UV Microbeam Instrument

SEIDEL Type

Leitz WETZLAR

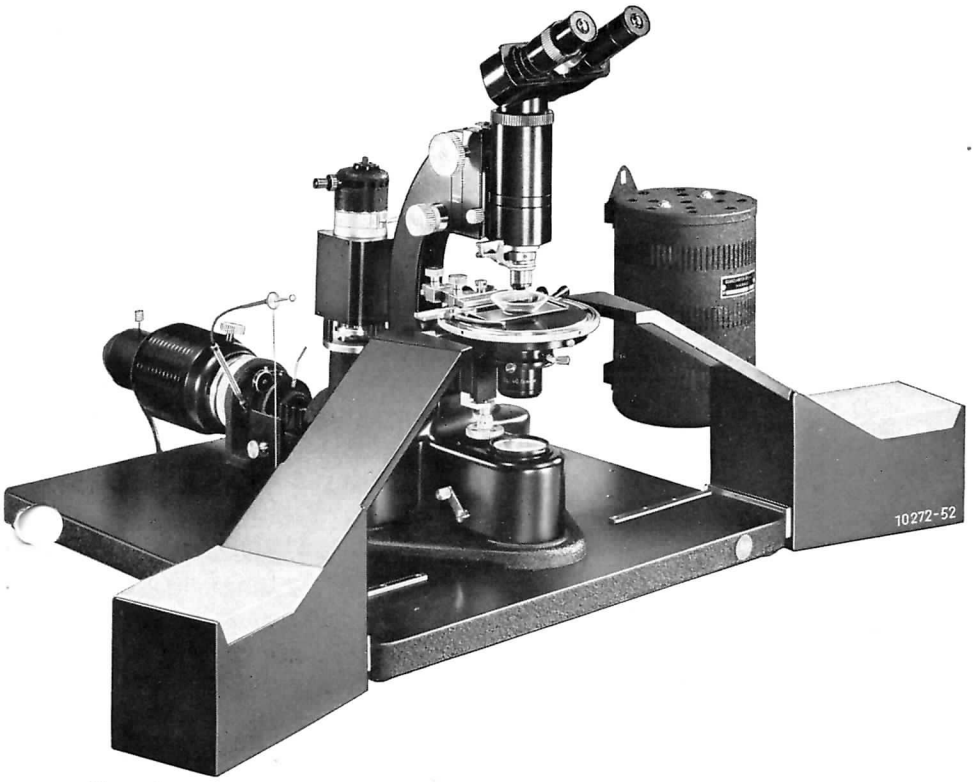


Figure 1

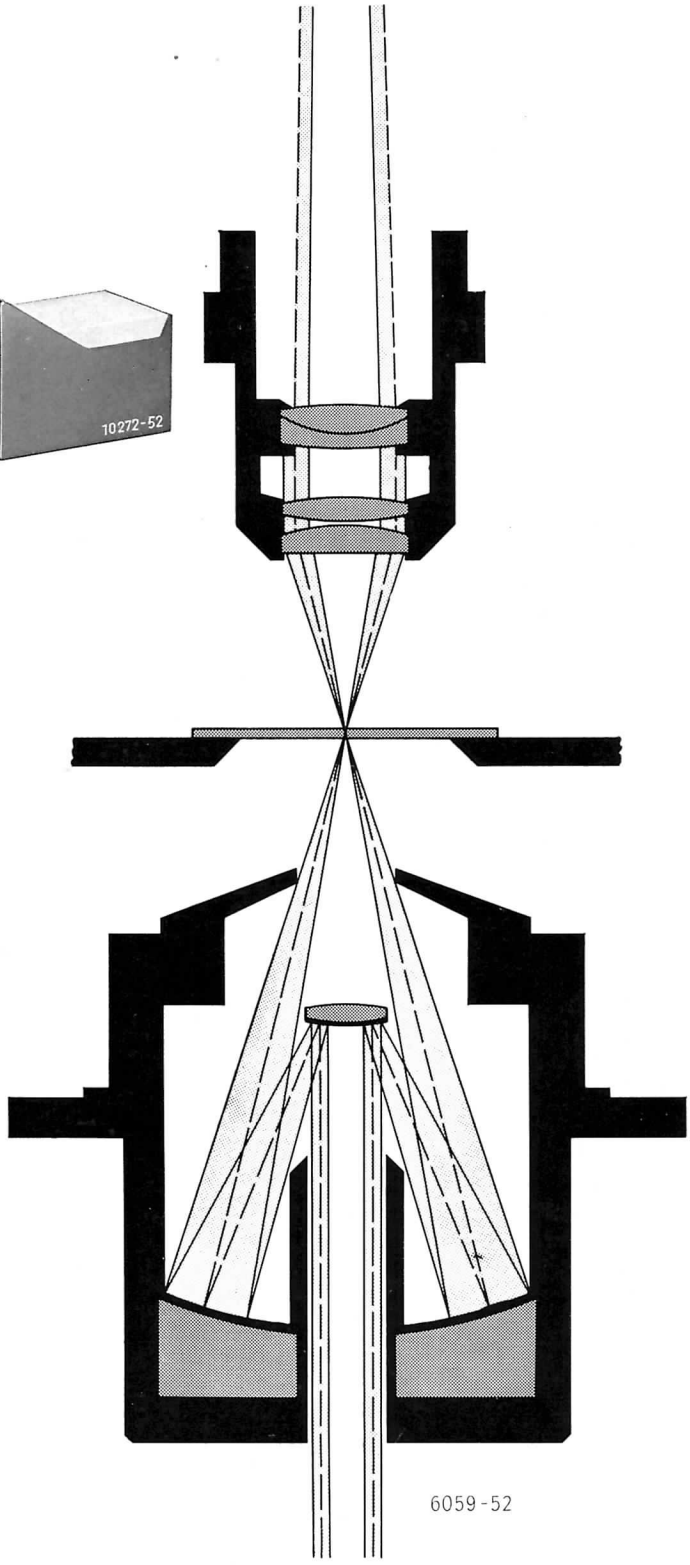


Figure 2 **Path of rays in mirror condenser and objective**

The UV Microbeam Instrument is used for experimental work in general physiology, ontogenetic physiology, cell physiology and in cytogenetics. The instrument directs a well defined UV-microbeam at a small portion of the specimen. The microbeam cross-section is rectangular and adjustable. A shutter permits exposures to known doses of UV-radiation. In this way it is possible to examine tissues or single cells for the direct reaction of their components to UV rays of given wavelength. Conclusions are possible regarding the extent of absorption or the physiological, physico-chemical and colloid-chemical changes produced in the tissues and cells. As the permeability of the cell membranes changes at the spot affected by the UV rays, it is possible to examine in vivo the behaviour of the cell content by alterations in the composition of the medium surrounding the cell, for example by the addition of certain types of ions or traces of cell poisons. With UV-microbeam irradiation it is also possible to stimulate eggs artificially to parthenogenesis. Similarly, by subjecting the chromosomes of the cell nucleus to UV rays, it is possible to produce mutations of the inherent characteristics of germ cells, single cells, cells of blastem and tissue cells. The use of a lethal ray intensity eliminates tissue parts, cells or cell constituents from the organism. By means of closing the diaphragm to a slit it is

Duration of radiation regulable by photographic shutter to any length up to 1/100 second. Size of the object field subjected to the rays continuously adjustable from 5 μm×5 μm to 250 μm×250 μm.

possible to split embryos, protozoans or explanted cells. One can alter the consistency of the cell membrane and thus cause cells to fuse.

Small radiation defects, which are carried along within the parts of an embryo during the formative movements, can be used as markers for clarifying the directions and speeds of movement of the cells and for the understanding of ontogenetical organisation. This kind of marking experiment for tracing the history of development is a substitute for dye marking in cases where the specks of dye do not adhere to the embryo or cannot be applied due to the thick sheathing around the embryo.

The UV-microbeam method is independent of manual insufficiencies. It permits finer and more accurately directed work than by mechanical means, in particular when it is wished to work inside a structure without damaging its sheathing. It is thus an optically effective micro manipulator which has become an important tool in ontogenetic physiology in which, following an exclusion or transposition experiment, attempts are made to draw conclusions from the resulting development regarding the significance of the effected portion in the process of development.

Technical description

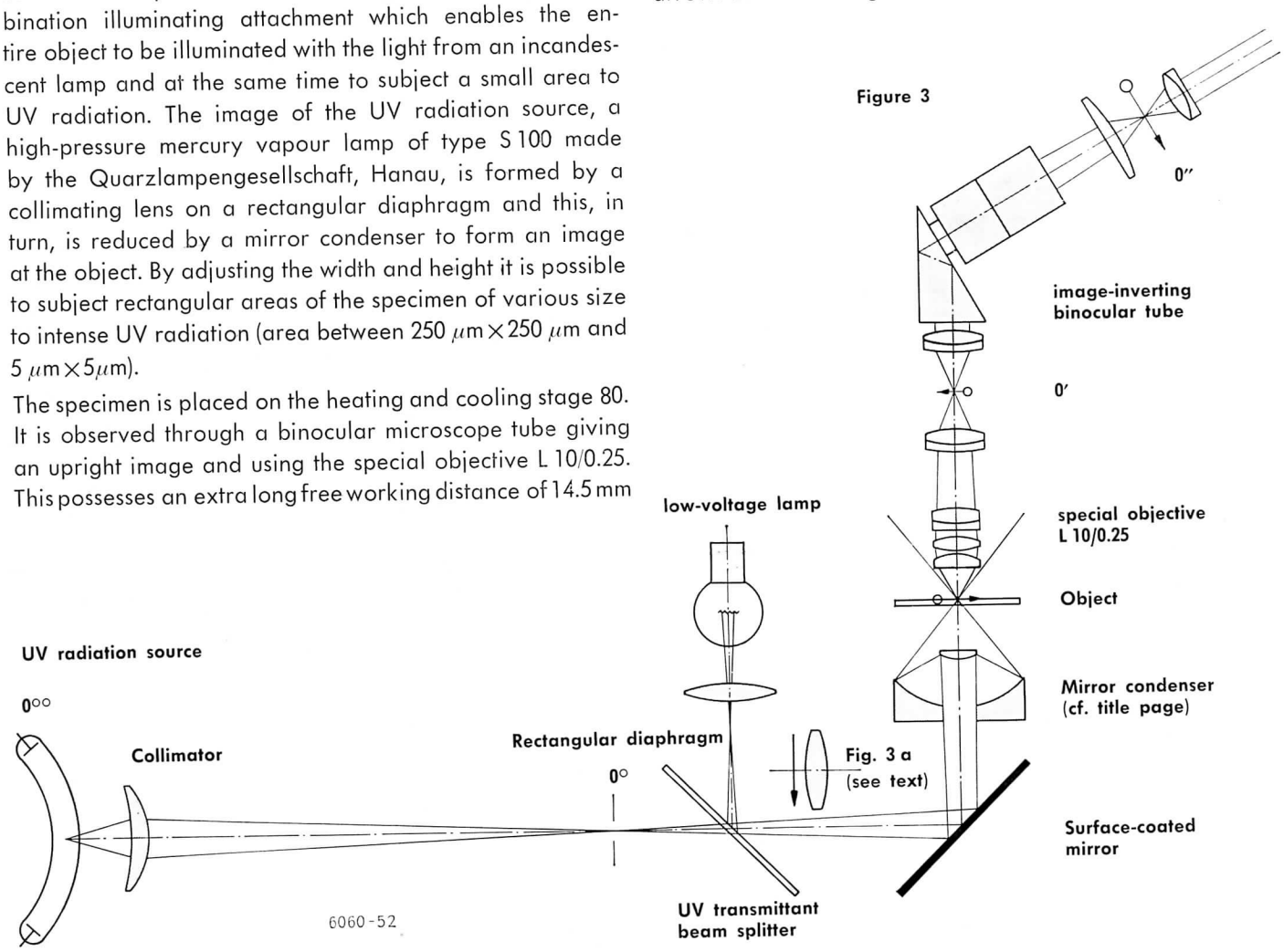
The Seidel UV Microbeam Instrument (figure 1) consists of a microscope of the LABORLUX II type with a combination illuminating attachment which enables the entire object to be illuminated with the light from an incandescent lamp and at the same time to subject a small area to UV radiation. The image of the UV radiation source, a high-pressure mercury vapour lamp of type S 100 made by the Quarzlampengesellschaft, Hanau, is formed by a collimating lens on a rectangular diaphragm and this, in turn, is reduced by a mirror condenser to form an image at the object. By adjusting the width and height it is possible to subject rectangular areas of the specimen of various size to intense UV radiation (area between 250 μm × 250 μm and 5 μm × 5 μm).

The specimen is placed on the heating and cooling stage 80. It is observed through a binocular microscope tube giving an upright image and using the special objective L 10/0.25. This possesses an extra long free working distance of 14.5 mm and is therefore very suitable for dissecting work. In conjunction with 6× eyepieces it is possible to obtain a 75× magnification. This greatly facilitates dissection and a micro manipulator is attachable to the instrument for this purpose. It is advisable to use stronger eyepieces for focusing and measuring the rectangular diaphragm. Thus, for example, the magnification can be increased to 320× by using the GF 25× eyepieces.

Liquid cells and filters can be inserted in the beam path for selecting suitable wavelength ranges from the UV lamp. The duration of radiation can be regulated by means of a photographic shutter.

The beam path diagram in figure 3 shows that the light from the UV source 0°° passes through a collimator to the rectangular diaphragm 0°. A 40 times reduced image of this diaphragm is formed by means of a surface-coated mirror arranged at 45° and the mirror condenser at the object. The image of the rectangular diaphragm and of the object can thus be observed simultaneously through the microscope. The microscope incorporates an intermediate image-inverting system so that, unlike a normal microscope, the images are upright and the right way round. This is of great importance in all dissecting work. Inversion of the image is effected by forming an intermediate image of 0′ at 0″ in the eyepiece diaphragm. The position of the object and of the two images is clearly apparent from the small arrows included in figure 3.

Figure 3



Since the rays coming from the rectangular diaphragm strike only a very small area of the object, particularly if this diaphragm is well closed down, illumination of the entire object field is also necessary. This is effected by means of a low-voltage 6 volts 30 watts lamp, the light from which is reflected by means of the beam-splitting plate which allows UV rays to pass. In this respect the collimator surface (0°) corresponds to the plane of the rectangular diaphragm and its image is formed at the object. Due to the heavy reduction in scale produced by the mirror condenser, the surface of the collimator does not fill the entire image field, and the field is therefore not fully illuminated. For this reason the swing-out lens in the microscope base (figure 3a) is brought into position for general observations and this then provides full illumination of the field.

When focusing the rectangular diaphragm and when the UV source is switched on, this swing-out lens should be in the inoperative position. The mirror condenser and objective were specially developed for the UV Microbeam Instrument. The illuminating aperture lies between A = 0.15 and 0.30. This means that the rays of light reaching the object are narrow, and this is of importance as regards damage afflicted to the areas above and below the point in the object receiving radiation is concerned. The working distance of the mirror condenser is so great that the details to receive radiation may lie up to 6 mm above the level of the heating stage. As a mirror condenser is completely free from chromatic aberrations, it is possible to focus the rectangular diaphragm at the object in any given wavelength of the visible range and then to subject the specimen to UV radiation of a given wavelength. This means that the inconvenience of re-focusing the condenser when switching from visible to ultra-violet light has been excluded.

With certain filter combinations, which permit not only UV but also parts of the visible spectrum to pass, the latter can be used as guide rays for centering and focusing the UV radiation.

The objects subjected to radiation are usually colourless and can therefore seldom be seen well in a normal bright field. For this reason the apertures of the mirror condenser and objective have been adapted to each other so that a mixed bright and dark field results in which the objects can be seen very well. The sectional view in figure 2 shows how part of the lens mounts are struck by the illuminating rays to produce the mixed bright and dark field illumination.

To ensure that the objects receive the highest possible UV intensity, all optical elements through which UV rays pass are made from quartz glass of specially good transmission and all mirrors are aluminium-coated. In addition, the liquid cells and object carriers are also made of the same material.

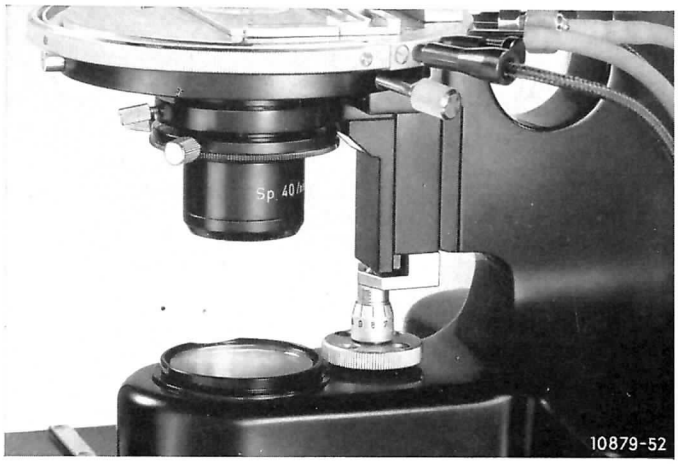


Figure 4

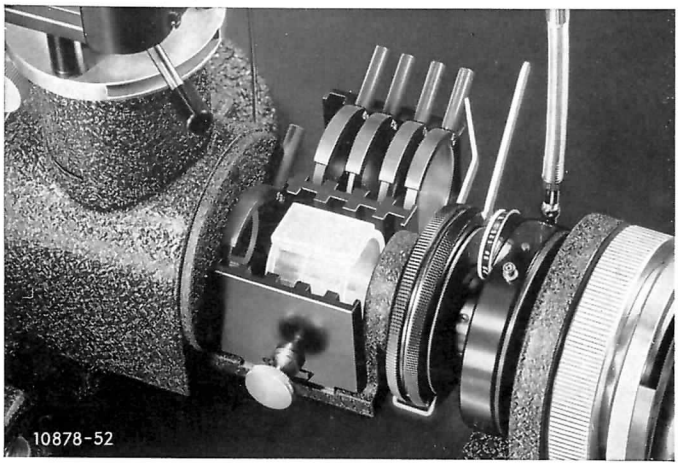


Figure 5



Figure 6

Figure 4
Mirror condenser Sp 40/0.15–0.30 in centering mount with fine vertical adjustment by means of micrometer screw (1 scale division = 0.05 mm)

Figure 5
Adjustable filter holder for filters and cells used as liquid filters

Figure 6
Heating and cooling stage 80 on rotating base and removable mechanical stage; culture dish with base of Ultrasil inserted in metal object holder

Outfits

Base plate with adjusting screw for correcting irregularities in bench surfaces; two arm rests; holder for cable release; bars for micro manipulator.

LABORLUX® II Microscope Stand (fixed to the base plate), aluminium-coated mirror with quartz protective layer, glass swing-out lens (for general observations), sealing glass of Ultrasil.

Image-inverting binocular tube with inclined eyepiece tubes and interpupillary adjustment, magnification = 1.25×.

Objective centering clutch on supporting bracket, with 3 centering rings.

Objective L 10/0.25, free working distance 14.5 mm, firmly centered in centering ring.

Heating and cooling stage 80 (range – 20° to + 80° C), on rotating base, regulating transformer, ready for connection (please state mains voltage when ordering).

Attachable mechanical stage.

Mirror condenser Sp 40/0.15–0.30 in centering mount with fine vertical adjustment by means of micrometer screw (1 scale division = 0.05 mm).

Eyepieces:

Paired eyepieces 6× with adjustable eyelens, one of them with 10 : 100 reticule micrometer.

Paired wide-field periplanatic eyepieces GF 25× M with adjustable eyelens, one of them with 10 : 100 reticule micrometer and cross lines.

Ray directing illumination mount (with cap nut, attachable to the stand), comprising:

Rectangular diaphragm with two jaws adjustable symmetrically to the center, each pair individually adjustable by lever to slit widths between 10.0 and approx. 0.2 mm (corresponding to a radiated object area of 250 μm×250 μm to 5 μm×5 μm); beam-splitting plate of Ultrasil and central shutter with cable release.

Adjustable filter holder for glass filters and cells used as liquid filters.

Cells:

Ultrasil cell with lid, path length 10 mm

Ultrasil cell with lid, path length 20 mm

Lamp housing with 6 volts 30 watts low voltage lamp; adjustable collimator; filter slit; ground glass screen and daylight filter; REDYX regulating transformer, ready for connection (please state mains voltage when ordering).

Housing for mercury high-pressure lamp (type S 100) with adjustable illuminating lens of Ultrasil.

Mercury high-pressure lamp (type S 100) in normal cap, insertable in the lamp housing by means of sliding sleeve; mains connecting unit for 220 volts a.c., connecting lead.

Accessories:

Metal object holder with circular cut-out portion for culture dishes and object carriers of 25 mm diameter.

Culture dish with cemented and optically plane 1 mm thick base of Ultrasil, to fit the metal object carriers, sterilizable.

Filters

	K: cut-off positions, λ for $\tau = 0.45$ [mμ] S: $\lambda_{max} - \tau_{max}$ – HW	material	transmitted lines of the mercury spectrum [mμ]
Neutral density filter 5%	–	2,3 mm NG 3 | 2 mm OG 2	578 focusing filter for the rectangular diaphragm
Orange filter	K 570		
Blue filter	S 400 – 65 – 110	3 mm BG 12	366 to 436
UV filter	S 330 – 85 – 100	1 mm UG 11	280 to 366
UV filter	S 330 – 90 – 140	2 mm UG 5	254 to 405
UV cut-off filter	K 405	Wratten 2 C (+ Mir.)	405 to 578
UV cut-off filter	K 355	1.5 mm WG 2	366 to 578
UV cut-off filter	K 295	3 mm plexi glass, colourless	297 to 578
UF filter for 313 HG	–	0.2 mm GG 9 + 2 mm WG 7	313, 405 to 578 (in conjunction with UV filter S 330–85–100 only line 313)

For cut-off filters the position of the cut-off (transmission $\tau = 0.45$) is stated.
Selection filters are marked with three figures:
position of maximum transmission λ_{max} [mμ]
transmission at this maximum τ_{max} [%],
band width at half maximum intensity in mμ

® = registered trade mark

Design subject to alterations without notice.

UV Microbeam Instrument,

complete outfit, with all accessories described above, including all the filters listed.

Code word IFZAK

List 52-18/Engl.

Printed in Germany
VIII/62/FLW/SD

ERNST LEITZ GMBH WETZLAR GERMANY

Subsidiary: Ernst Leitz (Canada) Ltd., Midland, Ontario

U.S. Distributors: E. Leitz, Inc. 468 Park Ave. S., New York 16 N.Y.