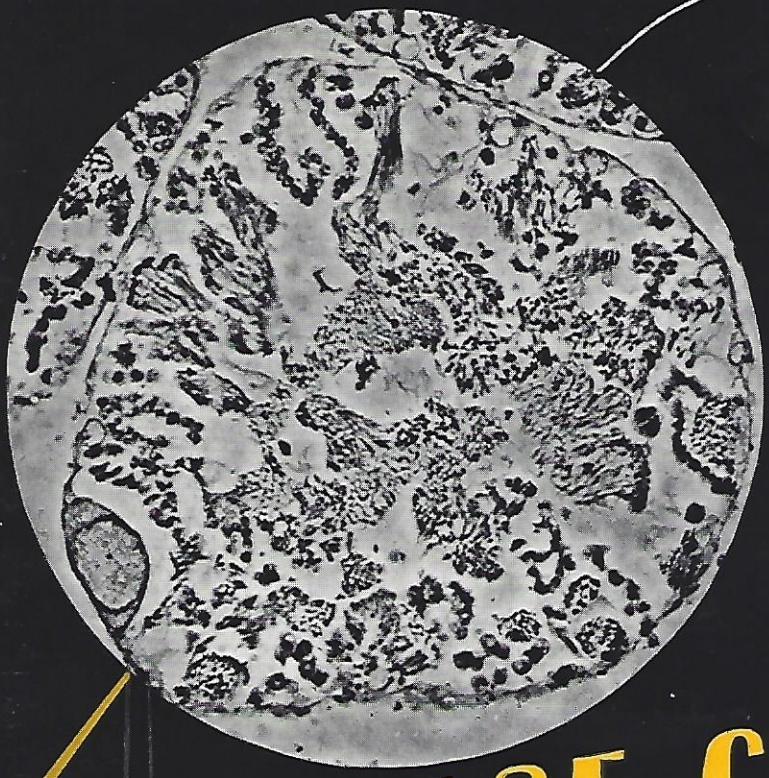
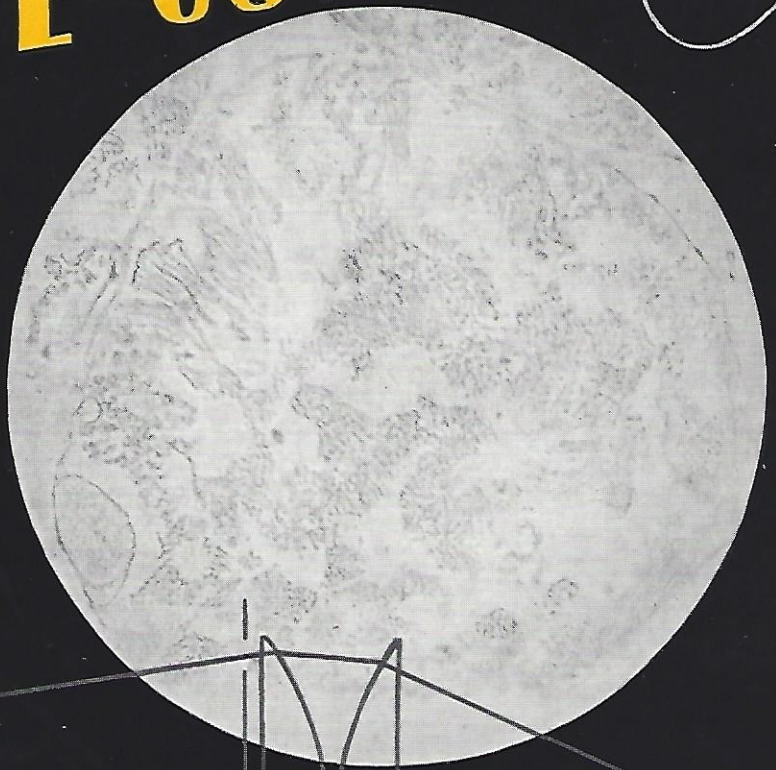


PHASE CONTRAST



WATSONS

THE PHOTOMICROGRAPHS ON THE FRONT COVER ARE OF

OVOTESTIS OF ARION EMPIRICORUM

in balsam

PHASE-CONTRAST ILLUMINATION BY TRANSMITTED LIGHT

The Phase-Contrast method of illumination in microscopy converts small variations in refractive index and thickness in the structure of transparent specimens into visible detail without any significant decrease in resolution. The Watson Phase-Contrast apparatus, developed from the work of Dr. John R. Baker and his colleagues,* has been designed to require the minimum modification to the microscope stand and provide the maximum ease in manipulation and quality of image.

To a modern Watson microscope fitted with the Universal No. 1 Achromatic Condenser, no modifications or additions are required other than the fitting of phase plates into the objectives and a front surface mirror to the substage to eliminate the inevitable spurious images due to the usual type of back silvered mirror.

BASIC PRINCIPLES

When a beam of light from the microscope condenser passes through a specimen, the structure of the object causes scattering or diffraction. A proportion of the incident light passes straight through without deviation (direct light) while the remainder (diffracted light) is deviated through angles which depend on the fineness of the object structure. The same effects occur whether the object is black and white or is perfectly transparent but with variations in refractive index and thickness. The only difference between the two cases is that with the transparent object a further retardation or acceleration of the phase of the direct light, relative to the diffracted light, occurs. Provided that the changes in refractive index and thickness in the transparent object are small, the relative retardation or acceleration of phase is very nearly constant and equal to a quarter wavelength of light.

Since the direct and diffracted beams are superimposed by the objective to form the final image, it follows that if the direct light can be separated from the diffracted light and altered in phase by the necessary quarter wavelength, then a transparent object may be made to give an image in good contrast, the variations of intensity in the image corresponding to variations of optical structure in the object. That is to say, the variations in refractive index and thickness within the transparent object can be converted into variations in light and shade in the image.

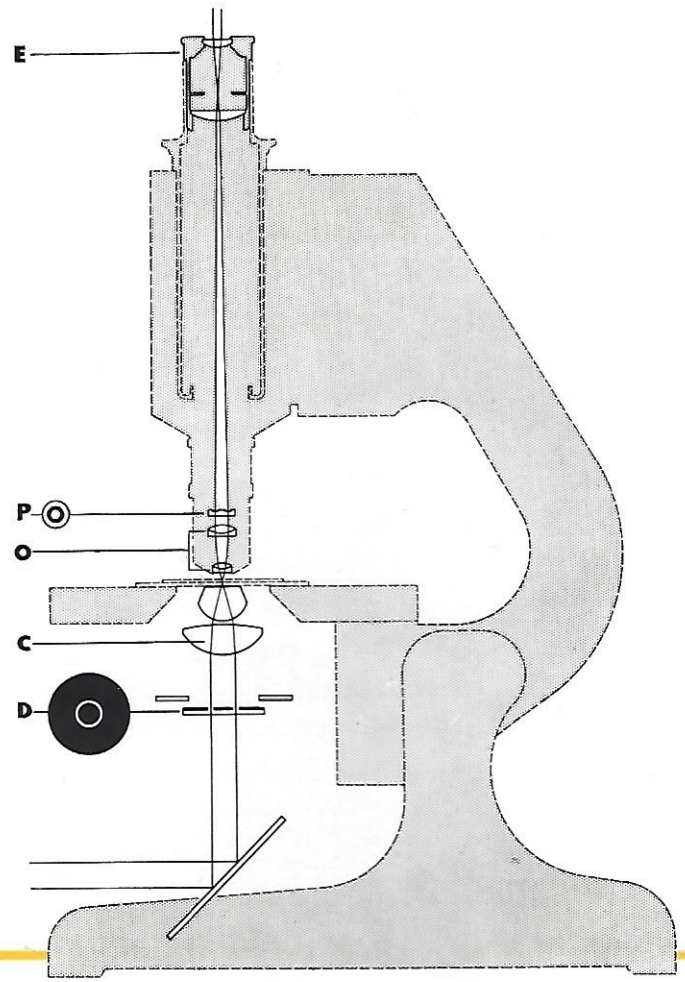
---

* D. A. Kempson, O. L. Thomas and John R. Baker, 1948. "A simple method for phase-contrast microscopy". Quart. J. Micr. Sci., Vol. 89, p. 351.
John R. Baker, D. A. Kempson and P. C. J. Brunet, 1949. "A simple method for phase-contrast microscopy; improvements in technique". Quart. J. Micr. Sci., Vol. 90, p. 323.

This is the basis of the Phase-Contrast method, given in a simple form, and the way in which this may be performed was applied by F. Zernicke as illustrated in Fig. 1.

The diaphragm D, located in the lower focal plane of the substage condenser C, limits the numerical aperture to a narrow annulus. The specimen is thus illuminated by a hollow cone of light the mean numerical aperture of which is about one half of that of the objective O. The objective is focused on the specimen and the light which it collects passes up through the eyepiece E into the eye or photographic camera. The condenser and objective together produce an image of the annular diaphragm in the upper focal plane of the objective at the point occupied by the phase plate P. This is a glass plate acting as a support to a ring of material exactly matching the image of the diaphragm in size and which accelerates the phase of the light passing through it by a quarter of a wavelength relative to any light passing through the remainder (supplementary area) of the plate.

FIG. 1



Since all the light illuminating the object has passed through the annular opening in the diaphragm and an image of the latter is formed on the phase plate ring, the direct light through the object must pass through this ring. Also, the scattered (or diffracted) light from the object which is collected by the objective will, except for a very small proportion, pass through the supplementary area of the phase plate. The direct light has thus been separated from the diffracted light and speeded up by a quarter of a wavelength. The result is an image in light and shade reproducing the optical structure of a perfectly transparent object.

Maximum contrast in the image is attained when the two beams are of the same order of intensity. In practice, the direct light is always the stronger and a thin layer of metal is therefore deposited over the ring on the phase plate and acts as a neutral filter to reduce its intensity.

PRINCIPLES OF THE WATSON PHASE-CONTRAST APPARATUS

The basic operating principles of the Watson Phase-Contrast apparatus are the same as those described above but as can be seen from Fig. 2, the application is different. The particular virtues of this system are that (1) Highest possible image quality is attained. (2) Modern Watson objectives are used. (3) A fully corrected condenser from the standard range is used. (4) The illuminating unit has as its basis a high intensity solid source lamp which is eminently suitable for all other forms of microscope illumination.

It should be noted that :—

(1) Provided an image of the illuminating annular diaphragm is focused on to the phase ring, the latter need not lie in the focal plane of the objective, and

(2) Provided the substage condenser provides hollow cone illumination of sufficient numerical aperture to illuminate the phase ring in the objective, whether or not the condenser is "focused" (in the conventional sense) is of no consequence.

Referring to Fig. 2, the optical arrangement is as follows :—

The source of light S, is a ribbon filament and the light is directed into the microscope by a large diameter aplanatic type lamp condenser L.C. The annular diaphragm D is placed close to the lamp condenser (and at a fixed distance from the microscope stage ST measured along the light path). The substage condenser C, is the Watson Universal No. 1, and for phase-contrast, always works at a level below the normally focused position.

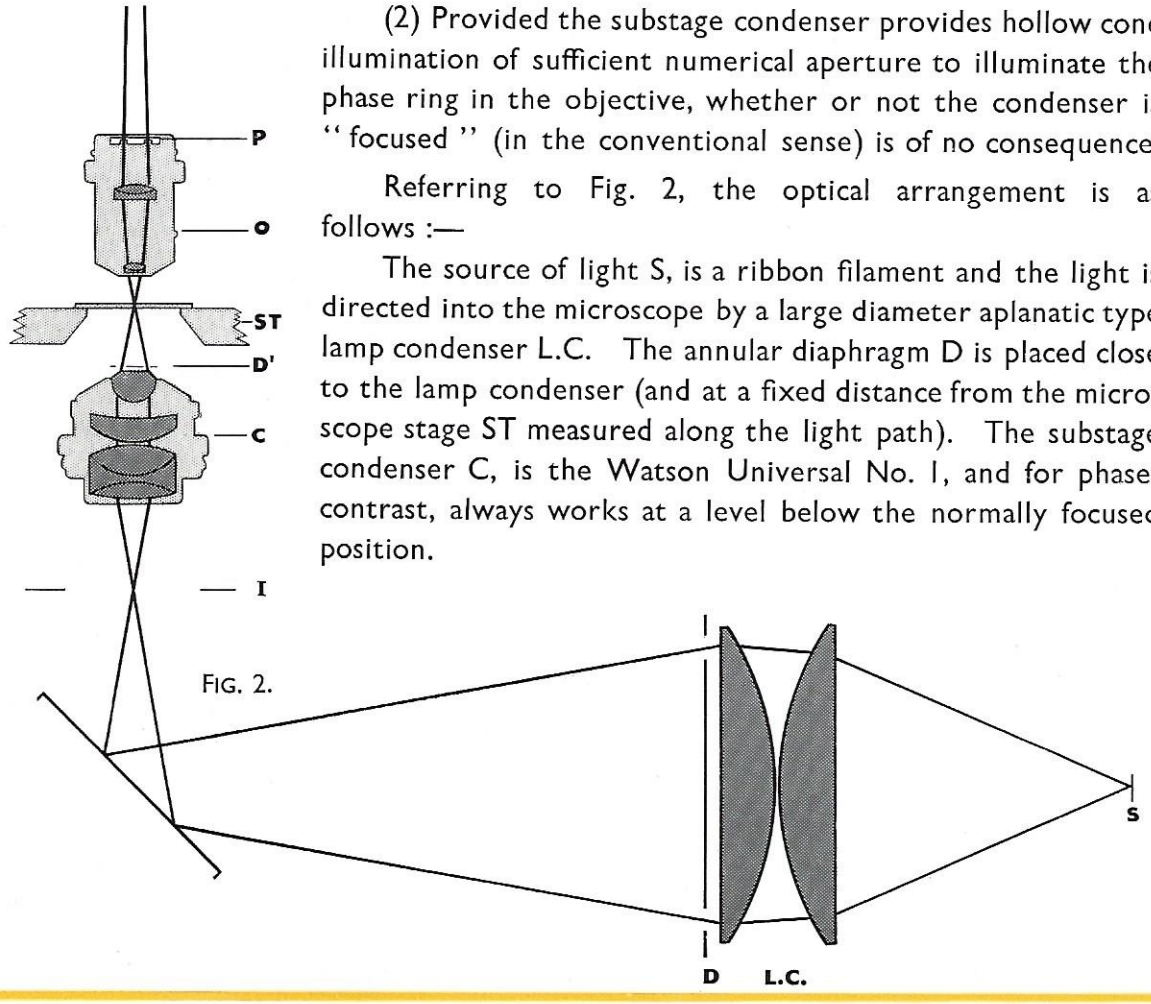


FIG. 2.

The phase plate P is located at a particular distance behind the lenses of the objective O. The lamp condenser forms an image of the source near the substage iris I and the substage condenser forms a second image approximately in the plane of the specimen on the stage. This condenser also produces an image of the annular diaphragm in the plane D and the objective forms a second image in the plane of the phase plate. The use of a fully corrected condenser materially contributes towards the final image quality since it takes part in producing on the phase plate an image of the annular diaphragm and the contrast in the microscope image depends on the exact superposition of the diaphragm image on the phase plate. This applies in all phase-contrast systems.

WATSON PHASE-CONTRAST APPARATUS

The complete equipment for phase-contrast comprises the following items :—

(1) *Lamp Unit* which consists of a lamp housing with focusing adjustment, a ribbon filament bulb 6 v. 18 amps, in a pre-centred cap, lamp condenser, annulus centring adjustments, tie-strap and tie-bar, all mounted on a horseshoe foot with tilting adjustment, one annulus disc for each objective.
(2) *Lamp Transformer and Regulating Resistance* (for use with 200–250V. A.C. mains only).
(3) *Front Surface, Plane Mirror* to replace normal back silvered mirror fitted to microscope.
(4) *Standard Objectives fitted with Phase Plates.*
(5) *Auxiliary Telescope* (with additional objective for Binocular Microscope) to check adjustment.
(6) *Universal No. 1 Condenser* unless already fitted to microscope.

A Mercury Green filter is neither included nor generally advised for use with the equipment since white light gives very satisfactory results and is less tiring to the eyes. Due to the inevitable slight change of phase-contrast effect with colour and high red content in the light from a filament lamp, an increase in contrast may be achieved by the use of a light blue (daylight type) filter.

The phase plate is contained in a cell which screws directly into the back of a standard objective ; each cell is engraved with the focal length and serial number of the objective to which it has been fitted. The processed side of the phase plate faces inwards, towards the lenses of the objective. Since the phase plate is not normally removed from its objective, this gives adequate protection to the somewhat delicate coated surface. The presence of the phase plate within an objective affects its performance with other forms of illumination to a very slight extent. If even this is objected to, the phase plate may be removed, but care must be taken that the surface of the plate is not damaged. The standard phase plates supplied give positive contrast (thicker or more highly refracting parts of the object appear dark) and the density of the light absorbing layer has been adjusted to give optimum contrast with an average specimen. Phase plates giving negative (reversed) contrast can be supplied to special order.

The following objectives can be fitted with phase plates :—

	Focal Length	Magnification	Numerical Aperture	Free Working Distance mm.
Parachromatic Series	16mm.	×10	0·28	7·0
	12mm.	×15	0·34	3·5
	4mm.	×40	0·70	1·0
	2mm. O.I.	×100	1·28	0·23
Fluorite Series	3·6mm. O.I.	×50	0·95	0·22
	2mm. O.I.	×90	1·30	0·12

THE BACTIL MICROSCOPE WITH PHASE-CONTRAST EQUIPMENT

PRICES OF PHASE-CONTRAST APPARATUS

SUGGESTED COMPLETE OUTFIT

Code No. £ s. d.

8A650 The Bactil Binocular microscope stand with :

Inclined binocular body to interchange with monocular body,
Rackwork coarse focusing adjustment,
Vertical lever fine focusing adjustment,
Controls for focusing adjustments on both sides of the limb,
Square built-in flat top mechanical stage with 75mm. lateral traverse, fitted with scales and reading by verniers,
Rackwork focusing substage with clamps for condensers,
Rotating quadruple nosepiece,
Surface aluminised mirror.

Complete with :

2 pairs of Huyghenian Eyepieces, ×6 and ×8,
Quadruple rotating nosepiece,
16mm. Parachromatic objective N.A. 0·28, ×10,
4mm. ,, ,, N.A. 0·70, ×50,
2mm. ,, ,, N.A. 1·28, ×100,
Universal No. 1 Achromatic Condenser N.A. 1·0, in centring mount.

Lamp unit which consists of a lamp housing with focusing adjustment, a ribbon filament bulb 6 v. 18 amps. in a pre-centred cap, lamp condenser, annulus centring adjustments, tie-strap and tie-bar, all mounted on a horseshoe foot with tilting adjustment - - -

Transformer and dimming resistance for A.C. mains voltage 200–250 -

Auxiliary telescope with object glasses suitable for both monocular and binocular bodies - - - - - - - - - - -

3 Metal Annular discs suitable for the 16mm., 4mm. and 2mm. Parachromatic objectives - - - - - - - - - - -

3 phase plates suitable for the 16mm., 4mm. and 2mm. Parachromatic objectives - - - - - - - - - - -

Mahogany case for microscope and equipment - - - - -
(This microscope outfit can equally well be used for ordinary microscopy).

The Phase-contrast apparatus can be fitted to existing Watson Microscopes provided the objectives with the instrument are of the Watson new series. The cost of the phase plates shown below would include fitting to existing objectives.

8A635 Lamp unit which consists of a lamp housing with focusing adjustment, a ribbon filament bulb 6 v. 18 amps. in a pre-centred cap, lamp condenser, annulus centring adjustments, tie-strap and tie-bar, all mounted on a horseshoe foot with tilting adjustment - - -

8A636 Transformer 18 amps. for A.C. current supply 200–250 volts - - -

Code No.		£ s. d.
8A637	Dimming resistance mounted on baseboard for adjusting the intensity of light	
8A632	60mm. Surface-aluminised mirror in mirror box (suitable for " Bactil " and " Service I " microscopes)	
8A633	50mm. Surface-aluminised mirror in mirror box (suitable for " Service II " microscope)	
8A638	Auxiliary telescope, for aligning the outfit and checking the centration of annulus to phase plate, complete with two object glasses for use with either the binocular or the monocular body of the " Bactil " Microscope	
8A639	Auxiliary telescope with one object glass only for the binocular body	-
8A640	Auxiliary telescope with one object glass only for the monocular body	-
8A641	Metal Annular disc for 16mm. Parachromatic objective	-
8A642	Metal Annular disc for 12mm. Parachromatic objective	-
8A643	Metal Annular disc for 4mm. Parachromatic objective	-
8A644	Metal Annular disc for 2mm. oil immersion Parachromatic objective	-
8A645	Metal Annular disc for 3·6mm. oil immersion Fluorite objective	-
8A646	Metal Annular disc for 2mm. oil immersion Fluorite objective	-
8A647	Fitted box to hold up to 4 Annular discs	
8A205	16mm. focal length Parachromatic objective ×10 N.A. 0·28	
8A651	Phase plate for 16mm. Parachromatic objective	
8A206	12mm. focal length Parachromatic objective ×15 N.A. 0·34	
8A652	Phase plate for 12mm. Parachromatic objective	
8A208	4mm. focal length Parachromatic objective ×40 N.A. 0·70	
8A653	Phase plate for 4mm. Parachromatic objective	
8A212	2mm. oil immersion focal length Parachromatic objective ×100 N.A. 1·28	
8A654	Phase plate for 2mm. Parachromatic objective	
8A234	3·6mm. focal length oil immersion Fluorite objective × 50 N.A. 0·95	
8A655	Phase plate for 3·6mm. Fluorite objective	
8A236	2mm. oil immersion focal length Fluorite objective ×90 N.A. 1·3	
8A656	Phase plate for 2mm. Fluorite objective	
8A180	Universal Condenser No. I optical part only	
8A159	Condenser centring mount	
8A648	Spare Ribbon Filament Bulb in pre-centred cap	

Plus P.T.

Service II Microscope

The 16mm. Parachromatic objective cannot be used on the Service II Microscope for phase-contrast. The 12mm. Parachromatic objective must be used in its place.

PHASE-CONTRAST PHOTOMICROGRAPHY

The HOLOPHOT photomicrographic equipment has proved itself to be the ideal equipment with which to use the Phase-Contrast Technique and this does not prevent the camera or microscope from being used for any other form of illumination. This apparatus is compact, free from vibration, complete in itself, operable without any difficulty and universal in its application.

COMPACTNESS

The apparatus occupies the small area of 41 cms. × 36 cms. at the base and is 90 cms. high. It fulfils the demand for visual microscopy with phase-contrast illumination transmitted light, dark-ground illumination, oblique top lighting or vertical illumination. In addition to these, photomicrographs can be made with any of these forms of illumination and this without interrupting the visual examination more than the length of time taken for the exposure of the plate. Furthermore, the equipment can be used for macro-photography up to a magnification of x10.

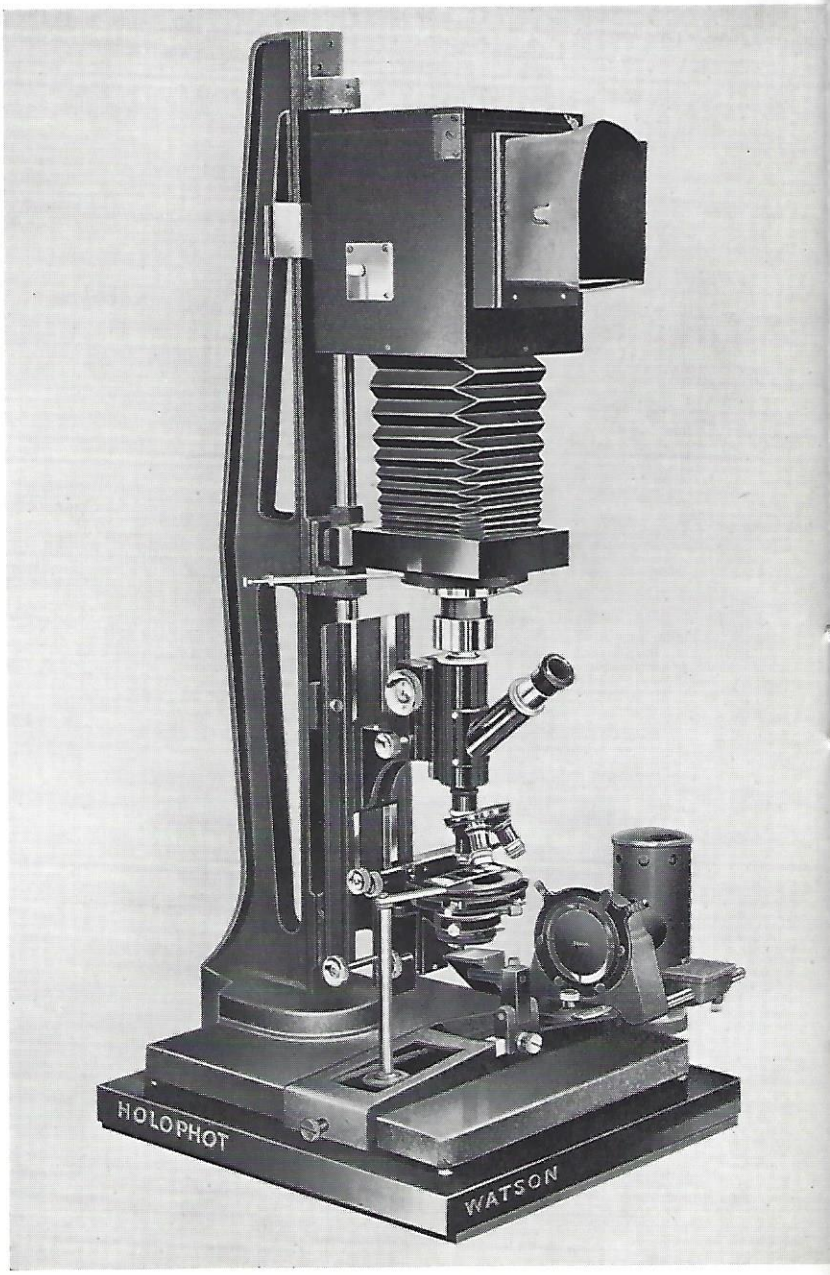
ELIMINATION OF VIBRATION

It will be seen from the illustration that the Holophot is robust in construction, the camera, microscope, stage and condenser being primarily mounted on a heavy vertical casting of a webbed pattern which is bolted on to a heavy metal base plate. This ensures not only the permanent alignment of the optical system, but that if the outfit is subjected to vibrations, it vibrates as a whole. An anti-vibration pad of sponge rubber is fitted under the metal base plate, but where the equipment is to be used adjacent to heavy machinery or traffic, causing considerable vibration, a special base can be supplied.

THE ILLUMINATING SYSTEM

The illuminating unit for phase-contrast is similar to that already described and illustrated but is mounted on a special bracket. This is interchangeable with the normal bracket on which is a Pointolite Lamp Unit, condenser, etc., with which all other forms of illumination may be obtained.

Full particulars of the Holophot Photomicrographic equipment may be obtained from List 5A.



CATALOGUES AVAILABLE

BACTIL BINOCULAR MICROSCOPES
SERVICE I MICROSCOPE
SERVICE II MICROSCOPE
KIMA MICROSCOPE
STEREOSCOPIC MICROSCOPES
MICROSCOPE ACCESSORIES
FENESTRATION MICROSCOPE
THE HOLOPHOT PHOTOMICROGRAPHIC EQUIPMENT
THE WATSON KONIMETER
THE ZOOM LENS

LIST 3538A. *Improvements in design are introduced from time to time, so that illustrations and specifications used in this list may not be correct in every detail.*

W. WATSON & SONS LTD.

Established 1837

Head Office and Showrooms :

313 HIGH HOLBORN, LONDON, W.C.1

Works :

BARNET, HERTS.

TELEPHONE: HOLBORN 2267/8. TELEGRAMS: "OPTICS, HOLB, LONDON."

AGENCIES IN

ARGENTINE	CZECHOSLOVAKIA	ITALY	SOUTH AFRICA
AUSTRALIA	DENMARK	MALAYA	SPAIN
BELGIUM	EAST AFRICA	MEXICO	SWEDEN
BRAZIL	EGYPT	MOZAMBIQUE	SWITZERLAND
CANADA	GREECE	NEW ZEALAND	SYRIA
CEYLON	HOLLAND	NORWAY	TASMANIA
CHILE	HONG KONG	PERU	TURKEY
CHINA	INDIA	PORTUGAL	URUGUAY
CYPRUS	ISRAEL	RHODESIAS	VENEZUELA

Addresses may be obtained on application at Head Office.

Printed in England