Epi - fluorescence condenser III RS

Operating Instructions

G 41 - 351 - e

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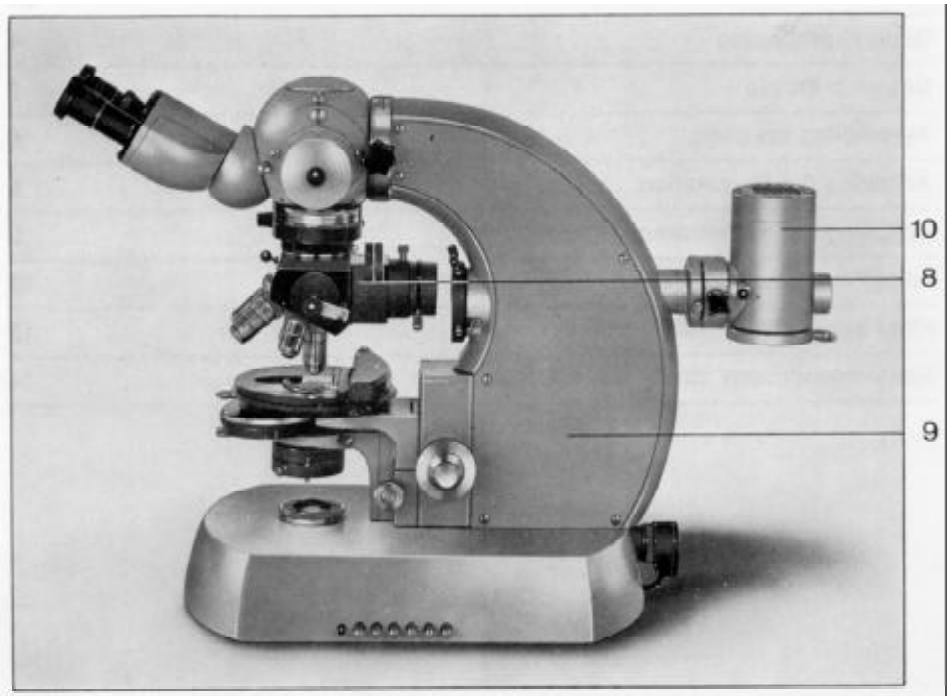
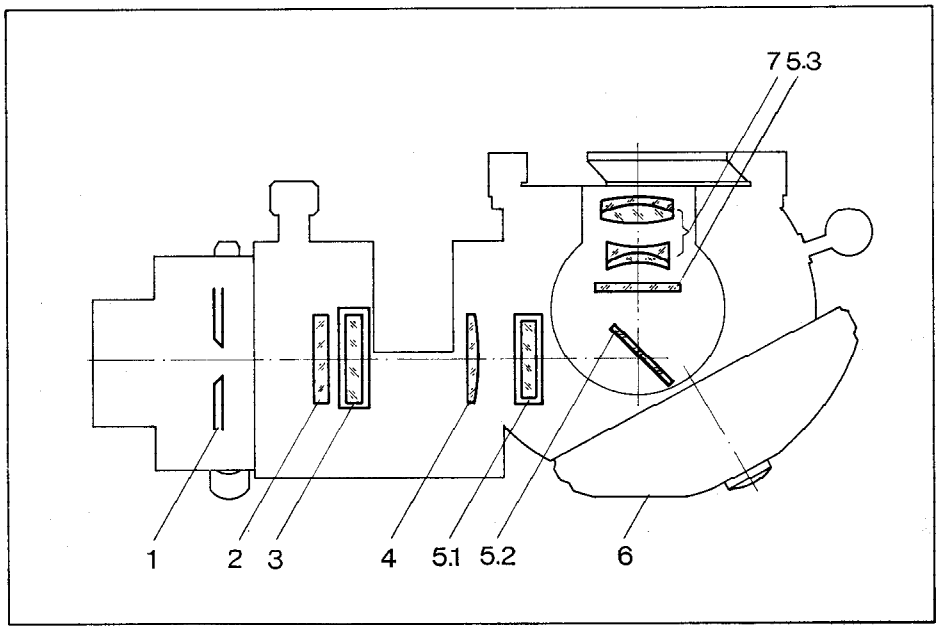


The epi-fluorescence condenser III RS (8) converts the large research microscopes UNIVERSAL (9), Photomicroscope, and Ultraphot into epi-fluorescence microscopes, to examine transparent specimens on slides, and opaque specimens as well.

The condenser III RS contains a slide with 4 filter sets for UV, violet, blue and green excitation. Other filter sets are easily compiled.

Depending on the application, there is choice of the following light sources: HBO 50 W, HBO 100 W/2, HBO 200 W/4 high-pressure mercury sources, XBO 150 W/1 xenon source, CSI 250 W metal halide source, and 12 V 100 W filament lamp (especially for FITC excitation). Depending on which light source is chosen, use the lamp housings 100 (10) or 250.



1. Lamp field stop

2. Heat-absorbing filter KG 1

3. Slide with 3 positions:
position 1 blocks the light passage
position 2 takes red-attenuation filter BG 38
position 3 is free for other filters of your choice

4. Collective lens

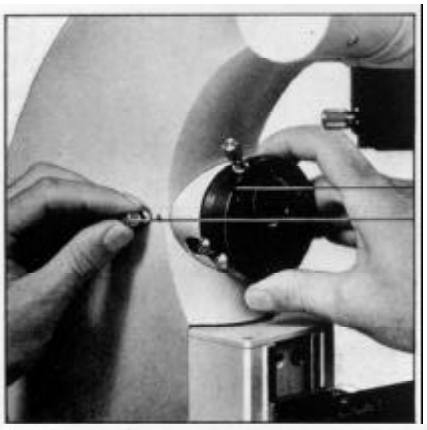
5. Slide holding a set of:
5.1 4 exciter filters on slide
5.2 4 reflectors (plane glasses 45° acting as chromatic beam splitters)
5.3 4 barrier filters on slide

6. Quintuple revolving nosepiece

7. Telan lens system with 1.3 magnification factor

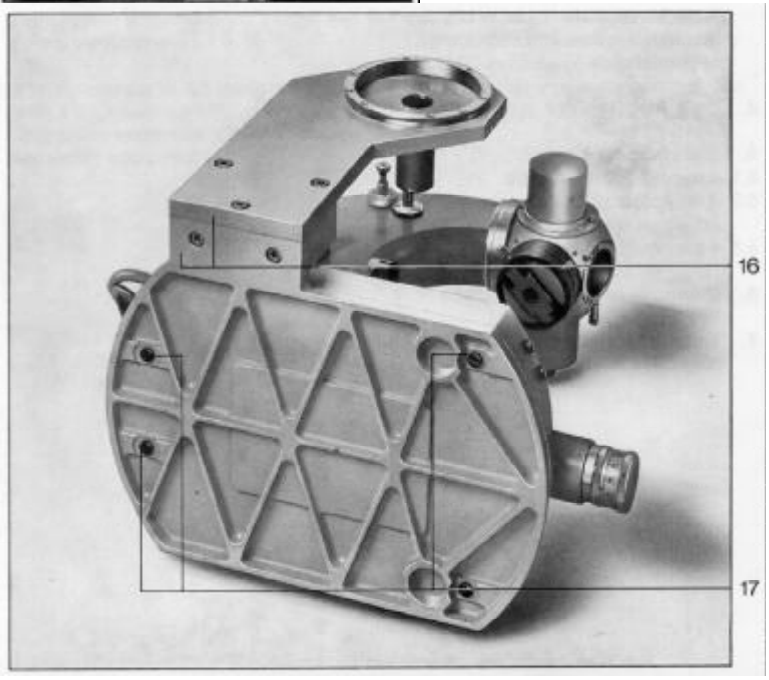
Assembling the fluorescence microscope with epi-fluorescence condenser III RS

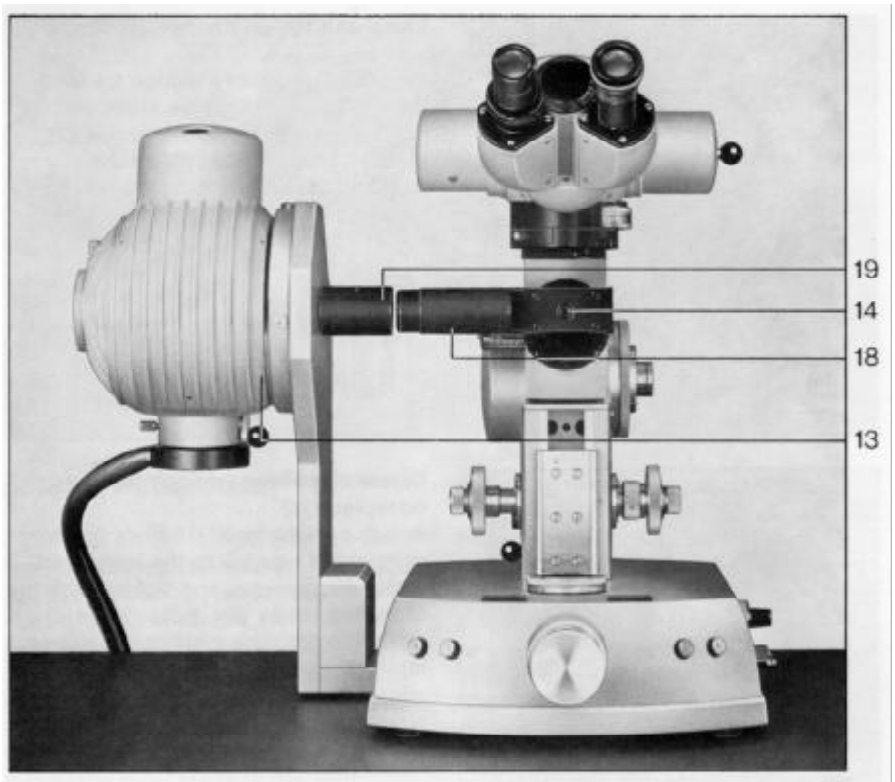
Insert reflected light aperture stop insert (47 20 71) (11) in stand of UNIVERSAL or Ultraphot, and fix with clamping screw (12). Attach the microscope illuminator 100 or 250 to the corresponding dovetails of the microscopes (see corresponding operating instructions).



Microscope illuminator 250 (13) with lateral light reflection (14) on Photomicroscope (15)

Tilt Photomicroscope and unscrew the four rubber supports. Fix the base plate with lateral holder (46 72 89) (16) with four screws (17) from below to the microscope base. With Photomicroscope II use in addition four washers (46 72 89-0107) to compensate for the lower base, and fix with four cylinder screws M 6 x 50 DIN 912-8.8. Set up Photomicroscope.
Insert the oriented aperture stop insert (14) with lateral light reflection in the stand and fix it.
Connect light-tight sleeve (18) to tube (19).
Mount microscope illuminator 250 (13) on dovetail and fix it.
If necessary, screw light shield (20) to the lateral holder.

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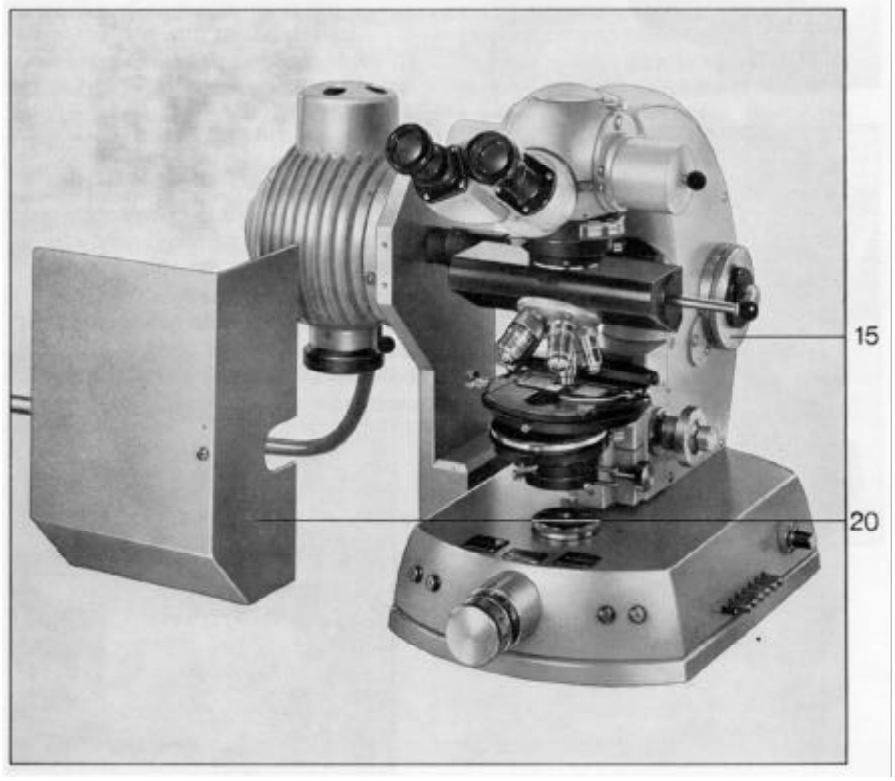
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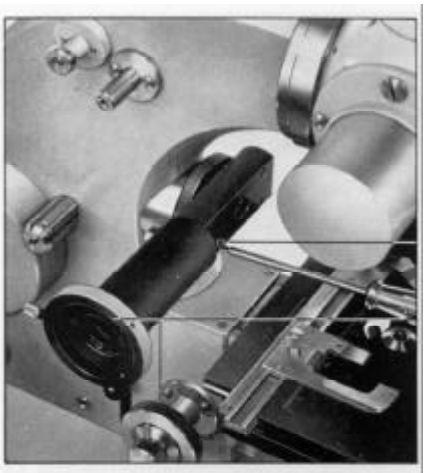
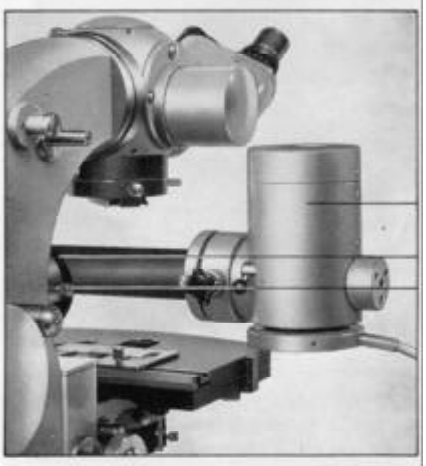


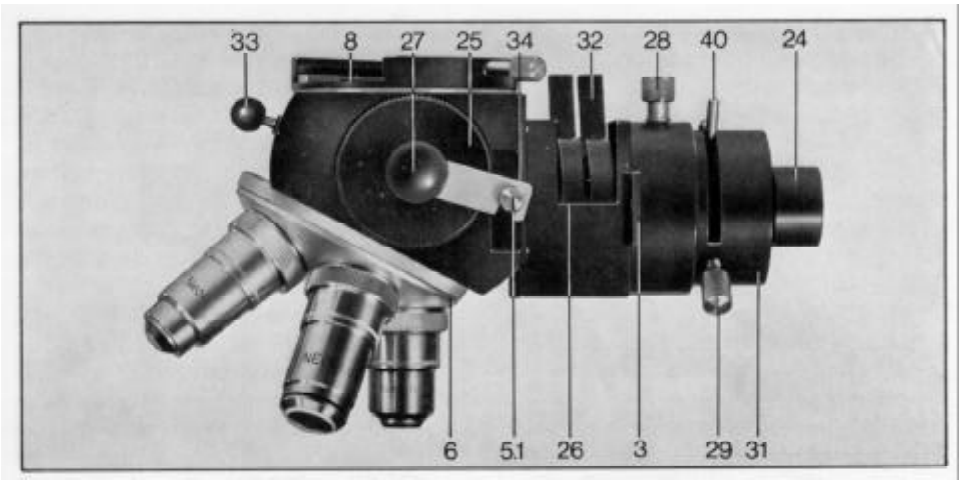
Lamp unit 100 on Photomicroscope

Insert lateral light reflection for lamp unit (46 70 34) (14) in the stand and fix with one socket head cap screw (21). Mount lamp unit 100 (10) on the dovetail (22) and fix it.



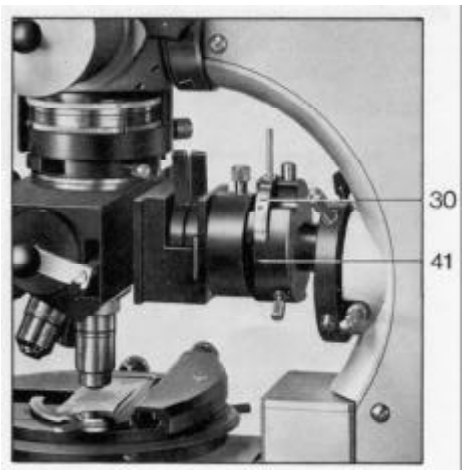
Screw objectives into quintuple nosepiece (6).
Attach condenser III RS from the front right to the rear left to the tube head of the microscope, and tighten with clamping screw (23). Note: when using the aperture stop insert with lateral light reflection on the Photomicroscope, unscrew the light-tight sleeve (24) before mounting the condenser III RS.

The Planapochromat 4/0.16 (46 02 40) is not intended for use with the epi-fluorescence condenser III RS. If, in spite of that, it is to be employed with it, it must be screwed only into the aperture of the nosepiece which is just in the beam path.
Caution! Do not rotate nosepiece after this as otherwise the parts of this objective which project into the nosepiece will be damaged.



Adjusting the illumination

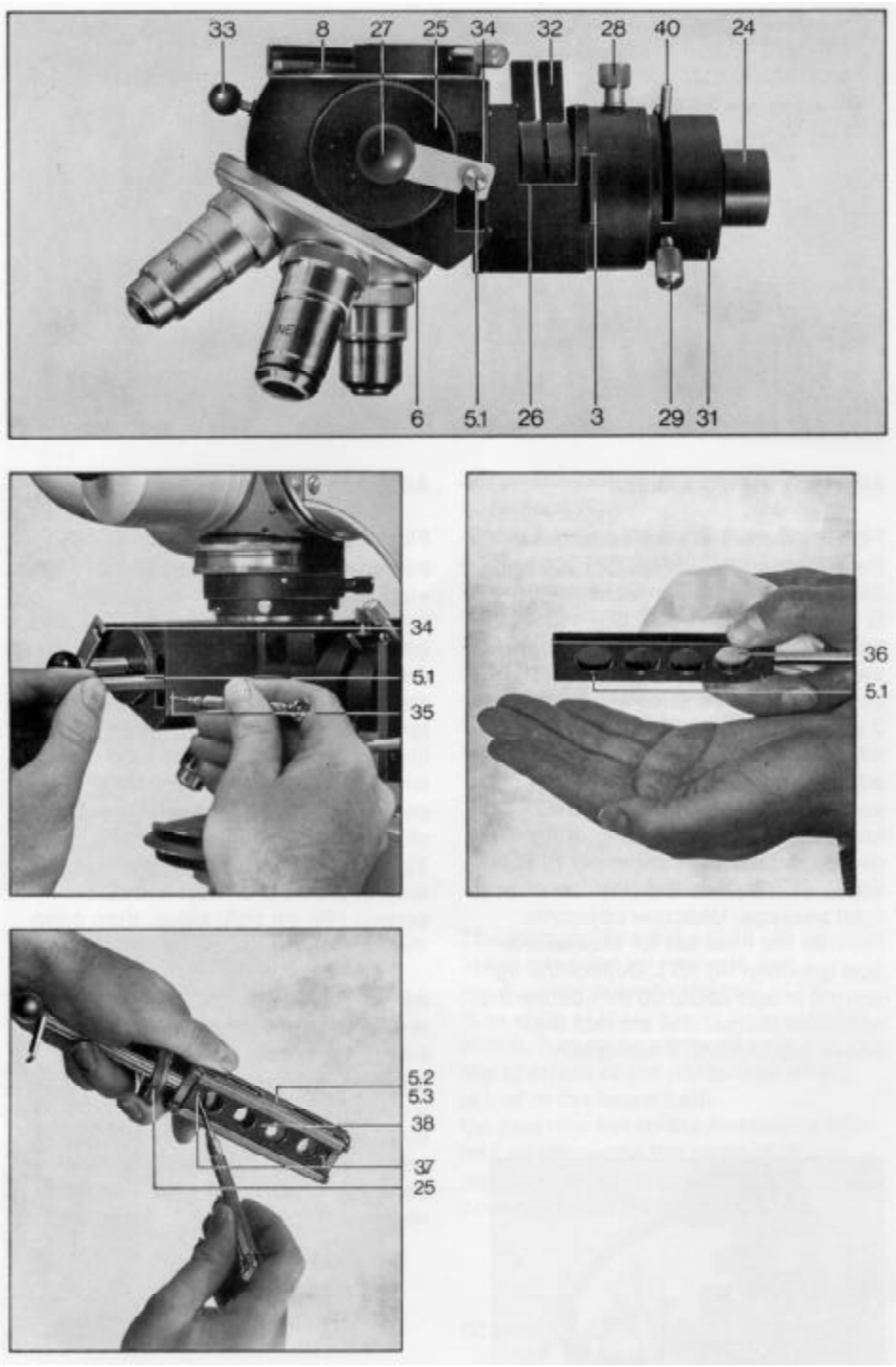
For insertion of the light sources in the microscope illuminators 100 and 250 see operating instructions G 41-310 and G 41-320. Connect the light source to its power supply and switch it on. Without condenser III RS, collect the light source image at about 2 m distance from the microscope; with the focusable lamp condenser, adjust the focal spots of the light sources and center them in the luminous field with the centering device. Attach the condenser III RS (8). Set the slide (3) to the position of free light passage. Unscrew objective. Provide the filter set for blue excitation (position III) (27). Collect the light source image about 20 mm below the objective flange, and correct the above adjustment, if necessary.



Adjusting the fluorescence image

Fluorescence observation will be easier when you observe the following: start with a low-power objective, preferably a NEOFLUAR 10/0.30, and use a strongly fluorescing specimen (adjust first to the paper label many specimens have, because this facilitates assessment of the uniformity of illumination). Close lamp field stop with lever (40). After loosening clamping screw (28), shift lamp field stop insert and focus the stop. Tighten clamp. Center the lamp field stop in the field of view with the screws (29) on both sides, then open the stop to the rim of the image field.

After loosening clamp (28), the centerable lamp field stop insert (31) can be exchanged for the centerable diaphragm insert (46 62 52) (41) with 6 openings in its turret (30) for diaphragms of the set of 8 (47 73 80). Insert the diaphragms in the turret, corresponding to the luminous field required for fluorometry.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The heat-absorbing filter (2) (see diagram on page 5) is a KG 1 solid glass filter. It should remain constantly in the light path for protection of the other filters and the specimens, so there is no facility for removing it. It is easily exchanged, if necessary, however, by removing the lamp field stop insert.

The light-tight and filter slide (3) has two free passages for filters of 18 mm dia. The BG 38 red-attenuation filter is supplied with the basic equipment. The second opening can take any filters of 18 mm dia. up to a thickness of 5 mm, such as the KP 600 or KP 560 minus-red interference filters. These eliminate the red more stringently than the BG 38 attenuation filter, but have reduced UV transmittance.

Additional exciter filters can be inserted in the filter slots (26). For filters with 32 mm dia. the holding ring (46 72 52) should be used; for those with 18 mm dia. the adapter for 18-to-32 mm dia. (46 78 93) as well. Maximum filter thickness for either holding ring is 5 mm.

Exciter filters, beam splitters and barrier filters are mounted in a slide which contains 4 filter sets, 2 of which can be rapidly exchanged. Pull knob (33) to bring in the other two filter sets alternately. When the individual filter sets are to be brought into the beam path successively, pull out knob (33) and turn it to the left; this overruns the stop position. In the basic equipment, the arrangement of the filters is such that with increasing wavelength the different types of excitation follow each other (see table on page 12).

All 18 mm dia. exciter and barrier filters listed in the table are easily exchanged. Unlatch lever (34) and loosen screw (35); the exciter filter slide (5.1) can be pulled out.

With a linnen cloth push the filter through the wider opening (36) and insert the new one. Exciter filters may be up to 6 mm thick. When using exciter filter sets, insert the interference filter first (coated surface towards the light source), then color glass or sandwich filters. Then insert slide (5.1); make sure that the filter flanges point towards the light source.

Unscrew cover (25) to pull the reflector-and-barrier-filter slide (5.2, 5.3) from the tube of the condenser III RS. After loosening screws (3), remove cover (38), and exchange the barrier filters. The barrier filters may be up to 2.2 mm thick.

The reflectors are multi-layer interference filters with steep edges, which are rigidly mounted and adjusted in our factory. We recommend to keep these beam splitters in their positions. Reflectors should be exchanged in our factory or in the workshops of our representatives only.

Filter sets for epi-fluorescence condenser III RS

Sets included in standard equipment

Methods Applications	Filter designation	Cat. No.	Filter type or function
Position I UV excitation for auto- fluorescence — SITS after Ploem BAO after Ruch, etc.	UG 1 UV-transmittant black glass FT 420 chromatic splitter LP 418 colorless UV barrier filter	 46 79 68 46 63 02 46 78 61	 exciter filter beam splitter barrier filter
Position II Violet excitation for autofluorescence and conventional fluorochromes	UG 51 violet glass BG 3 blue glass FT 460 chromatic splitter LP 478 yellow filter	46 79 67 46 79 65 46 63 03 46 78 63	exciter filter exciter filter beam splitter barrier filter
Position III Blue excitation for acridine orange, auramine and other conventional fluorochromes, FITC	BG 12 blue glass FT 510 chromatic splitter barrier filter 50	46 79 66 46 63 04 46 78 65	exciter filter beam splitter barrier filter
	alternately: blue interference filter set 455 . . . 490 FT 510 chromatic splitter LP 520 orange filter	 42 79 02 46 63 04 46 78 73	 exciter filter beam splitter barrier filter
Position IV Green excitation (selective), rhodamine derivatives MPS after Ploem Feulgen fluorescence	BP 546/7 green interference filter set FT 580 chromatic splitter LP 590 red filter	 42 79 01 46 63 05 46 78 69	 exciter filter beam splitter barrier filter

Special sets

Methods Applications	Filter designation	Cat. No.	Filter type or function
Selective SITS excitation and observation	UG 1 UV-transmittant black glass	46 79 68	exciter filter
	FT 420 chromatic splitter	46 63 02	beam splitter
	LP 418 colorless UV barrier filter	46 78 61	barrier filter
	KP 560 minus-red interference filter	46 79 62	barrier filter
Selective violet excitation, especially for detection of biogenous amines after Falck	BP 405/5 violet interference filter	46 79 51	exciter filter
	BP 405/14 violet interference filter	46 79 52	exciter filter
	FT 420 chromatic splitter	46 63 02	beam splitter
	LP 418 colorless UV barrier filter	46 78 61	barrier filter
Selective blue-violet excitation, especially for chromosome fluorescence after Caspersson	BP 436/5 blue-violet interference filter	46 79 53	exciter filter
	BP 436/17 blue-violet interference filter	46 79 54	exciter filter
	FT 460 chromatic splitter	46 63 03	beam splitter
	LP 478 yellow filter	46 78 63	barrier filter
Selective FITC excitation and observation	blue interference filter set 455 . . . 490	42 79 02	exciter filter
	FT 510 chromatic splitter	46 63 04	beam splitter
	green interference filter set 520 . . . 560	42 79 03	barrier filter
Selective rhodamine excitation and observation	BP 546 green interference filter set	42 79 01	exciter filter
	FT 580 chromatic splitter	46 63 05	beam splitter
	LP 590 red filter	46 78 69	barrier filter
	BG 18 red-attenuation filter	46 79 92	barrier filter

KP = shortwave pass filter which transmits light to a cut-off given by the wavelength with the transmittance 0.5

LP = longwave pass filter which transmits light from a cut-off given by the wavelength with the transmittance 0.5

BP = band pass filter which transmits light of a wavelength range limited by cut-offs on both sides. The first figure is the wavelength of the peak transmittance, the second the approximate bandwidth in nm.

In the interest of technical progress we reserve the right to alter the filter selection.

With the condenser III RS the fluorescence exciting radiation is directed from above on to the specimen through the same objective used for observation, i. e. reflected light brightfield excitation. This method results in two exceptional features in practice:

1. The objectives must have sufficient transmittance for the exciting radiation and be themselves weakly fluorescing.
2. Illumination aperture and observation aperture are identical.

The conditions under 1) are fulfilled by all Achromat and NEOFLUAR objectives. With the more highly corrected Planachromats and Planapochromats, some may be restriced in their application.

It follows from point 2) that low-power objectives with smaller aperture are less suitable. If there is a choice of equal-power objectives with varying aperture, the one with higher aperture is recommended. NEOFLUAR 10/0.30 is thus preferable to Achromat 10/0.22 and NEOFLUAR 40/0.75 preferable to Achromat 40/0.65.

In general the NEOFLUAR series is particularly to be recommended for reflected light brightfield excitation.

In fluorescence microscopy with the condenser III RS it is by no means necessary to use exclusively low-power eyepieces. Here, as with other methods, the best observation conditions are provided by medium-power eyepieces of about 8 to 12.5x magnification.

Epi-fluorescence microscopy can easily be combined with transmitted light methods such as phase contrast, darkfield, differential interference contrast and polarization. There is no restriction on the choice of transmitted light condenser.