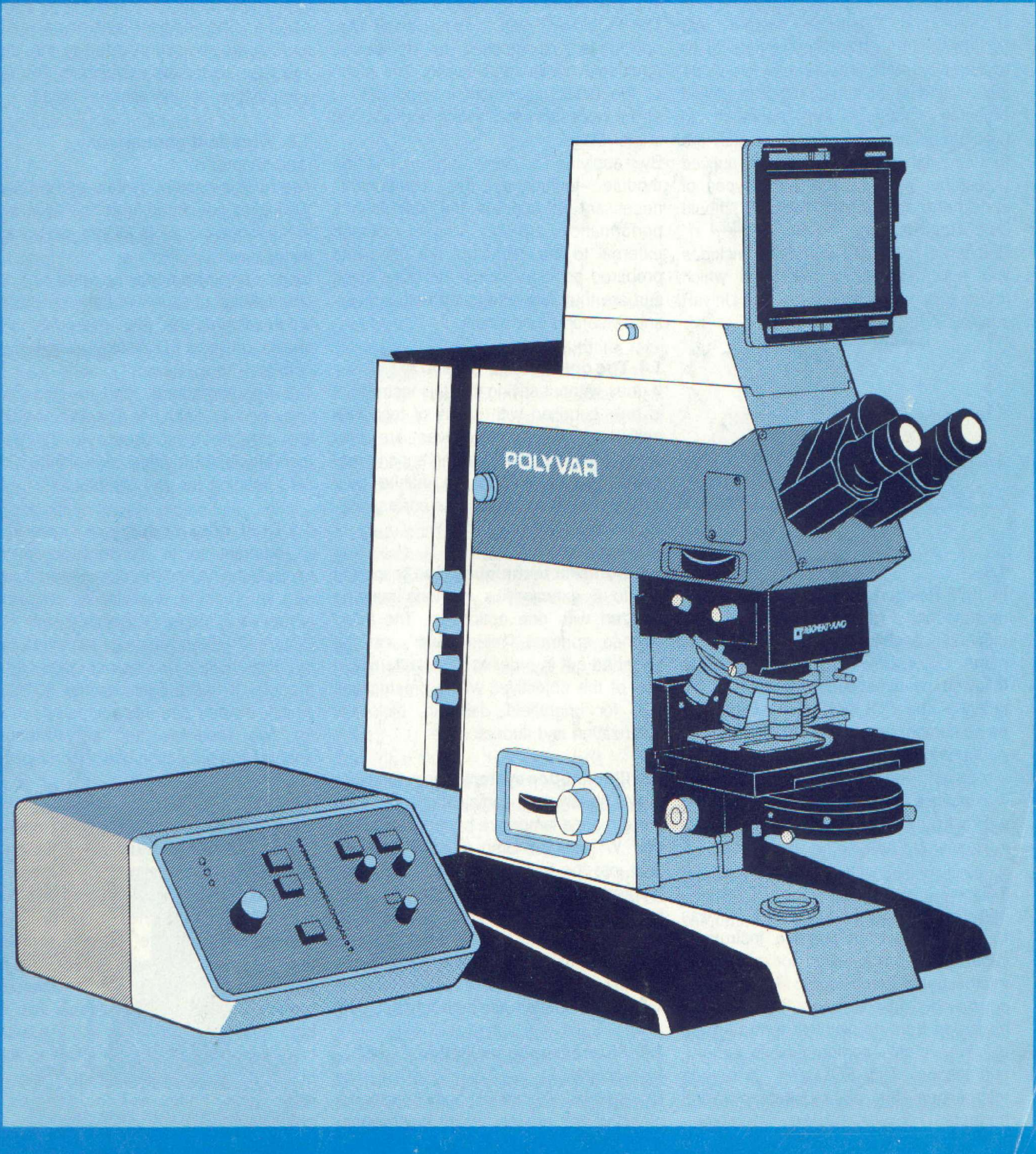
REICHERT-JUNG

POLYVAR

Widefield Photo Microscope



1. The Concept

The product development programme of C. Reichert Optische Werke AG recognises the time requirements of all research workers, whether they are working on fundamental problems or in applied research. Although the approach to problem solution may differ ultimately the fundamental researcher and the applied researcher examine the same types of specimens and their instruments should be compatible.
Reichert's product line now includes two new research microscopes which reflect this basic philosophy – the UnivaR and the POLYVAR.

1.1.
In 1973 Reichert introduced their "supermicroscope", the UnivaR, and its universal capabilities are expressed in its name. Now, in 1978, the UnivaR is followed by a second instrument in the family – the POLYVAR. Once again its name symbolizes its many applications in:
- Fundamental Scientific Research,
- Applied Research in Institutes and Hospitals,
- Practical Laboratory Applications of Research Work.

1.2.
The design concept for the POLYVAR was to produce a multi-purpose instrument capable of being used for all known microscopical techniques. Its modules construction also ensures that it can be upgraded from its simplest form right up to the most comprehensive photomicroscope. The POLYVAR is truly a microscope for all requirements and budgets.

1.3. The stand (see chapters 2 and 3)
The POLYVAR, which uses UnivaR type optics, is distinguished by its superb functional design and styling. The stand of the basic instrument incorporates all of the necessary facilities to upgrade it at a later stage.
By applying a new opto-mechanical module technique, the components necessary to upgrade the instrument's performance are no longer mounted external to the stand but are fitted into prepared positions within the basic stand and are thus fully integrated both appearancewise and functionally.

1.4. The optics (see chapter 4)
It goes without saying that this instrument is only supplied with optics of top class performance. The objectives are fully plane achromatically or plane apochromatically corrected – for an infinite tube length and for an object field corresponding to field of view 30.

1.5. Contrast techniques (see chapter 5)
Up to 5 examination methods may be realized with one optics set. The interference contrast "main prism" can be switched out in order to permit alternative use of the objectives without restrictions also for brightfield, darkfield, biological polarization and fluorescence.

1.6. Illumination system (see chapter 6)
Mirror housings in combination with high-performance lamps are unnecessary. The 100 W halogen lamp which is already built into the basic instrument is complemented by simply adding the big lamp housing and a deviating mirror. This configuration does not affect the excellent overall appearance of the instrument and its result is a perfect equipment for all illumination modes with both light sources.

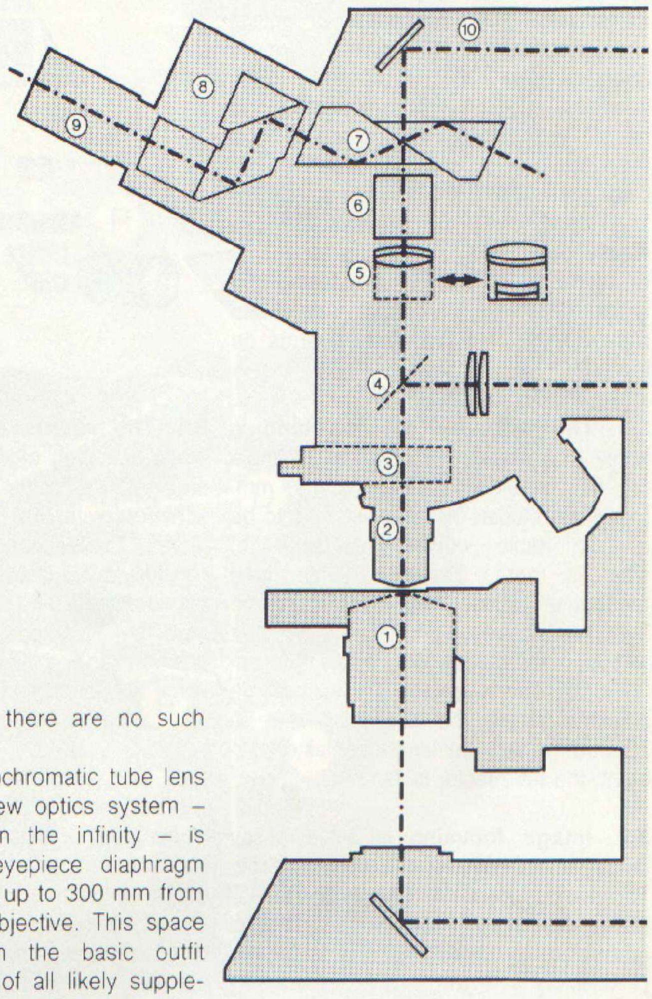
1.7. Fluorescence excitation
(see chapter 7)
No special illuminator and no special objective set are necessary for incident-light illumination for fluorescence examinations. The incident-light beam splitter mirrors are directly inserted to the stand and may be rapidly exchanged. The filter combination can be easily modified.

1.8. Widefield photography
(see chapter 8)
The built-in camera system of the POLYVAR does not impair flexibility. All formats in the range 4 × 5" to 16 mm can be used successively.
Light measurement, calculation and computation of exposure time are effected automatically and the computed time shown on the LED display (range 1/100 sec. to 1 hour).
The photographed object field corresponds to field 24. The POLYVAR is the only photomicroscope in the world market which provides consistantly widefield imaging for photography.

1.9. Dual reflex module (see chapter 9)
In contrast to all conventional microscopes the various intermediate tubes – e. g. for TV, cine, dual viewing or drawing – are no longer necessary with the POLYVAR. Their functions are taken over by the built-in swing-out dual reflex module. It has a beam splitter 50/50 % which divides the microscopical image for two receivers and simultaneously enables the projection of a second image.

1.10.
Thus the new concept has formed an instrument which is equally suitable for very special applications as well as for allround use as far as budget and performance are concerned.
The appearance, the form of operation and the working position as well as the optical parameters of the microscope remain unaltered. This means that the high quality of the optics and the extremely wide object field are effective without restrictions for all methods, for all illumination modes and for all types of objectives.

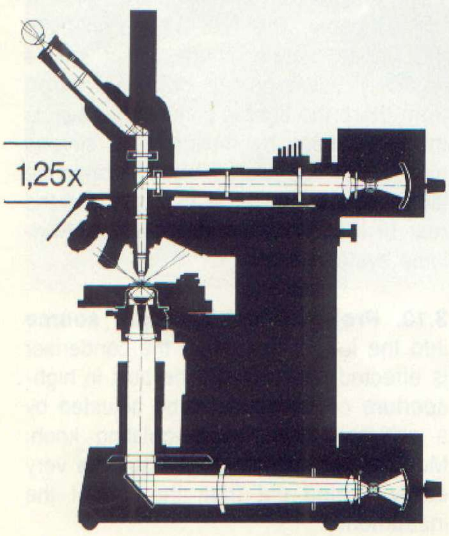
2. The Theory



1 – Condenser
2 – Objective
3 – IC main prism
4 – IL fluorescence
5 – Tube lens
6 – Dual reflex module
7 – Deviating prism
8 – Binocular tube
9 – Eyepiece
10 – to camera

The development of an absolutely novel optical system, including objectives corrected for infinite tube length, was the prerequisite for the design of the stand described in the introduction.

2.1.
Conventional microscopes have a finite tube length of 160 to 170 mm. In general, this distance corresponds to the sum of the lengths of the objective nosepiece, the optics carrier and the binocular tube. Additional optical or mechanical elements must be added and, therefore, increase the tube length by a factor of 1.25X or 1.6X. Indeed, the negative effects of these "tube factors" are usually neglected in relevant literature but they nevertheless have to be taken into account. For example, when using an incident-light fluorescence illuminator the nominal magnification increases, the image brightness decreases and the field of view is reduced accordingly. The intermediate tubes mentioned under 1.9. as well as the tube lens for phase or interference contrast – which has to be inserted in the objective nosepiece of some widefield microscopes – have the same effects.



2.2.
With the POLYVAR there are no such problems.
By means of an apochromatic tube lens the image of the new optics system – which is situated in the infinity – is transferred to an eyepiece diaphragm plane which may be up to 300 mm from the socket of the objective. This space already provided in the basic outfit allows incorporation of all likely supplementary optics. By this neither the optical and mechanical dimensions of the instrument nor tube length, magnification, brightness or field of view change.

2.3. The design of the instrument has been essentially influenced by this novel module principle.

2.3.1.
The objective – the tube lens – the binocular tube and the eyepiece are the basic optical elements of the instrument.

2.3.2.
The magnification of the system changes if the focal length of the tube lens is changed. A series of individual tube lenses mounted on a turret form the magnichanger of the POLYVAR (see point 4.6.).

2.3.3.
A deviating prism distributes the light to viewing tube and camera in the ratio 20 : 80. With dark specimens a second prism transmits 100 % of the light to the viewing tube.

2.3.4.
The ray path between objective and tube lens is parallel. If a dichroic beam splitter mirror is inserted at this position, incident-light fluorescence illumination is obtained without any optical artefact (see point 7.2.).

2.3.5.
For normal work an optically neutral wedge is placed between the tube lens and the deviating prism. If this is replaced by a beam splitter 50/50 % the possibilities of image splitting or image projection exist (see point 9.2.).

3. The Stand

The stand, with its heavy and stable design, offers the optimum prerequisites for correct incorporation of all optical and mechanical components. Consequently quick and purposeful image selection and focusing are possible and remain unchanged in every phase. Yet the instrument's dimensions are modest, it does not require excessive bench space. The stand is only 385 mm wide and weighs about 20 kg.
All elements necessary for focusing and image selection are clearly visible and within reach of the operator's arms on the rests.

3.1. The binocular viewing tube is firmly mounted to the stand at an angle of 26°, the viewing height is 440 mm. Extensive studies have shown this to be a comfortable working position and, what is more, it remains unchanged regardless of the outfit specified. The eyepiece sleeves are designed to accept widefield eyepieces with a diameter of 30 mm. The eyepoint height can be adjusted within the range 55 mm to 75 mm – the tube length remains constant, the tube factor is 1X.

3.2. Image focusing is achieved by setting the height of the square stage. The coaxial coarse/fine focusing mechanism is conveniently positioned and graduated for thickness measurements (1 division = 1 μm). The fine focusing control is effective without any restriction over the whole adjusting range of the stage.

3.3. Specimen and objective protection with the POLYVAR is achieved in many ways:
- All objectives with a working distance below 1 mm have spring mounts.
- All objectives are parfocally and parcentrally adjusted. After focusing with a low-power objective every objective, including the oil immersion objectives, can be swung in and refocussed with minimum adjustment of the fine control.
- An autofocus stop in the drive system automatically fixes the working position of the stage for any particular specimen. This position may then be reproduced by means of the coarse control at any time and without risks. This system saves working time and protects specimen and optics when the same specimen is repeatedly focussed – e. g. after cleaning of the optics – or when different specimens with the same object slide thickness are focussed.

3.4. The square stage permits systematic scanning of the specimen over an area of 50 x 75 mm. Adjustment is easily achieved with the low-positioned coaxial drives. The specimen is held safely and carefully by a damped spring finger. All manipulations in the specimen/objective area are made easy by the low working position and the large surface of the stage (140 x 200 mm).

3.5. The brightfield condenser is of a novel optical design which gives correct illumination of the wide fields as well as the corresponding illumination apertures for all magnification steps. When changing to lower magnifications not only the high-aperture front lens 0.90 of the condenser is swung out but simultaneously another optics with an aperture of 0.32 is swung in. For special requirements such as when apochromatic objectives are being used and for contrast microscopy we supply an apochromatic aplanatic immersion condenser N.A. = 1.30.

3.5.1. Height adjustment of the condenser is made by means of a rack and pinion drive with high ratio gearing which permits precise adjustment of the Köhler illumination even with high magnification steps. This is particularly important for adjusting interference contrast.

3.5.2. Centring of the condenser is almost unnecessary because of the precise construction of the instrument. However, every condenser is equipped with an easily accessible centring device for minor corrections of the position of the field diaphragm image.

3.5.3. Change of the condenser is effected very quickly and easily by means of a high-precision quick-change device.

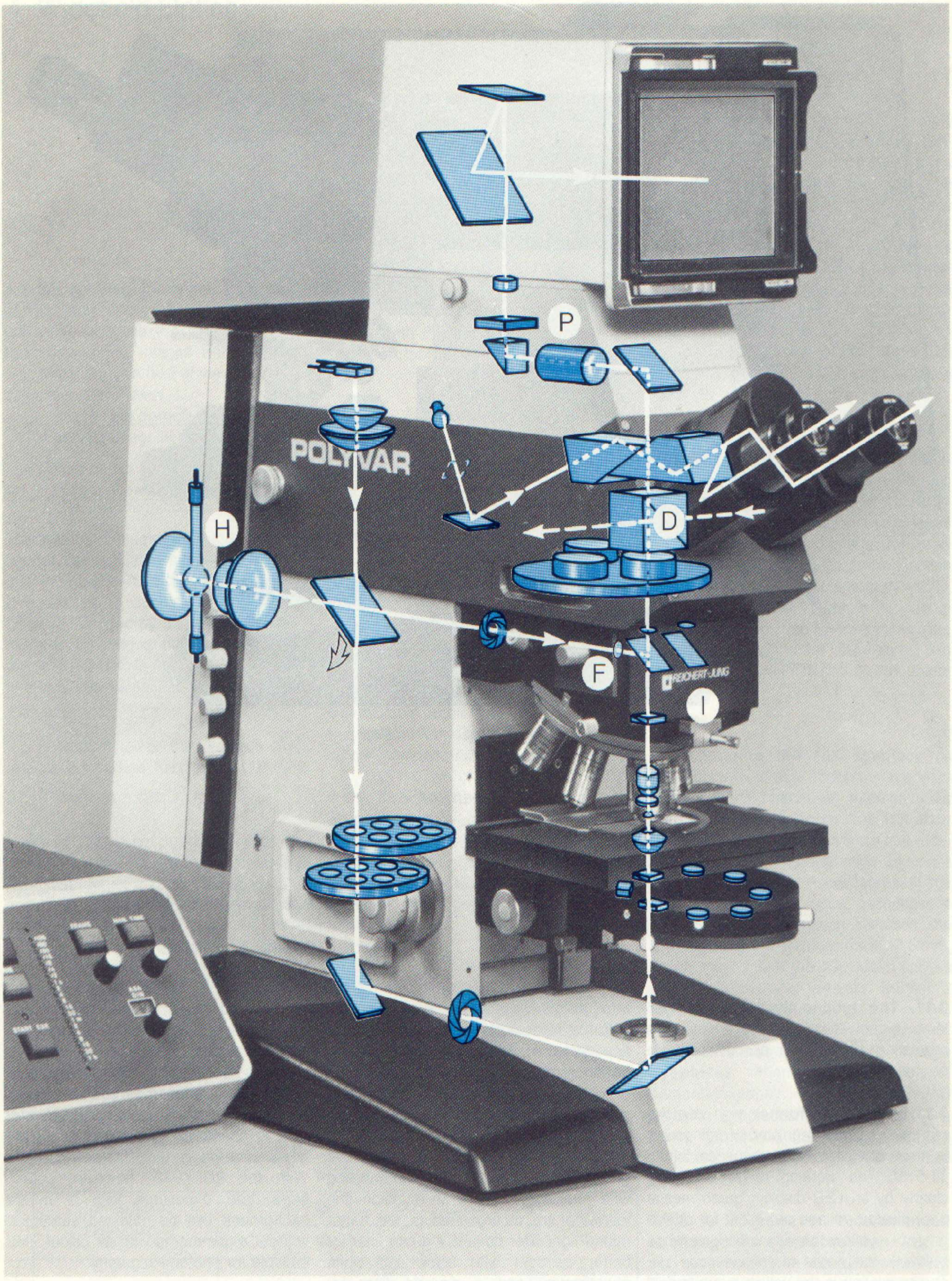
3.6. The field-iris diaphragm is built into the stand of the POLYVAR, it is very easy to operate and effective for all condensers. Its function is to reduce the light bundles coming from the condenser to the diameter corresponding to the object field imaged by the respective objective. Thus over-radiation of the image by light scatter is avoided and image contrast maintained.

3.7. Light source. In general a 100 W halogen lamp is used. This lamp type has the ideal characteristics for microscope illumination because of its even filament structure and high luminous density. The colour temperature is about 3200 K, the working life is normally several hundred hours.

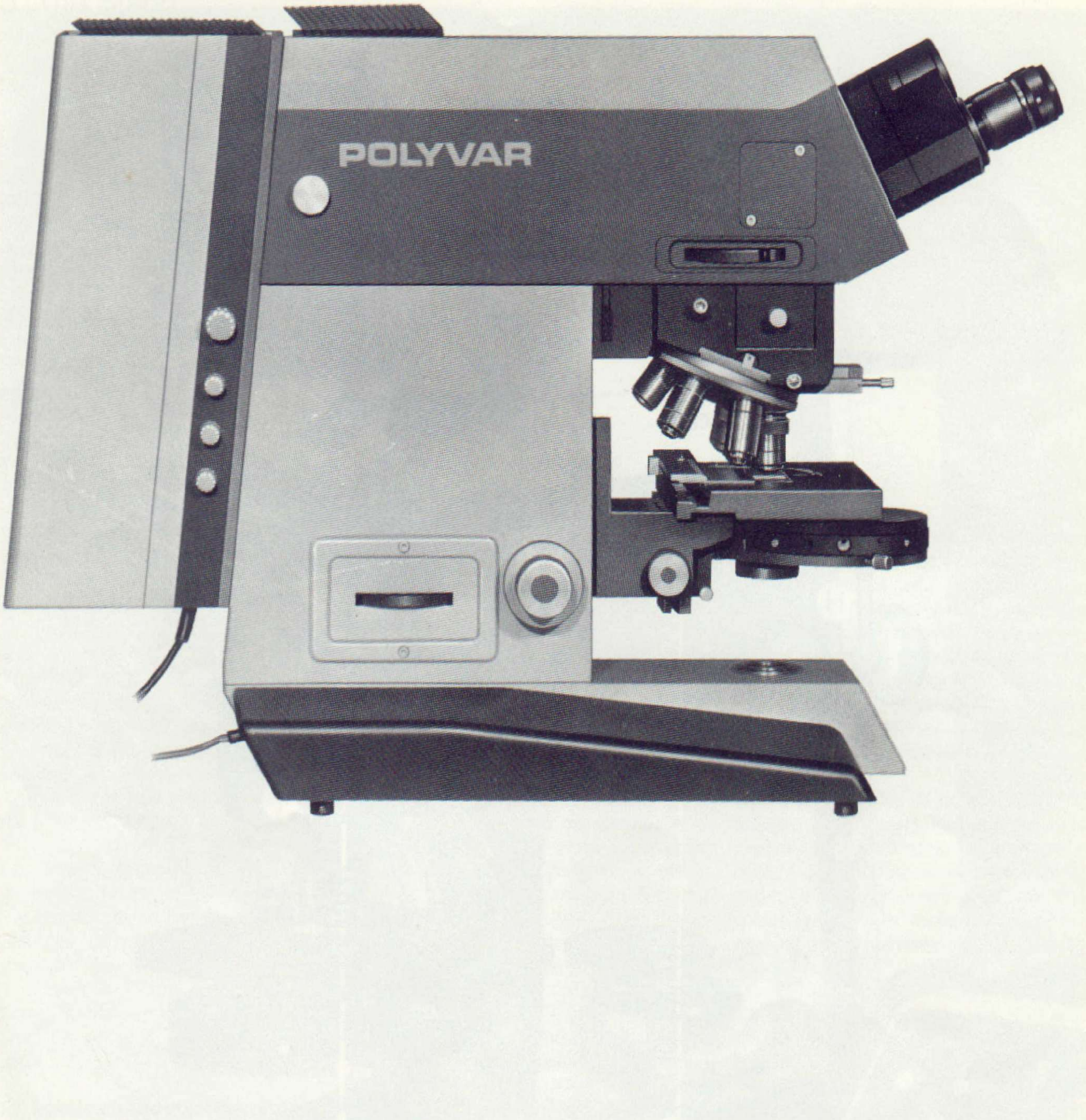
3.8. Lamp housing. For normal work with the 100 W lamp a separate external lamp housing is not required. The housing for the 100 W lamp is fully integrated in the basic microscope stand thus producing an extremely compact design.

3.9. The illumination system of the POLYVAR employs novel and very effective concepts. The 100 W lamp built into the upper portion of the stand shines vertically down onto a deviating mirror; from there the light is conducted towards the condenser by means of a second mirror. A separate high-performance lamp housing may be mounted on the rear of the stand to form a perfect twin-lamp system.

3.10. Projection of the light source into the focusing plane of the condenser is effected by means of the built-in high-aperture collector. It can be adjusted by a conveniently placed regulating knob. Mounting and centring of the lamp is very easily carried out from the top of the instrument.



Ⓟ POLYMATIC camera module
Ⓓ Dual reflex module
Ⓕ IL fluorescence equipment
Ⓘ Interference contrast/main prism
Ⓗ High power lamp housing



3.11. The luminosity of the halogen lamp is regulated either by means of a regulating transformer or by a built-in neutral filter.

3.11.1. The transformer with thyristor control can be regulated continuously in the very wide range between 5 and 11.5 V. The working range is shown clearly by an LED display: green means an indicated voltage of 11.2 V for photography – yellow the visual working range below – red warns of voltage over-ride. The regulation in the yellow range should only be used for visual observations or black and white photography, as with luminosity the colour temperature of the light changes. For colour photography where colour is of importance the halogen lamp should be adjusted to 11.2 V.

The above transformer also serves as a mains supply unit for the photo equipment and is mounted in a simple housing with the camera control unit. This simplifies operation, reduces costs and saves bench space alongside the microscope.

3.11.2. Colour-neutral light subduing is effected by the 3 neutral filters (T = 0.2 %, 1.6 % and 12.5 %) supplied with the basic outfit. In addition the following are incorporated in the transmitted-light filter holder: a green filter for better contrast with black and white photography and a daylight filter for visual observation. In case other filters are required, the dustproof turret may be taken out and filled with the optional filters.

3.12. The colour temperature of the light. Many factors influence the colour temperature of the light even when the correct voltage is applied to the lamp. For example, different types of glass, different optical coatings etc. These changes are hardly detected by the human eye but lead to colour changes with colour photography. Such changes can be countered by the use of gelatine filters such as Wratten filters – Kodak.

With the POLYVAR, however, colour-tinge free exposures with a neutral background can be obtained easily and without experiments with all colour films suitable for photomicrography.

The interference compensating filter of the POLYVAR is a new multi-layer filter which is not only matched to the light source but also to the entire optical system. It neutralizes all colour artefacts and modifies the light coming from the microscope to that required for normal photo illumination.

4. Widefield Optics

4.1. We speak of the widefield imaging of an objective if – compared with conventional types – a larger section of the specimen is imaged with the same magnification. The field of view number is calculated as follows:

$$S = \frac{\text{Image field diameter}}{\text{Eyepiece magnification}} = \frac{S'}{V_{OC}}$$

The visible object field D then results from

$$D = \frac{\text{Field of view number}}{\text{Objective magnification}} = \frac{S}{V_{Obj}}$$

The widefield imaging of the microscope cannot be evaluated simply by the field of view number S of the eyepiece but it is necessary to calculate the object field D* really imaged by the whole system. If there is an intervention in this system, the imaged field – though the values S and V_{Obj} are maintained – is modified by the factor $\frac{1}{q}$:

If q is coordinated to the eyepiece, there results a modified field of view number $S^* = \frac{S}{q}$ and from this $D^* = \frac{S^*}{V_{Obj}}$

Intermediate tubes or incident-light illuminators with conventional microscopes compulsorily give a factor $q_I = 1.25X \ldots 1.6X$ and thus a reduction of the field of view and the object field. With the POLYVAR in these cases always $q_I = 1X$, so that there is no unintentional modification of magnification and object field diameter.
Intermediate magni-changers in general are effective with a factor $q_C = 1X \ldots 2.5X$ which leads to further reduction of the object field.
In contrast to that on the POLYVAR there is imaged an object field with $q_C = 0.8X$ that equals the field of view number $S^* = 30$.

		V_{OC}	S	q_C	q_I	V^*_{OC}	S*
a	Conventional Instruments	8×	18	1×	1×	8×	18
b		6×	18	1×	1,25×	8×	14,4
c				0,8×		8×	30
	POLYVAR	10×	24	1×	1×	10×	24
				1,25×		12,5×	19,5

a/c Brightfield: with 8X magnification V^*_{OC} the ratio $S^*_a : S^*_c = 18 : 30$;
the informative content with the POLYVAR is 2.5X larger

b/c Incident light fluorescence: with V^*_{OC} = 8X the ratio $S^*_b : S^*_c = 14.4 : 30$;
the informative content with the POLYVAR is 4.35X larger

4.2. The optical system of the POLYVAR complements the performance of the objectives. All collectors, condensers, the magni-changer, the binocular tube and the photo optics are coated with achromatic multi-layers and are thus suitable for use with widefield objectives. These unique features make it possible to use all known examination techniques and illumination modes with the POLYVAR – with the same optical quality, the same eyepieces and the same fields of view.

4.3. The correction of the objectives permits the imaging of an object field corresponding to a field of view 30. This is based on an infinite tube length. The barrel length on the nosepiece is 45 mm, the screw-on thread has a standard diameter of 0.8 inches. These widefield objectives have been developed for the UnivaR research microscope and confer the same quality standards of this top-class instrument to the POLYVAR.

4.4. The choice of object types is dictated simply by the needs of the examination problem in hand and not by restrictions imposed when employing certain contrast or illumination techniques.

4.4.1. The Reichert plane achromatic objectives give a very contrasty and absolutely flat image right to the periphery of the wide field and at the same time give excellent resolution. Thus the quality of these objectives meets the needs of most users in practically all fields of science.

4.4.2. The Reichert plane apochromatic objectives represent the highest quality available in micro optics today. Therefore the use of these objectives is confined to special applications where a high degree of colour correction is necessary and where a higher numerical aperture is essential.

4.5. Change of objectives. A sextuple nosepiece with maintenance-free ball-bearings and precision click stops gives reproducible image centration within one micron. The orientation of the nosepiece on the optics carrier is dorsal, i. e. opposite the observer. This means a good view on the specimen, easy immersion and direct reading of the objective magnification. For most of the examination techniques an outfit of 6 objectives will be sufficient; for special techniques the nosepiece may be exchanged on a horizontal slide.

4.6. The change of total magnification can be augmented by a built-in turret magni-changer. This device modifies the total magnification by the factors 0.8X, 1X and 1.25X and is effective for all examination techniques, illumination modes and with all supplementary equipment. Furthermore, there is a built-in Bertrand lens for use when adjusting the annular phase contrast diaphragms and controlling the illumination of the objective pupil.

4.7. Changing the 10X eyepiece on the POLYVAR is only necessary in special cases – it is not necessary to do so under normal working conditions. The 10X plane compensating eyepieces are of a true widefield type, with a field of view 24 and a viewing angle of 51°, and represent the limit as far as physiological usefulness is concerned. The distance between the exit pupil and the eyepiece rim is 17.5 mm, which means that the eyepieces can be used by spectacle wearers without a reduction of the viewing angle.

4.7.1. It is not necessary to change eyepieces when changing over to photomicrography. With the POLYVAR focusing eyepieces are not used but a frame format and the focusing aids are projected into the normal eyepiece (see point 8.4.).

4.7.2. It is, however, recommended to change the eyepieces when examining specimens with weak fluorescence. The WPK 6.3X eyepiece gives the same field as the 10X but provides – in combination with the magni-changer 0.8X – the illumination of a 5X eyepiece and an object field corresponding to field of view 30.
For extremely high magnifications – e. g. chromosome research – a WPK 16X eyepiece is available.

5. Contrast Techniques

Some specimens do not have adequate natural structural details to provide informative images with brightfield techniques. Nor can these features be enhanced with staining techniques. The frequently practised expedient of reducing the illumination aperture produces an unacceptable loss of resolution and is not really an adequate solution. A more satisfactory solution is to use one of the contrast techniques – usually in combination with special objectives and condensers.

5.1. Darkfield illumination. With this technique no direct light enters the objective and hence empty specimen areas appear completely dark. Specimen details, however, show up brightly against the dark background. Using this illumination technique particles become visible even if their size is below the resolving power of the objective.

5.2. Phase contrast illumination converts – by means of an interference in the imaging system – the phase distinctions present in every specimen into amplitude variations. The structures become visible with a bright/dark contrast.

5.3. Polarized light causes a brightening of anisotropic particles in determined interference colours. These colours are specific for special physical characteristics and, therefore, permit conclusions to be made about condition and structure of the specimen.

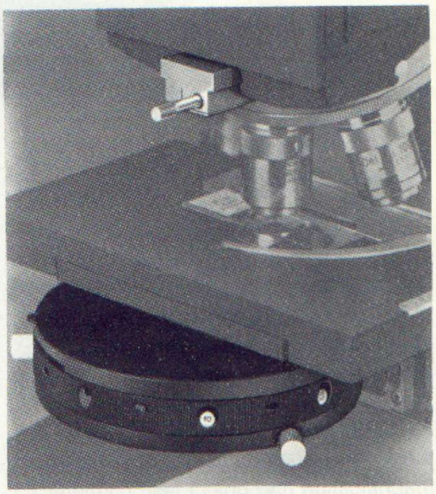
5.4. Interference contrast makes variations of refractive index and thickness within the specimen visible either as a black/white contrast with a pronounced three-dimensional effect or as interference colours. This technique produces clear images from poorly contrasted specimen structures. It permits the full use of the objective aperture, with unrestricted resolution and without the disturbing halo effect associated with phase contrast technique. The reduced depth of focus of this technique permits layer-wise scanning of the specimen.

5.5. Contrast techniques in practice. No single contrast technique produces optimum results for all specimens. Each method is suitable for certain types of specimens and the most suitable technique can only be selected after trials. Hence an instrument that permits a rapid change of technique is the "ideal" for many tasks in modern science.

The need to exchange optical outfits for many of the examination techniques makes the "ideal" difficult to achieve using conventional microscopes.

Furthermore, widefield images when employing phase contrast or interference contrast techniques have been impossible to obtain.

The POLYVAR, like the UnivaR, has the unique capability of permitting rapid changes from one technique to another whilst maintaining the same image quality – the same field of view – and without optical or mechanical changes to the focus.



5.5.1. Interference contrast equipment. Conventional microscopes employ special objectives for interference contrast (IC) techniques and each objective incorporates a separate IC prism. If the user wishes to carry out comparative examinations he either has to accept the image degradation caused by the IC prism (when used for other techniques) or use a second set of objectives.

The unique design of the POLYVAR with its single "main prism" inserted in a slideway above the nosepiece eliminates this problem. The "main prism" is inserted for IC techniques and withdrawn for all other techniques. Thus a single objective set can be used for brightfield, darkfield, IC, polarization and fluorescence examinations.

6. The Illumination System

5.5.2. For darkfield work all objectives from 25X to 100X are suitable, and those with a numerical aperture of 0,65 or higher are fitted with an iris diaphragm. For phase contrast we offer a set of plane achromatic objectives with magnifications of 10X, 30X and 100X. These objectives may also be used for less critical brightfield work but it should be noted that the image quality is reduced by the annular phase coating. In order to maintain the highest performance standards we recommend a combination of brightfield and phase objectives.
For example, a complete optical outfit on the sextuple nosepiece may consist of 3 phase contrast and 3 brightfield objectives with identical magnifications. This outfit is ideal for brightfield, darkfield, phase contrast, interference contrast, polarization and fluorescence – uncompromising as far as quality and field of view are concerned.

5.5.3. The universal condenser of the POLYVAR is perfectly matched to the wide capability of the objective set. In this version the turret of the contrast condenser is equipped with annular phase contrast diaphragms, 3 interference contrast prisms, 1 annular darkfield diaphragm and 1 empty opening for brightfield. The optics of the condenser have achromatic aplanatic correction, they may be used for oil immersion work (N. A. = 1.30) or used dry (N. A. = 0.90). The universal condenser is also suitable for polarized light techniques. The polarizer is fitted to the light exit opening of the stand; the position of the rotatable filter being read off from a scale.
The analyser is inserted in a slide above the objective.

5.5.4. Simpler optics may be chosen when only a limited number of examination techniques are to be used.
We also supply the universal condenser with limited optical elements for brightfield/darkfield/phase contrast techniques or for brightfield / darkfield / interference contrast techniques.
The immersion darkfield condenser (1.42/1.18) is recommended as a supplement for brightfield or transmitted-light fluorescence work.

The 100 W halogen lamp built into the basic instrument is extremely efficient and quite adequate for normal visual observations and photomicrography. The optional high-performance lamp will only be needed in special cases, e. g. micro projection, TV or cinemicrography and especially in fluorescence microscopy.

6.1. The 150 W xenon burner is about 3 times more efficient than the halogen lamp and the colour temperature is about 6000 K (daylight colour film). Luminosity and spectral distribution of the xenon light also allow it to be used for blue-light fluorescence excitation.

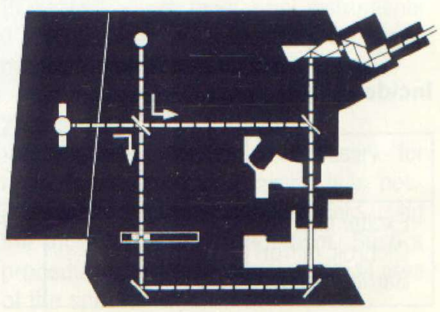
6.2. The HBO 200 high-pressure mercury vapour burner is the best and most economic light source for both transmitted and incident-light fluorescence microscopy. The spectral distribution of the burner permits – in combination with the appropriate filters – excitation of all wave-lengths necessary for modern fluorescence microscopy. In visible light the HBO burner produces an image which is about 7 times brighter than that from the halogen lamp. Colour reproduction is, however, not correct and this burner is only suitable for less critical specimens and black and white photography.
Some manufacturers offer the HBO 50 with their research microscopes – we believe that an instrument in the POLYVAR class justifies the enhanced performance of the HBO 200. It gives a brighter and more even illumination over the whole wide field. Furthermore, the higher initial cost will quickly be offset by the considerably longer working life obtained from the HBO 200.

6.3. The lamp housing is mounted at the rear of the stand and forms an integrated part of the microscope stand. The high-aperture collector and the loss-free light path provide optimum use of the light source. Collector adjustment and burner adjustment in 3 axes can be carried out from a comfortable working position with simultaneous visual control.

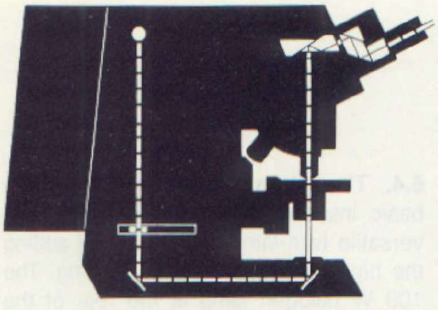
6.4. The illumination system of the basic instrument can be upgraded to a versatile twin-lamp unit by simply adding the high-performance lamp housing. The 100 W halogen lamp at the rear of the stand is directed down towards a deviating mirror and the high-performance lamp is directed horizontally – without deviation – to the incident-light illuminator. The two light paths cross – without interference – and depending on which paths are selected the following illumination modes are available:
- incident-light illumination with the HBO (XBO) burner,
- transmitted-light illumination with the halogen lamp,
- mixed illumination using both light sources.

6.5. A deviating mirror inserted at the intersection of the illumination paths produces exactly the opposite illumination modes:
- incident-light illumination with the halogen lamp,
- transmitted-light with the HBO (XBO) burner.

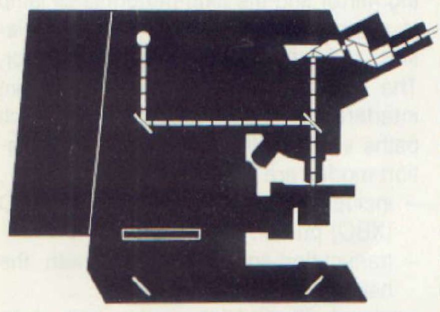
Therefore, in contrast to all normal instruments, no separate mirror housing, no additional twin-lamp unit and no fundamental modification of the illumination system is needed to equip the POLYVAR with a very flexible and versatile twin-lamp illumination.



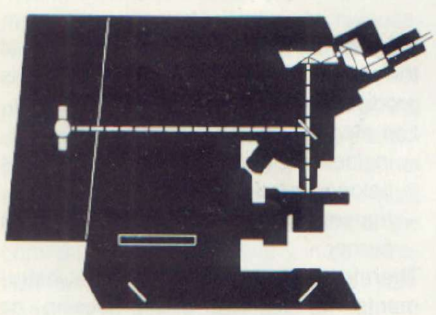
7. Fluorescence



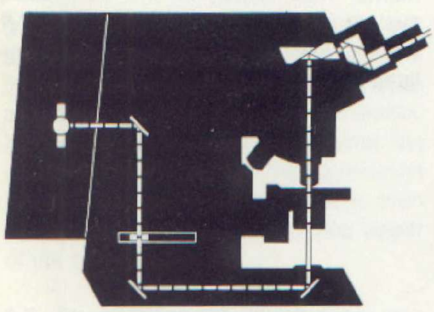
Transmitted light with halogen lamp



Incident light with halogen lamp



Incident light with HBO-burner



Transmitted light with HBO-burner

The unique illumination system of the POLYVAR also gives corresponding benefits for fluorescence techniques. Transmitted-light and incident-light excitation can be obtained alternatively using the same light source. The equipment is clearly visible and easy to operate. All filter combinations are carefully matched and clearly marked.

7.1. Transmitted-light fluorescence with blue excitation is easily achieved if specimen staining is correct. The built-in halogen lamp has only to be supplemented with an FITC exciter filter (blue) (inserted in the filter holder), the barrier filter module and the immersion darkfield condenser fitted.

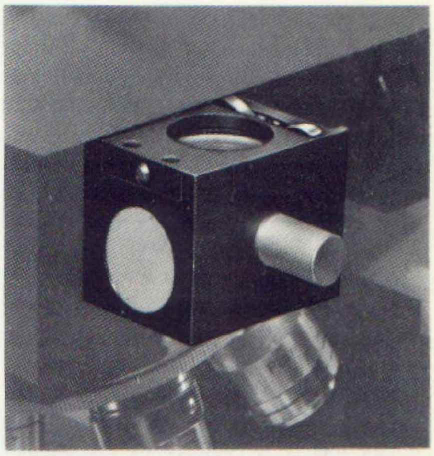
7.1.2. Transmitted-light fluorescence with differing excitation wave-lengths requires the use of the high-performance lamp housing, the HBO burner and the filter combination for the required excitation mode.

7.1.3. The filter holder for all transmitted-light fluorescence exciter filters is built into the stand as is the holder for neutral filters supplied with the basic equipment.

7.2. Incident-light fluorescence is obtained with the POLYVAR using a unique optical combination. The incident-light illuminator with its own set of objectives no longer has to be interchanged with the transmitted-light objective nosepiece. It is sufficient simply to insert a fluorescence filter cluster into the optics carrier – in addition to the already mounted objectives. This feature alone results in many benefits:

7.2.1.
The objective nosepiece and all objectives remain mounted on the stand and can be used without restrictions. Normally no second objective set is needed and the change of optics is eliminated.

7.2.2.
The working position of the microscope stage remains unchanged.



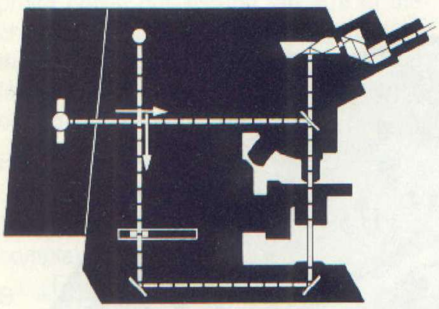
7.2.3.
The changeover from techniques in visible light to incident-light fluorescence can be carried out very easily and quickly. No optical or mechanical modifications of the instrument are necessary – the changeover to incident-light illumination does not introduce an inconvenient magnification factor. The image brightness and the nominal field of view remain unchanged.

7.2.4. The flexibility of the POLYVAR when used for incident-light fluorescence techniques is considerably enhanced by the plug-in module concept. Conventional microscopes incorporate up to four built-in interference beam splitter mirrors which are usually difficult to exchange. The POLYVAR is designed to accept any number of plug-in modules each incorporating matched exciter and barrier filters. Two plug-in modules can be housed in the stand at any one time to provide a very rapid changeover between excitation modes. It only takes slightly longer to exchange modules thus extending the range of possible excitation modes from the simplest single FITC mode up to any number of modes including those required for special examination techniques.

7.2.5.
We supply as **standard outfit** six different filter combinations which correspond to the most popular requirements. On demand, we adapt these filter combinations to personal customer requests.

Incident-light fluorescence modules

	U 1 UV	V 1 violet	V 2 blue violet	B 1 blue	B 2 blue narrow	G 1 green
Exciter filter Dichroic mirror Barrier filter	BP 330–380 DS 420 LP 418	BP 405/8 DS 420 LP 418	BP 390–450 DS 460 LP 475	BP 455–490 DS 500 LP 515	BP 455–490 DS 500 BP 515–560	BP 546/10 DS 580 LP 590



Mixed illumination –
IL–HBO-burner/TL–halogen lamp

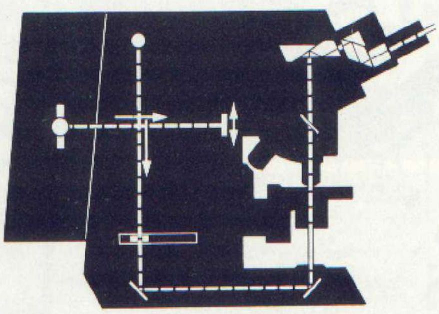
7.3. Mixed illumination with transmitted-light and simultaneously incident-light fluorescence is particularly suitable for cases where fluorescing details need to be localized in their – non fluorescing – surroundings. Mixed illumination may also be helpful in determining the numerical ratio between marked and unmarked particles.

7.3.1. Darkfield illumination initially shows the entire specimen. In combination with incident-light excitation and brightness adjustments the fluorescing details become visible.

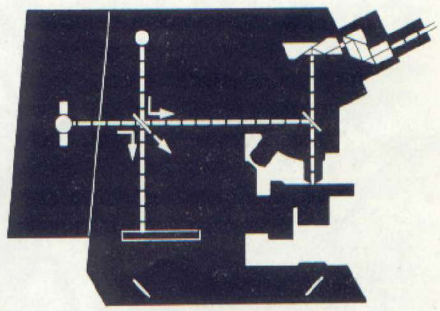
7.3.2. Fluorescence contrast can be obtained by combining phase contrast with incident-light fluorescence techniques. This combination is suitable when the darkfield optics do not reveal the non-fluorescing specimen details. Standard equipment is used for the phase contrast; incident-light excitation is then achieved using the same objectives.

7.4. The brightness of incident-light excitation is at its optimum because of the direct light path and the high-aperture collector. This brightness may be increased further by the use of the 6.3X plane compensating eyepiece which, in combination with the 0.8X step of the magnichanger, produces an overall magnification of 5X. It is important to note that even for fluorescence microscopy the POLYVAR is a true widefield microscope with an object field corresponding to a field of view 30.

7.5. Protection against fading of the specimen is one of the key problems in fluorescence techniques. Continuous increases of excitation energy are coupled with increasing rates of specimen fading. Therefore, critical specimens should only be exposed to full radiation for the shortest possible time. The POLYVAR has some very practical protective features which do not hinder the necessary image selection and focusing.



7.5.1.
After switching out incident-light excitation, the specimen can be scanned in transmitted-light darkfield in visible light. Under these conditions also non-fluorochromed particles show up clearly against the dark background. When the interesting specimen areas have been located the incident-light excitation is switched in again and will now show all fluorescing details.



7.5.2.
With suitable stains careful image selection and focusing can also be made by means of incident-light blue excitation using the halogen lamp. This light source has no short-wave light and, therefore, does not cause fading. For such examinations the deviating mirror is swung in, the HBO 200 is automatically switched out and the light from the halogen lamp is directed to the incident-light ray path. Image selection and focusing can then be made using the visible weak fluorescence. Swinging out the deviating mirror automatically switches in the HBO 200. This very convenient auxiliary illumination technique is very simple with the POLYVAR – with most other instruments it is impossible or at least very complicated.

7.5.3.
If full UV excitation is necessary for image selection and focusing it is possible to reduce the illuminated field using the incident-light iris diaphragm. Such a procedure ensures that only a small area of the specimen is affected.

Comparison Fields of view / Image fields:

POLYVAR

Visual: $V^*_{Oc} = 8\times$

$S^* = 30$

35mm Photo: $M_1 = 1{,}8\times$

$B^* = 24$

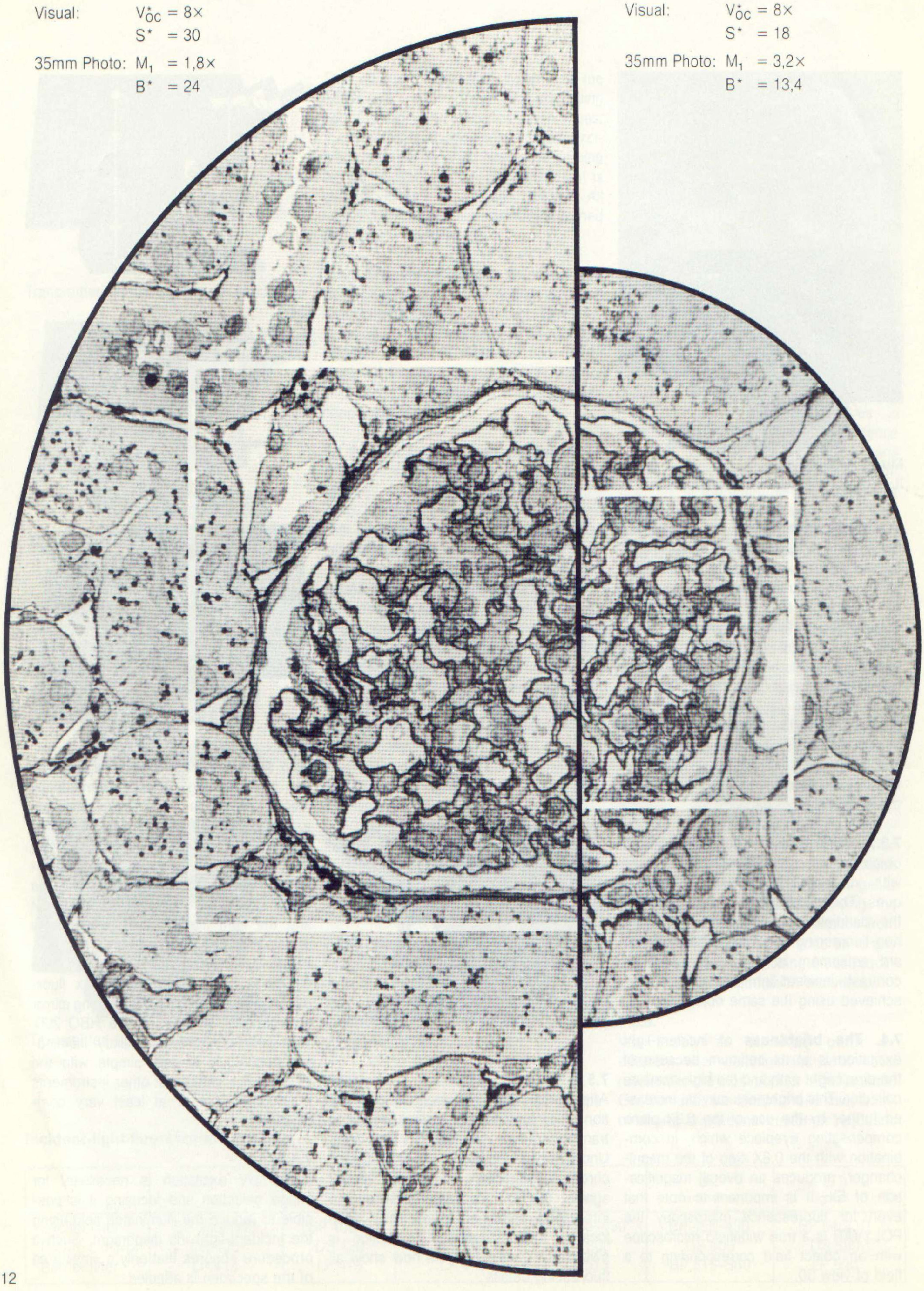
Conventional Microscope

Visual: $V^*_{Oc} = 8\times$

$S^* = 18$

35mm Photo: $M_1 = 3{,}2\times$

$B^* = 13{,}4$



8. Widefield Photography

Earlier we have referred to the possibility of creating the "widefield effect" in visual microscopy by using suitable high-quality optics. In fact, such techniques can be used to increase the eyepiece angle, that means the image field diameter S' (see 4.1.). In photography the size of the photographed field does not only depend on the photo optics but also on the size of the chosen camera format. Analogously to the field of view number also an image field number B may be calculated for a determined camera system:

$$B = \frac{\text{Image diameter (format diagonal)}}{\text{Camera Scale } M_1}$$

The image field number modified to the complete system is

$$B^* = \frac{\text{Format diagonal}}{M_1 \cdot q_C \cdot q_I}$$

As the format diagonal is determined by the film format, the imaging of a "wide field" can only be obtained by using photo optics with low magnification M_1 or by means of a magni-changer with $q_C < 1$.

On the other hand, intermediate tubes or incident-light illuminators cause, also with photography, a reduction of the imaged object field.

$$D_{CAM} = \frac{B^*}{V_{Obj}}$$

8.1. POLYMATIC – the widefield photo system

The POLYVAR and the UnivaR are the only microscopes in the world market that satisfactorily allow the user photographic "wide fields" onto 35 mm film. The POLYVAR offers true widefield photography with a photo field 24 for all film formats, with all types of objectives and using any examination technique.

The photographic reproduction of a large object field requires top quality optics. At the same time the low-power photo optics limit the magnification standard on the film and therefore cannot be used with all specimens. Because of these limitations there have been considerable problems in imaging wide fields.

The POLYVAR possesses all necessary prerequisites for consistent use of the widefield optics. In addition to that it is possible by means of the built-in magni-changer to change to standard magnifications with the usual object field sizes.

A picture with a scale 400 : 1 – using the 40X objective – therefore can be obtained in different ways:

1) POLYVAR – 4 × 5'' large format contact copy or Polaroid
   object field diameter 0.37 mm
2) Conventional microscope 35 mm camera
   subsequent enlargement to 7 × 10 mm
   object field diameter 0.34 mm
3) POLYVAR 35 mm widefield
   subsequent enlargement to 13 × 18 mm
   object field diameter 0.6 mm.
   This means a 2.6X higher informative content of the widefield exposure (see table 2).

	35 mm-Camera				4 × 5''-Camera			
	M_1	q_W	M_1'	B*	M_1	q_W	M_1'	B*
Conventional Instruments	3,2×	1×	3,2×	13,4	8×	1×	8×	19,5
POLYVAR	2,25×	0,8× 1× 1,25×	1,8× 2,25× 2,8×	24 19,5 15	8×	0,8× 1× 1,25×	6,3× 8× 10×	24 19,5 15

	Camera	V_{OBJ}	M_1	q_W	M	B*	D_{CAM}
a	4 × 5''-Camera POLYVAR	40/1,00	8×	1,25×	400×	15	0,37 mm
b	35 mm-Camera POLYVAR	40/1,00	2,25×	0,8×	72×	24	0,6 mm
c	35 mm-Camera Conventional Instrument	25/0,45 40/1,00	3,2×	1×	80× 125×	13,4	0,54 mm 0,34 mm

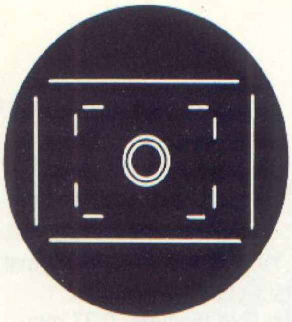
M_1 Camera scale
q_C Factor of the magni-changer
B* modified image field number of the camera
M Total scale on film ($M_1 \times q_C \times V_{OBJ}$)
D_{CAM} Diameter of the photographed object field

8.2. Detail resolution in photography depends not only on the photographic emulsion but also on the resolution of the objective. If suitable film material is selected the effects of the emulsion may be neglected. However, the selection of the objective has to be carefully made with regard to magnification, resolution, depth of focus and size of object field. Again, the POLYVAR offers additional flexibility by virtue of its widefield imaging and its magni-changer.

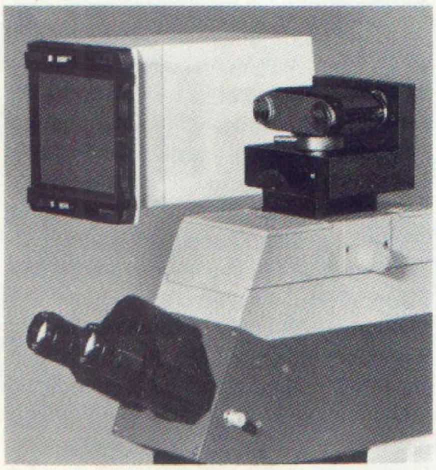
If in practice the imaging of a large object field is of no advantage, the system may be used in reverse to improve detail resolution, which means to improve image quality. For example, to photograph a specimen of a diameter of 0.54 mm with a conventional miniature camera system an 25X objective has to be used. With the POLYVAR a 40X objective can be used (see table 2/b + c).

In both cases magnification and imaged object field are nearly identical; when using the 40X apochromatic objective, however, the resolution is considerably improved.

8.3. Image selection and focusing as well as the necessary correction of visual acuity is normally achieved by means of graticules inserted either in the eyepiece or in a secondary image. Such graticules are difficult to see and very difficult to focus with dark specimens or with techniques having limited light, e. g. darkfield, fluorescence and interference contrast. Moreover, if the graticules are inserted in the eyepiece they interfere with normal work and must, therefore, continually be exchanged with standard eyepieces.



8.3.1.
On the POLYVAR image selection and focusing is simplified using the system developed and patented for the UnivaR. Format delineation and concentric circles are projected as luminous lines into the image of a normal eyepiece to enable the photo field to be selected. The luminosity may be adjusted continuously on the control unit to compensate for specimen brightness. If format projection is not required it can be manually switched out. When exposing, the switch-out is done automatically by the shutter. This new system eliminates the need for eyepiece changes and enhances the precision of the photography – irrespective of specimen and technique.



8.4. Film formats between 4 x 5" and 16 mm can be used with the POLYVAR photographic system.
The new concept of this system combines the stability of a built-in camera with the flexibility of an add-on unit. The optics as well as the measuring cell and the electronic shutter are built into the stand. The camera housings are interchangeable but, nevertheless, are rigidly mounted on the camera module. All cameras have the same photo field 24 and, therefore, show the same specimen section. Parallel exposures with different formats can be used to directly compare their informative content. When cameras are exchanged the film format and exposure time are automatically adjusted.
The following cameras are available:

8.4.1 35 mm miniature camera. The film magazine is easily exchangeable, the change of film types is made quickly and without loss of exposure.

8.4.2. 4 × 5" large format camera with vertical arrangement of photo cassette and ground glass screen. This camera is easy to operate from sitting position, the ground glass serves for image projection and can, therefore, be used for demonstrations to a limited number of observers.
All film types available for the international format 4 × 5" can be used:
4 × 5" cut film/Polaroid sheet film
$3\frac{1}{4} \times 4\frac{1}{4}$" roll film/ Polaroid roll film
9 × 12 cm cut film.

8.4.3. 16 mm Cine cameras are mounted laterally to the microscope stand by means of the DUAL reflex module (see point 9.2.). Image selection and focusing is made through the binocular tube; light measurement and display of exposure time calculated for the cine camera is made by the POLYMATIC. Any brightness modifications within the specimen or caused by the changeover to another examination technique are immediately measured and compensated. Independently of cine shooting the POLYMATIC cameras remain functional and may be used simultaneously for single exposures with large formats. A movable illuminated pointer may be projected to mark important specimen details.

8.5. Exposure practice on the POLYVAR differs from the conventional photomicroscopes in so far as the complete automatic systems offers considerably more flexibility.
The POLYMATIC is a fully automatic photo system. The measuring element built into the camera module is highly sensitive and reacts immediately to all changes in brightness within the specimen. Measured brightness, adjusted film speed and indicated camera format are automatically processed by the built-in computer, exposure time is determined and the electronic swing-out mirror shutter programmed. Simultaneously exposure time is indicated on the LED scale of the control unit.

8.5.1. For routine photography the best one can do is forget the photo equipment and concentrate on selecting specimen detail and optical image focus. When the "release" is touched it will certainly produce an excellent photograph.

8.5.2. Standard exposures are also possible with the POLYMATIC. It is well known that specific problems and very critical film types have their preferred exposure times. For such purposes the image brightness may be regulated until the required standard exposure time is indicated on the LED scale.

8.5.3. With difficult specimens the computed exposure time may be modified in the following ways:
- If exposure times are too short the light is subdued.
- If exposure times are too long the reciprocity failure of the film emulsion is compensated.
- If exposure times are much too long the light source is intensified.
- In darkfield or with structures having significant variations in brightness distribution the computed exposure time must be modified to suit the important features.
- If special effects are required the corresponding detail is deliberately over or underexposed.
- In cinemicrography the brightness is permanently controlled in order to compensate for unevitable changes.

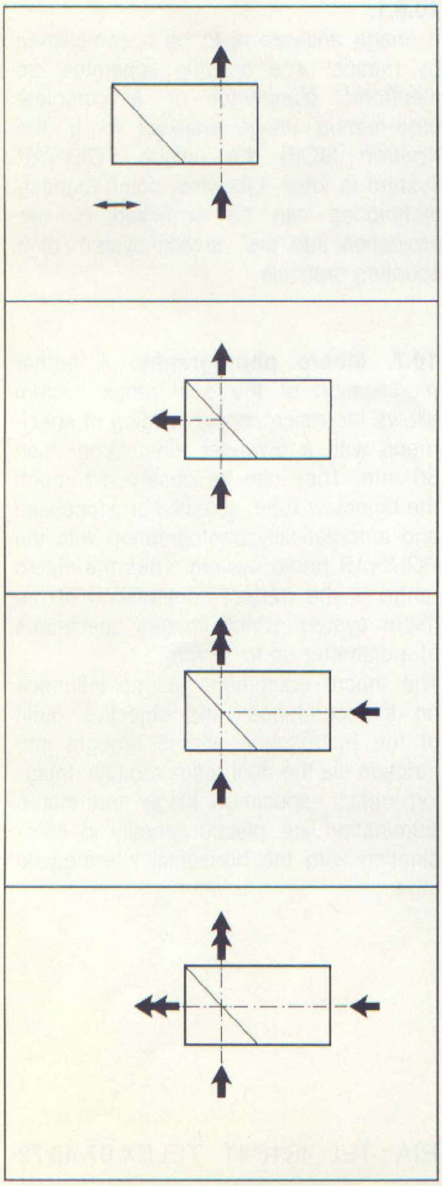
Only by the above advanced information – which is of a precision of about 1/2 shutter time – it is possible to effect these corrections.
The automatically computed exposure time may be manually overridden. Instead of the normal factor keys a regulator is provided on the control unit and it covers the complete time range (20 steps).

8.6.
The sensitivity of the silicon photo cell built into the control circuit is adequate for measuring very faint specimens. There is an easy-to-understand limiting case which can be defined as follows:
Every image still visible and focusable in the viewing tube – with full light from the beam splitter prism (100 %) – in a blacked-out room – with adapted eyes – may still be measured and automatically exposed with the POLYMATIC.
The POLYMATIC achieves all limits of performance that can reasonably be expected from a photo system. The measuring capabilities of the equipment include the selection of 26 light values, continuous automatic shutter adjustment and exposure time display in the range 1/100 sec. to 1 hour. Films with speeds in the range 9 to 42 DIN can be accommodated.

9. Dual Reflex Module

In practical microscopy there is often a need to transmit the image to some other receiving device apart from the normal viewing tube or the photographic equipment. The receivers may be a cine camera, a TV camera or a second viewing tube for dual observation. Similar problems arise with the need to project a second image into, and to view it simultaneously with, the regular image. This may be the case with a drawing apparatus or when projecting marking pointers or graticules and scales or reference standards.

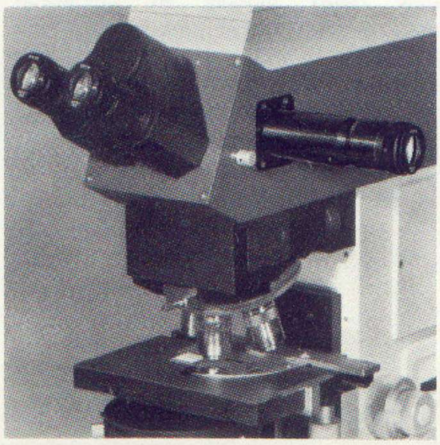
9.1. With conventinal microscopes these problems either cannot be solved at all or only by means of additional elements which have to be clamped between the stand and the viewing tube. Such intermediate tubes change the tube length, introduce a "tube factor", reduce the field of view and also change the viewing height of the tube. These devices cannot be swung in and out and cannot be combined in their functions. They have to be continuously mounted and dismounted which is extremely inconvenient, particularly if an add-on camera is being used.



9.2. The dual reflex module is an important component in the modular concept of the POLYVAR and it avoids all of these disadvantages. It essentially comprises an optical beam splitter which is inserted in the stand between the magni-changer and the viewing tube. This beam splitter – which can easily be swung in or out – is accessible from both sides of the stand and effective on both sides.

9.2.1. Functioning as an image divider, the beam splitter reflects 50 % of the light coming from the objective into a horizontal tube and, at the same time, passes on the remaining 50 % to the normal viewing tube. Thus the microscopical image is divided for the two receivers.

9.2.2. Functioning as a projection system, the opposite side of the dual reflex module reflects 50 % of the light falling on the beam splitter coating into the binocular tube and passes on the remaining 50 % to the horizontal tube. Thus the projected image is superimposed on both halves of the divided microscopical image.



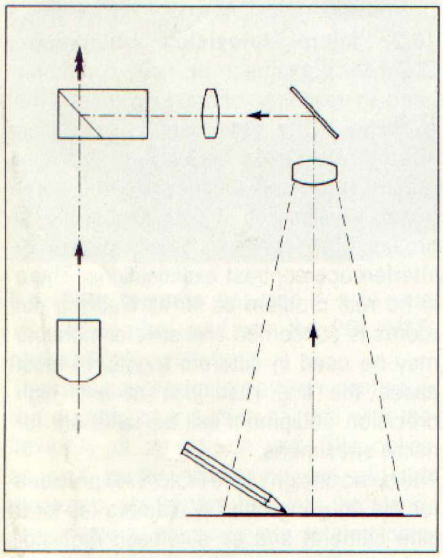
10. Accessories

The dual reflex module plus any one of the accessories produce a functional unit – what is more, two units can sometimes be used together.

10.1. Cinemicrographic equipment. The dual reflex module is coupled to a horizontal tube and a special eyepiece with an extremely high eyepoint. Any cine camera can be fitted to it using a simple table stand, and the camera will be operational immediately. Image selection and focusing is then made through the normal viewing tube and the light measurement is done in the normal photo ray path.

10.2. Micro television equipment. C.C.T.V. equipment is now commonly used in teaching laboratories where the expense of the basic camera installation and monitor(s) can be justified. The light requirements are modest and the electronic amplification makes it possible to project images from even phase or interference contrast examinations. There is no real problem as far as blacking out rooms is concerned and several monitors may be used in different rooms. In most cases the line resolution of any high precision equipment will be sufficient for micro specimens.
The basic design of the POLYVAR provides for the coupling of a T.V. camera (as for a cine camera) and an automatic regulator is provided to continuously control light levels. It also protects the camera against excessive light inputs.
The T.V. camera is mounted horizontally which improves the stability of the system and provides better operating conditions for the T.V. tube.
Again, the dual reflex module may be used to project pointers or scales into the image.

10.3. Dual viewing tube. Scientific workers fequently require to consult with colleagues – a projection screen is quite suitable for this purpose with brightfield images but usually too faint with dark specimen and contrast techniques. A T.V. equipment is ideal for demonstrations and teaching but too expensive for laboratory work. Therefore, a dual viewing tube offers the most reasonable solution to this problem. On the POLYVAR it is mounted laterally to the plug-in dual reflex module and does not result in any changes either of the stand or the comfortable working position of the operator. The second viewing position is about 60 cm away from the main binocular tube and the auxiliar binocular may be rotated to provide several partners with the opportunity of viewing the image. An adjustable illuminated pointer may be projected and used to mark interesting specimen details.



10.4. Drawing apparatus with image projection. In spite of the high performance of the photographic equipment the drawing of micro structures has, in certain cases, its advantages, for example:

- three-dimensional structures – the thickness of which exceeds the depth of focus zone of the objective – cannot be clearly photographed in their totality. When drawing the detail can be fully appreciated by refocusing.
- Very complicated structures may be simplified by drawing and thus enhanced in their didactic effect.
- Parallel to the micro exposure individual structures may be pointed out and marked. The drawing may then be used in addition to the photograph – eventually as a superimposed transparency.
- Interesting structures can be marked in the drawing plane before exposure and then be photographed together with these marks.

Image projection also permits the projection of large patterns from the drawing plane into the microscope, to superimpose them on the specimen structure, to compare them and to photograph them together.

The drawing apparatus of the POLYVAR is coupled to the dual reflex module and comprises a focusable projection eyepiece and a deviating prism which is directed towards the drawing plane. The eyepiece is interchangeable and projects the drawing plane in full format up to a diameter of 180 mm.

10.5. Graticule projection. Via the dual reflex module an illuminated graticule negative is projected into the microscopical image. Using this technique it is possible to superimpose on the microscopical structures graticules for simple measuring and counting, special patterns for stereometric measurements, grain size graticules etc.

Here again special optics as well as the mount for the graticule and a light source with illumination optics are coupled to the supplementary horizontal tube. This system has been specially developed for the POLYVAR and is unique.

10.6. Image analysis. It goes without saying that the laterally mounted T.V. camera can be used not only for image transmission but also for connection to an image analysing computer (e. g. QUANTIMET, OMNICON).

10.6.1.

If image analysis is to be accomplished by means of a drawing apparatus, an electronic planimeter or a complete opto-manual image analyser (e. g. the Kontron MOP) the unique POLYVAR system is ideal. Likewise, point counting techniques can be simplified by the projection into the viewing system of a counting graticule.

10.7. Macro photography. A further modification of the dual reflex system allows the macroscopic imaging of specimens with a diameter even larger than 50 mm. They can be observed through the binocular tube, selected and focussed and automatically photographed with the POLYVAR photo system. Thus the macro range is the perfect continuation of the micro system which images specimens of a diameter up to 12 mm.

The macro equipment has no influence on the condenser and objective outfit of the microscope and is brought into function via the dual reflex module. Imaging optics, specimen stage and macro illumination are placed laterally in combination with the horizontal intermediate tube.

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