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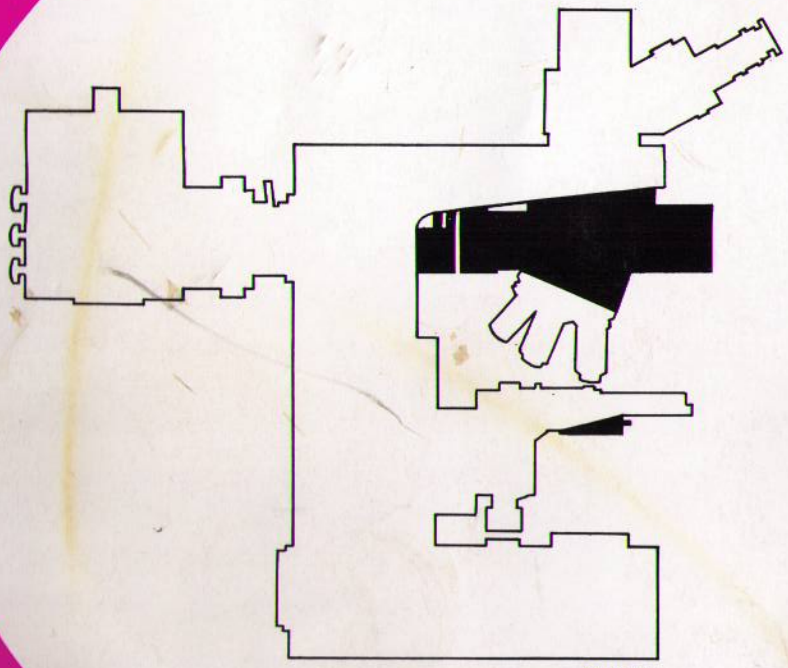
**ERNST LEITZ** GMBH **D-6330 WETZLAR**
Subsidiary: Ernst Leitz (Canada) Ltd., Midland, Ontario

List **513–143/Engl.** Printed in W-Germany IX/74/GX/B

# PLOEMOPAK® 2.1 a. 2.2

## Fluorescence Vertical Illuminator

## Instructions



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## Fluorescence Vertical Illuminator

Leitz WETZLAR

## Instructions


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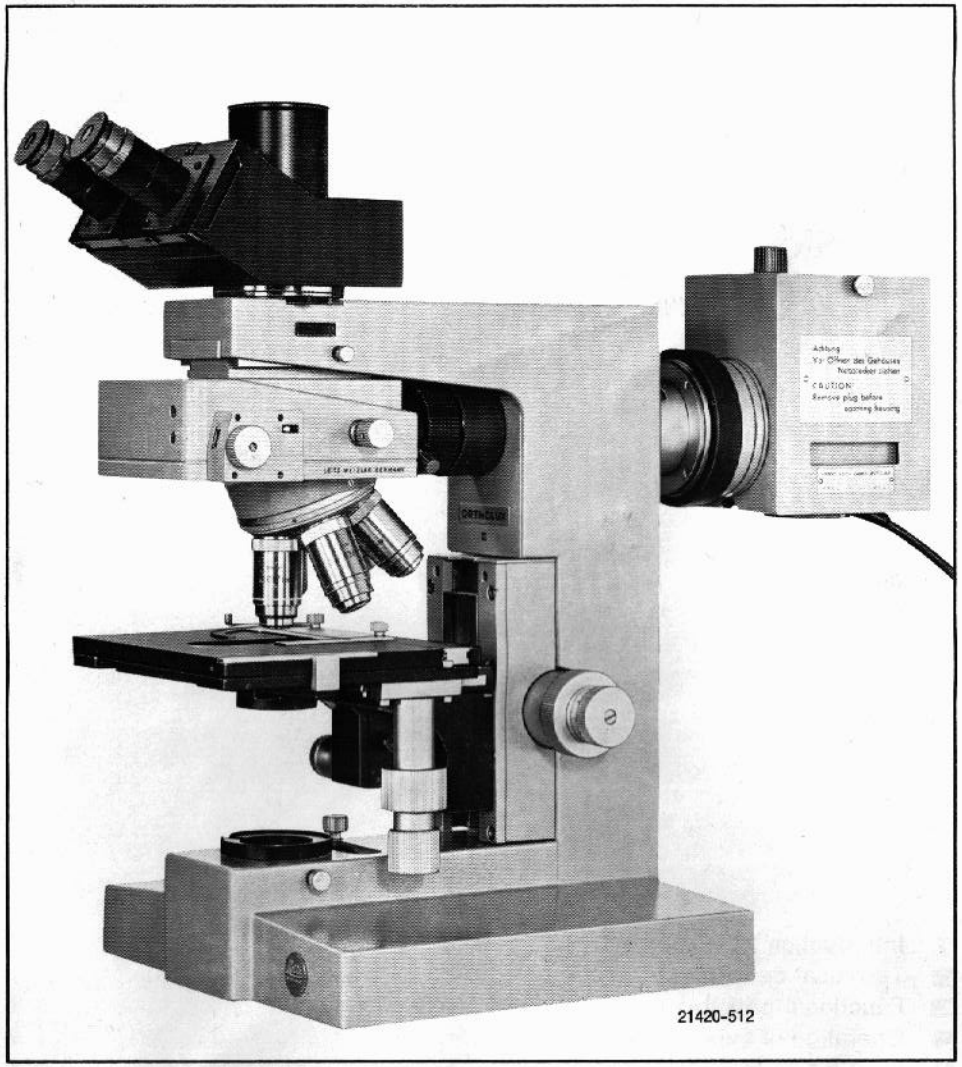


Fig. 1 ORTHOLUX® large microscope with PLOEMOPAK 2.2



# 1 Introduction

The PLOEMOPAK 2 serves for fluorescence excitation in incident light. It can be obtained suitable for the ORTHOPLAN®, ORTHOLUX and DIAVERT microscopes. The optical path is shown in Fig. 5. The designation of the PLOEMOPAK 2 indicates the following features:

PLOEMOPAK 2.1 for the ORTHOPLAN largefield microscope.

PLOEMOPAK 2.2 for the ORTHOLUX and DIAVERT/EPIVERT microscopes. Exciting filters, beam-splitting mirrors, and suppression filters are arranged on individually interchangeable filter systems. The wave length is changed by the adjustment of the filter system turret or exchange of the various systems. The filter systems have been selected so that the absorption and emission maxima respectively of the conventional fluorochromes and fluorophores can be covered with the filter combinations contained in them.

Since the PLOEMOPAK 2 is used for incident-light excitation with transmitted light objectives, it can also be combined with all transmitted-light methods, (dark-ground, phase contrast, etc.). The possibility also exists of simultaneous incident-light and transmitted-light fluorescence observation, which can be of value in the demonstration of two different fluorescences wihtin one and the same specimen.

# 4 Operation of the PLOEMOPAK 2

## 4.1 Insertion of the PLOEMOPAK 2.2

Release the clamping screw 6.1 on the microscope stand.
Insert the filter holder or centring tube 2.7 in the aperture on the stand.
Insert the PLOEMOPAK 2 fully into the guide, tighten clamping screw 6.1.
Insert the light trap in the dovetail guide below the object stage.

## 4.2 Preparing the PLOEMOPAK 2 for operation

Switch on the light source.
Fully pull out the exciting-light suppression. For the choice of one of the 4 positions set the lever 8.4 in the direction of the arrow (see Fig. 8). For rapid interchange between only 2 positions set the lever 8.4 towards the front (in the direction of the observer).

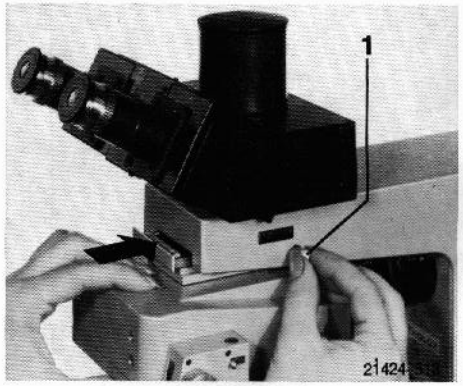


Fig. 6 Inserting the PLOEMOPAK 2.2
1 Screw for arresting the PLOEMOPAK 2

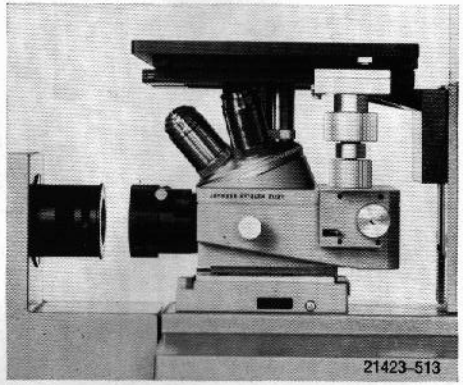


Fig. 7
PLOEMOPAK 2.2 on the DIAVERT/EPIVERT

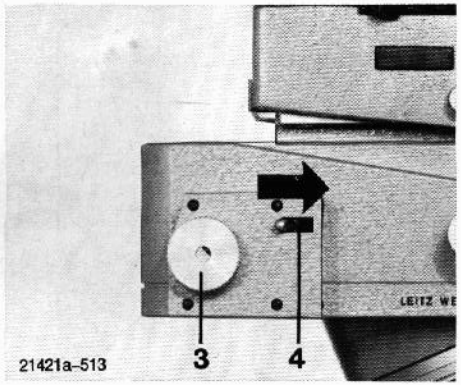


Fig. 8
Switching over the filter system
3 Rotary knob for switching over the filter system
4 Lever for limiting the switch-over facility to 2 alternative positions



## 4.3 Centring the light source

Turn an objective into the beam and unscrew it from the revolving nosepiece. Place a sheet of white paper on the object stage and adjust the centring knobs on the lamp housing until the patch on the paper is uniformly illuminated (see Instructions 514–119, 514–72, and 514–127). See Figs. 9 and 10.

Screw the objective into the nosepiece, place the specimen on the stage and focus it.
Adjust the condenser of the lamp housing until even in visual observation through the eyepieces the field of view appears uniformly illuminated.

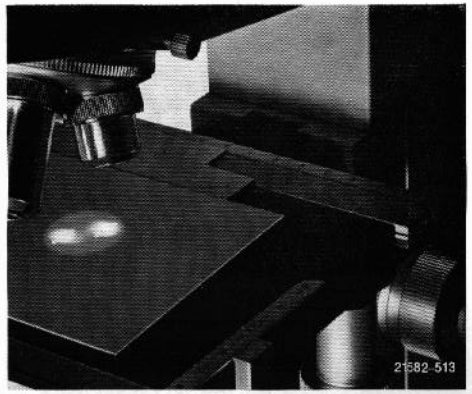


Fig. 9
Unadjusted light source

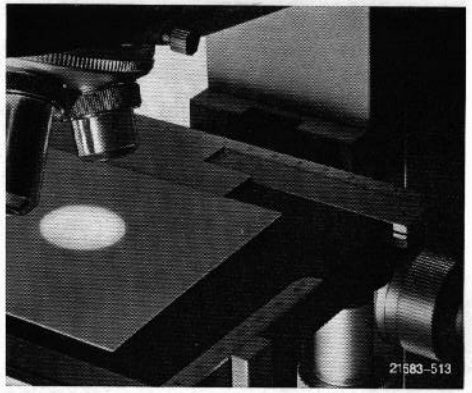


Fig. 10
Uniform illumination

### 4.4 Setting the field diaphragm

Close the field diaphragm with ring 2.6 until the rim of the diaphragm becomes visible in the field. Focus the rim of the diaphragm with knob 2.2 and centre it in the field with screws 2.5. Open the field diaphragm until it just dissappears beyond the edge of the field. To isolate small details and at the same time protect the surroundings from bleaching during observation open the field diaphragm only as far as necessary.

### 4.5 Fluorometry

It is generally possible to work with the field diaphragm built into the PLOEMOPAK to isolate small details in the illuminating beam.

With rectangular measuring diaphragms and always during the isolation of extremely delicate object details use the field diaphragm which can be inserted in the lamp carrier of the MPV. See MPV 2 Instructions (621–36).

Insert the centring tube in the microscope stand. For optical reasons only part of the field of view will now be illuminated. For photomicrography, temporarily remove, if necessary, the centring tube.
Adjustment on the MPV:

Insert the short-circuit lens in the photometry tube.

Place a test specimen on the object stage.

Switch on and adjust transmitted-light illumination (see Instructions MPV 2 621–36).
Focus the specimen, then switch over to incident-light illumination. Open the light suppression 3.8 on the PLOEMOPAK.

Open the photometer field diaphragm as far as possible.

Switch on filter system according to the fluorochrome of the specimen.

Close the field diaphragm 2.6, centre it in the field of view by means of the centring screws 2.5. Open the field diaphragm.

Close the photometer field diaphragm in the lamp carrier and centre it to the crosslines inserted in the eyepiece by means of the centring screws in the centring tube. Focus the rim of the field diaphragm by adjusting the focusing screw 2.2.



Only with the grating monochromator: insert the filter holder **without** exciting filter (only with the built-in dichroic beam-splitting mirror and suppression filter) in the PLOEMOPAK 2.
Insert the mirror combination in the mirror housing according to directions on the inside of the lid *. Switch Mirror Housing 500 to transmitted-light measuring illumination (see Instructions MPV 2) and centre the monochromator slit 0.5mm with adjusting screw on the mirror housing to the dust glass on the foot of the stand **.
Switch Mirror Housing 500 to incident-light photometer illumination.
Adjust the wave length of the monochromator until the test specimen becomes visible in the field of view.
Mount the pair of slits suitable for the desired half-value width of the exciting radiation on the light exit and entrance, of the grating monochromator.

### 4.6 Two-wave-lengths method

Arrange the required filter systems adjacently according to point 4.7 of these Instructions. Move lever 8.4 in the direction of the arrow. It is now possible to switch to and for rapidly between the positions.
Examples:
Positions 1 and 2 occupied. Let the catch engage at position 1.
Positions 4 and 1 occupied. Let the catch engage at position 4.

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* Here the mirror combination for reflection measurement must be used. Instead of the mirror housing a special lens (in preparation) can be used for forming the image of the slit.

** Particularly accurate slit adjustment is possible with the aid of a reflecting specimen (e.g. a glass surface) and the focusing telescope.

### 4.7 Exchanging the filter systems

Release clamping screw 2.1 and pull PLOEMOPAK 2 out of the stand.

For the interchange of the complete filter systems or for change of position of several filter systems open the lid of the housing 12.10. Take out the Allen key 12.21 clamped inside and release the screw (Fig. 13). The position number is engraved on the corresponding holder. Pull the filter system out of the housing as shown in Fig 14. Insert the new system in the dovetail guide and secure it with the screw. Clamp the key on the lid, place the lid in position and lock it.

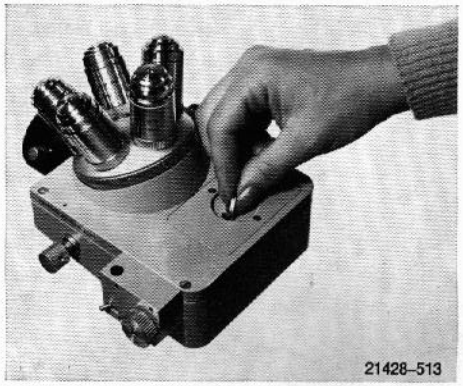


Fig. 11
Opening the lid of the housing on the PLOEMOPAK 2.2 to change the filter system

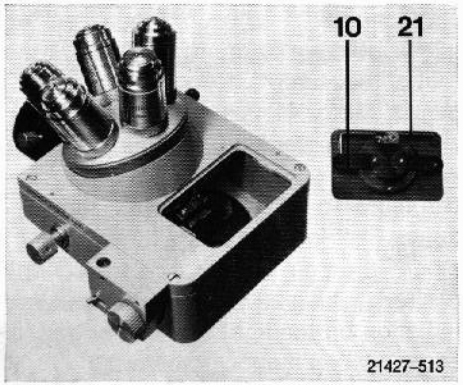


Fig. 12
PLOEMOPAK 2.2 with the housing lid removed

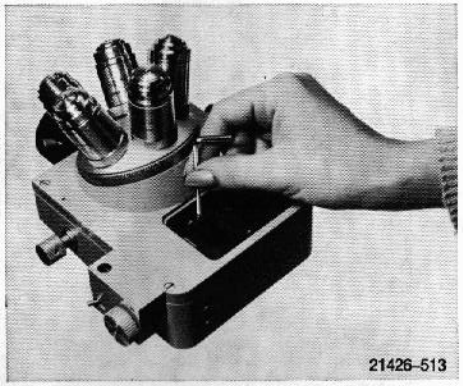


Fig. 13
Releasing the filter system

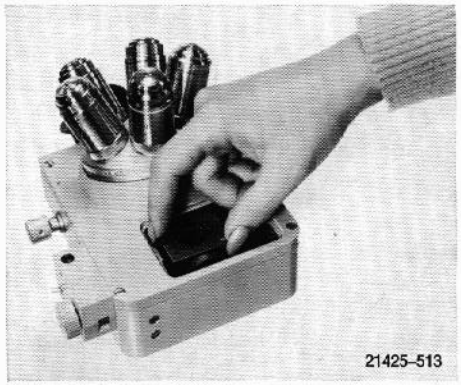


Fig. 14
Removal of the filter system



## 5 Practical hints

### 5.1 Optical system and light sources

Specially recommended for work with the PLOEMOPAK 2:

Eyepieces of low magnification; with the ORTHOPLAN, for instance, PERIPLAN® GW 4 x (high-point) or GW 6.3 x eyepieces.

Eyepieces of higher magnification can be used, but their images are dimmer.

Objectives of large numerical aperture, since the fluorescence intensity increases as the square of the aperture.

Example: Oil and water immersion objectives.

Ultra-high-pressure mercury lamps if excitation is to be carried out with various wave length regions.

High-pressure xenon or tungsten-halogen lamps if excitation is required in the region between 450 and 500nm.

### 5.2 Photomicrography

When the ORTHOMAT®-W fully automatic 35mm camera is used, the lower red sensitivity of the photomultiplier must be allowed for. This calls for an excessive exposure time and therefore overexposure of specimens of red fluorescence.

Guide value for the setting of the film speed:

Set the film speed scale of the ORTHOMAT-W 6 DIN (4x ASA value) higher than the stated value. Since these values may differ depending on the film used, it is recommended to make a series of trial exposures and thereby calibrate the ORTHOMAT-W. With double fluorochroming (two-wave-lengths method) with FITC (fluorescein isothiocyanate) and rodamine derivatives such as TRITC (tetramethyl rhodamine isothiocyanate) expose for the red fluorescent details of the object.