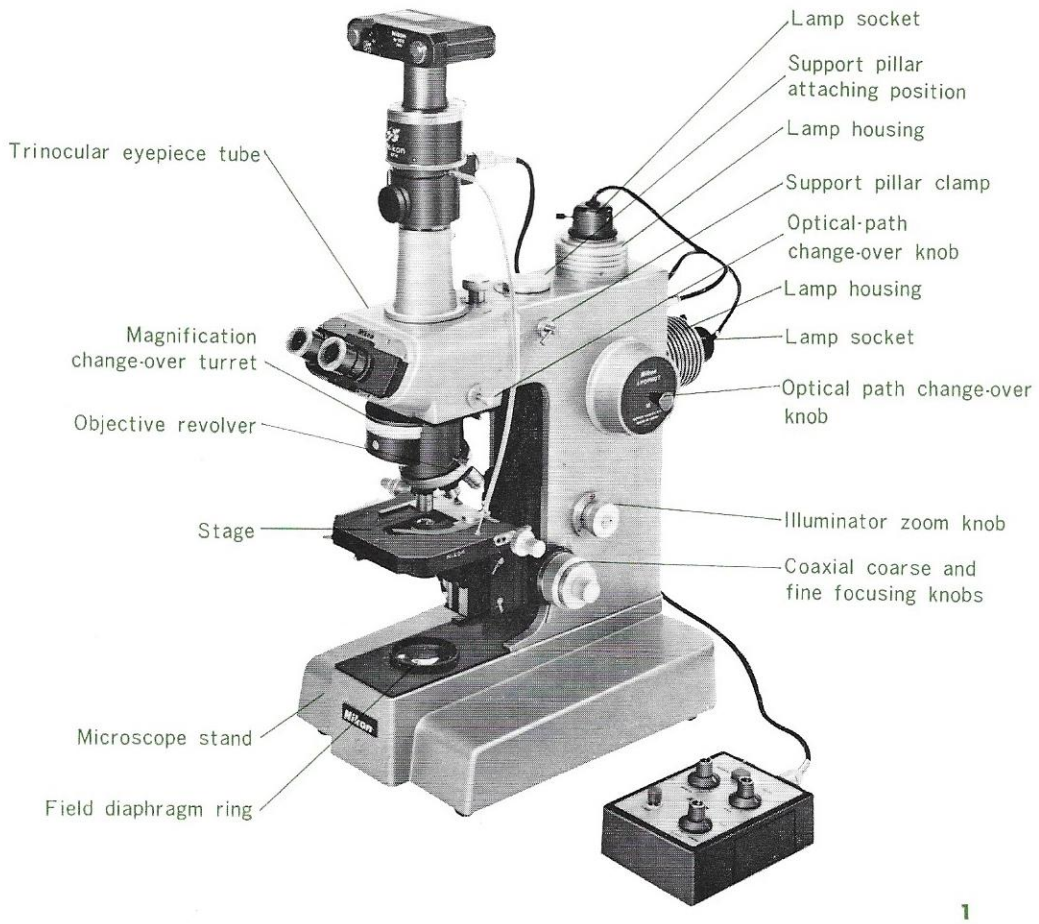
3. Polarizing Unit 30

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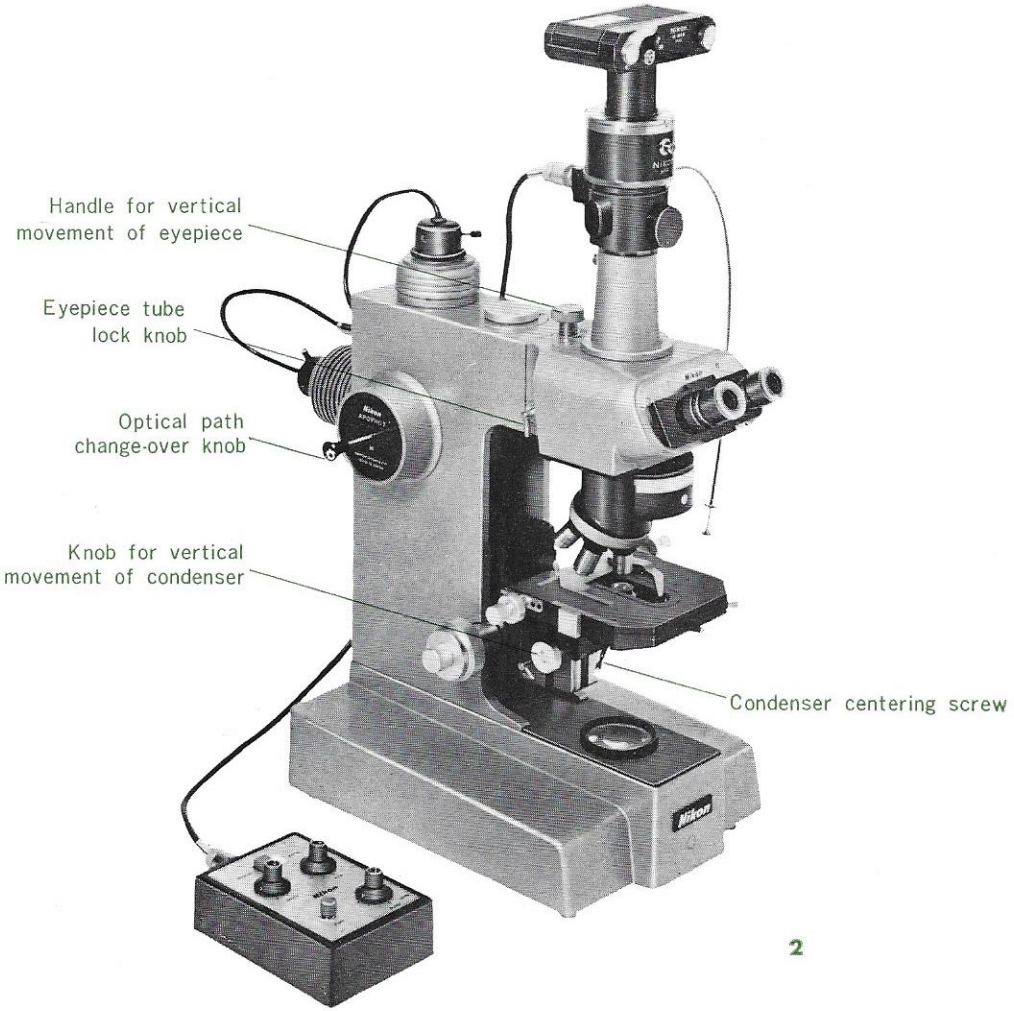
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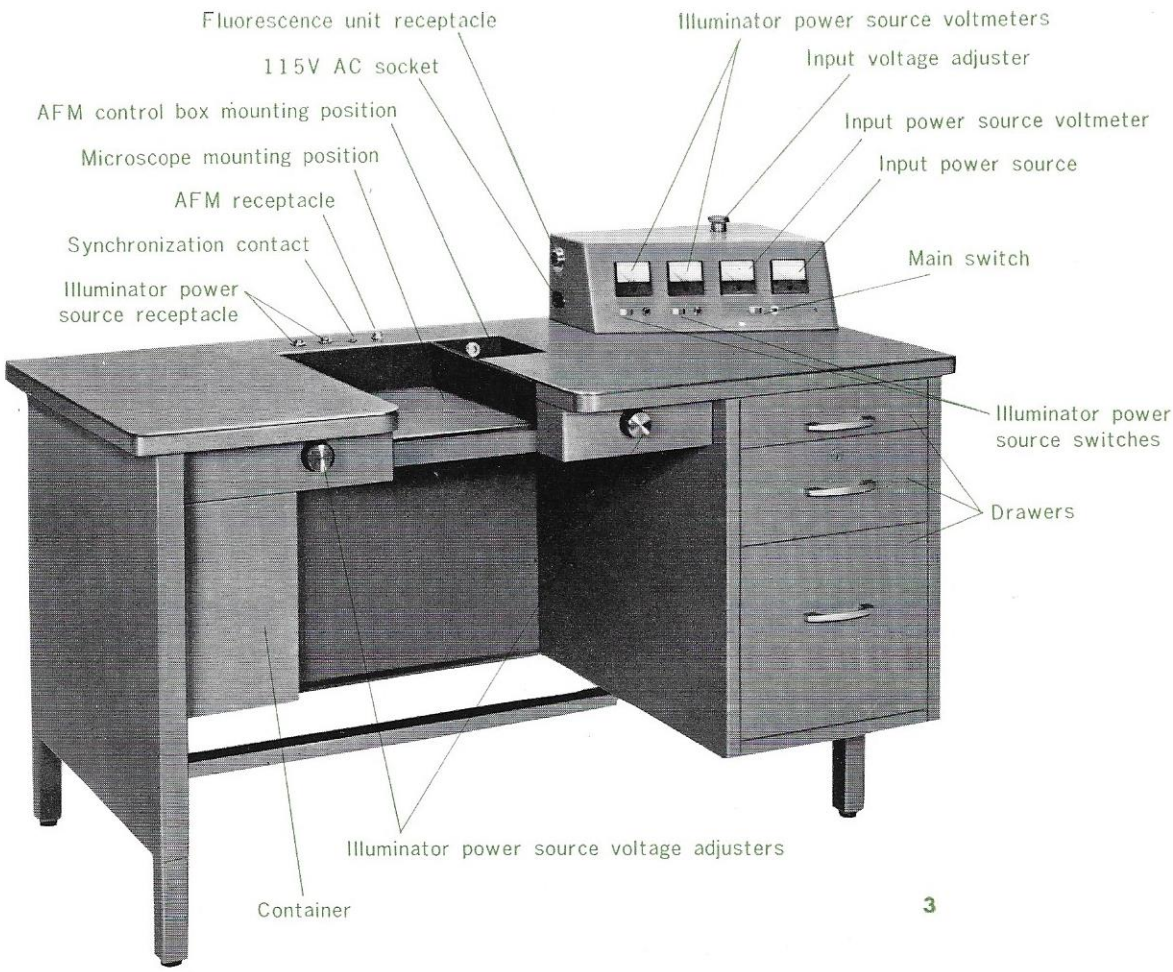
I. NOMENCLATURE



1

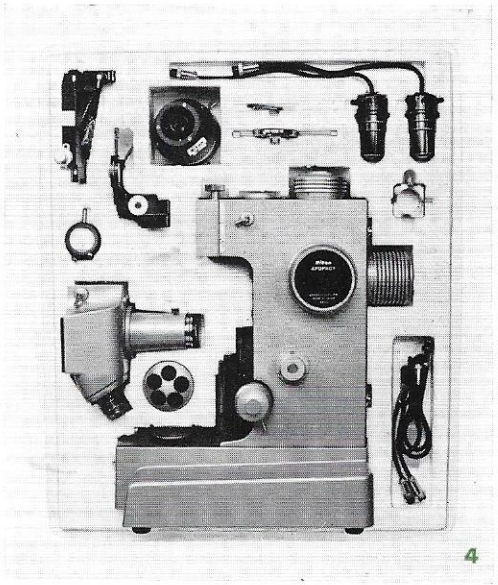

Handle for vertical movement of eyepiece
Eyepiece tube lock knob
Optical path change-over knob
Knob for vertical movement of condenser
Condenser centering screw

2


Fluorescence unit receptacle
115V AC socket
AFM control box mounting position
Microscope mounting position
AFM receptacle
Synchronization contact
Illuminator power source receptacle
Illuminator power source voltmeters
Input voltage adjuster
Input power source voltmeter
Input power source
Main switch
Illuminator power source switches
Drawers
Illuminator power source voltage adjusters
Container

3

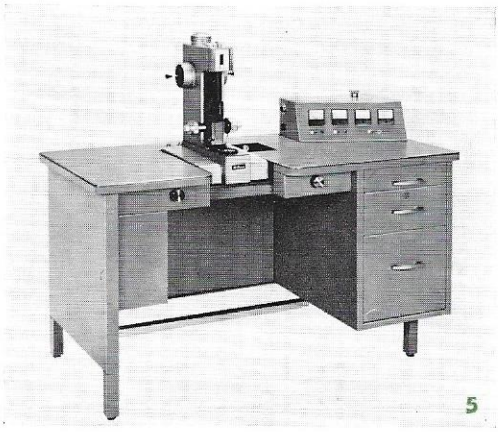
II. ASSEMBLY



4

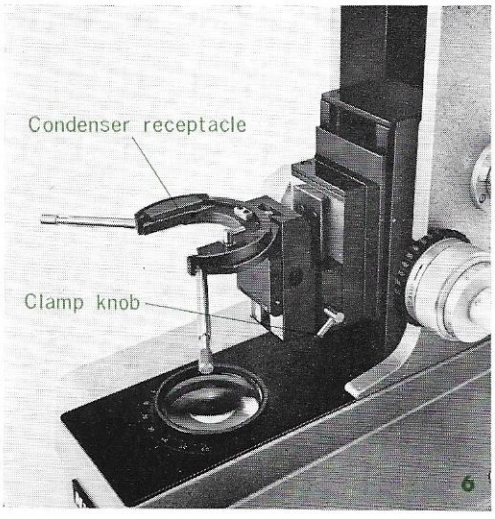
1. Attaching the microscope stand

● Remove the microscope stand from the transport case.



5

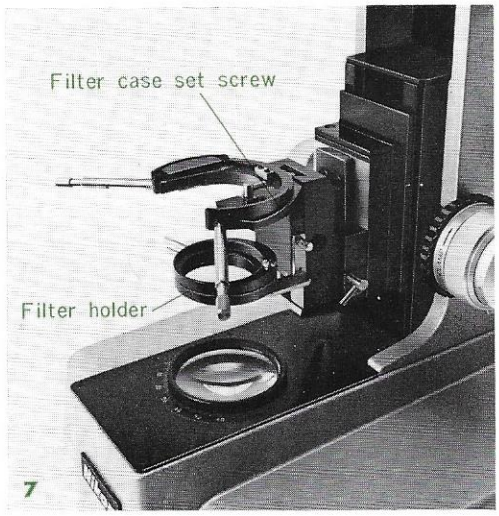
● When the desk, exclusively prepared for APOPHOT, is used, set the instrument up in the specified position on the desk.



6

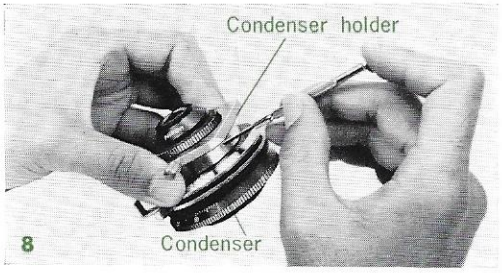
2. Attaching the condenser receptacle

● Attach the condenser receptacle to the microscope stand by sliding it in the dove-tail groove on the microscope stand as far as it will go, and clamp in position.



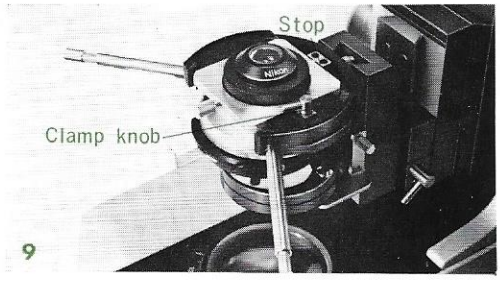
3. Attaching the filter holder

- Attach the filter holder to the side of the condenser receptacle by means of the pin and the set screw.

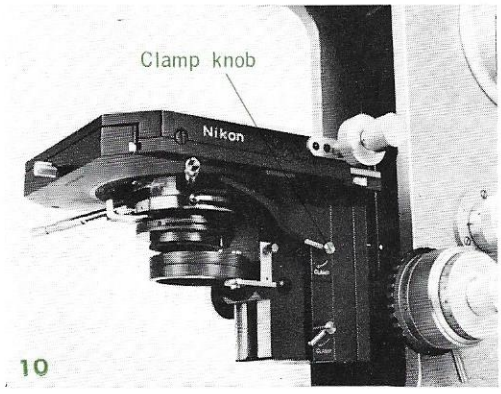


4. Attaching the condenser

- After releasing three set screws on the condenser holder, slide in the condenser and fasten it in position by means of the same screws.

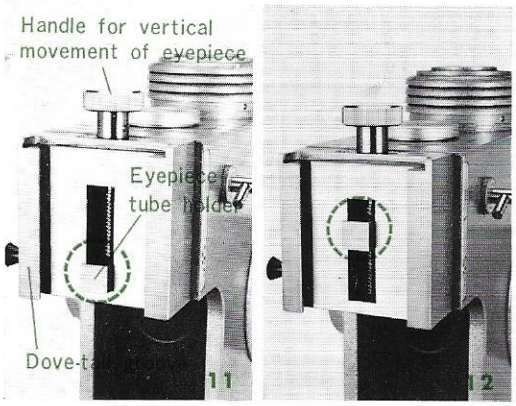


- Slide the holder on the condenser receptacle as far as possible and clamp in position.
- This procedure can be completed after the microscope has been completely assembled.

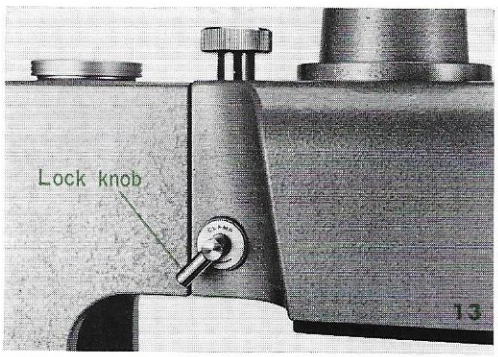


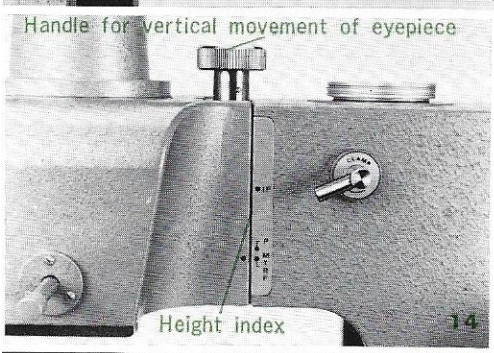
5. Attaching the stage

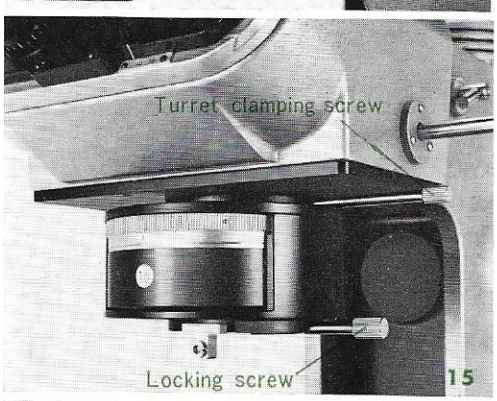
- Mount the stage in the dove-tail groove on the stand as far as possible, and clamp in position.



○ ×







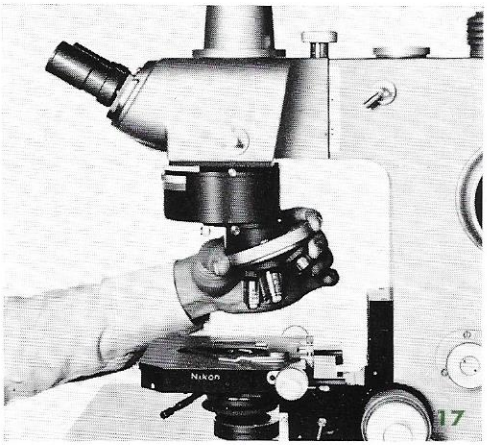


6. Attaching the trinocular eyepiece tube

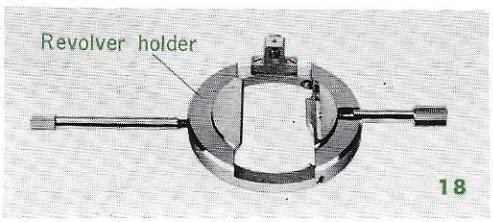
- Move the trinocular eyepiece tube holder to its lowest position.
- Release the lock knob on the eyepiece tube and slide it on the stand as far as it will go.
- Adjust the height of the trinocular eyepiece tube to that of the intermediate tube (magnification change-over turret, metallographic attachment, etc.) For details, see p. 23.
- After the trinocular eyepiece has been attached, insert the eyepiece to be used into the eyepiece sleeve.

7. Attaching the magnification change-over turret

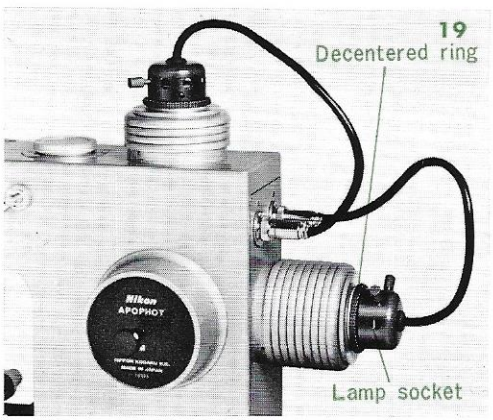
- Fit the magnification change-over turret into the circular groove under the trinocular eyepiece tube by means of the turret clamping screw.
- Various other accessories can be attached to this ring.
- If the clamping screw provided with spring is used, there will be no fear of damaging the specimen, etc. because dropping the turret is avoided even when the screw is released.



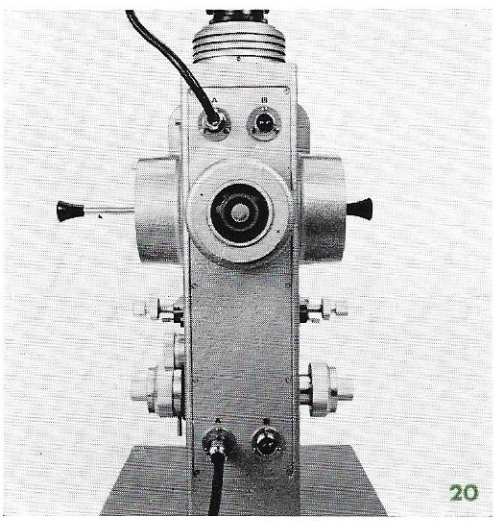
17



18



19
Decentered ring
Lamp socket



20

8. Attaching the objective revolver

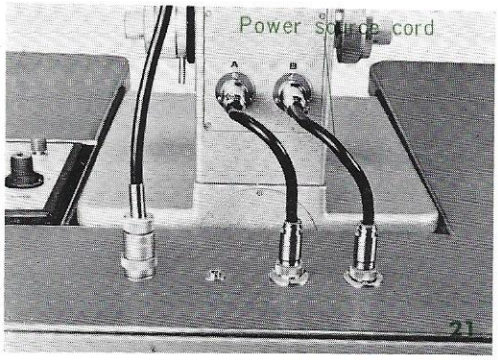
- Releasing the locking screw for the objective revolver on the magnification change-over turret, slide the revolver into the dove-tail as far as it will go.
- Do not clamp the revolver lock screw before the revolver is completely in, to avoid injuring the spring on the screw.
- Two types of revolver, one for four objectives and the other for five, are available.
- When other intermediate tubes (such as for fluorescence, polarizing observation, etc.) are used in place of the magnification change-over turret and epi-illuminator, attach the revolver below the revolver holder in the same way as above, after this holder has been attached below the intermediate tube.

9. Attaching the objectives

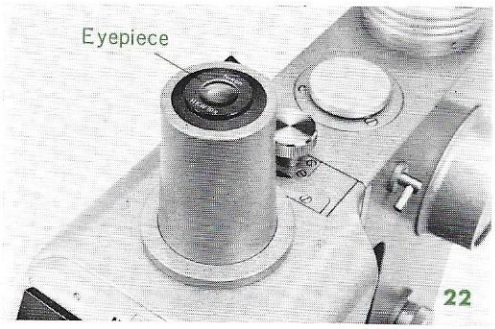
- It is recommended the objectives be arranged on the revolver so that the magnification is increased as the revolver is rotated clockwise, as viewed by the user.
- A series of objectives of the same type, such as metallographic or phase-contrast can be mounted on separated revolvers, for convenience.

10. Attaching the lamp socket

- Turning the decentered ring on the lamp socket, fit it in position at the rear of the stand.
- Connect the socket to the top receptacle on back of the stand.
  The power source cord is to be connected to the bottom receptacle at the bottom of the back of the stand.
  The receptacles A at the top and B at the bottom are connected to sockets A and B, inside the stand, respectively.
  Connect the power source cord to socket A, when the socket is connected to receptacle A.



● When using the APOPHOT Desk, make electric connection between the microscope stand and the desk, using the shorter cord available as accessory.



11. Attaching the photographic unit

● Unscrew the cap from the straight sleeve on the trinocular eyepiece tube.



● Insert the eyepiece. Clamp the eyepiece in position.

When using eyepiece 8×, use the long adapter for this eyepiece.

● When using the APOPHOT Desk and the photomicrographic attachment—Microflex AFM, connect the cord from this Microflex to the socket on the desk, and the AFM control box to the control box receptacle on the desk.

12. APOPHOT Table

● The electrical elements on the desk are as follows (For the numbers refer to the accompanying photo):

① Receptacle to be connected to ⑨, ⑩ and ⑲
② Receptacle to be connected to ⑪, ⑫ and ⑱ on the microscope stand
③ Synchronization contact to be connected to the electronic flash
④ AFM receptacle to be connected to the AFM body
⑤ Microscope stand mounting position
⑥ AFM control box mounting position provided with AFM control box connecting plug and synchronization contact, each connected to ③ and ④, respectively
⑦ Socket, for current indicated on the voltmeter ⑭
⑧ Fluorescence unit receptacle to be connected to the plug on the fluorescence unit starter
⑨ Illuminator power source switch to be connected to ①, ⑩ and ⑲

⑩ Illuminator power source voltmeter to be connected to ①, ⑨ and ⑲

⑪ Illuminator power source switch to be connected to ②, ⑫ and ⑱

⑫ Illuminator power source voltmeter to be connected to ②, ⑪ and ⑱

⑬ Input voltage adjuster

⑭ Input power source voltmeter, to be adjusted to the voltage to be used (e.g. 100V, 115V, 220V or 240V) by means of the adjuster ⑬

⑮ Input power source ammeter

⑯ Main switch

⑰ Drawer

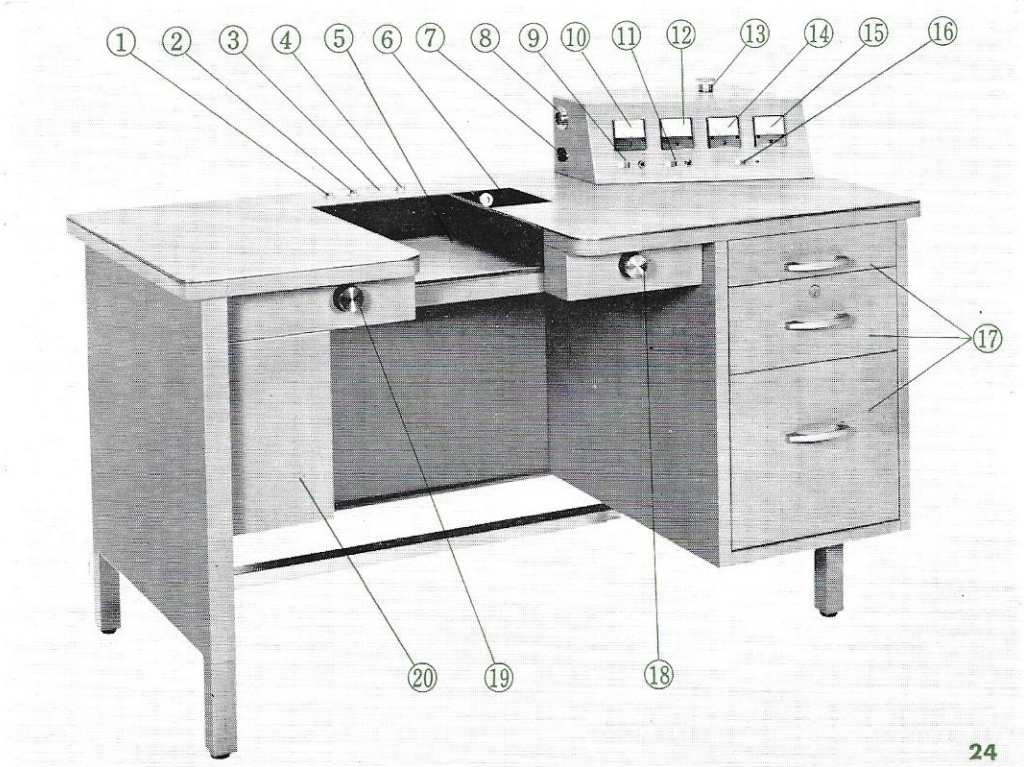
⑱ Illuminator power source voltage adjuster to be connected to ②, ⑪ and ⑫

⑲ Illuminator power source voltage adjuster to be connected to ①, ⑨ and ⑩

⑳ Cabinet, containing connector for input power source and fluorescence unit starter plug

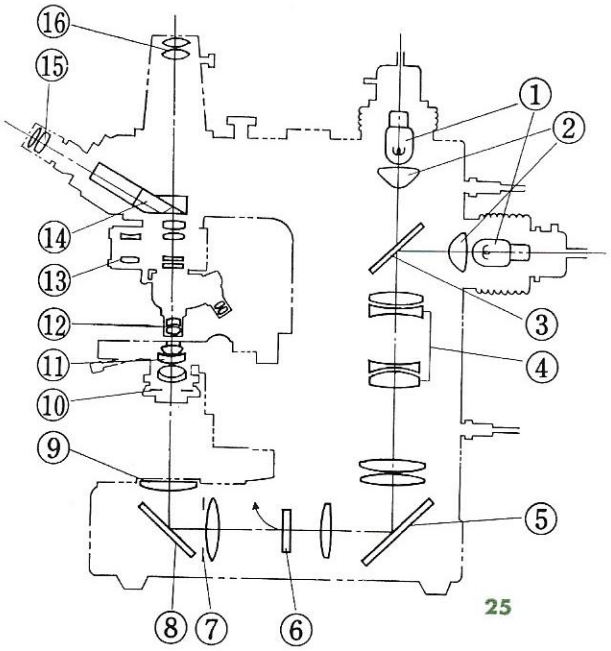
Others: ● Power source receptacle at the bottom on the rear right side of the desk

● Fuse, located at back of the meter box. Use 10A fuse.



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III. ILLUMINATION



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1. Transmitting (Diascopic) illumination

The elements used for transmitting illumination are as follows;
(Refer to the accompanying photo for the numbers).

① Light source
② Collector lens
③ Light source change-over mirror
④ Zoom lens system
⑤ Mirror
⑥ Matte filter
⑦ Illumination field diaphragm
⑧ Mirror
⑨ Field lens (condenser)
⑩ Aperture diaphragm
⑪ Condenser lens
⑫ Objective
⑬ Magnification change-over turret lens
⑭ Optical path change-over prism
⑮ Eyepiece
⑯ Photographic eyepiece

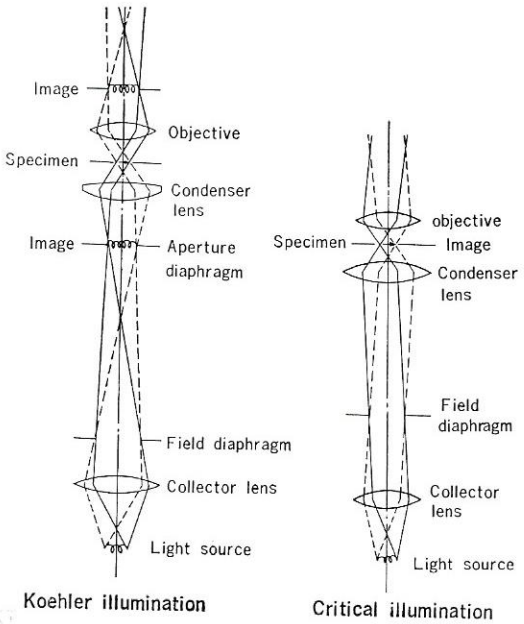
★Principle of Koehler Illumination

Two methods of illumination are available for the microscope: one termed "critical" and the other "Koehler".

The method is called "critical", in which the image of the light source is produced on the plane of the specimen to be observed. It offers bright, but uneven illumination.

On the other hand, in the Koehler method, by which the entire viewfield can be illuminated with uniform brightness, the light source is imaged through the collector lens on the aperture diaphragm in front of the condenser lens, and the illuminating rays, made parallel to each other, pass through the specimen, producing an image of the light source at the exit pupil of the objective. The image of the light source is then finally projected through the eyepiece onto the observer's eye.

Since, in Koehler illumination, a diaphragm placed behind the collector lens, is imaged through the condenser lens sharply on the plane of the specimen, the area of the illuminated field on the specimen



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can be controlled by adjusting the opening of the diaphragm to that of the viewfield of the microscope.

As a result, the Koehler method can fulfill the following requirements:

(1) It enables the specimen to be illuminated with an aperture angle large enough to cover that of the objective being used, thus ensuring 100% of the resolving power of the objective.

(2) It enables the entire field of view of the microscope to be illuminated with uniform brightness, because the light source is not focused on the specimen but on the exit pupil of the objective.

(3) It permits limiting the aperture angle of the illuminating cone so as not to let the light overflow the objective aperture to avoid reduction of contrast or flare in the microscope image.

(4) It permits adjusting the extent of the illuminated field so as not to let it exceed that of the microscope viewfield. This is also important for increasing contrast of the image.

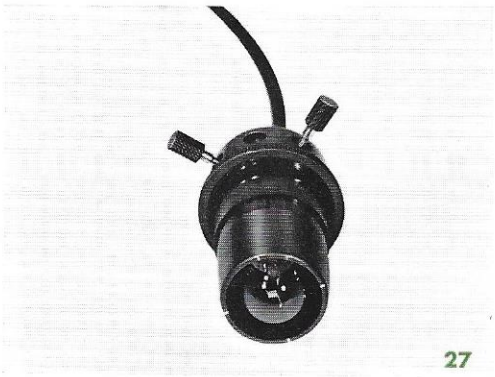
To satisfy requirement (1), the APOPHOT has a zoom lens system to change the size of the image of the light source on the condenser diaphragm continuously to correspond to the numerical aperture of the objective, which widely varies with the magnification of the objective being used.

The requirement (2) is evidently accompanied by the necessity for centering the illumination. For this purpose, the APOPHOT is equipped not only with a centering device for the illumination field diaphragm, but also with that for moving the position of the light source for exact centering of its image on the exit pupil of the objective.

(3), the best result is generally obtained when the aperture of the illuminating light is stopped down to 60—70% of that of the objective. The APOPHOT permits snch an adjustment of the opening of the condenser diaphragm.

Requirement (4) deals with the size of the illuminated field. If illuminating light covers an area larger than the viewfield on the specimen plane, the excess light may produce flare or other contrast reducing factors. The microscope is provided with an adjustable illumination field diaphragm to limit the illuminated field on the specimen, that is, to bring the area to coincidence with that of the viewfield.

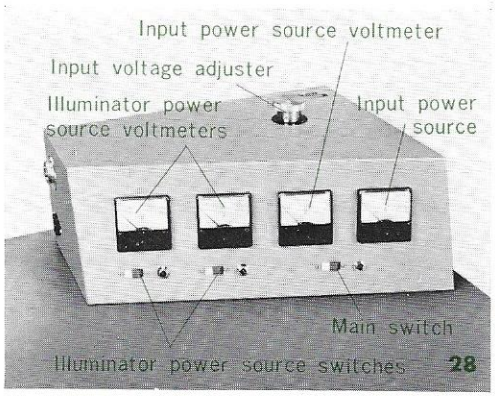
★Koehler Illumination



27

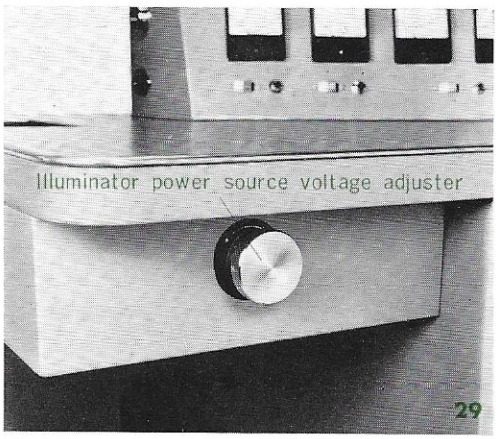
(1) Attaching the lamp bulb

- Remove the lamp socket from the microscope stand. Remove the light baffling tube and install the lamp bulb (6V, 30W).
  After the bulb has been fitted, replace the light baffling tube. Bring the center of the white cross into coincidence with the lamp filament. Then, replace the lamp socket in the microscope stand.
- Although the lamp housing and the socket should be about 2mm apart, it will be necessary to change this distance for most uniformly illuminating the viewfield. Fasten them in this position.



(2) Lighting the lamp

- When the APOPHOT Desk is used, connect the power source cord and turn on the main switch.

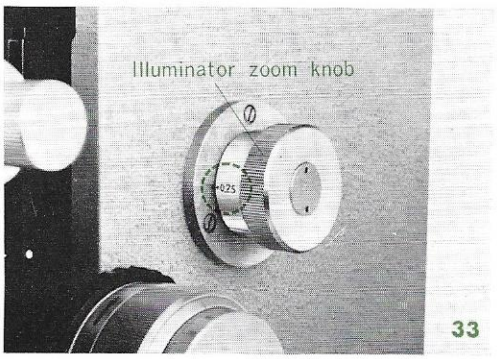


- Switch on the illuminator power source and regulate the voltage by turning the voltage adjuster.
- The switch to the right makes connection with the righthand voltmeter, the illuminator voltage adjuster and the socket.



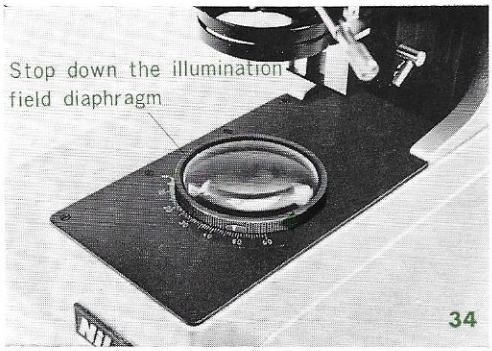
(3) Setting the optical path change-over mirror

- When using the light source on the top, pull out both the knobs M and H.
- When using the light source on the back, push in the knob M. (See Table p. 19)

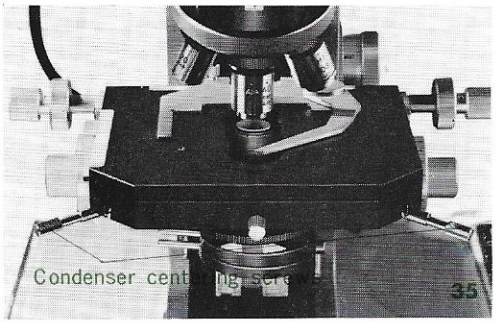


(4) Observation with Koehler illumination

- Set the numerical aperture on the illuminator zoom knob approximately to that of the objective being used.

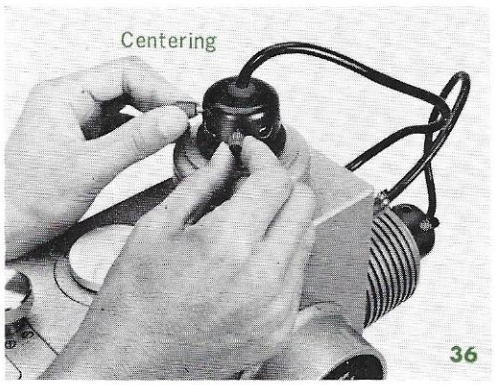


● Bring the specimen into focus.
Stop down the illumination field diaphragm, so that the image of the diaphragm is visible within the viewfield.

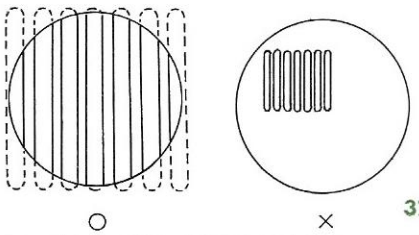


● Raise or lower the condenser until the image of the illumination field diaphragm appears sharp. By means of the two condenser centering screws bring the image of the field diaphragm to the center of the viewfield.

● Open the diaphragm up to the full size of the viewfield.



● Draw out the eyepiece. View into the eyepiece tube to see an image of the lamp filament on the exit pupil of the objective. Manipulate the centering screws on the lamp until the image of the filament comes to the center.



● Turn the illuminator zoom knob to adjust the size of the filament image, so that the filament image fully covers the exit pupil of the objective.

● Replace the eyepiece into the eyepiece tube.
Stop down the condenser diaphragm to an appropriate size (Generally corresponding to 60%—70% of the numerical aperture of the objective being used.)

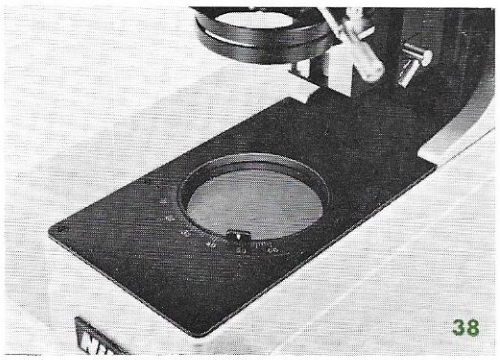
● The maximum opening of the condenser diaphragm for the objective being used is such that the exit pupil of the objective is not stopped down further.

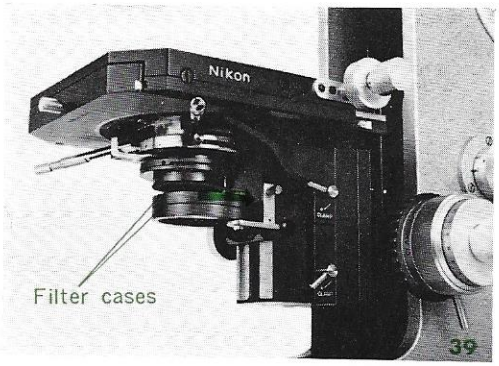
● When using a low power objective, such as 3× or lower, set the illuminator zooming knob to numerical aperture 0.1, and remove the field lens.
The standard condenser is to be replaced by the low power condenser.

● Lower the stage about 27mm from the position for higher magnifications.

● Set the lefthand knob at the base of the microscope stand to L.

● The other arrangements for low magnifications are the same as for high magnifications.

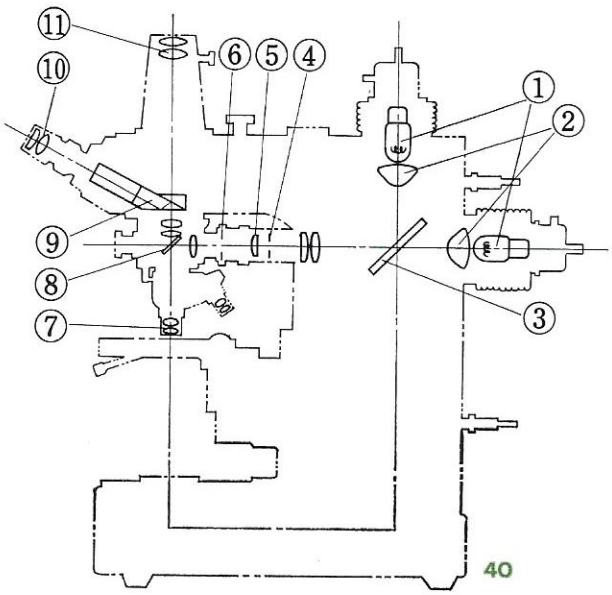




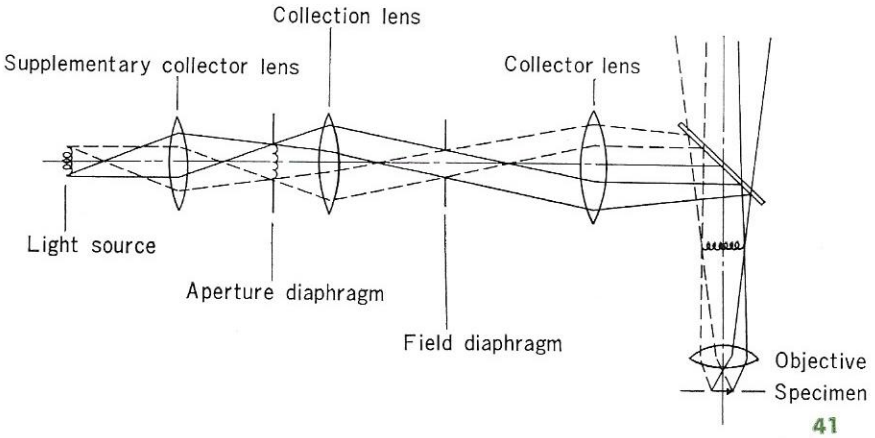
(5) Filters

- Two types of filters, one 45mm and the other 40mm in diameter are available.
- When the polarizing unit is attached, use the matte or lemon skin filter, because Koehler illumination is not applied in this case.

2. Reflecting (Episcopic) illumination



① Light source
② Collector lens
③ Light source change-over mirror
④ Aperture diaphragm
⑤ Correction lens
⑥ Illumination field diaphragm
⑦ Objective
⑧ Half-reflecting glass
⑨ Optical path change-over mirror
⑩ Eyepiece
⑪ Photographic eyepiece

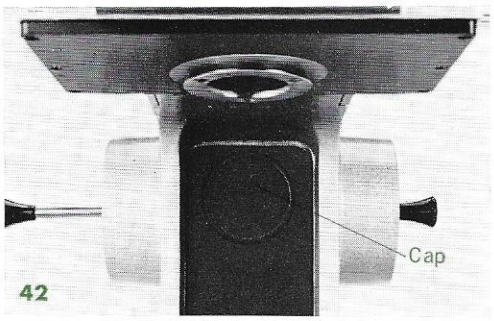


★ Koehler illumination is also applicable for reflecting (episcopic) illumination.

Optically, reflecting illumination differs from transmitting (diascopic) in that the role of the condenser lens is assumed by the objective. The light source image produced on the exit pupil of the objective is reflected from the specimen and imaged again on the exit pupil of the objective, and then through the eyepiece projected onto the pupil of the observer's eye.

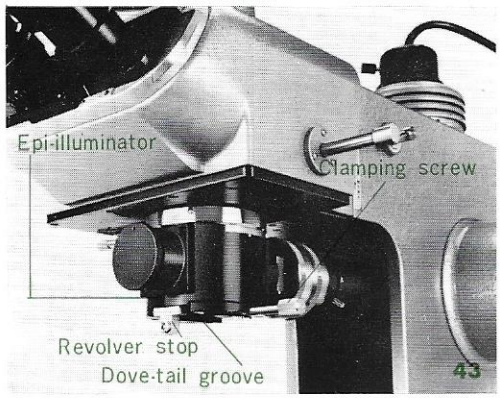
The size of the light source image on the exit pupil is adjusted by moving the correction lens in the illuminating system. For the principle of Koehler illumination, refer to the previous section (p. 13)

★ Practice

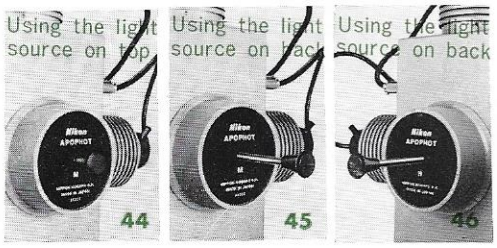


(1) Attaching the epi-illuminator

- Remove the cap from the opening for introducing episcopic illuminating light.

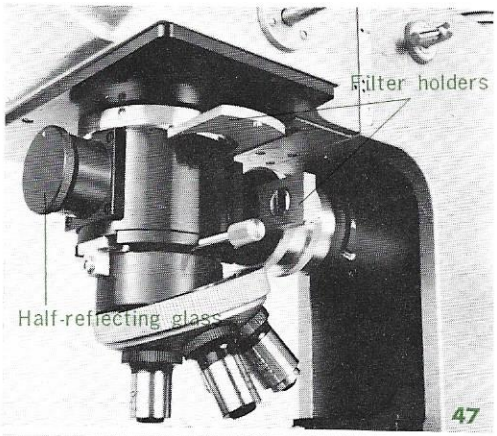


- Remove the magnification change-over turret and attach the epi-illuminator in its place.
- Place the stray-light prevention ring found at the rear end of the epi-illuminator onto the illuminating light opening.
- Fully slide in the revolver and clamp in position.
- Use the M-type objective for episcopic illumination.



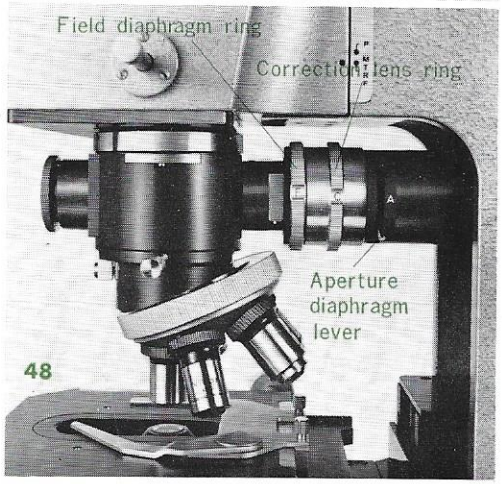
(2) Setting the optical path change-over mirror

- When using the light source on top, push in the knob M.
- When using the light source on back, pull out both knobs M and H. (See Table p. 19)



(3) Observation

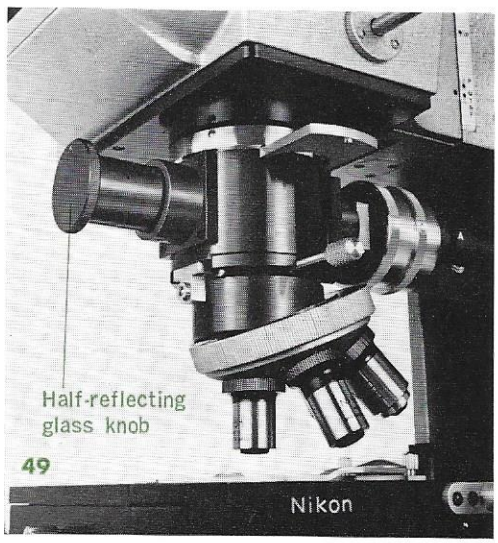
- Push in the half-reflecting glass.
- Bring the specimen into focus.
- Draw out one of the eyepieces from the eyepiece tube to see that the image of the lamp bulb filament is centered on the exit pupil of the objective. If not centered, manipulate the centering screws on the lamp.



- Stop down the illumination field diaphragm until its image appears in the viewfield.
- Move or turn the half-reflecting glass holder so that the image of the illumination field diaphragm is centered in the viewfield.
- Open the illumination field diaphragm up to the full extent of the viewfield.
- Turn the correction lens ring until uniform brightness is obtained over the viewfield.
  When an objective of high magnification is used, turn the ring toward H, and when an objective of low magnification is used, turn the ring toward L.
- Stop down the aperture diaphragm to the extent that the best illumination without flare is attained.

(4) Filters

- Filters of 16mm diameter are placed in the filter holders at the top and bottom.
- The polarizing filters for the metallographic illuminator on Model S Microscope can also be used.



(5) Changing over to transmitting illumination

- Merely by pulling out the half-reflecting glass, the illumination can be changed over from reflecting to transmitting.

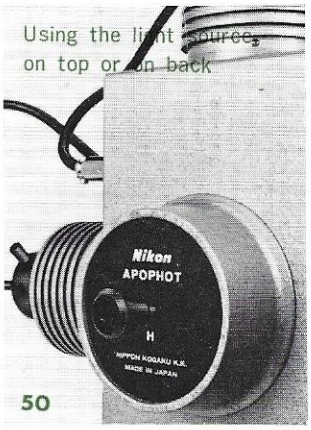
(6) Magnification factor

- The magnification factor for reflecting illumination being 1.5× and that for the trinocular eyepiece tube 1.25×, the intermediate magnification will be 1.5×1.25=1.875×.
  Therefore, the total magnification will be the combined magnification of the microscope (magnification of eyepiece×that of objective) multiplied by the above intermediate magnification. For example, when 8× eyepiece is used, the total magnification will be magnification of objective ×8 ×1.875=magnification of objective ×15.

3. Simultaneous illumination

The APOPHOT permits diascopic and episcopic illumination at the same time. This combined illumination is suited for observing semi-transparent specimens. It enable using two filters of different colors, each for either type of illumination.

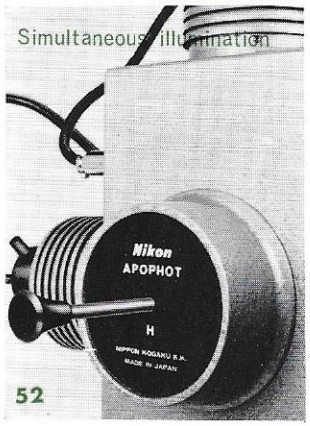
The optical arrangement for such simultaneous illumination is no more than a combination of both illuminations heretofore described. (The magnification change-over turret is not used.)



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(1) Setting for simultaneous illumination

- Arrange the dia. and epi. illumination systems.

(2) Setting the optical path change-over mirror (See Table below).

- When either of the top and back light sources is used, push in the knob H.
- When both light sources are used simultaneously, push in the knob M or pull out both knobs M and H. In this case each illuminator can be used with different brightness.

(3) Observation

- Observe the specimen under the two types of illumination as mentioned above.

(4) Objectives

- When observing a transparent specimen, use objectives of the biological type.

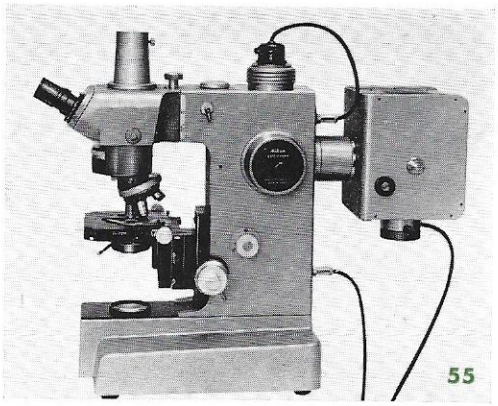
Setting of Optical Path Change-Over Mirror

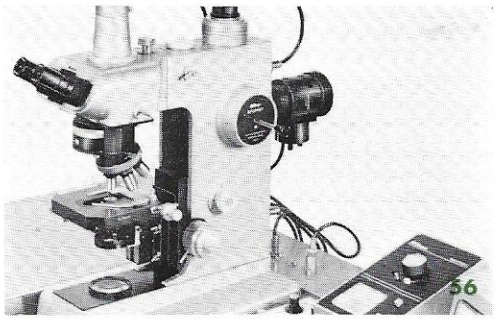
Illumination	Using Top Light		Using Rear Light		Simultaneous Use of Both Lights	
	Knob M	Knob H	Knob M	Knob H	Knob M	Knob H
Transmitting (Diascopic)	Pull	Pull	Push	Push	Not needed When two different lights are used, same as given at left.	
Reflecting (Episcopic)	Push	Push	Pull	Pull		
Simultaneous	×	Push	×	Push	Push Pull	× Pull

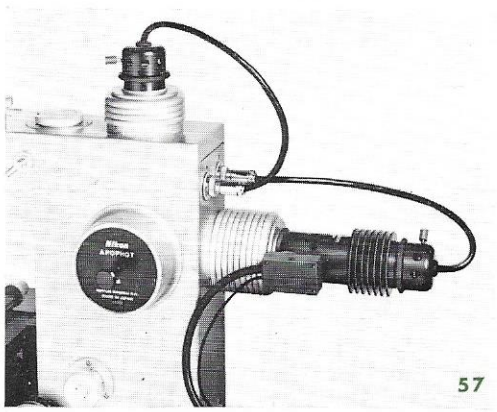
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4. Special light sources

Special light sources such as a high intensity lamp, quartz iodine lamp, electronic flash can be used in place of the regular tungsten lamp.


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(1) High intensity lamp

- Releasing three screws at the back of the microscope stand, remove the tungsten lamp housing, and attach in place the high intensity lamp.
- This light source is available as a xenon lamp, high pressure mercury lamp or metal halide lamp. Each lamp is used in combination with its socket and lighting device.
- For details, refer to the instructions for using the high instensity light sources.

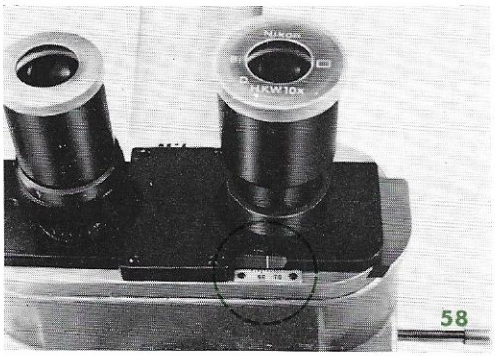
(2) Iodine lamp

- In place of the tungsten lamp housing, mount the iodine lamp housing with its bulb turned upward or sideways (not downward).
- As a transformer use the one exclusively designed for the iodine lamp. For details, refer to the instructions for using the iodine lamp. For details, refer to the instructions for using the iodine lamp.

(3) Electronic flash

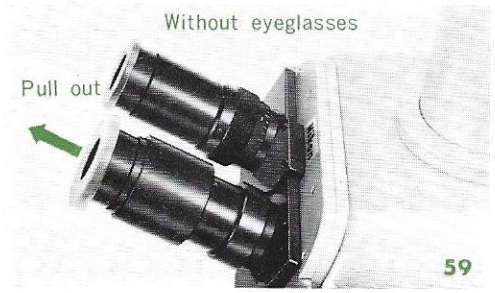
- Mount the electronic flash unit in place of the tungsten lamp housing. Attach the tungsten lamp socket onto the rear of the electronic flash unit.
- The synchronization cord is to be connected to the synchro-contact on the APOPHOT Desk.
- The flash unit emits light of a color temperature of 6000°K with an output of 200W.
- For details, refer to the instructions for using the electronic flash.

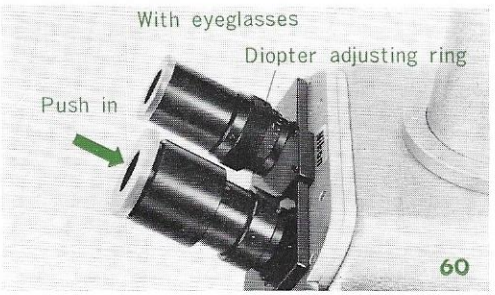
IV. FOCUSING

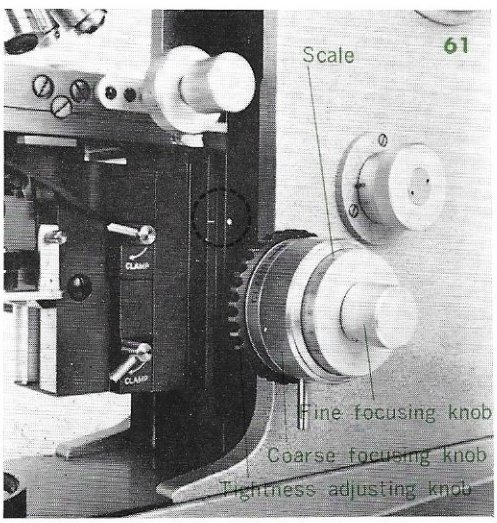


1. Eyepiece

- In binocular observation, adjust the interpupillary distance between the two eyepieces to that between the observer's eyes. With the distance scale set to the red dot (for 64mm distance), the mechanical tube length of the microscope becomes exactly 160mm.
  For adjusting the eyesight (diopter), see below (2. Coarse Focusing).
- The HK eyepieces are designed with high eyepoints for permitting observation using eyeglasses. For those who do not use eyeglasses, the use of the eye guards which can be drawn out is recommended.







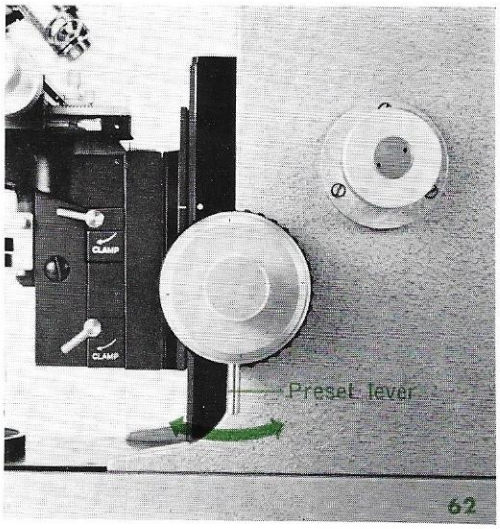
2. Coarse Focusing

- By means of the coarse focusing knob, first perform approximately.
- View and focus through the right side eyepiece. Then, turning the diopter adjusting ring on the lefthand eyepiece. bring the specimen into focus through both eyepieces.
- When using the DHKW 10× eyepieces, first bring the cross-hair in the eyepiece into sharp focus, and then, proceed with binocular focusing.
- The focused position using a slide of standard thickness is indicated by a white dot lined up to the white line on the slideway.

- All the objectives being parfocal, it is convenient for focusing to use lower power objectives with wider viewfields prior to higher power ones.
- Tightness of the coarse focusing knob can be adjusted by means of the tightness adjusting knob.
- The coarse focusing movement ranges from 16mm upward to 35mm downward from the standard position.

3. Fine Focusing

- One rotation of the fine focusing knob moves the stage 0.2mm, direct reading to 0.001mm being possible on the circumferential scale.
- The fine motion range is infinite owing to a specially designed single-axis differential gear.

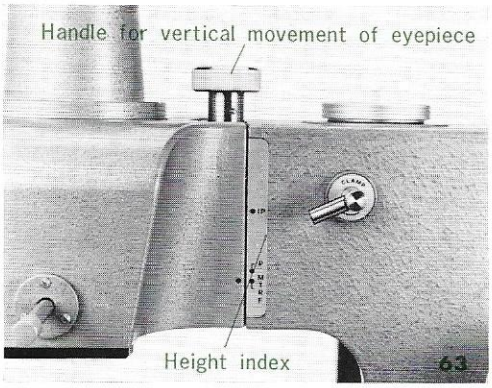


4. Preset Lever

- After the stage is brought to a focused position, turn the preset lever to " SET STOP ". Then, the stage cannot be raised further, even though the focusing knobs are actuated. Thus, the focused position will be easily obtained after the stage is lowered for exchanging the objective and revolver, or for applying immersion oil. Then, refocusing can be rapidly and simply accomplished by means of the fine focusing knob only.
- As long as parfocal objectives and a slide of standard thickness are used, the preset lever serves to avoid damaging of the specimen and troublesome refocusing.

5. Oil Immersion

- When the oil immersion objective 100× is used, special care should be taken in focusing, because of its extremely shallow depth of focus and short free working distance (0.10mm)
  The use of the preset lever will facilitate focusing in this case.
- For oil immersion, first, lower the stage by means of the coarse focusing knob. Change over the objective to that of 100×. Apply a drop of immersion oil onto the top of the condenser lens and the cover glass. Raise the stage up to the position previously set by the preset lever.
- The oil immersion objective may sometimes show unsatisfactory images. One of the causes can be attributed to air bubbles contained in the immersion oil. Remove the eyepiece and look into the microscope tube. Turn the revolver back and forth, and a slight amount of immersion oil, or use a needle or feather.
  Another cause may be attributed to the use of oil of incorrect refractive index (standard=1.515). Do not use cedar oil. The use of Nikon Cargille immersion oil is recommended.
- Remnants of oil on the objective surface will also cause bad seeing. After use, therefore, wipe off the immersion oil completely using a slight amount of xylol moistened cleaning cloth.

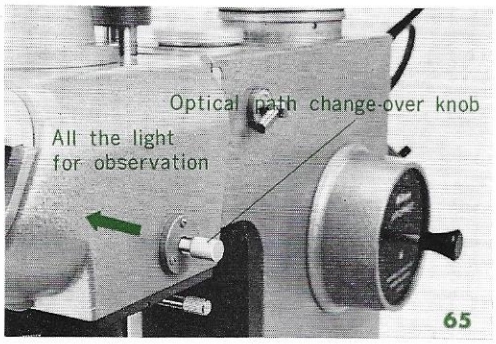


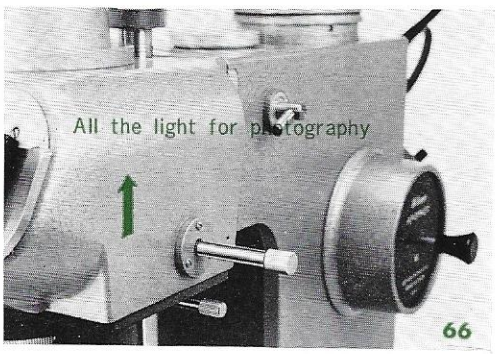
6. Trinocular Eyepiece Tube

- Since the adjustment of the interpupillary distance between the two eyepieces for binocular observation on the trinocular tube for APOPHOT is automatically coupled to the mechanical tube length for the photographic strait tube, there is no need for any correction when using the photographic tube.
- However, if other accessories for Model S Microscopes and for the APOPHOT are used on the bottom of the trinocular eyepiece tube, adjust the position of this eyepiece tube as below:

Setting position	Accessories
IP	Interference phase-contrast unit for Microscope Model S
P	Polarization unit for Microscope Model S
MT. RF.	Magnification change-over turret for APOPHOT Epi-illuminator for APOPHOT Metallographic attachment for Microscope Model S Differential interference unit T and R for Microscope Model S Fluorescence unit for Microscope Model S

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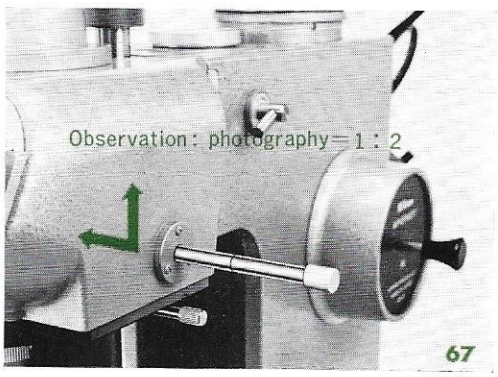


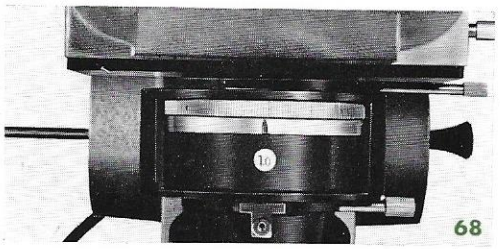
- Changing over the optical path

  By means of the optical path change-over knob the optical path can be changed in three ways: (1) for observation using all the light through the binocular tubes, (2) for photography using all the light through the straight tube and (3) for observation and photography using the amount of light divided in proportion 1 : 2 respectively.

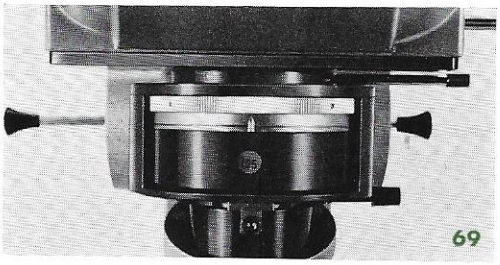
- Caution

  Take care not to touch the lens surfaces at the bottom of the trinocular eyepiece tube, when assembling and changing over various accessories on the trinocular eyepiece tube, to avoid leaving finger marks.

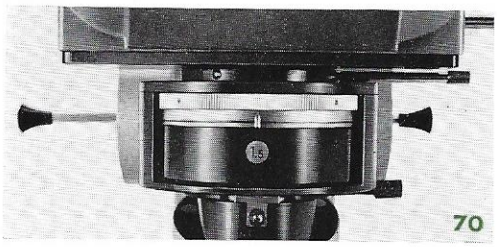




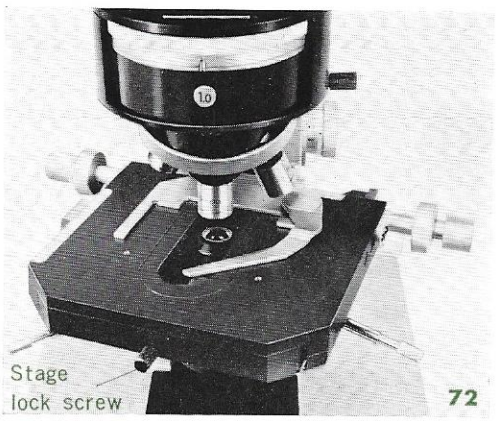
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7. Magnification Change-over Turret

- By manipulating the turret, the intermediate magnfication can be changed to 1×, 1.25× and 1.5× without changing the magnifying power of the objective and of the eyepiece.
- Furthermore, a centering telescope, provided in position Ph, permits centering of the phase-contrast ring in phase-contrast observation. It can also be used for examination of the exit pupil of the objective.



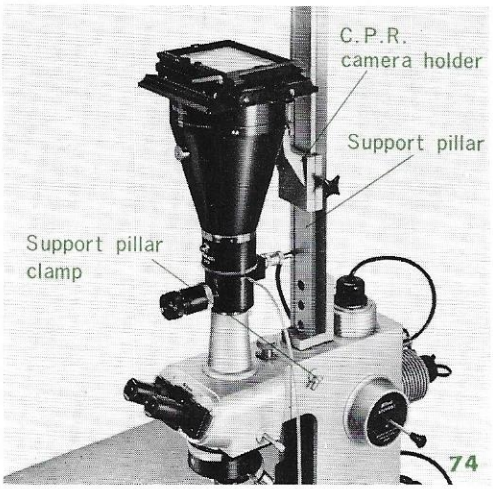
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8. Stage

- Three types of stages; biological rectangular mechanical stage, metallographic rectangular mechanical stage and circular rotating stage for polarization observation are available.
- The biological rectangular mechanical stage, being rotable, is convenient in trimming the picture field, etc. when taking photomicrographs.
- When the stage is attached in reverse position, it can be rotated 180°.

V. PHOTOMICROGRAPHY AND CINEMICROGRAPHY

1. Photomicrography



(1) Attaching the photomicrographic unit

- Refer to II-11 (p. 10).
- Although the microscope stand of the APOPHOT is sufficiently sturdy and vibration-free, it is recommended, when a large format camera such as 4″×5″ is mounted, to use a support pillar, which enables the equipment to be set absolutely free of vibration.
- In this case attach the C.P.R. camera holder to the tripod sockets on the C.P.R. cone adapter with the lens 1.3× at the bottom, and slide the C.P.R. holder into the deve-tail groove on the pillar and clamp it in position.

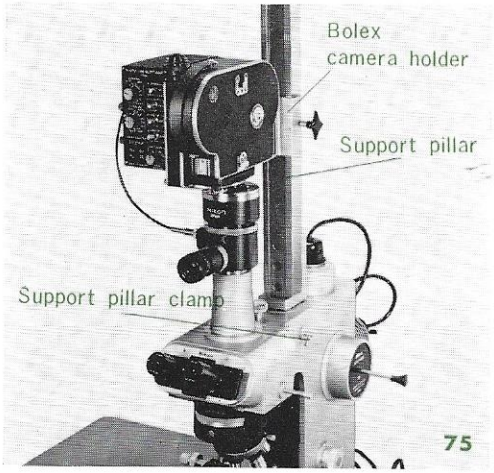
(2) Photography

- Refer to the instructions for using each photomicrographic attachment.

(3) Photographic attachments

- Three types of attachments are available: Microflex AFM, EFM and PFM.
  - AFM: With a built-in electronic shutter, which permits automatic setting of shutter speed depending upon the brightness of specimen. It requires only to depressing the button for exposure.
  - EFM: A simplified Microflex which, coupled to the exposure meter to the shutter, simplifies the photographic procedure.
  - PFM: A standard Microflex with built-in shutter which is coupled to the movement of the inside prism to introduce 100% of the light for photography or for observation.

2. Cinemicrography



(1) Attaching the cinemicrographic attachment

- The attachment can be mounted in the same way as the photomicrographic one.
- Screw the 1/4 relay lens into the 16mm cine camera. Slide the camera on the camera holder and clamp in position.
- Use the pillar described previously (Section 1) to enhance sturdiness and vibration-proofness.
- The cine camera holder available with the pillar is used for mounting the Bolex camera. Attach the holder to the tripod socket on the camera. Then slide into the groove on the pillar and clamp.

(2) Cinemicrography

- For operating the cinemicrographic attachment and for filming, refer to the instructions for each cine attachment.

(3) Types of cinemicrographic attachments

- Two types are available: "CFMA" with automatic exposure meter and "CFM", a standard type.
  - CFMA: In combination with the Bolex camera, permits continuous filming and intermittent single frame exposures at 1 sec.—30 min., the exposure being determined automatically and manually.
  - CFM : A standard type cinemicrographic attachment permitting viewing through the finder, while filming.

In addition, a standard slow speed attachment is available, which, in combination with the CFM and the Bolex camera, permits continuous filming and intermittent exposures at 2 sec.—20min. automatically and manually.

The Microflex AFM, EFM or PFM can be used as a finder.

VI SPECIAL OBSERVATIONS

The APOPHOT is a universal microscope designed to fulfill various special requirements such as phase-contrast, interference, polarizing microscopy by attaching the accessories below. Refer also to the instructions for respective accessories.

1. Phase-contrast Unit

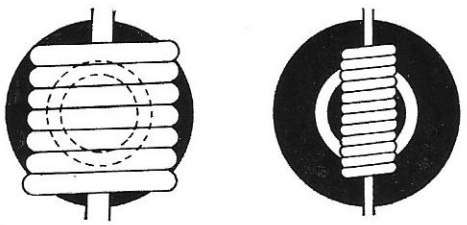
(1) Phase-contrast condenser turret

- The turret is slid onto the condenser holder, the same way as the condenser turret, previously mentioned (p. 7), and clamped in position.

(2) Objectives

- Attach the objectives, chosen from the DM, DLL and BM series, all for phase-contrast observation, on the revolver in such an order that the objective of the same magnification as the condenser ring diaphragm comes in play as the revolver and the turret are revolved in the same direction.

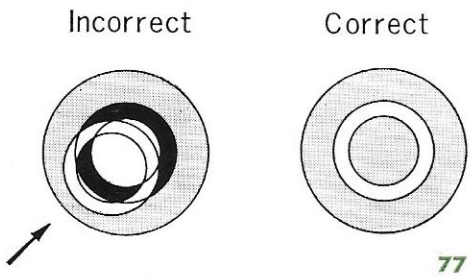
(3) Illumination



○ × 76

- Illumination brightness in phase-contrast observation is generally much darker than that in the brightfield, because some amount of light is not only blacked by the ring diaphragm located in the focal plane of the condenser but also absorbed by the phase-contrast plate in the objective.
- Use the Koehler method as the most efficient illumination and be specially careful to center the light source, so that the image of the light source filament fully covers the ring diaphragm of the condenser.

(4) Centering



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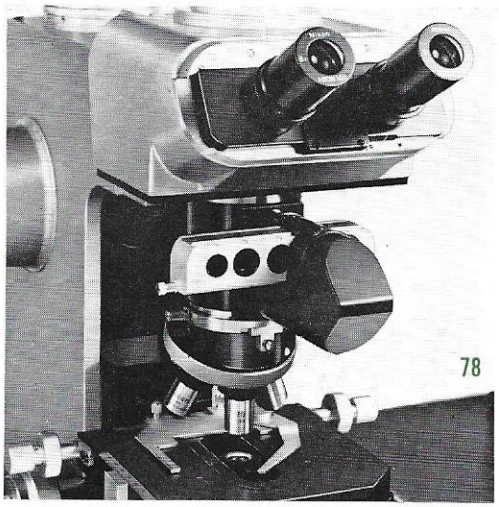
- Use the centering lens, turret position Ph, after bringing the image of the phase ring diaphragm in the condenser into sharp focus on the phase ring in the objective, without removing the eyepiece.
- If the image of the condenser ring diaphragm is displaced from the ring, move the former. This adjustment is most important for phase-contrast observation, because, if it is not correctly done, poor image contrast will result.
- When using the objective 100×, apply immersion oil between the specimen and the objective as well as between the specimen and the condenser, and then proceed to centering.
  When centering is done with a specimen in position, it may occur that the image of the ring diaphragm does not appear clear.

● If so, change the position of the specimen or remove the specimen and then center. However, note that such a specimen is basically not suitable for phase-contrast observation, or in some cases the cause may be attributed to water drops on the surface of the condenser or of the slide. Clear them away. Observation is performed the same way as with the ordinary microscopy.

(5) Filter

- Use a heat absorbing filter to avoid changing of the specimen, especially death of living ones by over heating. The heat absorbing filter is indispensable for phase-contrast observation where the observation of living organisms occurs most frequently.
- For microscopy or photomicrography with monochromatic film, a color filter (preferably green), is to be used.
- Filters of 33mm in diameter can be placed in the filter holder of the condenser and those of 45mm or 40mm in diameter in the filter holder of the microscope stand.

2. Interference Phase-contrast Unit



(1) Interference phase-contrast unit

- Remove the magnification change-over turret and raise the trinocular eyepiece tube by means of its height adjustment knob, so that the index dot is brought to IP.
- Mount the interference unit under the trinocular eyepiece tube using the attaching screw provided with spring. (Photo 18, p. 9).
- Then, attach the objective, revolver (Photo 18, p. 9) under the interference unit, and slide in the revolver on the bottom of the holder as far as it will go, and clamp it in position.
- Should the clamp screw on the revolver holder be fastened before the revolver has been fully slid in, the spring on the attaching part could be injured.

(2) Condenser turret

- The condenser holder with groove is attached to the condenser turret the same way as the standard condenser (see p. 7), and slid into the groove on the condenser receptacle of the microscope stand as far as it will go and clamped in position.

(3) Illumination

- The illumination for interference observation necessitates more brightness than that for phase-contrast. The Koehler illumination method is the best. If necessary, a high intensity light source such as an iodine lamp or xenon lamp is to be used.
- Centering of the light source should be carried out so that the image of the filament covers fully the ring diaphragm in the condenser (Photo 76, p. 27)

(4) Centering

- Remove one of the eyepieces from the eyepiece tube and in place insert the centering telescope.
- The ring plate to be used on the interference unit should be of the same magnification as the objective and the condenser.

- Viewing through the centering telescope, see if the condenser ring diaphragm image is correctly centered to the ring plate. If not, move the ring diaphragm (Photo 77, p. 27)
- This centering is most important for interference phase-contrast observation. If it is not correctly done, the image will not show good contrast.
- When using the 100× objective, apply immersion oil between the specimen and the objective as well as between the specimen and the condenser and thereafter conduct centering.
- If the image of the ring diaphragm is not clear, with the specimen in place, change the position of the specimen or remove it and then center again. However, basically such a specimen is not suitable for phase-contrast observation. Furthermore, water drops may sometimes cause such an unclearness. Clean them thoroughly.
- Thereupon the interference observation proceed the same way as with ordinary microscopy.

(5) Color contrast observation (with white light)

- For color contrast observation, use the polarizing unit and white light for illumination.
- Set the analyzer to 45° on the absorption scale, beforehand. Slowly move the polarizer from the position of about 90° on the phase scale, until the best color contrast is obtained. Thereafter, rotate the analyzer slightly so that the clearest color is visible.

(6) Changeable contrast observation (with monochromatic light)

- Place a green filter on the filter holder of the condenser turret or on that of the microscope stand.

  More effective illumination will result by using a sodium lamp or other high intensity light source.
- Bring the analyzer to the position 45° and the polarizer to 120°–130°, then the viewfield will be darkfield-illuminated.

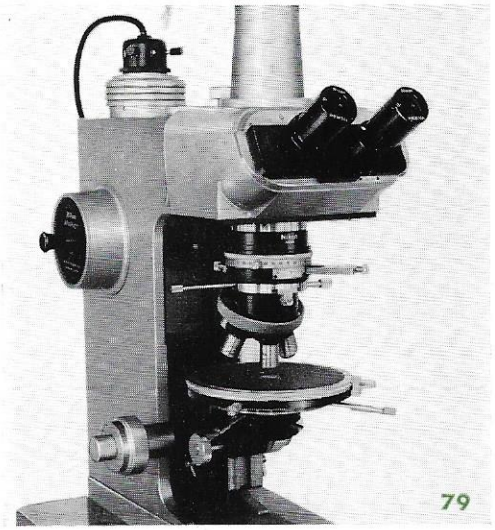
  Starting from this position, if the polarizer is rotated in the direction in which the reading number increases, a dark contrast image will appear.

  Conversely, if the polarizer is rotated in the reverse direction, a bright contrast image will be visible.
- The contrast will be further improved by rotating the analyzer slightly.
- In this way by rotating the polarizing plates, it is possible to change the contrast continuously from dark contrast through dark field observation to bright contrast until the best contrast is obtained, depending upon the property of the specimen. This is an outstanding feature of the APOPHOT.

(7) Cautions to be taken in photography

- Since in photographing living organisms and in color photography more intense illumination is required, the current for the lamp should be intensified and a rapid shutter speed used.
- In this case, however, it is necessary to use a heat absorption filter to avoid death or change of the living specimen.
- The rotation of the polarizing plate will have a great influence on the exposure. It is recommended, for determining correct exposure, to use the Microflex AFM, EFM with automatic built-in exposure meter, or an exposure meter or to make trial exposures.

3. Polarizing Unit



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(1) Rotating stage

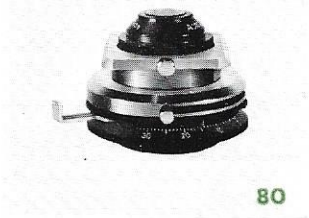
- Remove the rectangular, coaxial mechanical stage after releasing the clamp screws. Attach the rotating stage in place.

(2) Polarizing intermediate tube

- Remove the magnification change-over turret. Raise the trinocular eyepiece tube by means of the height adjusting knob until the index dot reaches the position P.
- Mount the polarizing intermediate tube under the trinocular eyepiece tube by means of the screw provided with spring (Photo 16, p. 8).
- Then, attach the objective revolver below the polarizing intermediate tube and slide in the revolver at the bottom on the holder and clamp it in the deepest position.
- When centering the objective is necessary, manipulate the centering screws on the revolver provided with centering device (available as accessory).

(3) Objectives and eyepieces

- The objectives specifically designed for the Nikon Polarizing Microscope are of strain-free type and identified by the figure "P" engraved on the outer barrel.
- The objectives are to be arranged on the revolver so that, as this is revolved clockwise, the magnification increases.
- The eyepiece chosen is to be inserted with its orientation pin fitted into the groove.



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(4) Condenser

- Attach the S-Po condenser to the condenser holder the same way as the ordinary condenser (p. 7) with its orientation line set to the horizontal position.
- The flap-top type condenser for the Polarizing Microscope Model II can also be used. In this case, the holder which is attached to the condenser is to be directly slid in the condenser receptacle as far as it will go and clamped in position.

(5) Filters

- It is not necessary to apply the Koehler method. Simply place the matte filter in the filter holder.

- Viewing through the centering telescope, see if the condenser ring diaphragm image is correctly centered to the ring plate. If not, move the ring diaphragm (Photo 77, p. 27)
- This centering is most important for interference phase-contrast observation. If it is not correctly done, the image will not show good contrast.
- When using the 100× objective, apply immersion oil between the specimen and the objective as well as between the specimen and the condenser and thereafter conduct centering.
- If the image of the ring diaphragm is not clear, with the specimen in place, change the position of the specimen or remove it and then center again. However, basically such a specimen is not suitable for phase-contrast observation. Furthermore, water drops may sometimes cause such an unclearness. Clean them thoroughly.
- Thereupon the interference observation proceed the same way as with ordinary microscopy.

(5) Color contrast observation (with white light)

- For color contrast observation, use the polarizing unit and white light for illumination.
- Set the analyzer to 45° on the absorption scale, beforehand. Slowly move the polarizer from the position of about 90° on the phase scale, until the best color contrast is obtained. Thereafter, rotate the analyzer slightly so that the clearest color is visible.

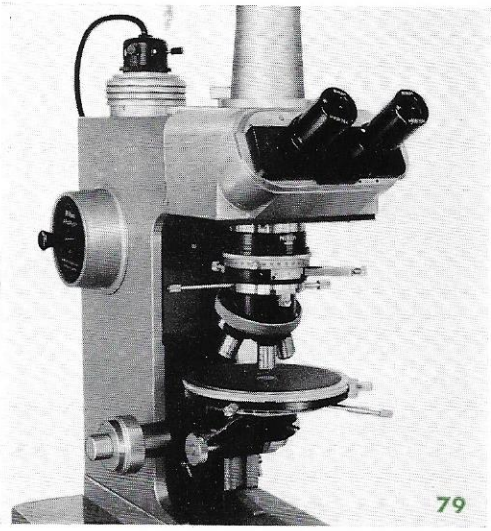
(6) Changeable contrast observation (with monochromatic light)

- Place a green filter on the filter holder of the condenser turret or on that of the microscope stand.
  More effective illumination will result by using a sodium lamp or other high intensity light source.
- Bring the analyzer to the position 45° and the polarizer to 120°–130°, then the viewfield will be darkfield-illuminated.
  Starting from this position, if the polarizer is rotated in the direction in which the reading number increases, a dark contrast image will appear.
  Conversely, if the polarizer is rotated in the reverse direction, a bright contrast image will be visible.
- The contrast will be further improved by rotating the analyzer slightly.
- In this way by rotating the polarizing plates, it is possible to change the contrast continuously from dark contrast through dark field observation to bright contrast until the best contrast is obtained, depending upon the property of the specimen. This is an outstanding feature of the APOPHOT.

(7) Cautions to be taken in photography

- Since in photographing living organisms and in color photography more intense illumination is required, the current for the lamp should be intensified and a rapid shutter speed used.
- In this case, however, it is necessary to use a heat absorption filter to avoid death or change of the living specimen.
- The rotation of the polarizing plate will have a great influence on the exposure. It is recommended, for determining correct exposure, to use the Microflex AFM, EFM with automatic built-in exposure meter, or an exposure meter or to make trial exposures.

3. Polarizing Unit


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(1) Rotating stage

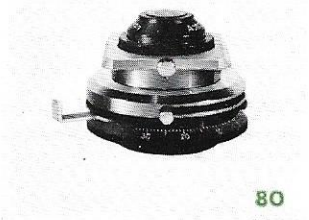
- Remove the rectangular, coaxial mechanical stage after releasing the clamp screws. Attach the rotating stage in place.

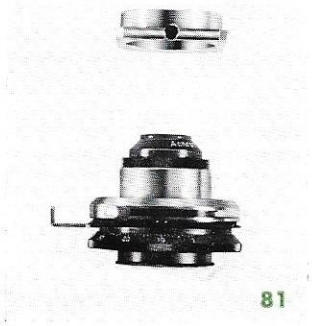
(2) Polarizing intermediate tube

- Remove the magnification change-over turret. Raise the trinocular eyepiece tube by means of the height adjusting knob until the index dot reaches the position P.
- Mount the polarizing intermediate tube under the trinocular eyepiece tube by means of the screw provided with spring (Photo 16, p. 8).
- Then, attach the objective revolver below the polarizing intermediate tube and slide in the revolver at the bottom on the holder and clamp it in the deepest position.
- When centering the objective is necessary, manipulate the centering screws on the revolver provided with centering device (available as accessory).

(3) Objectives and eyepieces

- The objectives specifically designed for the Nikon Polarizing Microscope are of strain-free type and identified by the figure "P" engraved on the outer barrel.
- The objectives are to be arranged on the revolver so that, as this is revolved clockwise, the magnification increases.
- The eyepiece chosen is to be inserted with its orientation pin fitted into the groove.


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(4) Condenser

- Attach the S-Po condenser to the condenser holder the same way as the ordinary condenser (p. 7) with its orientation line set to the horizontal position.
- The flap-top type condenser for the Polarizing Microscope Model II can also be used. In this case, the holder which is attached to the condenser is to be directly slid in the condenser receptacle as far as it will go and clamped in position.

(5) Filters

- It is not necessary to apply the Koehler method. Simply place the matte filter in the filter holder.

(6) Adjustment

The following adjustment is needed prior to polarizing observation.

● Crossed-Polars :

Line up the positions of the polarizer and of the analyzer to 0°.

Bring the orientation line on the trinocular eyepiece tube opposite to the index line on the polarizing intermediate tube. Viewing through the eyepiece without mounting any specimen, the viewfield will be dark. In this case, remove the compensator and Bertrand lens out of the optical path. If the viewfield is not sufficiently dark, rotate slightly the polarizing intermediate tube slightly to the right or to the left, until the darkest viewfield is obtained. Clamp it in this position. Now, a position of crossed-polars has been attained, in which the direction of the cross-hair in the eyepiece coincides with that of polarizer and analyzer.

● Centering :

The center of the cross-hair and that of the rotating stage must be coincident with each other. For this purpose, manipulate the centering screws on the rotating stage until a point on the specimen image comes to the cross-hair center in the eyepiece, and does not move when rotating the stage.

(7) Photography

● Set the photographic attachment (Microflex) in such a position that its finder is 45° to the plane of vibration of light at all times.

(8) Observation

A) Orthoscopic observation

Illumination by parallel rays at right angles to the specimen is to be used as a rule.

First, stop down the condenser diaphragm to the position B, where both the polarizer and the analyzer are set to crossed-polars, that is, to 0°. In this position, the viewfield will be darkest, if the specimen is not mounted.

However, when using the 40× objective, in which the elliptical component of polarization produced by the spherical surfaces of the lens is enlarged, the viewfield will be slightly brighter.

Since dust on the specimen also emits polarized light to some extent, it should be cleared away thoroughly to avoid misjudgment.

B) Conoscopic observation

For conoscopic observation it is necessary to illuminate the specimen with a conic light bundle as large as possible.

Raise the condenser to the highest position and enlarge the opening of the condenser diaphragm to a position farther than indicated by A).

After an approximate focus is obtained, bring the Bertrand lens into the optical path (using the 40× objective in this case), and a conoscopic image will appear.

For details, refer to specialized literature on polarizing microscopy.

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If a uniform interference color does not cover the whole area of the viewfield, slightly rotate the compensator housing.

- This rotation of the compensator is not necessary every time the magnification is changed. Only one rotation at the beginning. Take care not to rotate too much.
- Aperture diaphragm:
  Open the aperture to 60–70% of that of the objective by clockwise turning of the knob on the aperture diaphragm. The contrast will improve.
- To change the color contrast, rotate the knob on the compensator.
- With the background darker, so called darkfield contrast will be obtained, which gives an interference image similar to the image of bright phase-contrast.
- When the color of the dark ground is made slightly gray, the best contrast will appear, showing a relief, as it were, produced by shadowing in the electron microscope, thus giving a birds-eye view of the phase difference distribution over the whole specimen.
- When the background color is made reddish-violet (tint of passage), an interference color will appear depending upon the inclination of differences of refractive index and thickness in the specimen resulting in the highest color contrast.
- Rotate the compensator further until a background of sky blue is obtained. An interference image will appear which is similar to the image produced by the use of the DLL objective for phase-contrast microscope.
  The heretofore described methods are applied, when a good contrast background is desired from a general specimen of slight phase difference.
  In specimens of great phase difference, the background can be turned to other colors depending the desired contrast.

5. Fluorescence Unit

(1) High intensity light source

- Refer to the chapter (p. 20) on the high intensity light sources.
- Use a high pressure mercury lamp as the light source.

(2) Condenser turret

- For fluorescence observation, attach the condenser holder to the condenser turret the same way as the normal condensers (p. 7). Slide the holder in the condenser receptacle and clamp in the deepest position.

(3) Intermediate tube

- Remove the magnification change-over turret.
- Attach the intermediate tube under the trinocular eyepiece tube by means of the spring screw (Photo 16, p. 8)
- After attaching the objective revolver below the intermediate tube (Photo 18, p. 9), slide in the revolver at the bottom of the revolver holder. Clamp it in the deepest position.

(4) Filters

- Place the filter holder on the field lens.
  Clamp it in position by means of the clamp screw.

(5) Observation

- As a rule, for antigen-antibody observation by fluorescence, darkfield illumination is to be used. For the illumination, generally use both the UV excitation and BV excitation methods.
- To use darkfield condensers, attach the darkfield ring diaphragm onto the condenser turret.

  In case the specimen produces a dark fluorescence or one of low contrast, use the immersion darkfield condenser.

  Otherwise, use the ring diaphragm attached to the condenser turret for the darkfield illumination.
- Illumination :

  The UV excitation method for discriminating primary fluorescence from the reaction by exciting UV light of a wavelength around 3655 Å, and the BV excitation method for mainly observing the particular reaction by an exciting blue light of a wavelength around 4047 Å or 4348 Å, are performed as shown in the following table :

		UV Excitation	BV Excitation
Filter near the light source		Set to " UV "	Set to " BV "
Condenser		Use the darkfield condenser for weak fluorescence. Change over the darkfield ring diaphragm on the turret condenser depending upon the magnification.	
Filters on the eyepiece	at the top	Set to " 0 "	Set to " 1 "
	at the bottom	Set to the center	Set to the center

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VII SPECIAL ACCESSORIES

The following accessories, not included in the regular equipment of the APOPHOT, are available on order:

1. For Biological Observation (under transmitting—diascopic illumination)

◆Phase-contrast unit ..... Same as for the Microscope Model S. No centering telescope is necessary.

◆Interference phase contrast unit .. Same as for the Microscope Model S. No centering telescope is necessary.

◆Differential interference unit .... Same as for the Microscope Model S.

◆Polarization unit..... Same as for the Microscope Model S.

◆Fluorescence unit ..... Fluorescence intermediate tube for the Microscope Model S, in combination with the filter case and the fluorescence light source, both designed for the APOPHOT.

◆Low temperature unit ..... Attached to the APOPHOT using an adapter, for observation at temperatures to −120°C.

2. For Metallographic Observation (under reflecting—episcopic illumination)

◆Epi-illuminator..... Same as for the metallographic unit on the Microscope Model S.

◆Differential interference unit R.... Same as for the Microscope Model S.

◆Epi-dark-field device ..... Now in preparation.

3. For Photomicrography

◆Microflex AFM ..... Using an electronic shutter, permits photography only by pushing the shutter release button.

The following six types are available:

AFMF: for Nikon or NIKKOR camera
AFMB: for Leica mount camera
AFMD: for 35mm double camera box
AFMR: for roll-film camera
AFMC: for sheet-film camera
AFMP: for Polaroid camera

◆Microflex EFM..... Provided with built-in exposure meter, same number of types as the AFM and, in addition, the type EFM 16-C for 16mm cine cemera (Bolex) are available.

◆Microflex PFM..... A traditional type without built-in exposure meter. Same number of types as with the EFM are available.

◆Support pillar ..... Can support the 1.3× lens holder and Bolex cine camera.

4. For Cinemicrography

◆Cine-Microflex CFMA ..... Accepts Bolex cine camera, enabling slow speed filming with automatic exposure measurement.

◆Cine-Microflex CFM ..... Permits connection of the APOPHOT with a 16mm or 8mm cine camera.

5. Light Source

◆High intensity light source .....A universal type, permitting use of xenon lamp, high pressure mercury lamp or metal halide lamp.

◆Iodine lamp housing.....For quartz iodine bulb of 12V, 100W.

◆Electronic flash unit.....Permits change-over to five different brightnesses: 50, 70, 100, 140 and 200W

6. Stage

◆Circular rotating stage.....Used for polarizing, differential interference observation, etc.

◆Metallographic rectangular mechanical stage..With provision for cross traveling by means of coaxial knobs.

◆Circular rotating mechanical stage..A mechanical stage, permitting 360° rotation in addition to cross-traveling.

7. For Demonstration

◆Small type P screen.....Convenient for demonstration and lecture given to a small group.

Nikon

NIPPON KOGAKU K. K.

Fuji Bldg., 2–3, 3-chome Marunouchi, Chiyoda-ku, Tokyo 100, Japan

NIPPON KOGAKU (U. S. A.) INC.

623 Stewart Avenue, Garden City, New York, 11530, U. S. A.

NIKON EUROPE N. V.

Freeport Bldg., Schiphol-Centrum, The Netherlands

NIKON VERTRIEBS G. m. b. H.

4000 Düsseldorf Uerdingerstrasse 96–102, West Germany

NIKON AG.

Kirchenweg 5, 8008 Zürich, Switzerland

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