



Carl Zeiss

Geschäftsbereich
Mikroskopie
7082 Oberkochen

**Epi-fluorescence
condenser IV FI**

Operating instructions

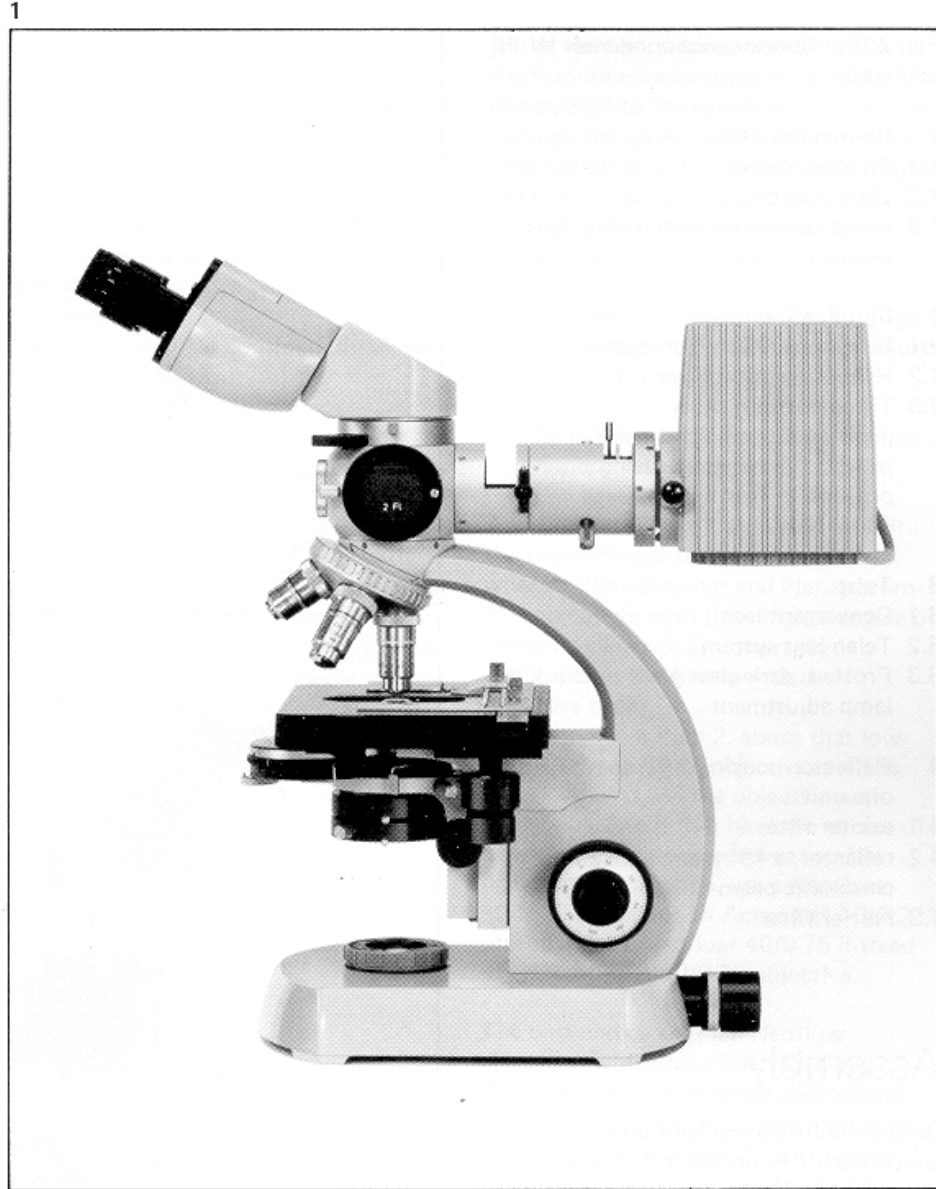
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Every Standard microscope can be converted into a fluorescence microscope for incident-light excitation with the epi-condenser IV FI (46 63 00-9901)¹⁾. Although primarily suited for transparent specimens on slides, it can be used to examine opaque specimens as well.

Depending on the application, you can choose from the following lamp types as exciting-light sources: HBO 50 W AC (38 16 19) or HBO 100 W/2 (38 00 18-4060) high-pressure mercury lamp, XBO 75 W/2 (38 00 79-9190) high-pressure xenon lamp, or 12 V 100 W halogen lamp (38 00 59-1660). Microscope illuminator 100 (see operating instructions G 41-310/III) is used as lamp housing of all above-mentioned lamp types.

A special feature of epi-fluorescence condenser IV FI is a 2-position sliding sleeve in the tube, which accepts reflector housing 2 FI with 2 complete filter sets each (see Table on p. 7).

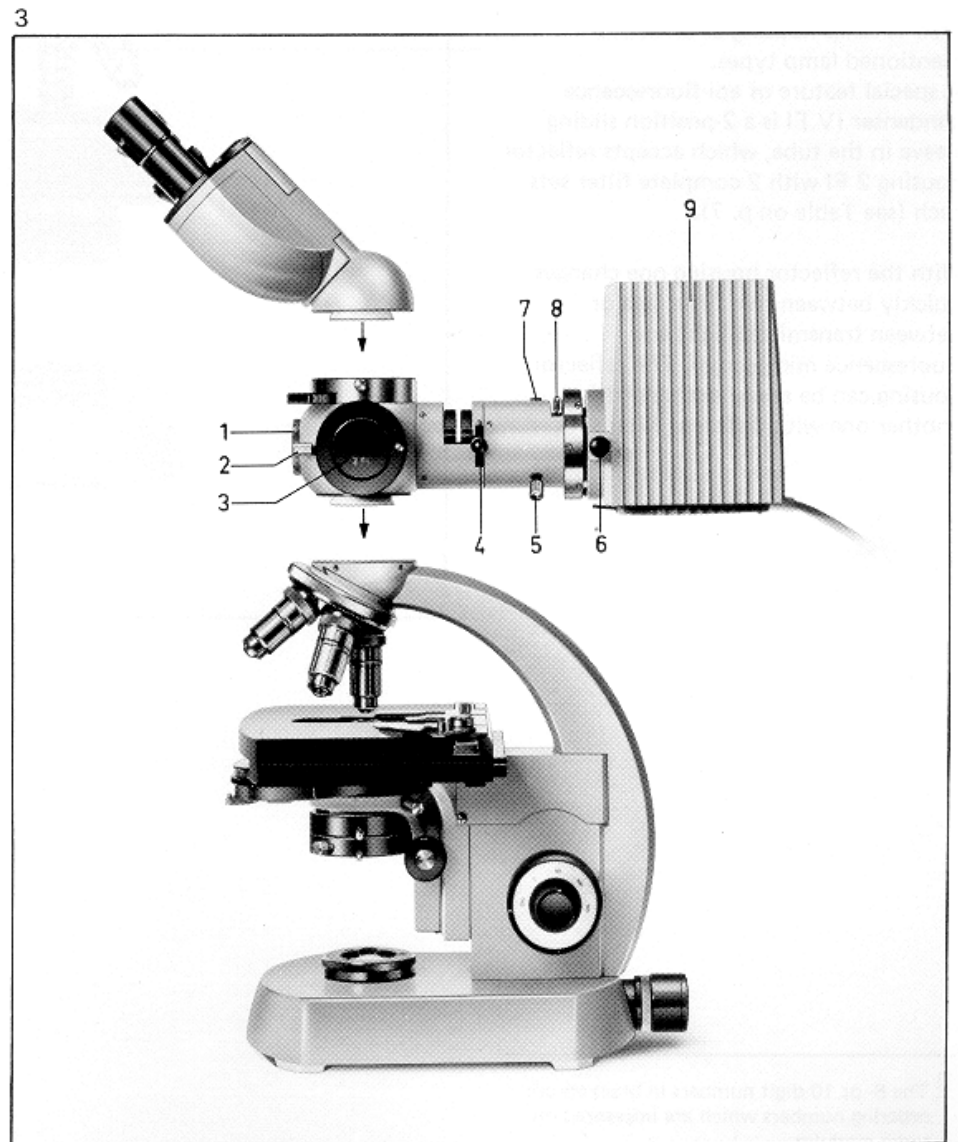
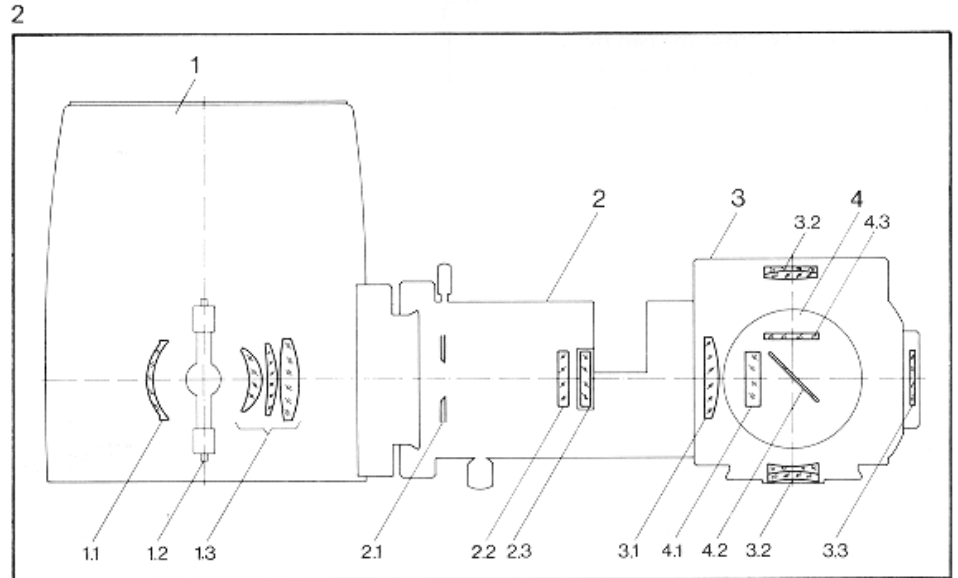
With the reflector housing one changes quickly between two filter sets or between transmitted-light and fluorescence microscopy. The reflector housing can be easily exchanged for another one with different filter sets.



¹⁾ The 6- or 10-digit numbers in brackets are ordering numbers which are imprinted on some modules.

Fig. 2: Epi-fluorescence condenser IV FI, schematic

- 1 Illuminator 100
 - 1.1 Concave mirror
 - 1.2 Light source
 - 1.3 Lamp condenser with several lens elements
- 2 Illuminating tube
 - 2.1 Luminous field diaphragm
 - 2.2 Heat-absorption filter
 - 2.3 Three-position slider
 - position 1: no light passage
 - position 2: red-attenuating filter
 - position 3: free light passage (filter holder)
- 3 Tube
 - 3.1 Convergent lens
 - 3.2 Telan lens system
 - 3.3 Frosted, dark glass plate to check the lamp adjustment
- 4 Reflector housing 2 FI, combining in one unit:
 - 4.1 exciter filter
 - 4.2 reflector, a 45° plane glass acting as chromatic beam splitter
 - 4.3 barrier filter



Assembly

Before mounting the epi-fluorescence condenser IV FI on the microscope, fit illuminator 100 (3.9)¹⁾ to dovetail ring of illuminating tube and secure with clamping screw. Insert the entire unit in the tube port of the microscope, and mount the viewing tube on top. Secure all dovetail connections with knurled screws. Slide reflector housing 2 FI (3.3) into sliding sleeve of tube as far as it will go and secure with screw (3.2).

¹⁾ (3.9) means Fig. 3, item 9

Adjusting the illumination with HBO 50 W high-pressure mercury lamp

The filter set for green or light-blue excitation is the best choice for adjustment.

Connect lamp to its power supply and switch ON. The HBO 50 ignites automatically, and is ready for adjustment after 2 to 3 minutes warm-up time.

Set slider (3.4) to position 3 (free light passage). Focus on the feature on groundglass (3.1) by focusing the lamp condenser with knob (3.6). The sharpness of the light-source images can be improved by closing the luminous field diaphragm (3.8). Center direct and reflected light-source images next to one another on groundglass (3.1) by moving the lamp socket vertically and laterally, and by focusing and tilting the concave mirror (Fig. 4) (see also operating instructions G 41-310/III).

The centering of the illumination can be controlled on the groundglass but need hardly ever be corrected. For fluorescence observation change the focusing of the lamp condenser so that the image of the object field is uniformly illuminated.

Adjusting the fluorescence image

Fluorescence observation will be easier when the following hints are observed: Start with low-power objective, preferably Neofluar 10/0.30. Use a strongly fluorescent object. Use as reference for the adjustment at first the paper label which sticks on many specimens. Such specimens help considerably when checking the uniformity of the illumination.

Close luminous field diaphragm with lever (3.8). Focusing of the diaphragm is possible after loosening slot screw (3.7). Center the diaphragm in the field with centering screws (3.5) on both sides. Open and close luminous field diaphragm as necessary; because of the parfocalization of the objectives, the focus of the luminous field diaphragm is maintained when objectives are exchanged.

In the epi-fluorescence condenser IV FI the fluorescence-exciting radiation is directed on to the specimen from above through the objective which is used for observation as well. Incident-light bright-field excitation is thus realized. With this method, the following factors must be considered in practice:

1. The objectives must let pass through sufficient exciting radiation and must have little intrinsic fluorescence.
2. The illuminating aperture is equal to the observation aperture.

Achromat and Neofluar objectives fulfill the requirements under item 1. above, whereas Planachromat and Planapochromat objectives with their higher degree of correction are not as universally applicable as the first-mentioned objective types.

It follows from item 2. above that low-power, low-aperture objectives are less suited. Always use the objective with higher aperture if you have the choice of objectives of equal magnification but different aperture, i.e. use a Neofluar 10/0.30 instead of an Achromat 10/0.22 objective, or a Neofluar 40/0.75 instead of an Achromat 40/0.65 objective.

The best choice are Plan-Neofluar objectives which have been specially developed for fluorescence microscopy. We recommend to use a combination of a high-power objective and a low-power eyepiece, because the brightness also depends on the microscope magnification. But, as with other methods in microscopy, the best observation conditions are achieved with medium-power eyepieces in the range between 8x and 12.5x.

Epi-fluorescence microscopy is possible in conjunction with transmitted-light techniques such as phase contrast, dark-field, differential-interference contrast and polarization. Any transmitted-light condenser can be used for the purpose.

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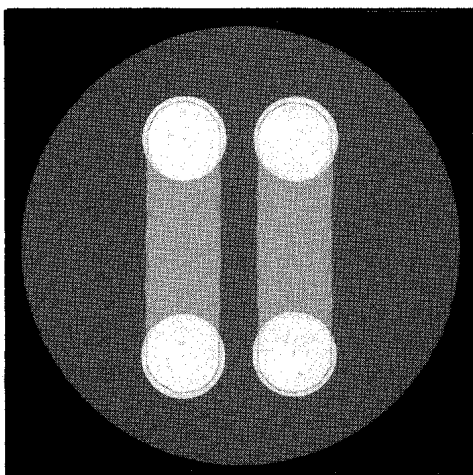
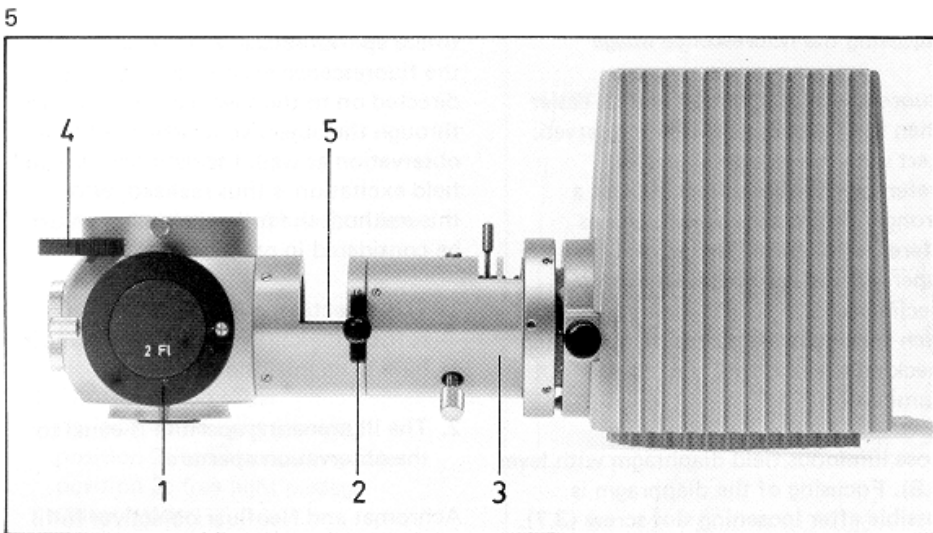


Fig. 4: Centered focal spots of HBO 50 W high-pressure mercury lamp and their mirror image



A solid-glass filter KG 1 is provided as heat-absorption filter (2.2) in the light path to protect the other filters and the specimen. It is easily exchanged by removing the tube part (5.3) carrying the luminous field diaphragm.

The shutter and filter slider (5.2) accepts two 18 mm dia. filters. The basic equipment includes a red-attenuating filter BG 38 in one opening of the slider, whereas an 18 mm dia. filter, max. 5 mm thick fits into the other opening.

Additional exciter filters can be loosely fitted in filter holders (5.5). Use ring (46 72 52) for 32 mm dia. filters, and, in addition, adapter ring (46 78 93) for 18 mm dia. filters. Max. filter thickness for each ring 5 mm.

The most frequently used exciter filters, chromatic beam splitters and barrier filters are exchangeably mounted in reflector housing 2 FI (5.1). All exciter and barrier filters listed on p. 7 are 18 mm in diameter. They are kept in place by plastic rings with dogs. The filter holders accept max. 6 mm thick exciter filters (6.2) and max. 4 mm thick barrier filters. Thinner filters are supplied with spacer rings.

To exchange a filter, disengage both dogs (6.3) from their notches and pull out plastic ring (6.1). The loose filters and spacer rings, if any, can be easily exchanged.

Insert the ring of max. 2 mm thick filters with the seating edge on the inside, and that of thicker filter rings on the outside. If a filter has play, place a spacer ring between filter and ring.

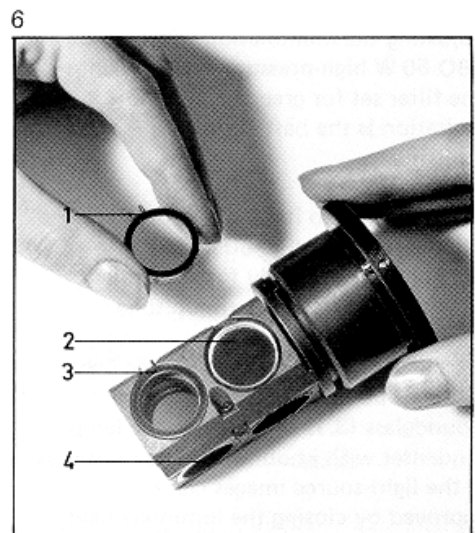


Fig. 6: Filter exchange of double reflector housing; cover plate removed

The 45° interference filters which act as chromatic beam splitters, have diameters of 22 mm. They are loosely fitted in their holders and accessible after loosening two screws. The highly sensitive interference layers being unprotected on the top surface of the beam splitters, these should be left in place.

Shutter and filter slider (5.4) 20x6 mm, above the barrier filters accepts additional 18 mm dia. barrier filters max. 5 mm thick.

Excitation ¹⁾		Filter set	Exciter filter	Chromatic beam splitter	Barrier filter	Cat. No.	Characteristics
Ultraviolet	G 365	02	G 365	FT 395	LP 420	48 77 02	Filter sets with conventional filters (mostly colored glasses), key letter G. Exciter filters with wide transmission range, transmission curve almost bell-shaped. Barrier-filter passband starting further or nearer exciter filter peak transmittance.
Blue-violet	G 436	07	G 436	FT 510	LP 520	48 77 07	
Ultraviolet	H 365	01	BP ²⁾ 365/12 ³⁾	FT 395	LP ⁴⁾ 397	48 77 01	High-performance narrow-bandpass filters (selective excitation) (interference filters), key letter H. Transmission curve of exciter filters adapted to Hg-spectrum lines, with sharp cut-off on both sides. Barrier filter passband starting near exciter filter peak transmittance.
Blue-violet	H 436	06	BP 436/10	FT 460	LP 470	48 77 06	
Blue	H 485	16	BP 485/20	FT 510	LP 520	48 77 16	
Blue IFB ⁵⁾	H 485	19	BP 485/20	FT 510	LP 515	48 77 19	
Blue SB ⁶⁾	H 485	17	BP 485/20	FT 510	BP 515-565	48 77 17	
Green	H 546	15	BP 546/12	FT 580	LP 590	48 77 15	
UV-violet	390-420	18	BP 390-420	FT 425	LP 450	48 77 18	High-performance wide-bandpass filters (most intensive excitation) (interference filters), type of excitation designated by spectral band without key letter. Transmission curve of exciter filters sharp at least for longwave range. Barrier filter passband adjacent.
Blue-violet	395-440	05	BP 395-440	FT 460	LP 470	48 77 05	
Blue	450-490	09	BP 450-490	FT 510	LP 520	48 77 09	
Blue IFB ⁵⁾	450-490	11	BP 450-490	FT 510	LP 515	48 77 11	
Blue SB ⁶⁾	450-490	10	BP 450-490	FT 510	BP 515-565	48 77 10	
Green	515-565	14	BP 515-565	FT 580	LP 590	48 77 14	

1) The filter sets are named by the type of excitation.

2) BP = bandpass filter

3) The number following the stroke indicates the spectral bandwidth

4) LP = long bandpass filter

5) Interference observation

6) Blue excitation with selective passband transmittance of the barrier filter. Use red-attenuating filter BG 38 (5.2) and heat-absorption filter KG 1 (2.2) as supplements in the illuminating light path in case of violet and blue excitations.

The table below lists fluorescence techniques which use time-tested and newly developed fluorochromes, and their fields of application.

Each filter set consists of exciter filter, chromatic beam splitter and barrier filter, included in a double reflector housing (Fig. 6) according to specification.

Different filter sets can be used for the different types of excitation (UV, violet, blue, green). The table on p. 7 lists the filter sets which have supplied the best results. Other sets from the same type of excitation are, however, equally well suited.

Field of application	Method (fluorochrome)	Specifically for	Excitation range	Recommended filter sets
Immunology	FITC (fluorescein isothiocyanate)	detection of antigen-antibody reactions	blue	48 77 09 (general use) 48 77 10 (selective, especially for photography) 48 77 16 48 77 18 (especially for observation)
	Evans blue	red contrasting of FITC stainings	green	48 77 15
	TRITC (tetramethyl-rhodamineisothiocyanate)	detection of antigen-antibody reactions	green	48 77 14 (general use) 48 77 15 (selective)
	Rhodamine B 200 (lissamine-rhodamine B)	detection of antigen-antibody reactions	green	48 77 15
	DNAS (dinaphthyl-aminosulfonic acid)	detection of antigen-antibody reactions	UV	48 77 01 or 48 77 02
Cytology and related fields	BAO (acc. to Ruch) (bisaminophenyl-oxadiazole)	quantitative DNA determination	UV	48 77 01 or 48 77 02
	acriflavine	nucleic acids	blue-violet	48 77 07
	pararosaniline (acc. to Feulgen)	small amounts of DNA (quantitatively)	green	48 77 15
	FIF (acc. to Falck) (formaldehyde-induced fluorescence)	biogenic amines, especially catecholamine	violet	48 77 18
	fluorescamine (FLURAM)	primary amines	UV	48 77 01 or 48 77 02
	mepacrine	thrombocytes	blue-violet	48 77 05
	DAPI	nucleic acid (DNA)	UV	48 77 01 or 48 77 02
	H 33258 bisbenzimidazole Hoechst	nucleic acids	UV	48 77 01 or 48 77 02
	dansylchloride	lysines	UV	48 77 01 or 48 77 02
	sulfaflavine	histones	UV	48 77 01 or 48 77 02
	euchrysin	lymphocytes, leucocytes	blue-violet	48 77 07
	thioflavine S	lymphocytes, leucocytes	blue-violet	48 77 07
	coriophosphine	lymphocytes, leucocytes	blue	48 77 09
	MPS (acc. to Ploem) (methyl green pyronine stilbene)	blood differentiation (double staining)	UV green	48 77 01 48 77 15

Field of application	Method (fluorochrome)	Specifically for	Excitation range	Recommended filter sets
Cells Cell nuclei Cell walls	acridine orange	cell nuclei	blue	48 77 09
	berberine sulphate	cell nuclei	blue-violet	48 77 07 or 48 77 05
	mithramycine	cell nuclei	UV-violet	48 77 18
	phosphine 3 R	cell nuclei	blue-violet	48 77 07
	coriphosphine	cell walls, cell nuclei	blue	48 77 09
Albumin Mucus	aurophosphine G	mucus	blue-violet	48 77 07
	acridine orange	mucus	blue	48 77 09
	euchrysine	mucus	blue-violet	48 77 07
	coriphosphine	mucus	blue	48 77 09
	thiazine red R	albumin	green	48 77 14
	sulfaflavine	albumin	UV	48 77 01 or 48 77 02
Tuberculosis Leprosy	auramine	TB acid-resistant rods	blue	48 77 09
	acridine yellow	TB acid-resistant rods	blue blue-violet	48 77 09 or 48 77 07
Bacteria in general	coriphosphine	diphtheria	blue	48 77 09
	acridine orange	various bacteria	blue	48 77 09
	berberine sulphate	various bacteria	blue-violet	48 77 07 or 48 77 05
Wood	euchrysine	cellulose	blue-violet	48 77 07
	coriphosphine	cellulose	blue	48 77 09
	primuline O	cellulose	blue-violet	48 77 05
Bones	tetracycline	bone tissue	blue-violet blue	48 77 05 or 48 77 09
	calceine	polychromes	UV	48 77 01 or 48 77 02
	xylene orange	sequential labelling of bones	green	48 77 15
	acid fuchsine	osteons	green	48 77 15
Chromosomes	Atebrin quinacrine quinacrine mustard	chromosome bands "drumsticks" (sex determination)	blue-violet	48 77 06

Exciter filter: left side of spectrum
 Barrier filter: right side of spectrum
 Chromatic beam splitter: curves with key letter FT

1) Filter set no longer available

