ZEISS

West Germany

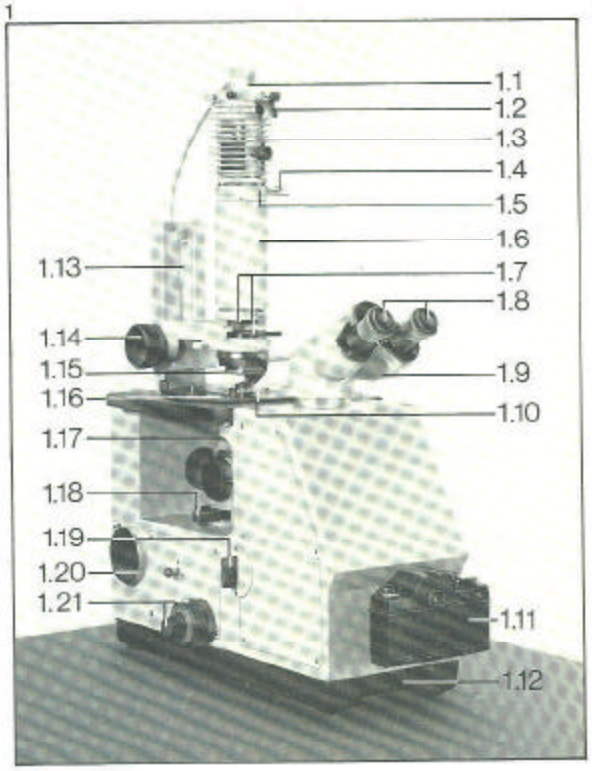
IM 35 and ICM 405* inverted microscopes for transmitted light and reflected light fluorescence

Operating Instructions

Contents

* Photomicrography with ICM 405 see G 41-126

1



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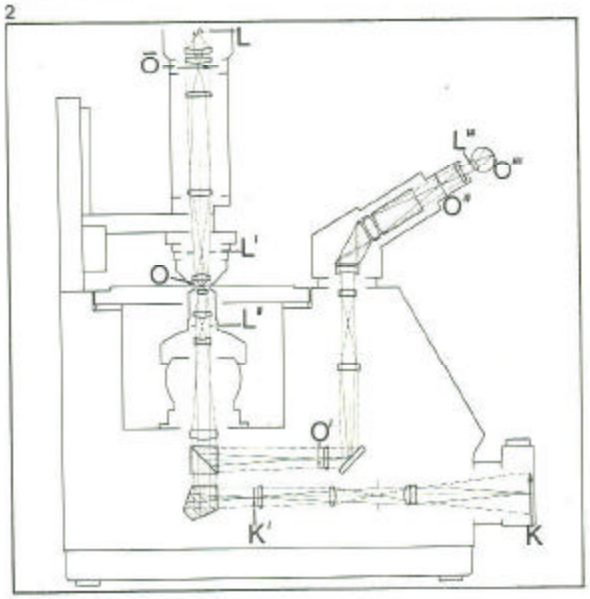


Note: Do not actuate rotary knobs (**1**.21 = in Figure **1**, Part 21) unless item **1**.9 Page 17 of these operating instruction has been taken care of.

In order to facilitate mounting of the revolving nosepiece and specimen stage and provide better access to the specimen the transilluminator (**1**.13) can be tilted back.

Figure 1
Inverted microscope IM 35 for brightfield and 35 mm photomicrography

1.1 Lamp socket 60/1 *(468015)** with 12 V 60 W filament lamp *(380018-2520)*
1.2 Clamping screw for lamp socket
1.3 Lamp housing 60 with lamp condenser *(467257)* (see G 41-305)
1.4 Clamping screw for illuminator 60
1.5 Intermediate piece with iris diaphragm *(467227)*
1.6 Spacer tube with auxiliary lens *(475638)*
1.7 Receptacle for filter in holder
1.8 Kpl wide-angle eyepiece with focusing eyelens
1.9 30° inclined binocular body IM *(473016)* producing upright, unreversed image
1.10 Transmitted-light specimen, cover glass facing down
1.11 Reflex camera housting CONTAX *(416165)* with fully automatic exposure control on special adapter T 2 *(476089)*
1.12 Front recess for carrying the instrument
1.13 Carrier for transilluminator *(471750)*
1.14 Control knob for illuminating system
1.15 Condenser
1.16 Gliding stage *(471720)*
1.17 Revolving nosepiece with transmitted-light objectives (not shown)
1.18 Empty slide or analyzer *(473668)*
1.19 Insert with photo reticle 35 *(471781)*
1.20 Push rod for beam splitter
pushed in: 100% light to observer
pulled out: 20% light to observer
and 80% to camera
1.21 Rotary knobs for coarse and fine focusing with revolving nosepiece

Figure 2:
Image-forming ray path (O):

Ō luminous field diaphragm
O specimen
O' intermediate image plane for reticles
O" field diaphragm, eyepiece diaphragm
O"' image on retina

Camera ray path (K):
K intermediate image plane
K' intermediate image plane

Illuminating ray path (L):
L light source
L' condenser (aperture) diaphragm
L" objective exit pupil
L"' exit pupil of the microscope

1.1
Place carton upright and open it.

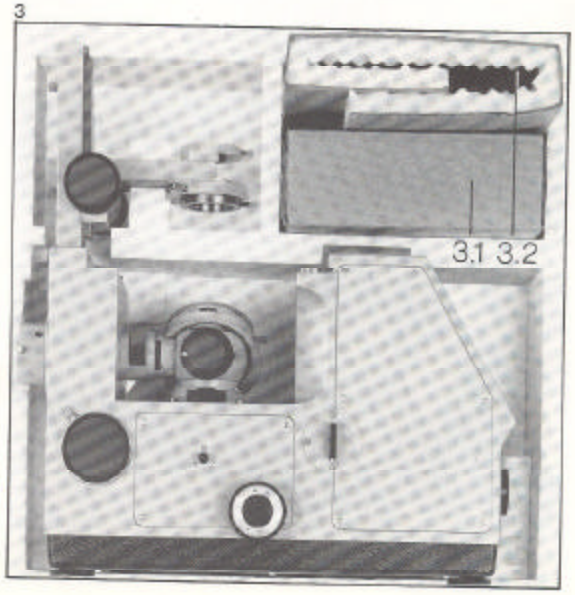
1.2
Take out Styropor container, lay it flat, cut adhesive tape and remove top.

Fig. 3 shows the IM 35 in the opened Styropor container. Specimen stage (**3.**1) and binocular body (**3.**2) are in a separate compartment and packed individually.

1.3
Take microscope by the two recesses (**1.**12) front and back and set it on the worktable; carefully unpack accessory parts and lay them out for mounting.

* The 6 or 10 figure numbers in brackets denote the order number. Every component has an order number.

3



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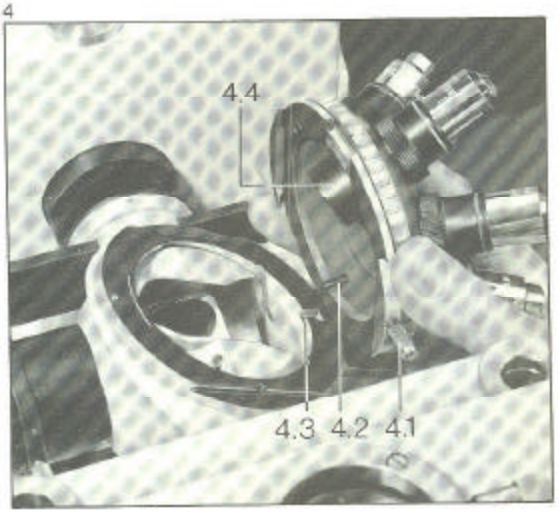

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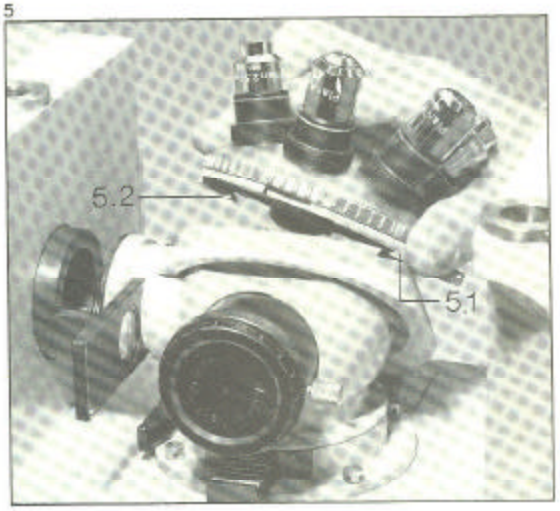

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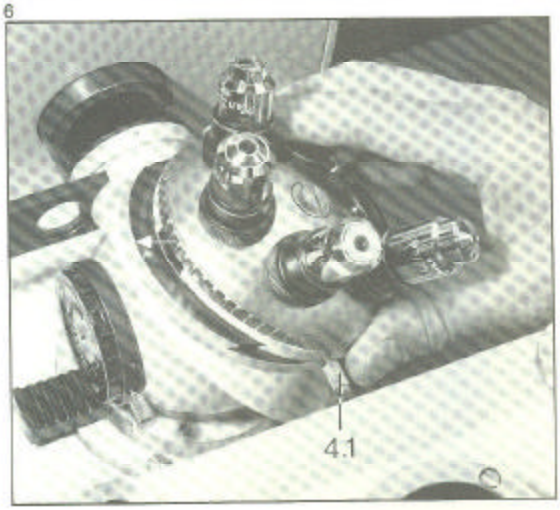


1.4
If the **revolving nosepiece*** is attached, remove dummy plugs amd screw in objectives. If you mount the revolving nosepiece yourself or exchange it later, proceed as follows:

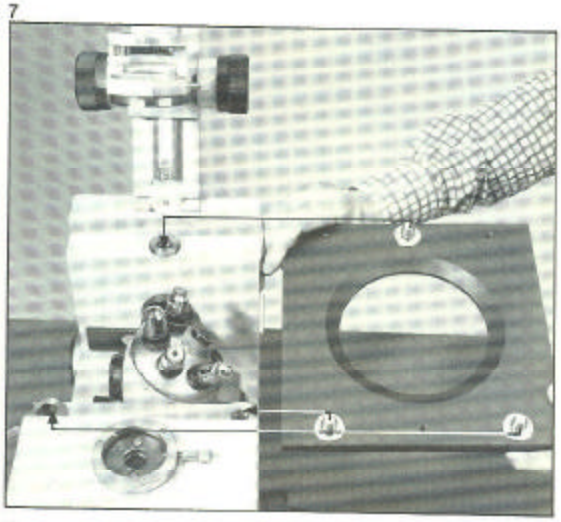
a) Hold revolving nosepiece so that the clamping screw (**4.**1) points toward the front.

b) Attach the revolving nosepiece, front dovetail surface first (**5.**1), and then lower the back (**5.**2). Pin (**4.**2) and matching groove (**4.**3) prevent it from sliding down.

c) Turn the revolving nosepiece to the left **as far as stop** – as indicated in figure by arrow – and fasten in this position with clamping screw (**4.**1).
In removing nosepiece loosen this clamping screw and also pull out spring locking pin. Otherwise proceed in reverse order.

Warning! A safety precaution is necessary when attaching or removing the revolving nosepiece, engraved 0.8x *(47 17 11)*: in order to avoid damaging the optics (**4.**4) please raise the nosepiece carrier as far as possible with the coarse adjustment.

7



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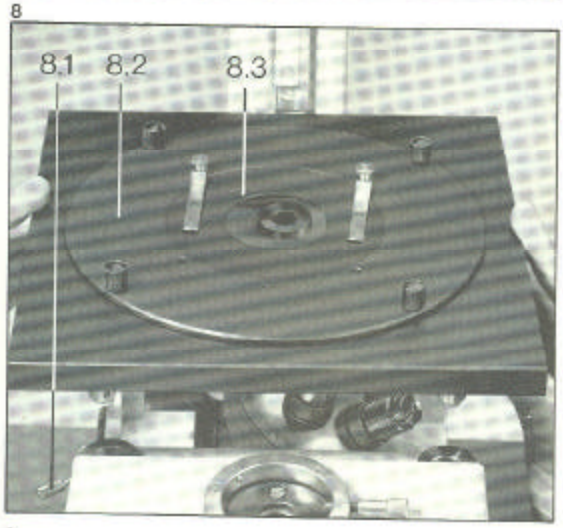

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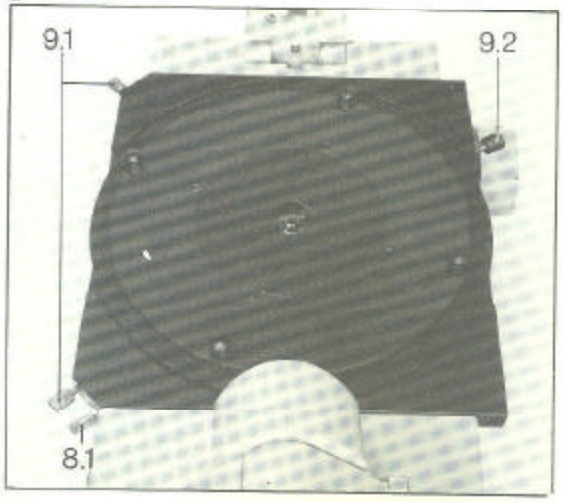

1.5
Mounting **specimen stage:** Insert the 3 studs on the stage into the matching openings of the microscope stand (**Fig. 7**). After inserting the specimen stage (perpendicularly from above), tighten clamping screw (**8.**1) (self-alignment). The stage insert (**8.**2) is placed into the opening of the base plate together with one of the two reducing plates (**8.**3) (inner diameter depends on size of cover glass).

Fig. 9: Gliding stage Z *(471722)*, rotatable and centerable, including 2 reducing plates, 24 and 48 mm dia.
Centering screws (**9.**1)
Clamping screw (**9.**2) for stage rotation.

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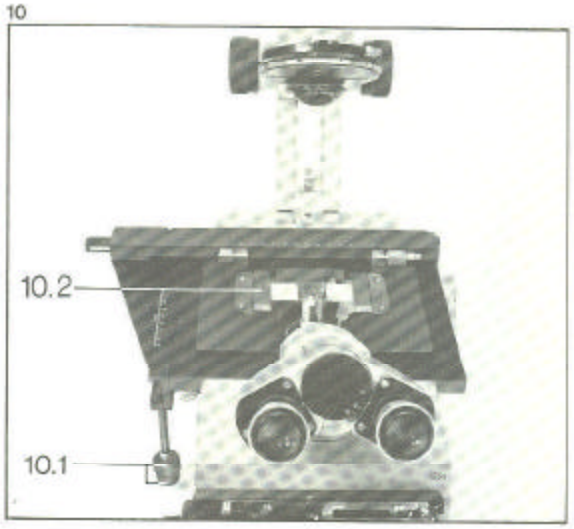

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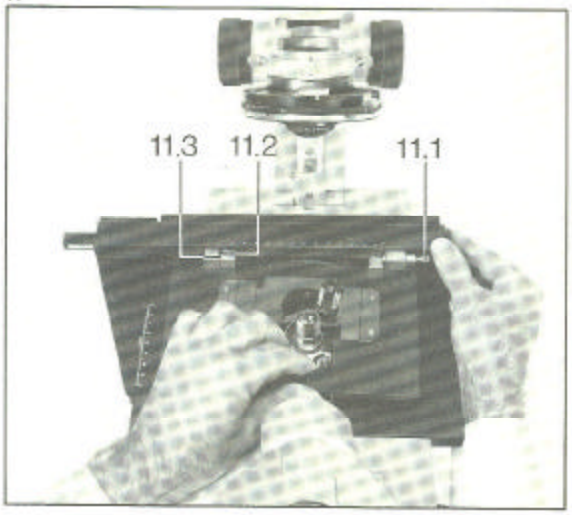

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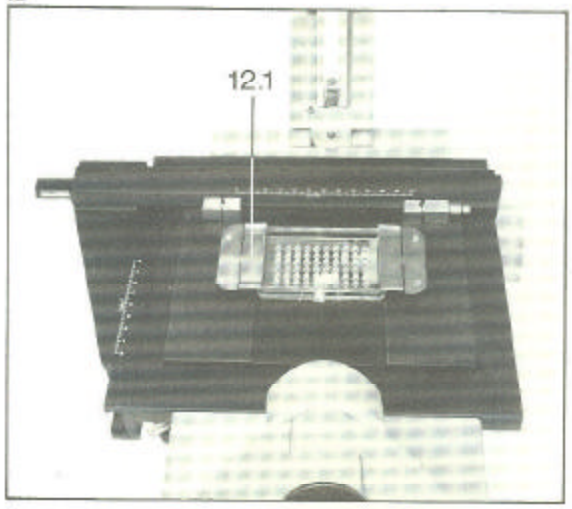

Fig. 10
Rectangular mechanical stage 71 x 110 mm *(471724)* with low-mounted controls (**10.**1).
Specimen holder *(471730)* **for specimen slides 76 mm** (**10.**2). In its place the holder *(471734)* for plankton chambers can be used.

Fig. 11
Mounting a specimen holder:
Unscrew hex socket screw (**11.**1) about 5 mm with special key supplied. Place left V-groove (**11.**2) of specimen holder on ball support of stage (**11.**3) and then swing in right side whereby the spring ball engages the appropriate groove.
Tighten screw (**11.**1).

Additional specimen holders for mechanical stage *(471724)*: Specimen holder (**12.**1) for **micro test plates,** 56 x 82 mm, 8.5 high *(471731)*, i.e., low Terasaki test plates; specimen holder 11 mm high *(471732)* for higher Terasaki test plates.
Specimen holder *(471733)* (**13.**1) **for microtiter plates** (82 x 127 mm) (**Fig. 13**).
Specimen holder *(471738)* (**14.**1) **for Petri dishes** (**Fig. 14**).

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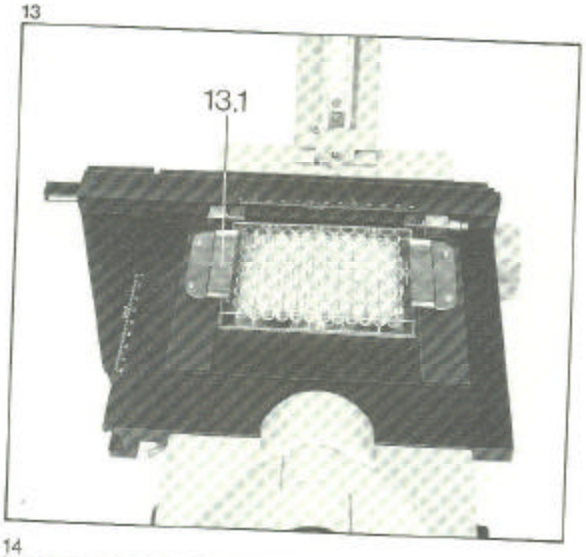

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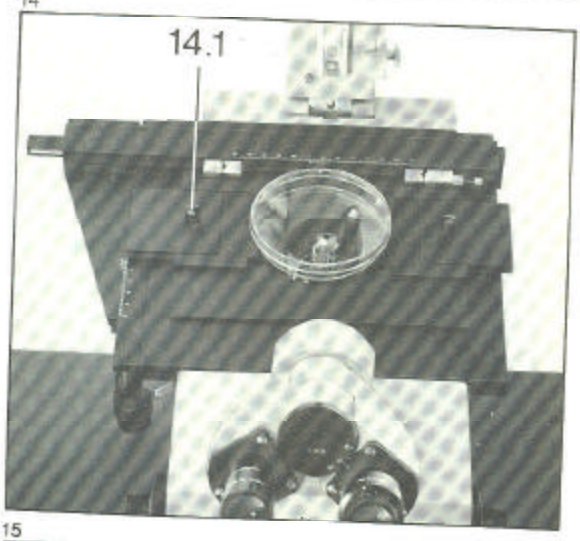

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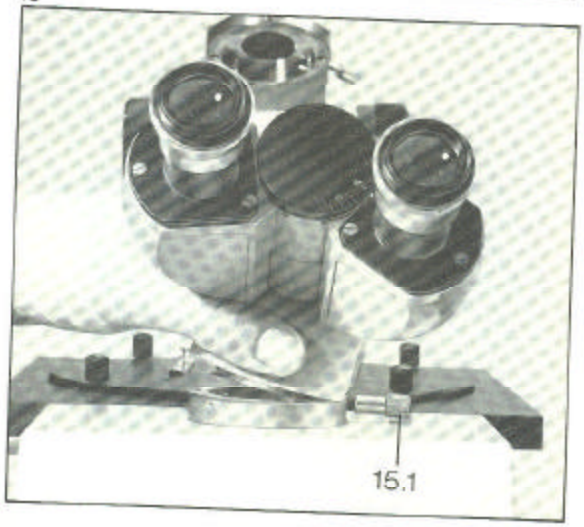
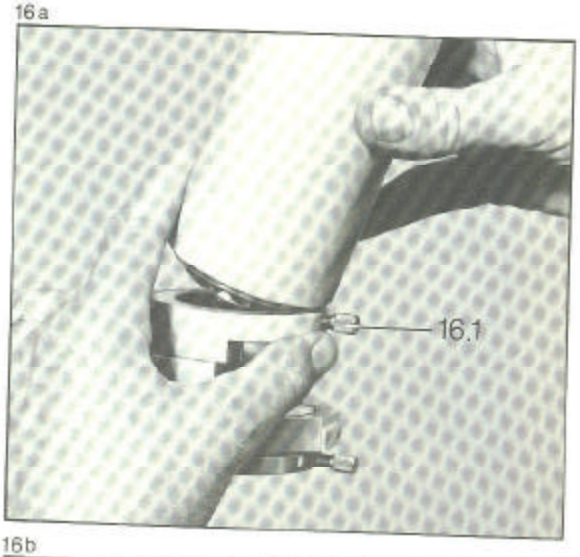


1.6
Attaching **binocular tube:** Remove protecting cover and loosen clamping screw (**15.**1). Attach by slotting in the dovetail ring, first pressing back spring bolt of clamping screw. Tighten clamping screw while still holding tube. – Attach eyepieces.

16a



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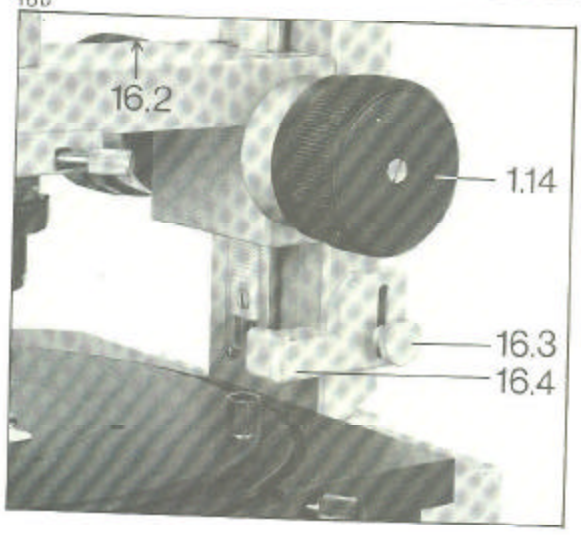

1.7

Illumination: Figure 1 shows the arrangement of the parts: spacer tube with auxiliary lens (**Fig. 16**), intermediate piece with iris diphragm, illuminator. In all cases attach to the already mounted parts as described above for binocular tube. Tighten with clamping screw (**16.**1).

After inserting filament lamp into socket wipe off possible fingerprints on bulb to prevent burning in. – Before inserting lamp socket into illuminator 60 switch in diffusion disk (black plastic knob with slit) (**20.**2), push in socket up to stop and slightly raise it, then clamp. The diffusion disk can now be moved freely.

The fine focussing drive (**1.**14) for the illumination can be stiffened by turning the Allen key in a clockwise direction in the ring (**16.**2) (not shown in the figure). An Allen key is supplied with the instrument.

An adjustable stop is available as an accessory *(431701)* (**16.**4) and is tightened on the carrier for the trans-illumination by the screw (**16.**3). It limits the lowest position on the condensor or the carrier, so that the specimen or e.g. a culture chamber cannot be damaged.

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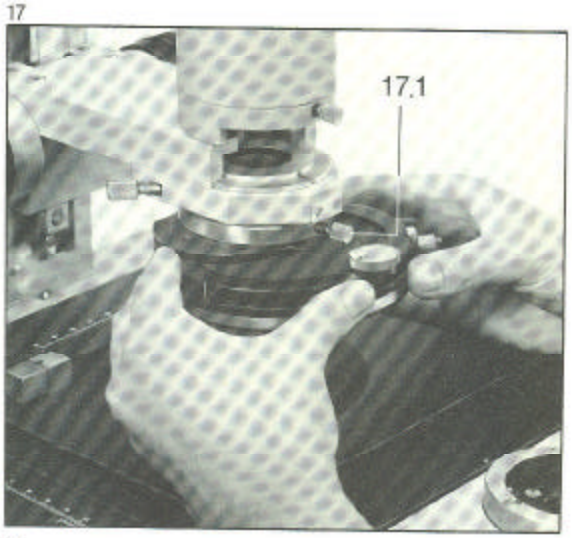

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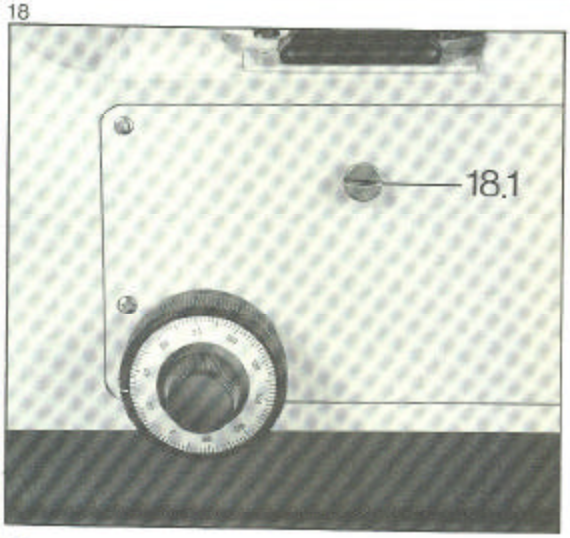

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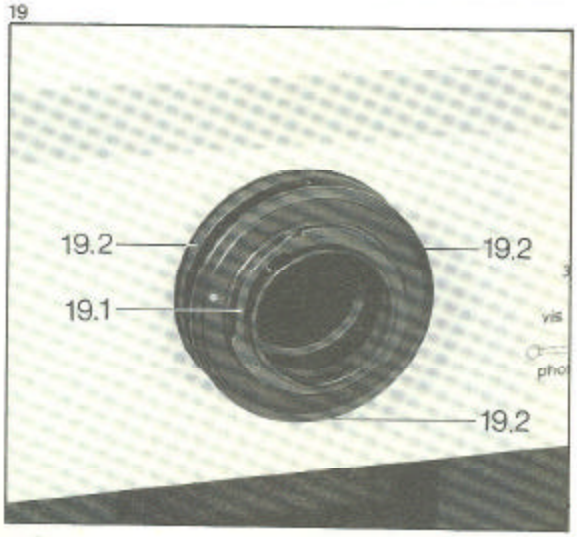

1.8
Bring the conical surface of the **transmitted-light condenser** dovetail up to the illuminator unit which has been tilted. Insert the dovetail of the condensor with groove in the spring bolts. After pressing in the spring bolt the condenser can be pressed up from below and fixed with screw (**17.**1).

1.9
Removing **transport locking:** Remove screw marked red (**18.**1) on right side of instrument and replace by gray plastic cap provided. Prior to transport first turn the coarse drive into the position where the hole in the movable nosepiece carrier coincides with bore (**18.**1) and reinsert locking screw.

1.10
Attaching **CONTAX housing:**
Screw special adapter T 2 *(476089)* (**19.**1) to camera receptacle (M 42 x 0.75 thread) of the microscope unless it was mounted at the plant. Attach camera so that the red point coincides with the red point of the adapter, turn camera to the right until engaged. By loosening the 3 screws (**19.**2) and turning the camera receptacle the camera (**1.**11) can be aligned. Retighten screws. Adapters for other camera makes are available.

Introduce insert with **photo reticle 35** *(471781)* (**1.**19). The reticle is imaged in the binocular tube and defines the 35 mm camera format in the image. The photo reticle (**1.**19) (for example with format outlines) is calculated in the light path. If it is removed for any reason the parfocalisation is slightly affected. This means that when a lens is changed the sharpness must be readjusted. There are no further disadvantages.

2.1
General preparations

- Place specimen on specimen stage:
normal specimens with cover glass facing down.
Specimens in dishes are imaged through the dish bottom.
- Switch in 10x or 16x objective.
- Insert pair of eyepieces with focusing eyelenses into binocular tube.
- Connect illuminator 60 (**1.3**) via transformer to mains
- Uniformly illuminate objective exit pupil:
Insert centering telescope *(464822)* in place of an eyepiece into binocular tube; the objective pupil is thereby magnified, not visible in normal size in the empty tube. Fine-focus pupil image by vertically adjusting eyelens.

- Open iris diaphragm (**1.5**) and condenser aperture.
- Center lamp filament in pupil center:
With knob (**20.2**) remove diffusion disk from beam path, loosen lamp socket (**1.1**) with clamping screw (**1.2**), shift it vertically until lamp filament uniformly fills pupil image. Reclamp lamp socket and, if necessary, center filament with screws (**20.1**).
Swing diffusion disk back in.
- Insert eyepiece in place of centering telescope.
- Adjust binocular tube by bending to interpupillary distance of observer.
- Focus each eyepiece individually on photo reticle by turning the eyelens.
- Focus specimen with coarse and fine controls.
- When working with low-power objectives (6.3x and below) a large field is imaged; to illuminate the entire field swing out or screw off condenser front lens and open condenser aperture; iris diaphragm (**1.5**) then acts as contrast (aperture) diaphragm.

2.2
Magnification

Microscope magnification = $M_{objective}$ x nosepiece factor x $M_{eyepiece}$
Example: 400 = 40 x 0,8 x 12.5
Magnification on 35 mm film = $M_{objective}$ x nosepiece factor x 3.2
Example: 100 = 40 x 0.8 x 3.2

2.3
Illumination with large working distance

For the examination of objects in chambers or micro test plates free space is required between specimen plane and illumination equipment. This is obtained by omitting the condenser. The light bundle emitted by the microscope illuminator in most cases has a smaller aperture than the objective. Therefore the illumination equipment should be attached as close as possible above the object. Stopping down for contrast enhancement is done with the iris diaphragm.

2.31
Brightfield

Brightfield illumination for large working distance (**Fig. 20**)

1.1 Lamp socket 60/I *(468015)* with 12 V 60 W filament lamp *(380018-2520)*.
1.2 Clamping screw for lamp socket
1.3 Lamp housing 60 *(467257)*
1.4 Clamping screw for illuminator 60
1.5 Iris diaphragm in intermediate piece *(467227)*. For more straightforward usage this is turned towards the front. Thus the iris diaphragm functions as an aperture diaphragm.
20.1 Centering screws for filament lamp
20.2 Control knob for diffusion disk
20.3 Support ring *(467252)* for light filter
20.4 Container with examination material

Adjust brightfield illumination according to Section 2.1.

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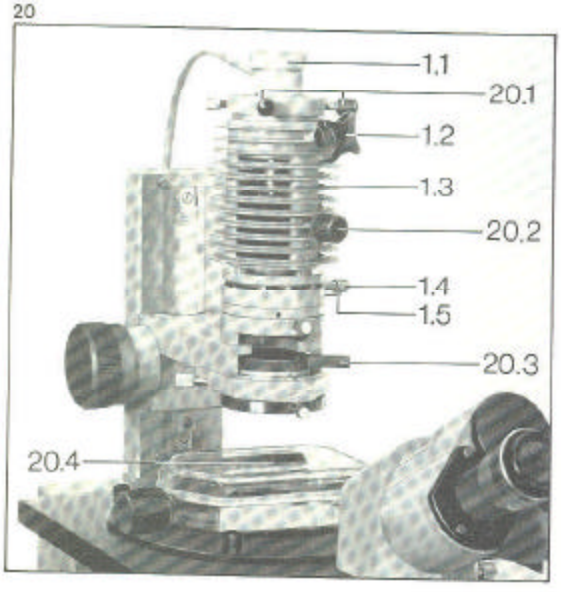

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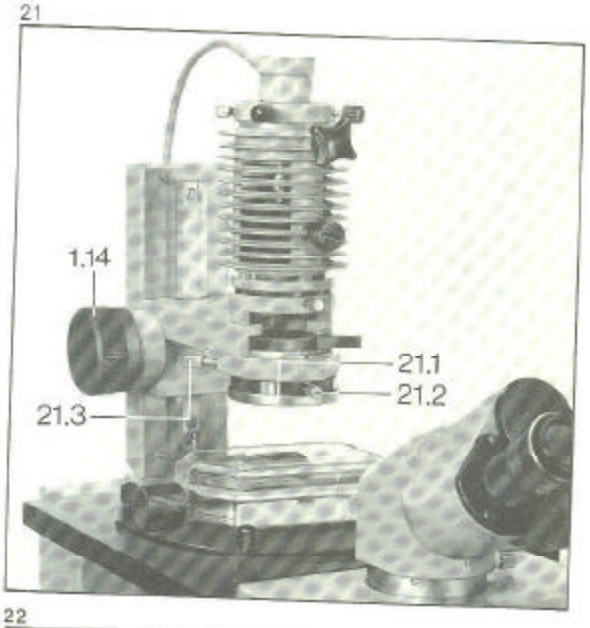

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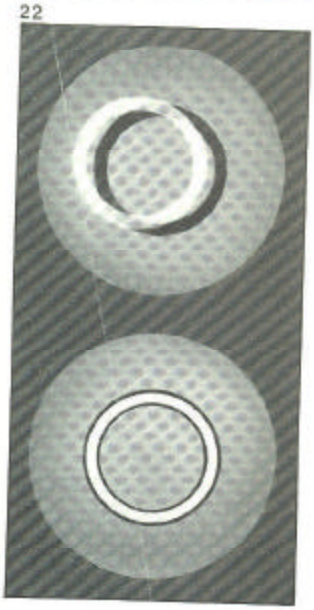

2.32

Phase contrast

Equipment (**Fig. 21**) as for brightfield illumination Section 2.31, but without iris diaphragm 1.5.
Additional parts:
Planachromat 6.3/0.16 Ph *(460311)*
with annular diaphragm Ph 1 *(475715)*
Planachromat 16/0.35 Ph *(460511)*

Planachromat 40/0.65 Ph *(460711)*
with annular diaphragm Ph 2 *(475716)*

Centering telescope *(464822)*

Adjustment:

- Screw objectives Ph into revolving nosepiece.
- Attach annular diaphragm Ph (**21.**1) matching the switched-in objective Ph 1 or Ph 2 to dovetail ring and fix with clamping screw (**21.**2).
- Proceed with work according to Section 2.1 unless already done.
- Replace on eyepiece by centering telescope. By vertically adjusting its eyelens focus bright diaphragm ring and dark ring.
- With centering screws (**21.**3) center both rings (bright and black) to each other.
- With knob (**1.**14) of illuminator carrier adjust size of bright ring until it superimposes black ring (**Fig. 22**).
- Replace centering telescope by eyepiece.

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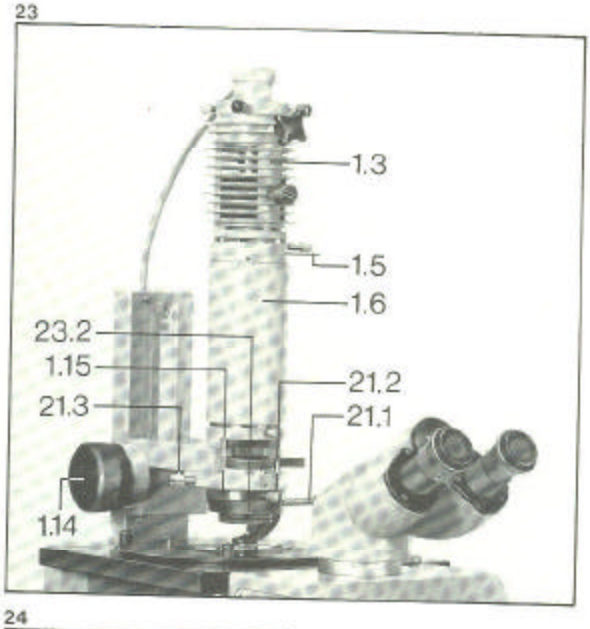

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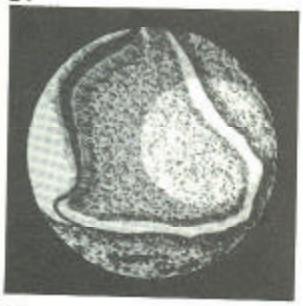

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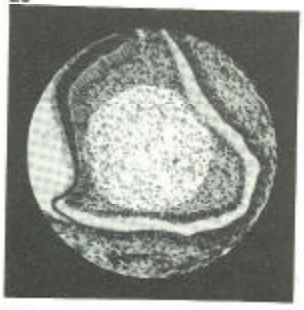

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2.4

Work with condenser

The condenser serves to illuminate the specimen with the required illuminating aperture. The resolution of higher power objectives is only fully utilized with a condenser. The Köhler illumination described in the following Section gives a uniformly illuminated specimen field, a brilliant image without reflections or glare and optimal specimen protection.

2.41

Brightfield

Equipment:
Attach condenser (**1.**15), spacer tube with auxiliary lens (**1.**6), iris diaphragm in intermediate piece (**1.**5) and illuminator (**1.**3) as described in Section 1.
Screw objectives into revolving nosepiece.
Make general preparations according to Section 2.1 unless already done.

- Close luminous field diaphragm (**1.**5) about half way.
- Focus image of luminous field diaphragm in specimen plane by raising the condenser with drive (**1.**14) from its position just above the specimen until image of diaphragm is sharply defined (**Fig. 24**).
- Precenter luminous field diaphragm in field of view (**Fig. 25**) with the two centering screws (**21.**3).
- Open luminous field diaphragm almost to edge of field of view, center if necessary and then open further until its edge just disappears behind the edge of the field of view (**Fig. 26**).
- Adjust image contrast with condenser aperture (**23.**2). Open condenser aperture so that about ⅔ of the objective exit pupil (point 2.1, page 18) is illuminated.
- With special condensers (Ph, DIC) set turret to position "J" (Iris). Its aperture diaphragm (**27.**5) can be centered with knurled knob (**27.**2) and lever (**27.**3).

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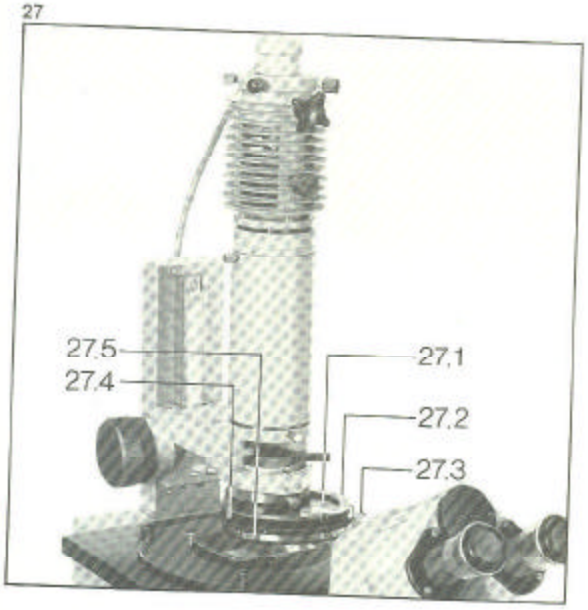

2.42

Phase contrast

Equipment (**Fig. 27**) as for brightfield illumination, in addition:

Screw objectives Ph into revolving nosepiece, attach phase contrast condenser in place of brightfield condenser to illuminator carrier; centering telescope *(464822)* and green filter VG 9/32x3 *(467805)* are also useful.

Adjustment:

- Switch in Planachromat 16/0.35 Ph 2.
- Focus specimen in brightfield according to Section 2.41 with Ph condenser in position "J" (Iris). Switch condenser turret to phase ring "2" (**27.**4).
- Exchange one eyepiece for a centering telescope and move its eyelens until bright and dark ring are in sharp focus.
- With knob (**27.**2) and lever (**27.**3) on condenser Ph adjust bright ring so that it is superimposed on the black ring (**Fig. 22**).
- Replace centering telescope by eyepiece.

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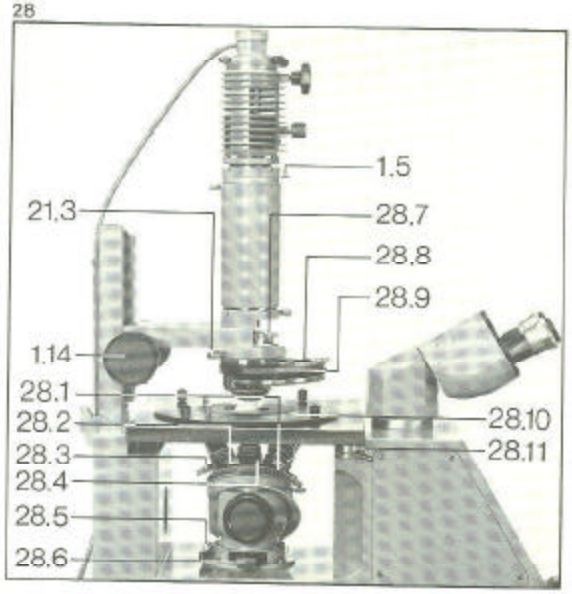

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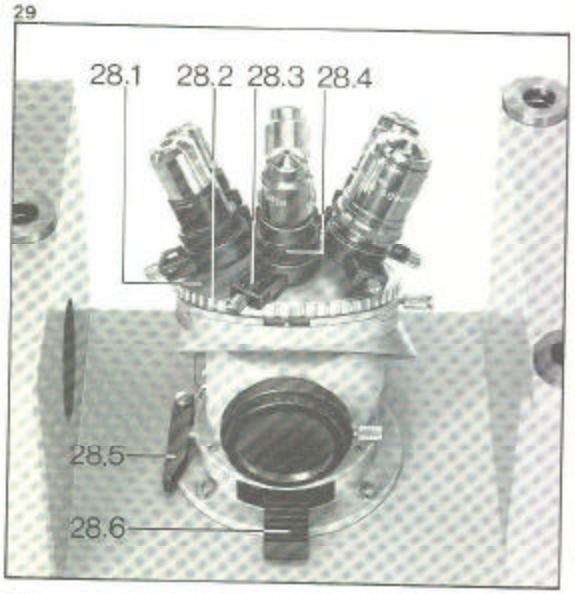

2.43

Differential interference contrast (DIC)

With this method differences in the optical length in the specimen (product of mechanical length and refractive index) are made visible as relief images. The contrast depends, among other things, on how the structure to be viewed is oriented in the field of view (azimuth effect). It is therefore recommended to turn the specimen around the optical axis, preferably with a rotating microscope stage.

Equipment as for brightfield, Section 2.41, in addition:

- Mount quintuple revolving nosepiece DIC, factor 1 *(471714)* (**28.**1), in place of the normal revolving nosepiece. Five oriented DIC intermediate rings are screwed into the nosepiece.
- Screw objectives (table page 28) into DIC revolving nosepiece.
- Insert DIC slide (**28.**3) (table page 28) matching respective objective – engraving face down – into the DIC intermediate ring (**28.**4). The slide is inserted from rear left to right front (**Fig. 29**).
- Adapt stage height to the 11 mm longer objectives with DIC slide by screwing 3 spacers *(471729)* (**28.**11) between stage plate and bolts.
- Insert polarizer *(473616)* (**28.**7) into filter opening and set to zero.
- Attach DIC-Ph-H-condenser (**28.**8) to carrier.
- Pull analyzer with exchangeable λ-plate *(473668)* (**28.**6) out to stop. To remove the analyzer slide, simultaneously press stop lever out of rest position.

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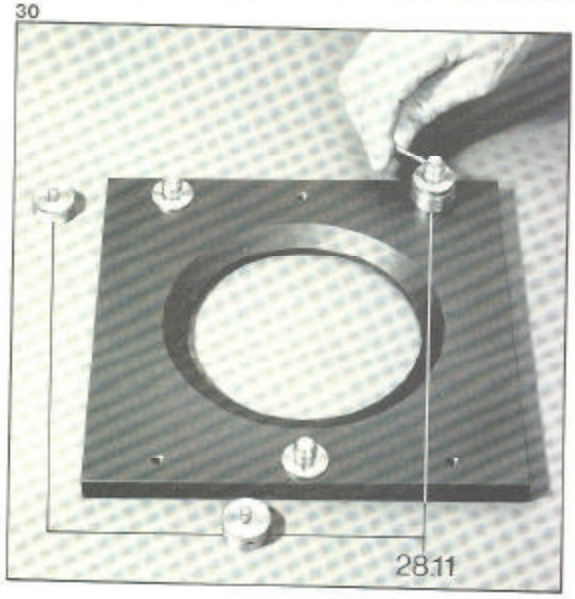
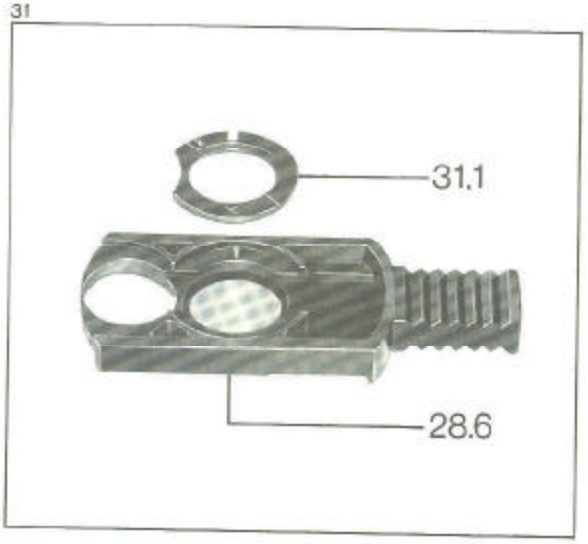


31



Adjustment:

- Focus specimen in brightfield according to Section 2.41, DIC condenser in position "J".
- Switch in objective with appropriate DIC slide.
- Swing in analyzer to stop.
- Adjust optimum black-white contrast with screw (**28.**2) of DIC slide.

Color contrast is producted by inserting λ-plate (**31.**1) into analyzer (**28.**6) towards the microscope.

- Lower stop lever (**28.**5), take out analyzer slide and place λ-plate on analyzer. Replace analyzer slide.

Objective	DIC slide (**28.**3) Cat. No.	DIC condenser IV Z/7 aperture 0.63 *(465273)* position (**28.**9)	DIC condenser IV Z aperture 1.4 *(465285)* position (**28.**9)
Plan 6.3/0.16	474531	I	I*
Plan 16/0.35	474551	I	I
Plan-Neofluar 16/0,5 W Oil	474555	I	I
Plan-Neofluar 25/0,8 W Oil	474560	II	II
LD-Plan 40/0.60 corr.	474564	II	II
Plan 40/0.65	474571	II	II
Planapo 63/1.40 Oil	474581	II	II
Plan 100/1.25 Oil	474591	II	II

* Field of view is not entirely illuminated.
Condenser position "J" (Iris) only for brightfield.
Condensor position 2 or 3 for objectives Ph 2 or Ph 3.
Condensor position III has been provided for special prisms.

Note
The DIC condenser prisms are correctly oriented in the turret if the white point coincides with the orienting pin.

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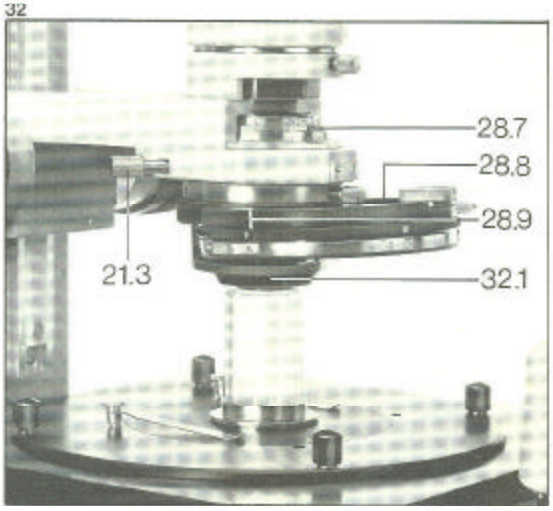

2.431
Differential interference contrast (DIC) with long back focal distance
This method is especially suited for examining objects in chambers or micro test plates which require a great illuminating distance. With the 6.3x, 16x and 40x Planachromats 20 to 40 mm working distance is obtained with good image contrast.

Equipment as for normal differential interference contrast, Section 2.43

Special feature: Use condenser DIC-Ph, IV Z/7 *(465273)*, unscrew its front lens and set condenser to position II (**28.**9).

Adjustment:
- Switch in low-power objective, Planachromat 6.3 or 16. Focus specimen, slightly close down luminous field diaphragm (**1.**5) and with knob (**1.**14) adjust condenser vertically so that the diaphragm appears in sharp focus in the specimen;
if necessary, bring this image to center with centering screws (**21.**3).
- The condenser may remain in this position (Köhler illumination) for all objectives provided the working distance of about 22 mm thus obtained is sufficient.
- Move analyzer (**28.**6) with or without λ-plate (page 29) and polarizer (**28.**7) into beam path.
- By turning the knurled screw (**28.**2) on the DIC slide and by slightly closing the condenser aperture optimum contrast can be obtained.
- When changing up to the next power objective, readjust contrast with DIC slide and condenser aperture only.
- If working distances larger than 22 mm are required, do not apply Köhler illumination but raise the condenser as much as necessary with knob (**1.**14). The condenser aperture remains completely open.

2.44
Darkfield

The **equipment** for darkfield is the same as that for brightfield (**Fig. 23**), except for using a darkfield condenser in place of a brightfield condenser. The darkfield condenser illuminates the specimen with a hollow cone of rays whose inner aperture must be higher than that of the objective. Only the light diffracted by the specimen enters the objective, the image background remains dark.

Adjustment
- Center condenser V Z *(465277)* in brightfield illumination and set turret to D or use darkfield condenser.
- Switch in Planachromat 16 objective.
Slightly close down luminous field diaphragm (**1.**5).
- With knob (**1.**14) adjust the condenser vertically, so that the light spot in the image appears as small, bright and sharply defined as possible.
- Using centering screws (**21.**3) bring this image of the luminous field diaphragm to the center of the field of view. Open luminous field diaphragm until its edge just disappears behind the edge of the field of view.
- If an **immersion objective** is used:
Apply immersion oil bubble-free to cover slide.
Close iris diaphragm of objective 100, focus image, improve centering of luminous field diaphragm.
Open iris diaphragm of objective just enough to keep the background dark.

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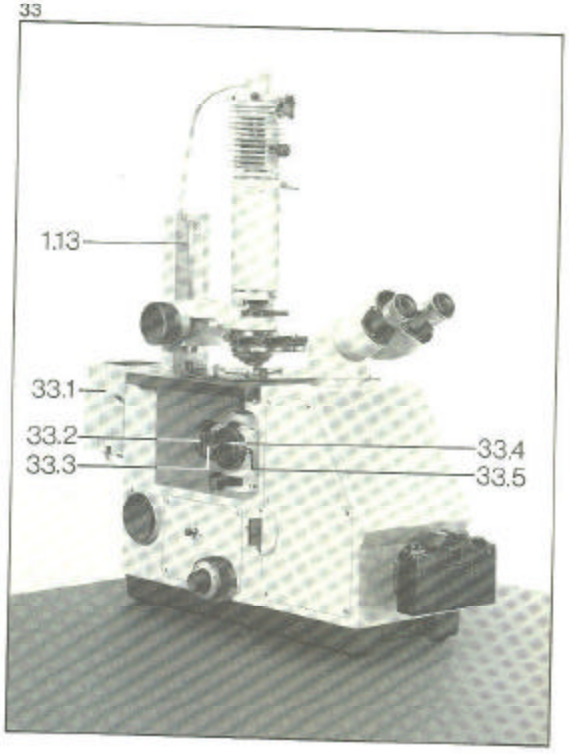

2.5

Epi-fluorescence

With the epi-fluorescence equipment the IM 35 inverted microscope for transmitted light can be converted to a fluorescence microscope for epi-excitation which mainly serves to examine transparent specimens but can also be used for opaque objects.

Epi-fluorescence equipment

33.1 Microscope illuminator 100 (see G 41-310) with HBO 50 W high-pressure mercury source as exciter lamp supplies good results with all fluorescence techniques; there is also the 12 V 100 W halogen source fot FITC-excitation.

33.2 Illumination attachment *(471761)* for epi-fluorescence.

33.3 Shutter and filter slide with 3 switch positions:
Position 1 blocks light passage
Position 2 red-attenuating filter BG 38
Position 3 free opening (filter receptacle)

33.4 Double reflector housing 2 FI allows changeover from UV to violet excitation by moving the reflector housing in or out. This double reflector housing can easily be exchanged for another one, e.g. for blue and green excitation, after loosening clamping screw (**33.**5). Other filter combinations (see K 41-005) can be chosen as required.

Double reflector housing 2 FI *(466301)* as a functional unit comprises:
Exciter filter
Reflector, a 45° plane glass acting as chromatic beam splitter and barrier filter.

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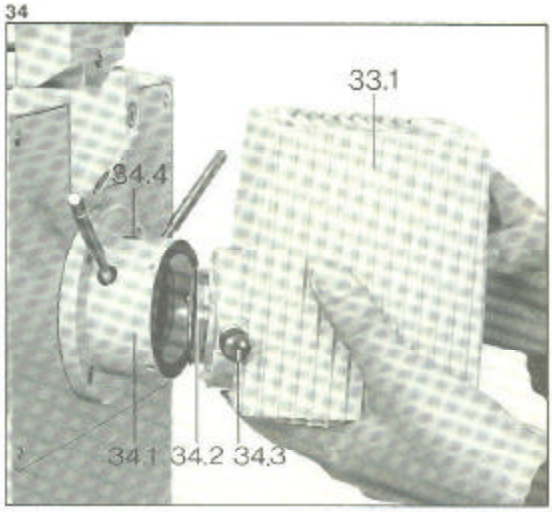
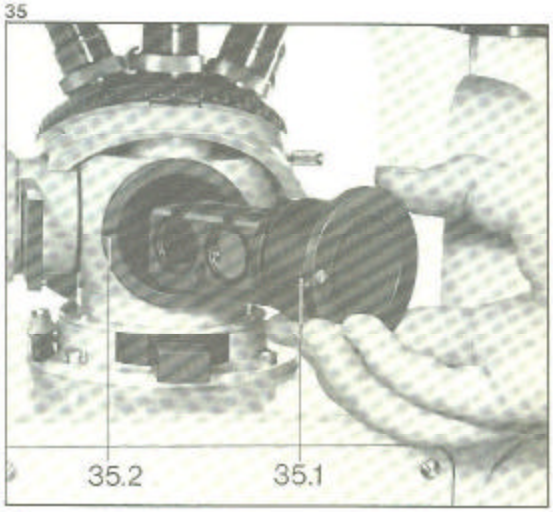
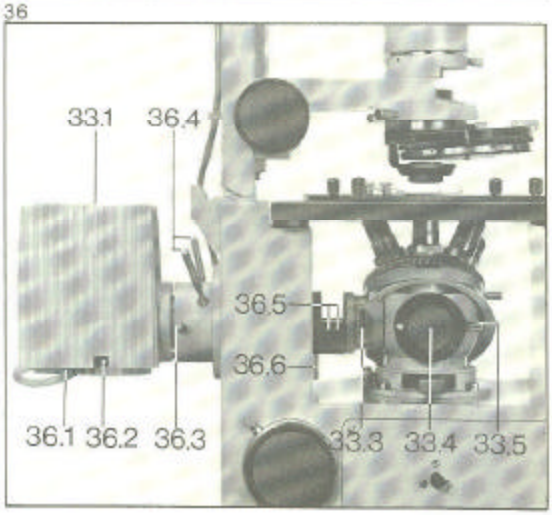


35



36



Mount **microscope illuminator 100** to epi-illumination attachment FI (**34.**1). If opening is covered, remove protective cap and loosen clamping screw (**36.**3). Attach dovetail ring (**34.**2) as previously described and tighten clamping screw (**36.**3) before letting go of the illuminator.

Insert **reflector housing 2 FI** into opening below revolving nosepiece: Loosen clamping screw (**33.**5). Introduce reflector housing into the sliding sleeve up to stop making sure that pin (**35.**1) fits into groove (**35.**2). Lock with clamping screw (**33.**5).

Adjustment

- Connect light source to its power supply unit and switch on. The HBO 50 W high-pressure mercury lamp ignites automatically. It can be used after 2–3 minutes warm-up time.
- Switch in filter combination for green or blue excitation

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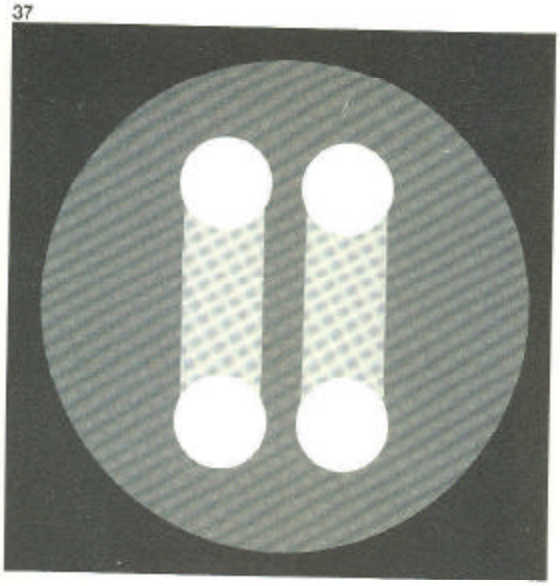

- Bring slide (**33.**3) (**Fig. 36**) into position for free light passage. Remove an objective from revolving nosepiece and swing in empty nosepiece position. Place ground glass or transparent paper on specimen stage. Center stage, if necessary. Focus light seen on ground glass by turning lamp condensor knob (**34.**3). Improve sharpness of light source images by narrowing luminous field diaphragm (**36.**6). If necessary center the direct and the reflected light source images side by side on the ground glass (**Fig. 37**) by vertically and laterally adjusting the lamp socket, and focusing and tilting the concave mirror (see G 41-310).
- Switch in low-power objective, preferably NEOFLUAR 10/0.30. Focus on strongly fluorescing objects.
- With lever (**36.**6) close luminous field diaphragm. The latter can be focused after loosening slotted screw (**34.**4) and centered with screws (**36.**4).
- Swing in suitable filter combination (see K 41-005, Page 37 and GE 41-351.1) in reflector housing (**33.**4). Heat-absorbing filter KG 1 is built into illumination attachment FI *(471761)*.

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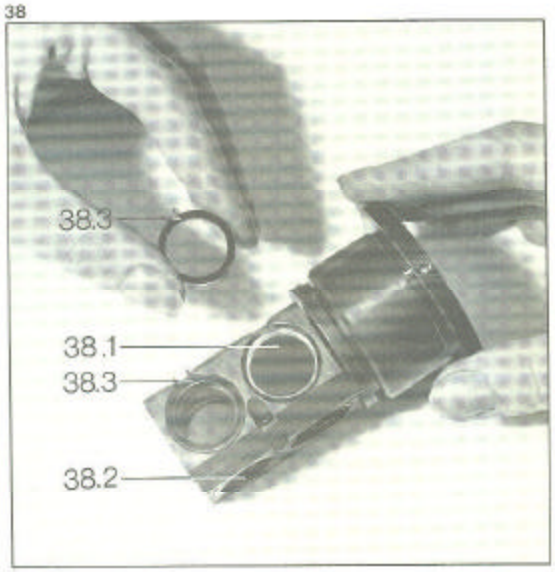

Exciter filter, chromatic beam splitter and barrier filter

in double reflector housing 2 FI are exchangeable. Exciter filter (**38.**1) and barrier filter (**38.**2) are 18 mm in dia. They are held by plastic rings with cams (**38.**3). To change filters press both cams out of notches and pull plastic ring (**38.**2) out of receptable.
The filters, and spacer ring, if any, now are loose in the filter receptable and can be easily exchanged.
With filters up to 2 mm thick insert support ring with contact rim inwards, with thicker filters with contact rim outwards. If the filter has too much play, place spacer ring between filter and support ring.
The reflector housing takes exciter filters up to 6 mm thick and barrier filters up to 4 mm thick. Thinner filters are supplied with spacer ring.

The 45° interference filters acting as chromatic beam splitter have a diameter of 22 mm. They also held loosely in their receptacles and are accessible after removing two screws. Since the interference coatings of the chromatic beam splitter lie unprotected on the surface and are very delicate, we recommend not to touch the chromatic beam splitter.

Additional exciter filters can be placed loosely into filter pockets (**36.**5). Use support ring with 32 mm dia. filters, for 18 mm dia. filters use 18 mm dia./32 mm dia. spacer ring *(467893)* in addition.

Epi-fluorescence microscopy can be combined with transmitted-light techniques such as phase contrast, darkfield or differential interference contrast. Any transmitted-light condenser may be used.

A CONTAX camera housing is well suited for this purpose because of its automatic exposure control (see camera operating instructions).
Other camera housings with focal-plane shutter can also be attached; adapters are available.
Before working with the equipment make test exposures at various DIN/ASA settings in order to make sure that all influences of the setup used here are taken into account.

- Attach camera to microscope according to Section 1.10
- Pull out push rod (**1**.20) as far as stop:
In this position 80 % light is directed to the camera and 20 % light to the binocular tube.
- Introduce insert with photo reticle *(47178)* (**1**.19). The reticle visible in the binocular tube defines the image section covered by the 35 mm camera.
- So that each user can bring the format outlines into sharp focus, eyepieces with adjustable eye lenses are required.
- Adjust microscope illumination and image sharpness by carefully following directions given in Section 2.
- Adjust image contrast with condensor aperture, image brightness with gray filters.
- When using color film for artifical light and illuminating with filament lamp operate lamp at rated voltage (6 V, 12 V and 2.5 A, 8 A, respectively via transformer.

Black and white photomicrography
Microscopic specimens are usually lacking in contrast as compared with normal photographic objects.
This can be remedied:

1. Use a colour filter for stained specimens. (A green filter is generally useful for many histological stains).
2. Narrow the aperture diaphragm (not too much).
3. Develop the film in a more concentrated developer (i.e. to a higher degree of gradation).
4. Optical contrast methods, such as darkfield, phase contrast or differential interference contrast, can be used.

Universal films of medium speed are suitable for almost all purposes. One need not hesiate to use the most sensitive films for flash photography, since the resolution of the film, even in this case, is better than that of the microscope.

Colour photography
Usually reversal film is used, which produces a transparency after being developed. (Suffix "-chrome" indicates a reversal film and "-color" negative film which yields paper positives.) For both there is one type for day light and another for artificial light. If the photomicrography will be interspersed with, for example, flash macrophotography then daylight film can be used and a filament lamp as light source with a (blue) conversion filter CB 12, or an artificial light film can be used, which needs no filter for the comparatively long exposure times.

While contrast can be enhanced in black and white photography by using colour filters and/or special developing, this is not possible with colour film; therefore the specimens should be more strongly stained.

Colour casts are caused by the following: wrong choice of film, variations between batches of film (note emulsion batch number), particulary long exposure times (Schwarzschild effect), wrong colour temperature (use nominal voltage), coloured inbedding medium, transmission value of optics not neutral (e.g. including heat protection grey and polarisation filters).

Graduated blue filters (CB 3, CB 6, CB 12) are very useful for correcting the colour of light, since filament lamp light is usually too "warm" (no problem with halogen lamps).

Other colour casts can be corrected with colour complementary filters (foils) e.g. Kodak CC filter; with strength 10 almost all casts due to batch variations can be corrected.

Colour cast	Filter col. reqd.
yellowish	blue (B)
reddish	blue-green (Cyan, C
greenish	purple (Magenta, M)
blueish	yellow (Yellow, Y)
blue-green	red (R)
purple	green (G)

Contrary to belief, the microscope objectives of more simple construction (Achromats) are suitable for colour photography, but the superiority of higher correction objectives is marked e.g. Plan Neofluar type.

Further hints on photomicrography can be found in leaflet A 41-400.1.

Accessories for plankton examinations are described in G 41-129, p. ff.
Subject to change.