

Inverted
Microscope
INVERTOSCOPE D

Operating Instructions

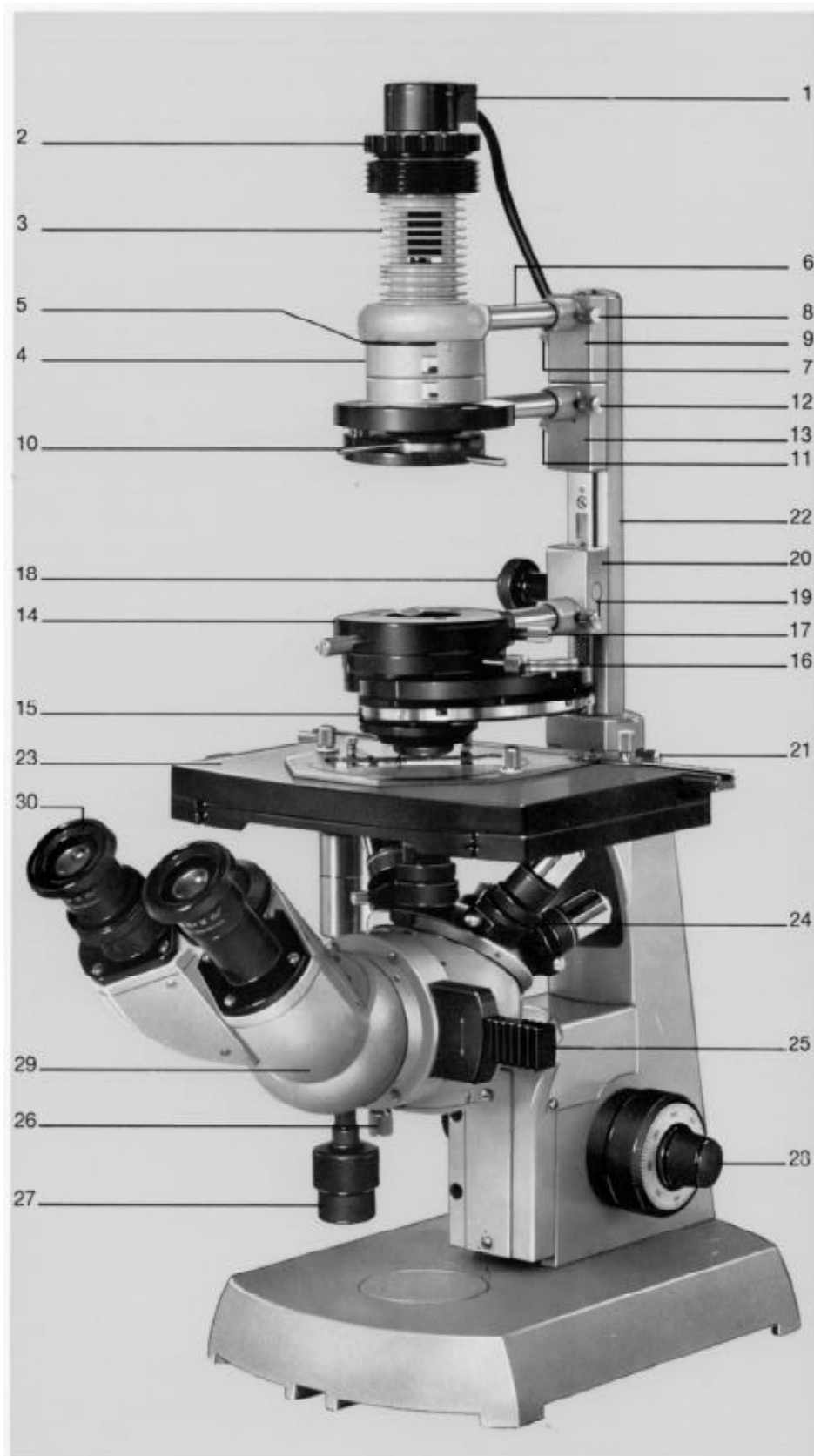
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INVERTOSCOPE D

with differential interference contrast equipment



- 1 Socket for 6 V 15 W tungsten filament lamp
- 2 Clamping ring for item 1
- 3 6 V 15 W tungsten filament lamp
- 4 Filter turret for holding rings (46 72 52) with filters and annular phase contrast diaphragms
- 5 Lamp field iris
- 6 Lamp holder
- 7 Clamping screw for slide 9
- 8 Clamping screw for lamp holder 6
- 9 Lamp slide
- 10 Holder with rotary, swing-out polarizer
- 11 Clamping screw for slide 13
- 12 Clamping screw for holder on slide 13
- 13 Polarizer slide
- 14 Holder with condenser carrier
- 15 Condenser (differential interference contrast-phase contrast condenser rigidly connected to condenser carrier)
- 16 Clamping screw to fix the condenser
- 17 2 centering screws for condenser
- 18 Knob for vertical condenser adjustment
- 19 Clamping screw to fix the condenser carrier on slide 20
- 20 Slide for condenser carrier
- 21 Clamp for column with illuminating system
- 22 Column for illuminating system
- 23 Large mechanical stage with special specimen clips
- 24 Quintuple revolving nosepiece with DIC slides and adapter rings
- 25 Analyzer
- 26 Clamping screw for analyzer setting, avoiding erroneous pulling out of the analyzer slide
- 27 Coaxial drive for 50 x 75 mm specimen movement on the mechanical stage
- 28 Coaxial drive for coarse and fine focusing
(one index line of the precision drive graduation corresponding to a vertical stage motion of $5\ \mu\text{m} = 0.005\ \text{mm}$)
- 29 Inclined binocular tube
- 30 Eyepieces

1. Assembling the microscope



Hold the tube **(29)** slightly inclined, with its annular dovetail press down the spring bolt of the loosened clamping screw **(31)**, insert the tube, and tighten the clamping screw.

Loosen the clamping screw **(21)** and slide the column **(22)** for the illuminating system into the dovetail guide to the stop. Tighten clamping screw **(21)**.

Working with condenser:

Insert condenser carrier **(14)** in slide **(20)** and fix with clamping screw **(19)**.

Bring the condenser carrier **(14)** into uppermost position with knob **(18)**, and loosen the clamping screw **(16)** of the condenser holder.

Insert the condenser from below:
Hold the condenser slightly inclined, and with its annular dovetail press down the spring bolt of the clamping screw **(16)**. Insert the condenser and turn it to the stop. Tighten the clamping screw **(16)**.

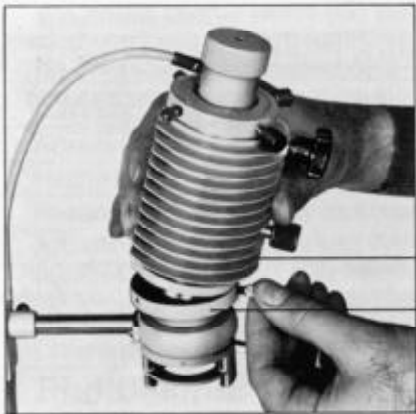
(When working with **DIC equipment**, the condenser need not be mounted on the condenser carrier **(14)**, because both parts are rigidly connected).

Lower the condenser with knob **(18)**.

Only for work with DIC equipment: insert slide **(12)** from above in column **(22)** and clamp it.

Fix the holder with polarizer **(10)** to the slide.

Screw the 6 V 15 W lamp to the lamp holder.



The connecting piece 46 70 42 **(33)** with annular dovetail is required to mount the 12 V 60 W lamp **(32)**.



Press the 6 V 15 W low-voltage tungsten filament lamp (38 00 18-1740) into the lamp socket **(1)** (red dot opposite red dot), and turn it clockwise. Remove fingerprints on the bulb. Slide the black sleeve **(34)** over the lamp bulb to avoid dazzling.

Loosen clamping ring **(2)**, insert the lamp socket and fix it.

Connect the 6 V 15 W lamp to step-up transformer 110-127-220-240 V/3-4-5-6-7-8 V, 50 . . . 60 Hz, 25 VA.



Insert slide **(9)** from above into column **(22)** and fix it.

Fix lamp holder **(6)** with lamp to the slide.

Screw objectives into revolving nosepiece **(24)**.

(When working with the **DIC equipment**, screw the objectives into the adapter rings on the revolving nosepiece, and push in the DIC slides corresponding to the objectives (engraving underneath) to the stop).

**For work with the
DIC equipment only:**

Loosen clamping screw
(26), pull out the dummy
slide, if any, insert the
analyzer slide (25) and
tighten the clamping screw
(26). Insert the eyepieces
in the tube.

Clamp the specimen clip on the
specimen stage in a position which
allows farthest lateral screening of
the specimen.

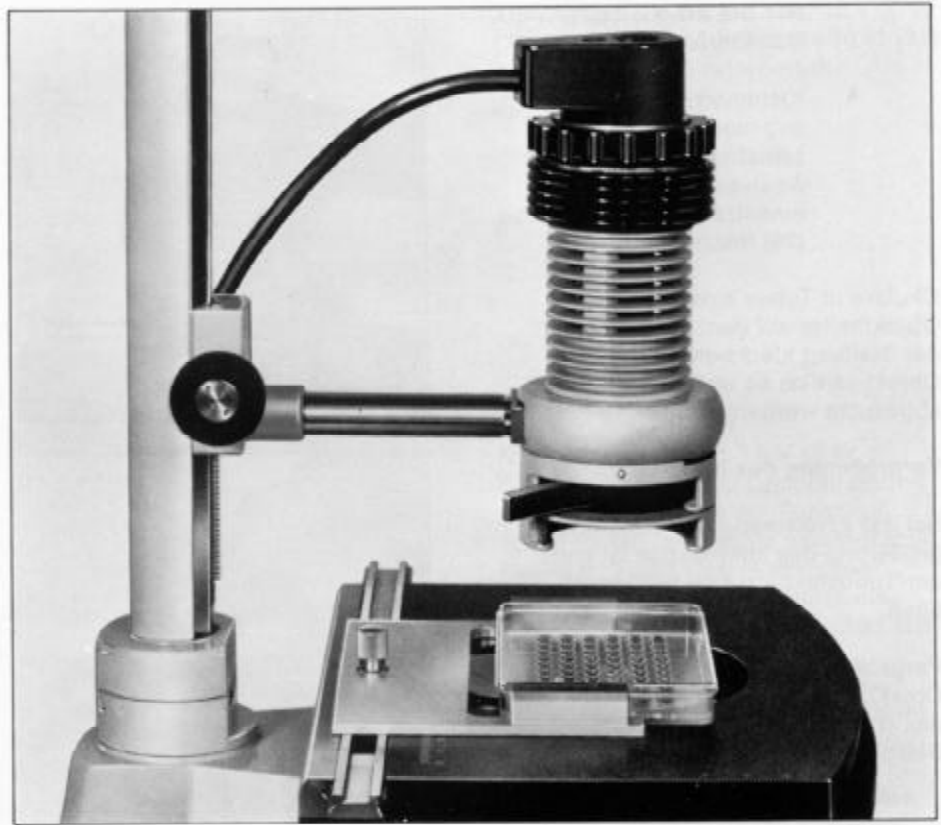
Microscope magnification

Always consider the tube factor 0.8
to determine the total magnification
of the microscope.

Magnification = objective magnifi-
cation x factor 0.8 x eyepiece
magnification.

Example: $12.5 \times 0.8 \times 10 = 100$.

2. Adjusting the microscope



2.1 Working without condenser

Specimens in vessels and chambers are investigated without condenser.

(The aperture of the light bundle emitted by the lamp is in most cases much smaller than the objective aperture. This produces the same contrast of the specimen as a stopped-down condenser).

Brightfield

Place the specimen on the stage.

NB

Place covered specimens with the coverglass underneath.
Clamp the lamp **(3)** as nearly above the specimen as possible.

Switch on the lamp.

Open the iris diaphragm **(5)** of the lamp.

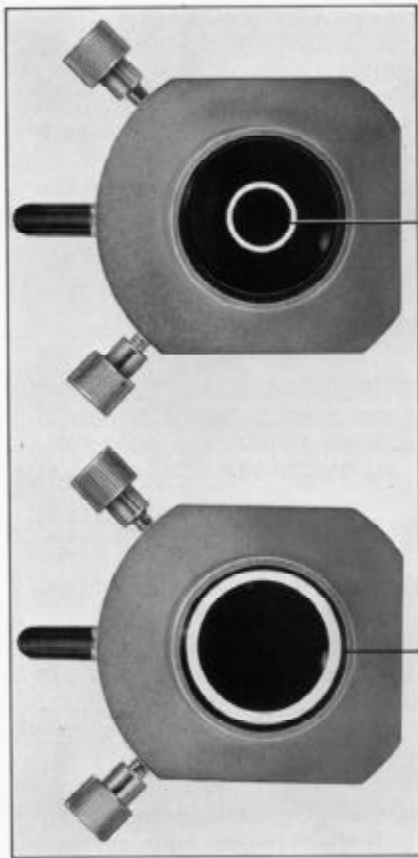
Loosen the clamp **(2)** and pull out the lamp socket **(1)** until the image of the specimen is brightly and evenly illuminated.

Move in the objective 10 — 25.

With the coaxial drive **(28)** focus to the specimen image with the right eye through the binocular tube. Focus with the left eye by turning the eyepiece tube.

Adjust the illumination on the transformer or with filters. Insert the filters in holding rings 46 72 52 in the filter turret **(4)**.

(Protect heat-sensitive specimens by using heat-reflecting filter 46 78 32. Insert the filter so that the filter surface marked "L" points towards the light source).
Adjust contrast with diaphragm **(5)**.



35

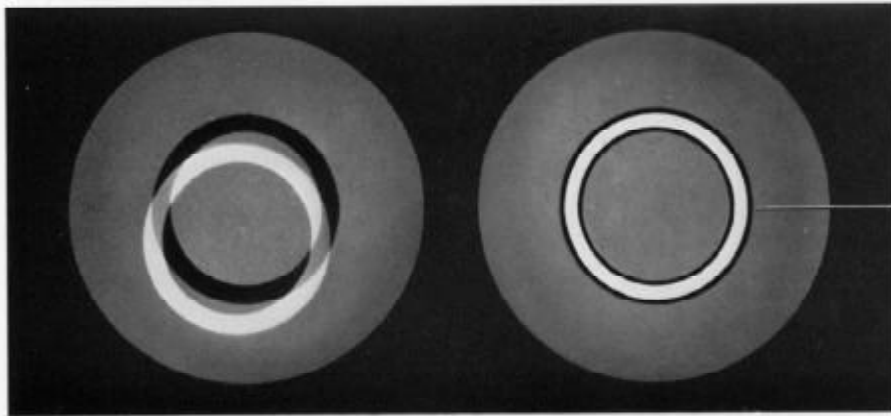
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37



39



38

Phase contrast

Phase contrast diaphragms Ph 1 **(35)**
and Ph 2 **(36)**.

Adjust brightfield illumination
according to section "Brightfield"
on page 9 with Achromat 10 Ph 1.

Exchange one eyepiece for the
centering telescope 46 48 22 **(37)**.
Push in the eyelens until a dark and
a luminous ring are in focus.

Adjust the lamp vertically until the
luminous ring fits into the dark one
(38).

Fit the luminous ring exactly into the
dark one with the centering screw
(39) on the phase contrast diaphragm
or by shifting.

Exchange the centering telescope
again for the eyepiece.

The phase contrast image is visible.

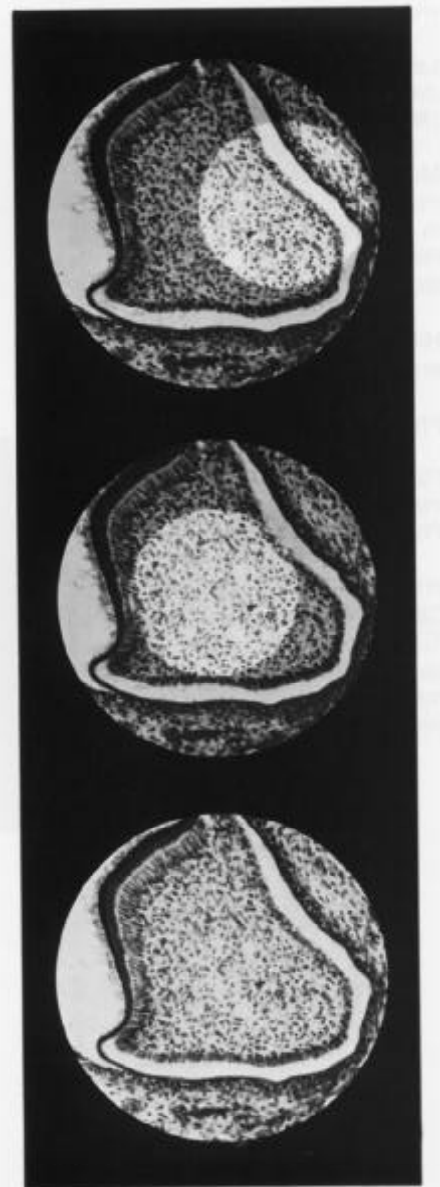
Use phase contrast diaphragm Ph 2
for Ph objectives with apertures
between 0.40 and 0.70.

If much space is needed (e. g. for
vessels) with a Ph 1 objective, phase
contrast diaphragm Ph 2 can be used
instead; fix the lamp correspondingly
higher.

2.2 Working with the condenser

The condenser supplies the necessary aperture for specimen illumination. Only with condenser will the resolving power of high-power objectives be fully utilized.

(The exit pupil of the objective can be optimally illuminated. With centering telescope 46 48 22 it appears enlarged, or in normal size through the empty tube, even with diopter).



Brightfield

Place the specimen on the stage.

NB

Place covered specimens with the coverglass underneath.

Fix lamp (3) to upper part of column (22).

Switch on the lamp.

Lower the condenser until it is just above the specimen slide.

Open the iris (5) of the lamp.

Set disk (43) of the phase contrast condenser to "I" (iris).

Close the iris (40) of the condenser. Loosen clamp (2) and shift the lamp socket (1) until the plane of the condenser diaphragm is brightly and evenly illuminated.

Open the condenser aperture diaphragm (40).

Move in the objective 10 — 25.

With the coaxial drive (28) focus to the image of the specimen with the right eye through the binocular tube. Focus with the left eye by turning the eyepiece tube.

(Protect heat-sensitive specimens by using heat-reflecting filter 46 78 32 which in its holder is inserted in filter turret (4). Make sure that the filter surface marked "L" points towards the light source).

(With condensers up to aperture 0.6, e. g. condenser with swing-out front lens, diaphragm (5) on the illuminator acts as aperture (contrast) diaphragm. Do not stop down this diaphragm further than required to detect details in the image. With higher aperture condensers, diaphragm (5) is the lamp field stop).

Stop down diaphragm (5).

With knob (18) adjust the condenser so that the image of the lamp field stop is visible together with the specimen.

Center the lamp field stop image with the centering screws (17) with regard to the center of the field of view.

Open the lamp field stop until its rim just disappears behind the rim of the field of view (see the three illustrations on page 12).

Only with phase contrast condensers:

Center the aperture diaphragm.

Close the diaphragm (40) of the condenser.

Exchange one eyepiece for the centering telescope, and shift its eyelens until the image of the stopped down diaphragm is in focus.

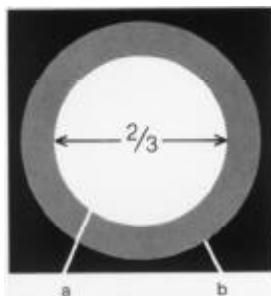
Without centering telescope, the diaphragm image is visible through the empty tube.

Center the rim of the diaphragm with regard to the objective aperture with knob (41) and lever (42).

Adjust the aperture diaphragm (40) so that the diameter of the image is about $\frac{2}{3}$ to $\frac{3}{4}$ of the diameter of the objective aperture (see Fig. below).

The adjustment described above is the adjustment for Koehler illumination, which meets even most exacting demands. Such an exact beam path is generally not required for routine work.

Provide eyepiece and objective for observation.



a) Aperture diaphragm image
b) Luminous surface with open diaphragm

Phase contrast

Adjust brightfield with the phase contrast condenser in position "I" of the revolving nosepiece, and with a low-power objective Ph, according to section "Brightfield" on page 13.

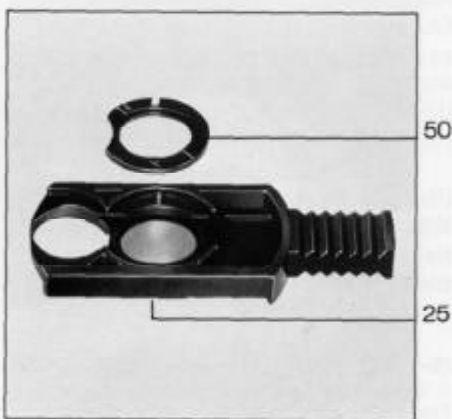
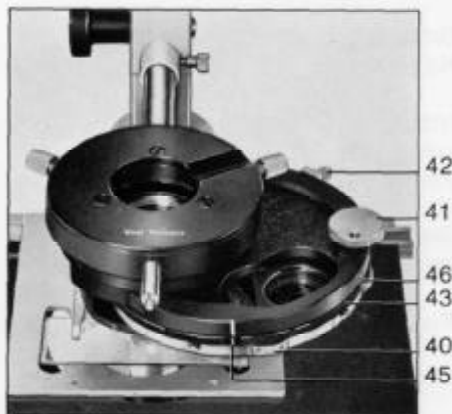
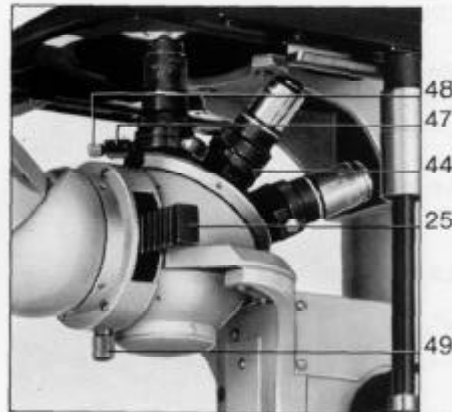
Bring the turret of the phase contrast condenser (**43**) into the position corresponding to the objective (for objective 16 Ph 2 in position 2).

Exchange one eyepiece for the centering telescope (**37**) and shift its eyelens until a bright and a dark ring are in focus.

With lever (**42**) and knob (**41**) on the phase contrast condenser, shift the bright ring exactly into the dark ring (**38**) (see Fig. on page 10).

Exchange the centering telescope for the eyepiece.

The phase contrast image is visible.



Differential interference contrast (DIC)

With this method differences in height and refractive index in the specimen are made visible as pseudo three-dimensional images.

The following requirements must be fulfilled for DIC: the DIC adapter rings (44) are screwed into the revolving nosepiece and adjusted. The rotary polarizer (47 12 75) (10) is adjusted in the factory so that it is crossed with the analyzer (25) in stop position zero.

Swing the polarizer (10) into the beam path and turn it to stop position zero. Lever in front!

Pull out the analyzer (25) to the stop.

Set the position corresponding to the objective used on the DIC condenser turret (47 12 74):

Condenser setting I (45) for:	
Planachromat 6.3/0.16	46 03 10
Planachromat 16/0.35	46 05 10

Condenser setting II (46) for:	
LD Planachromat 40/0.60 corr.	46 07 15
Planachromat 40/0.65	46 07 10
Planapochromat 63/1.40 oil	46 18 40
Planachromat 100/1.25 oil	46 19 10

The turret features an opening for a third DIC prism (e. g. a special prism for objectives other than those mentioned here); this prism is in the beam path when the turret is in position III.

Lid (82, page 14) covers the opening (83) which gives access to the turret with the DIC prisms (85, page 16). Spring rings hold the prisms in the turret. The prisms are pulled out through the opening with the aid of a tube with external thread (84). The pin must be inserted in the slot of the spring ring so that the white line (85) points in the direction shown in the Fig. Only then is the DIC prism correctly adjusted with regard to the oscillation directions of analyzer and polarizer.

With brightfield illumination produce an image of the specimen in the eyepiece.

Make adjustment according to the preceding section.

Slide the DIC slides (47) corresponding to the objectives into the adapter rings (44) — engraving underneath.

DIC slide for Planachromat		
6.3/0.16		47 45 31
DIC slide for Planachromat		
16/0.35		47 45 51
DIC slide for LD Planachromat		
40/0.60 corr.		47 45 64
DIC slide for Planachromat		
40/0.65		47 45 71
DIC slide for Planapochromat		
63/1.40 oil		47 45 81
DIC slide for Planachromat		
100/1.25 oil		47 45 91

Swing in analyzer (25).

Slide the DIC slide to the stop in the slot of the DIC adapter. When turning the knurled screw (48) of the DIC slide, the DIC prism is shifted diagonally in a plane perpendicular to the microscope axis. This changes the image contrast and allows optimum adaptation to specific structures.

Color contrast is produced by inserting the λ -plate (50) in front of the analyzer (25) towards the microscope. After loosening the screw (49) pull out the analyzer slide (25) and put the λ -plate (50) on the analyzer (25) in slide. Return the analyzer slide and fix with screw (49).

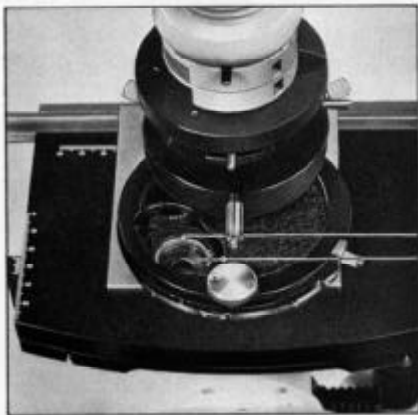
With weakly stained specimens, **amplitude contrast** * is produced by turning the polarizer (10). To achieve maximum contrast, turn the polarizer by small amounts in each direction.

* G. B. David, B. S. Williamson: Amplitude Contrast Microscopy in Histochemistry. *Histochemie* 27, 1 - 20 (1971).



The adjustment described on the opposite page is required when the microscope is subsequently equipped with the DIC system.

Make the adjustment separately for each objective when its adapter ring is screwed into the revolving nosepiece. Constant orientation of the DIC adapter ring is guaranteed only when the ring is in one and the same opening of the revolving nosepiece.



1.
Coarse adjustment of the DIC slide
(pre-orientation)

Remove condenser carrier (14) from the slide (20).

Screw the DIC adapter ring (44) into the revolving nosepiece. Loosen the two opposite inner screws (51) (apply the screwdriver from above).

The upper slotted part of the DIC adapter ring can be turned now.

Slide the DIC slide (47) corresponding to the objective — engraving underneath — from the front right into the DIC adapter ring, and align it in the direction NW — SE seen from above.

2.
Checking the cross position of polarizer and analyzer

Remove the DIC slide from the beam path.

Turn the polarizer into stop position zero.

Slide in analyzer (25).

Switch on the lamp. Remove the objective.

Look through the tube without eyepiece. The background must be dark. If this is not the case, i. e. if the background becomes darker when turning the polarizer from its stop position, turn it until maximum darkness is achieved.

3.
Aligning the DIC slide with regard to the polarizer (precision orientation)

Screw the objective corresponding to the DIC slide into the DIC adapter ring. Mount the condenser carrier (14) with DIC condenser on slide (20).

Bring the turret of the condenser into the position corresponding to the objective (position I or II, see preceding section). Pull out analyzer (25) and DIC slide.

With brightfield illumination produce an image of the specimen in the eyepiece according to section "Working with the condenser in brightfield" on page 13. Shift the specimen laterally until no more structures are visible in the field of view.

Remove the eyepiece and observe the objective pupil, or exchange one eyepiece for the centering telescope.

Push in its eyelens until the image of the stopped-down condenser diaphragm is in focus.

Open the condenser diaphragm.

Eliminate inhomogeneous illumination by axial shifting of the lamp (after loosening clamp (2)).

Insert the DIC slide corresponding to the objective — engraving underneath. Bring polarizer and analyzer into the beam path. Slowly turn the DIC adapter ring with the slide alternatively to the right and to the left, and turn with the screw on the DIC slide until most of the surface in the objective aperture (pupil) is evenly gray. (Adjust to optimum uniform illumination of the entire pupil).

Completely even illumination can generally not be achieved.

Maintain the position of the DIC adapter ring, while unscrewing the objective, and fix the rotating part of the DIC adapter ring by tightening the clamping screws (51).

Remove the centering telescope.

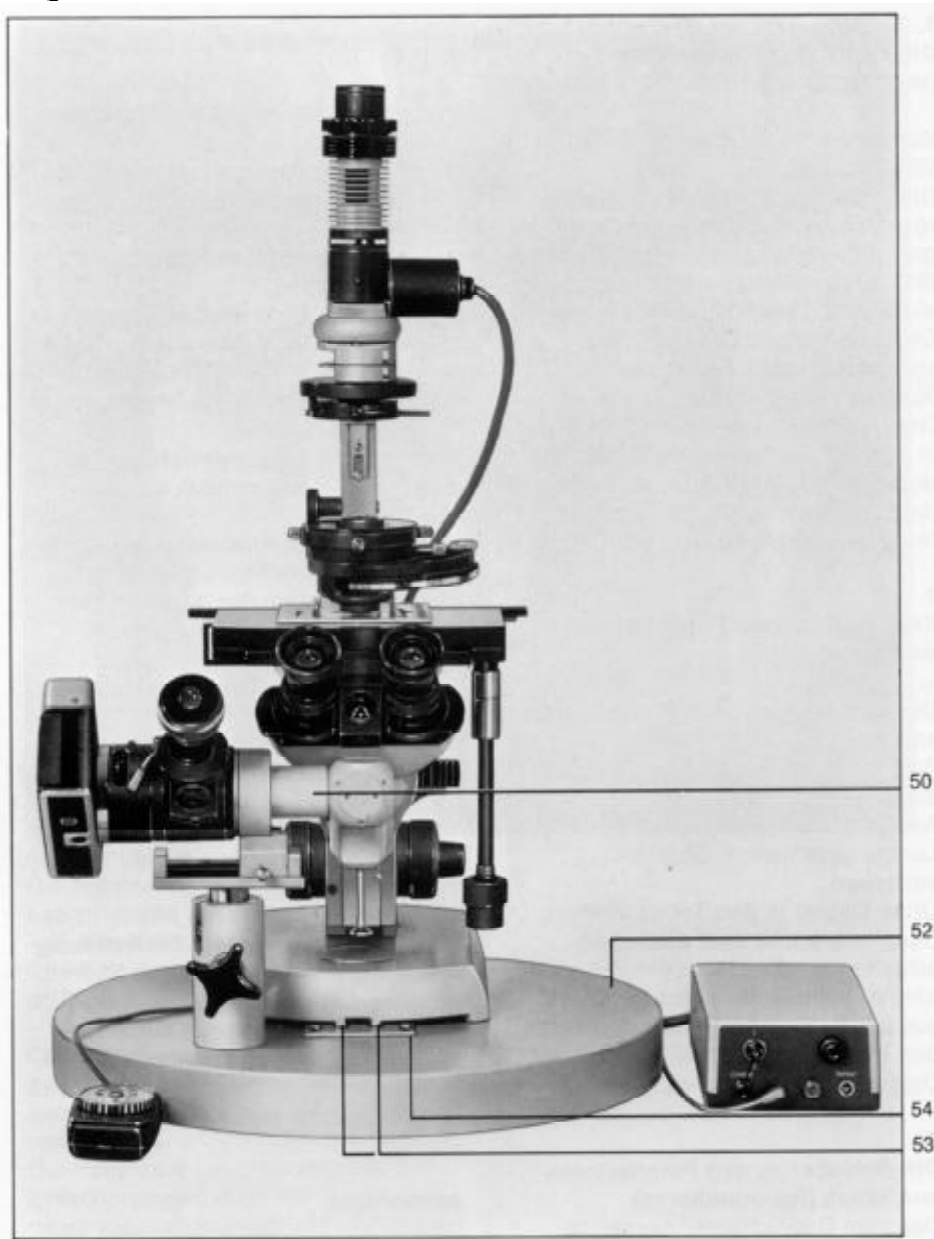
Insert the eyepiece.

Note: If, in spite of the adjustments and settings of the DIC equipment described here there is only low contrast, check the DIC prisms in the turret for correct adjustment: the pin must be set to the white line (85), **not** to the white dot!

If the lid (82) cannot be pulled out, loosen slot screw (86) which fixes the condenser to the centering piece, remove the condenser from the centering piece, and pull out the lid. When fixing the condenser again to the centering piece, make sure that the turret housing is oriented as before the removal.

The DIC adapter ring increases the distance between specimen and flange of the revolving nosepiece by 11 mm, i. e. the entire microscope tube (or the camera column) must be lowered by this amount from the setting for examinations without DIC adapter ring. To ensure equal height of all objectives, equip also those objectives with DIC adapter rings, which are not used for DIC.

3. Photomicrographic system



Assembly and adjustment of the photomicrographic system

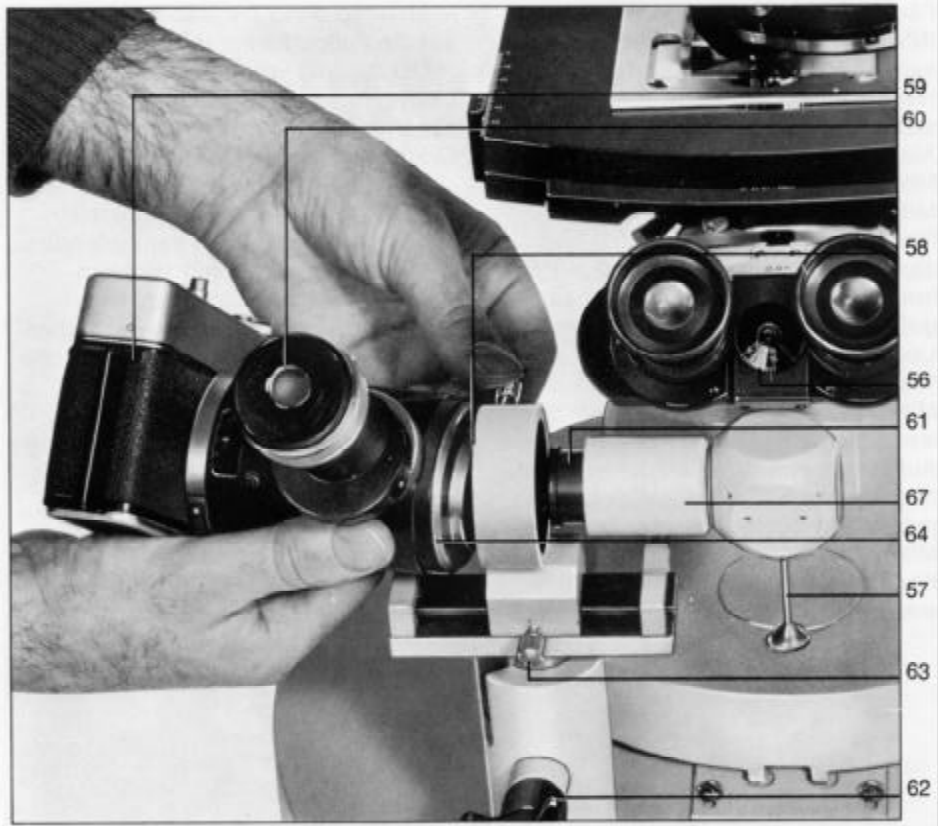
All photomicrographic cameras can be used for photomicrography with the INVERTOSCOPE. Operation and specifications see operating instructions G 41-410. Light sources: 6 V 15 W tungsten filament lamp, lamp unit 60 W (G 41-305), and micro flash II (G 41-330). All these light sources can be mounted on column (22) with the lamp holder (6). A condenser is required for photomicrography to fully utilize the resolving power of high-power objectives. Any of our transmitted light condensers on condenser carrier (16) can be used for the purpose.

Place the microscope without column (22) on the base plate with holder 47 61 00 (52), so that the two pivots (53) of the microscope base fit into the boreholes of the plate (54).

Connect INVERTOSCOPE and base plate from below by two screws. Tighten the screws only slightly so that the microscope can still be moved on the base plate. The plate (54) should also be movable.

Slide column (22) into the dovetail guide and tighten the clamping screw (21).

Exchange the binocular tube for the 90° binocular photo tube (55).



Adjust the interpupillary distance on the binocular tube. Set both eyepieces to the same value on the small disk (56). Push in lever (57) — all light is directed to the eye — and adjust the specimen image.

Mount the photomicrographic camera on the dovetail of holder (58) and clamp it.

Align the camera housing (59) parallel to the focusing eyepiece (60). (Red dot opposite red dot).

Pull lever (57) downwards. The prism directs all light to the camera. Insert eyepiece, e. g. Kpl 10 x (61) in the photo tube.

After loosening knob (62), vertically adjust the photomicrographic camera so that the optical axes of the eyepiece (61) and of the optical system of the photomicrographic camera coincide (coarse centering). After this centering tighten knob (62).

Loosen clamping screw (63), move the camera on the slide to the right until the basic body (64) almost touches the eyepiece (61).

Clamp screw (63).

View the image through the focusing eyepiece (60).

Microscope and camera are correctly adjusted, when there is no vignetting of the image field, and when the image of the centered lamp field stop is centered in the field of view. If this is not the case, adapt the camera height to the exit pupil of the eyepiece (61). Move the light-tight sleeve (67) towards the photomicrographic camera to the stop.

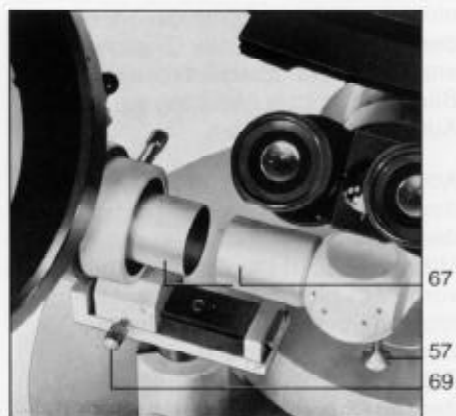
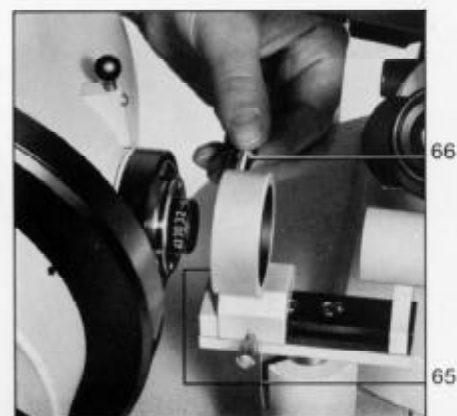
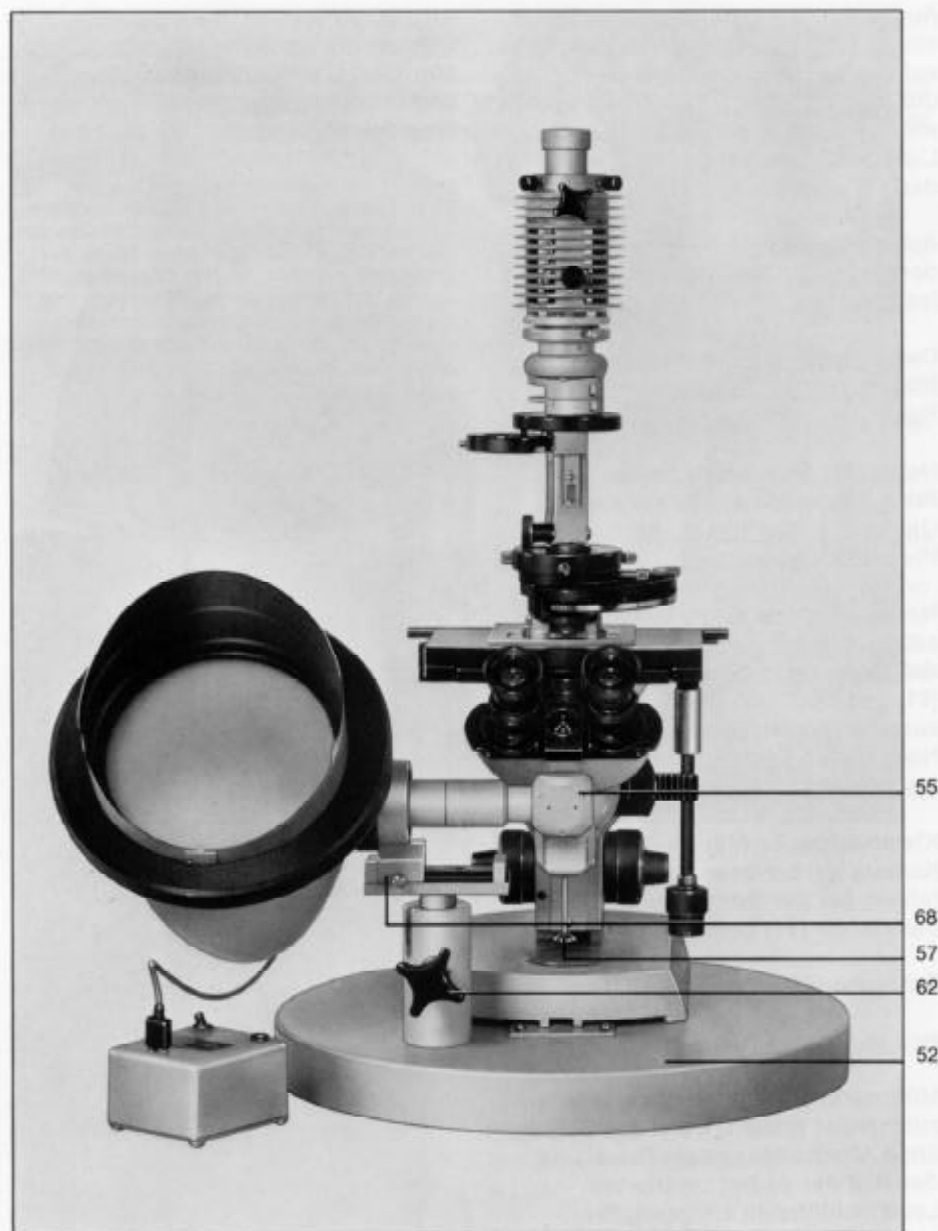
Eliminate vignetting of the image field by vertically moving the column or by moving the microscope on the base plate back and forth.

If the image field in the binocular tube is not correctly centered with regard to the image field in the focusing eyepiece, turn the entire microscope around its vertical axis to remedy the fault (precision centering). Fix microscope and plate (54) on the base plate.

If the aforementioned faults cannot be corrected by the indicated measures, please ask our representatives for advice. If microscope and camera are supplied together, correct centering is ensured by the fixed plate (54).

4. Projection attachment

with projection lens (43 30 32-8031)



With the projection attachment (43 30 32), the INVERTOSCOPE can be converted into a projection microscope (operation of the projection attachment see G 41-476). The magnification factor of the projection attachment is 16. (Total magnification = objective magnification x 0.8 x 16).

The base of the instrument is the base plate with holder 47 61 00 (52). Align the INVERTOSCOPE on the base plate and fix it according to section "Assembly and adjustment of the photomicrographic system" on page 21.

Exchange the normal binocular tube for the 90° binocular photo tube (55). Remove the eyepiece from the 90° eyepiece sleeve. (The optical system built into the projection attachment replaces the eyepiece). Mount the projection attachment on the dovetail (65) of the carrier arm and fix it with clamping screw (66).

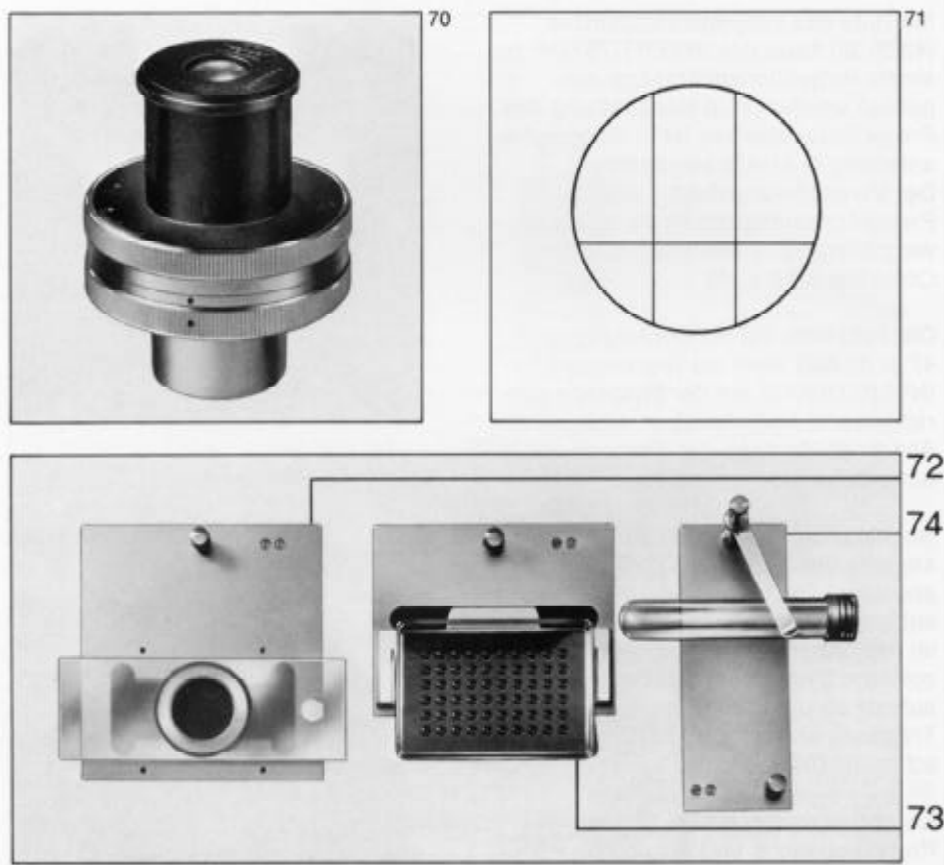
Plug the light-tight sleeves (67) on the projection lens and the 90° tube.

Push in lever (57), and adjust the image of the specimen through the binocular tube. A high-power light source, e. g. lamp unit 60, is required for projection.

Pull out the lever (57) and observe the projection image. If microscope and projection attachment are not correctedly adjusted, loosen screw (62), adjust the projection attachment vertically, and tighten clamping screw (62).

Move the slide (68) until the image on the projection screen has the same sharpness as that viewed through the binocular tube. The correct slide position is marked by a lockable limit stop (69).

5. Accessories for plankton examinations



8x Kpl eyepiece with adjustable counting lines (46 39 70) (70)

Use this eyepiece on monocular tube 47 30 00 only.

Insert into tube when both red dots are opposite each other.

Lock the eyepiece in the tube by turning the knurled ring.

Turn the eyelens of the eyepiece from the outside to the inside until the lines in the field of view are sharp.

Two parallel lines limit the counting line (71) whose width is variable. Turn the eyepiece in the tube to adjust the lines parallel to one of the motion directions of the transverse slide.

While moving the transverse slide, count all (plankton) particles within the two parallel lines when they cross the horizontal line in the field of view.

Count particles which are divided by the parallel lines only once to avoid double counts.

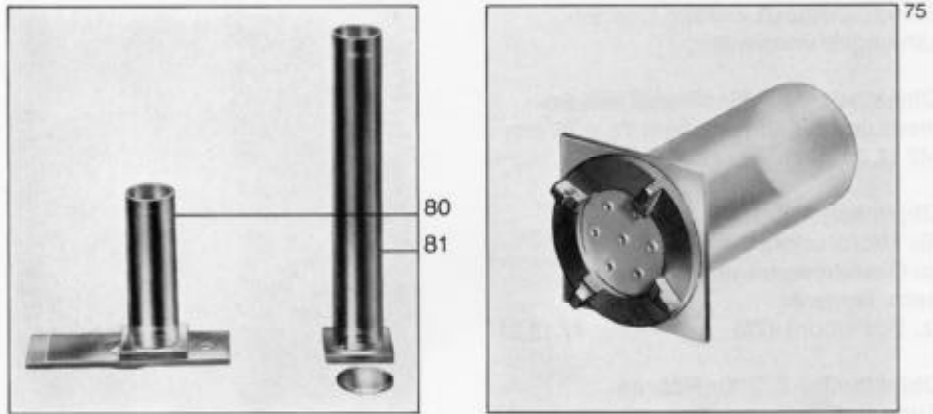
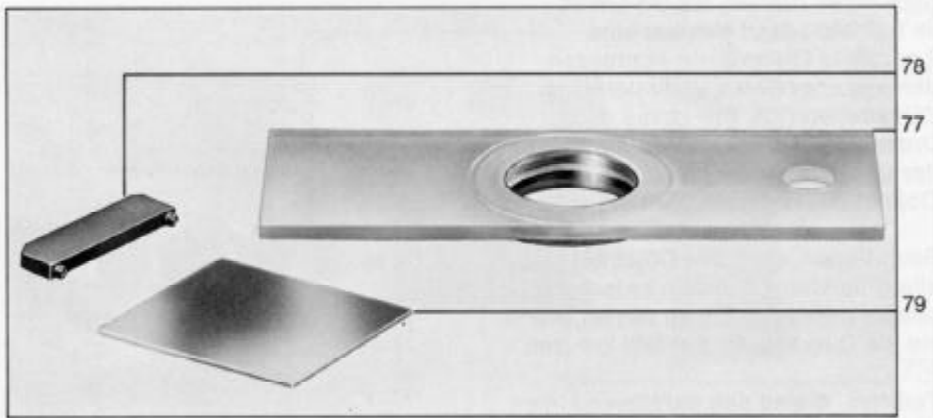
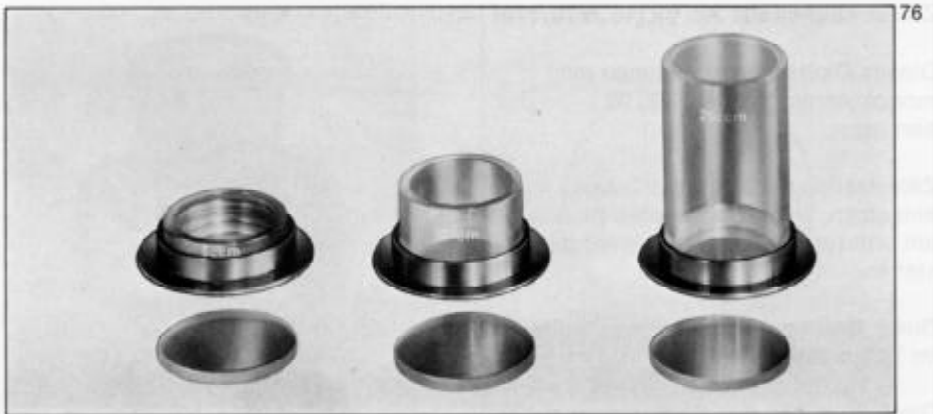
Specimen holder for plankton counting chambers and for 26 x 76 mm specimen slides (72) 47 12 50

Specimen holder Z/1 for micro test plates for Terasaki tissue compatibility tests (e. g. Falcon) (73) 47 12 51

Specimen holder Z/2 for cylindrical chambers and test tubes (74) 47 12 52

Rotatable specimen holder 47 34 90

Stage clip 47 33 72



Vessels for plankton examinations

Place the funnel chamber (47 86 20) (75) on the vessel and pour in the sample.

Cylindrical, plate and combined chambers

The size of these chambers for sedimentation and counting must correspond to the sample volume. Small chambers are sufficient for samples containing much plankton. The chamber should not be larger than absolutely necessary, because otherwise the counting requires too much time. Because of the fluctuation of the plankton content, several chambers of different sizes are required.

Cylindrical chambers (76)

They are closed at the bottom by a 0.2 mm thick glass plate which for cleaning can be removed after unscrewing the securing ring. Two spare plates 47 86 09 belong to each cylindrical chamber. It is closed on top by a cover plate.

Cylindrical chamber with cover plate 5 cm ³	47 86 00
Cylindrical chamber with cover plate 10 cm ³	47 86 01
Cylindrical chamber with cover plate 25 cm ³	47 86 02

The **plate chamber** 47 86 19 (77) is closed at the bottom by a 0.2 mm thick plate.

Spare bottom plate for cylindrical or plate chamber (0.2 — 0.3 mm thick)	47 86 09
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Spare bottom plate for cylindrical or plate chamber for immersion objectives (0.16 — 0.18 mm thick)	47 86 10
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To exchange the bottom plate, unscrew the securing ring with the socket wrench (78), exchange the bottom plate and screw on the securing ring.

During sedimentation close the plate chamber with a square glass cover (79).

Combined chambers (80) consist of two parts, i. e. the plate chamber described above as the lower part, and a cylinder (81), open at both ends, with a square flange at the bottom as the upper part. The chamber is closed by a cover plate.

Combined chamber 10 cm ³	47 86 11
Combined chamber 50 cm ³	47 86 13
Combined chamber 100 cm ³	47 86 14

6. Sample preparation for plankton counting

To ensure reliable results, the plankton particles must be evenly distributed. When after fixation and shaking the sample is poured into the chamber, the plankton is unevenly distributed after sedimentation. Especially with flat chambers this is almost eliminated by the **funnel chamber (75)**. The flowing liquid is dispersed by 1.5 mm dia. nozzles into several thin jets. The liquid settles more quickly.

Place the funnel chamber on the vessel and pour in the sample. To avoid additional streams, use only so much sample fluid that it forms a vault without spillage through the four recesses of the funnel chamber. Close carefully with the cover plate directly from above (not from the side or obliquely).

To fill the **cylindrical chamber (76)**, place it in a Petri dish, shake the storage bottle with the plankton sample, and pour the sample into the chamber to the point of overflow. Close the chamber with the cover plate and dry it with a soft cloth or with tissue paper without shaking it. Let the plankton settle for at least 24 hours. Then place the cylindrical chamber into the corresponding recess of the specimen stage.

The **combined chamber (80)** consists of a plate chamber and a cylinder. To fill it, place the lower part on a mirror glass plate with the thin bottom plate down. Press the cylinder firmly on to the plate chamber, and pour in the sample (shake well before) through the funnel chamber. Remove the funnel chamber and close the chamber with the cover plate. Let the plankton settle, which takes about 24 hours.

For counting under the microscope, separate cylinder and plate chamber without stirring the sediment. With a cover plate move the cylinder slowly and evenly towards the borehole of the plate chamber where it is emptied. The cover plate closes the plate chamber as the cylinder moves sideways.

When electrolytic water or plankton samples settle for a longer time, lime or salts may deposit between cylinder and plate chamber. To avoid this, grease all contact surfaces with a very thin film before mounting the cylinder. This is not necessary with soft water.

Air bubbles may deposit on the chamber walls, especially when the water heats up during sedimentation. These air bubbles can be avoided when the samples are warmed up beforehand.