WATSON

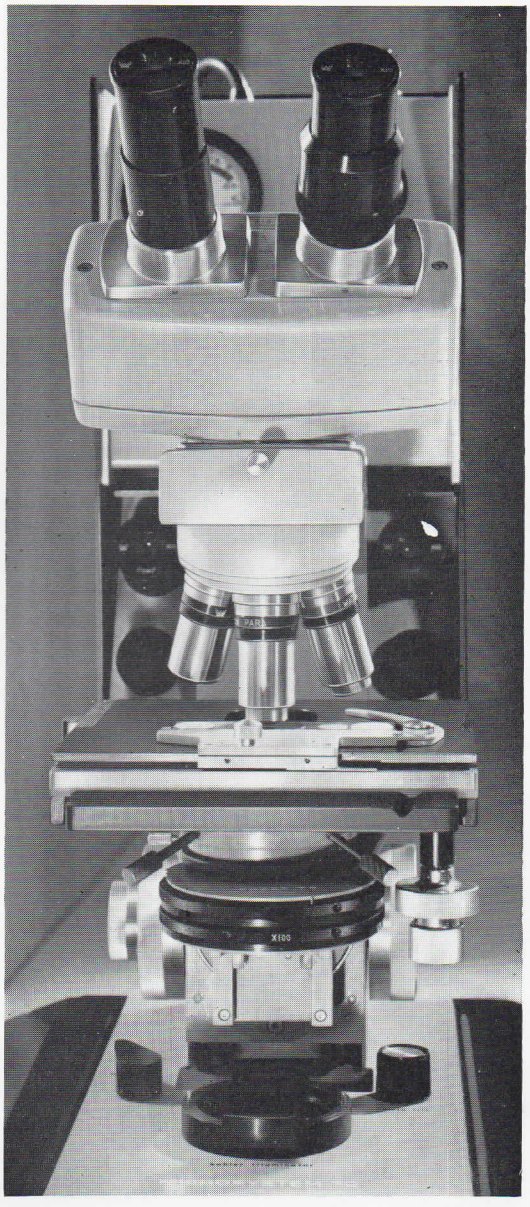
MICROSYSTEM 70

PHASE CONTRAST

PHASE 70

and

STUDENT PHASE



for true simplicity and excellent results . . .

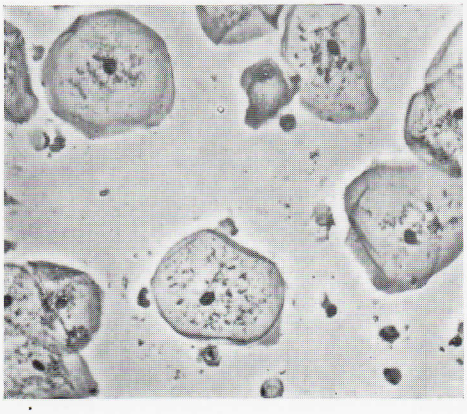
in both

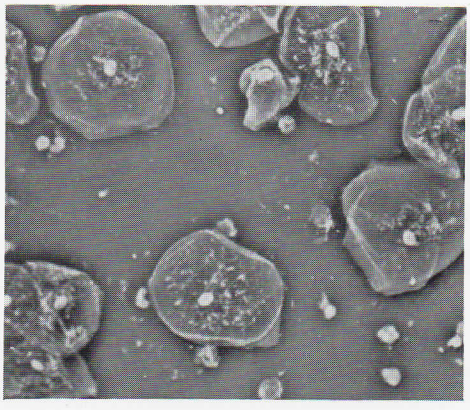
POSITIVE

and

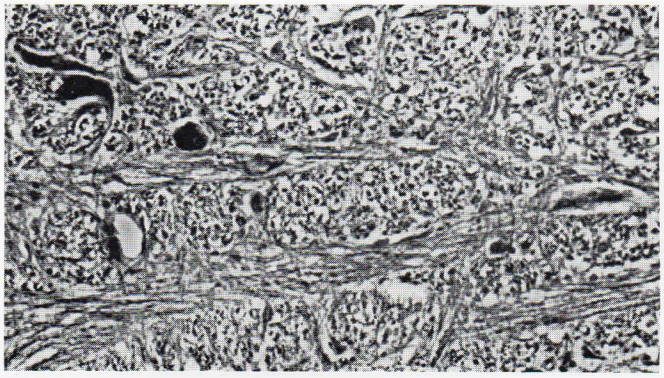
NEGATIVE

phase

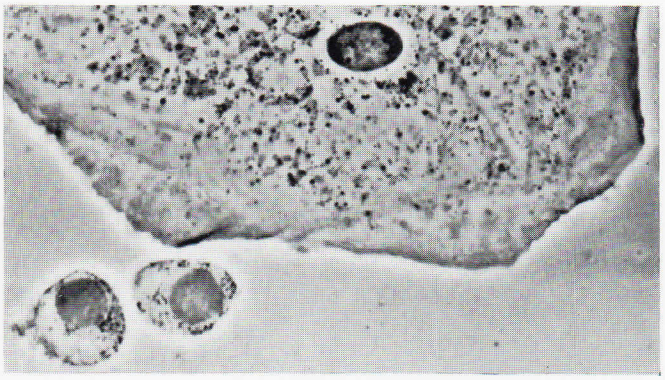




Phase contrast is one of the most effective ways of observing unstained biological material under the microscope. The equipment now available for System 70 microscopes is a highly developed form of the original Zernike[1] method giving an image with good contrast and less pronounced halos than many other systems. It is very easy to set up and use and stays in adjustment throughout long periods of use.



Human spinal cord, transverse section, unstained; positive phase; x150



Human salivary cells with epithelial cell; positive phase; x750

Principles of phase contrast

In the phase contrast microscope light diffracted by a transparent specimen is made to interfere with direct light (undiffracted) to provide an image with contrast. The optical principles are discussed in detail by Bennet *et al*[2] but these following explanatory notes may help those unfamiliar with this form of microscopy.

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. These effects occur whether the object is perfectly transparent, having variations in refractive index and thickness, or is black and white. The only difference between the two cases is that with the perfectly 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 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 changes of brightness in the image.

The faint image seen under a microscope without phase contrast indicates that residual aberrations and insufficient aperture are creating some contrast in the image. It is well known that the contrast can be considerably increased by reducing the aperture of the illumination and slightly refocusing the microscope but the resolution is so poor that the method is of little use.

In the dark ground microscope we eliminate the undeviated beam and, usually, half of the diffracted light, by limiting the illumination to apertures greater than the objective aperture so that no direct light can pass through the objective. The dark ground method has some inherent disadvantages which are overcome in the phase contrast microscope. The most important of these is the difficulty of observing fine detail when it is near to or part of gross detail which floods the image with light. In addition, the reduction of aperture of the high power immersion objective (by funnel stop or iris) in order to prevent direct light entering, considerably reduces the resolving power.

In the phase contrast microscope, by placing an annular stop near the back focal plane of the condenser, the specimen is illuminated by a hollow cone of light and the phase ring is positioned near the upper focal plane of the objective on which an image of the annulus is produced. The phase ring exactly matches the size of the annulus image and is formed by a special dielectric deposit which accelerates the light passing through it by a quarter of a wavelength relative to any light passing through the supplementary area. Since all the light illuminating the object has passed through the annulus and an image of the latter is formed on the phase ring, the direct light through the object must pass through this ring. Also, the scattered (or diffracted) light from the specimen which is collected by the objective will, except for a very small proportion, pass through the supplementary area. The direct light has thus been separated from the diffracted light and accelerated 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 direct light and the diffracted light are of the same order of intensity. In practice the direct light is always the stronger and a thin layer of metal is deposited over the ring to reduce this intensity by absorption. It is important not to have excessive absorption as this increases the contrast of gross detail more than that of fine detail, and although the result may appear to provide greater contrast at first glance, fine detail may be lost.

In negative phase contrast the phase ring is made a quarter wavelength thicker than the supplementary area so that dense particles show in bright contrast against a darker background. It is usual to provide higher absorption coatings to negative phase rings which then become particularly suitable for observing fine detached structures.

There are many possible kinds of phase rings, differing in performance characteristics dependent upon retardation, absorption and geometry. It would be prohibitively expensive to provide a large variety, and the delicate balance between contrast and resolution achieved with the two kinds offered performs extremely well with a wide variety of material.

1 Zernike, F., 1942. *Phase contrast, a new method for microscopic observation of transparent objects.* **Physica 9**: 686—698, 974—986.

2 Bennet, Jupnik, Osterberg & Richards, 1951. *Phase Microscopy.* John Wiley, New York.

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EQUIPMENT REQUIRED FOR PHASE CONTRAST WITH MICROSYSTEM 70

Necessary equipment

Phase contrast objective(s)

and either

Phase 70 unit
with annuli in turrets

or

Student phase condenser unit
with plug-in annuli

Optional equipment

Auxiliary telescope
Long-working distance condenser
Special etched bulbs
Filters

For descriptions of the last three items see overleaf

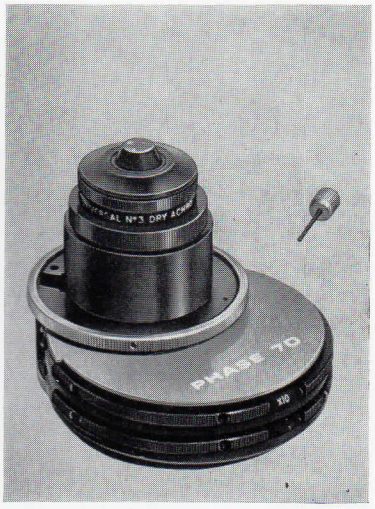
Phase contrast objectives

Optically similar to the standard Watson objectives but containing the phase plate, either positive or negative. They are clearly marked with a black engraved band below the standard marking band. The objectives differ from earlier phase objectives, having the phase plates deposited directly on to a lens surface so avoiding the additional air to glass surfaces necessary with a separate plate. They are remarkably free from glare and compare very favourably with other phase objectives when used for bright field.

The objectives available with phase plates are listed in the summary of available equipment shown on page 4.

Phase 70 unit

The Universal No 3 achromatic condenser is set in a special mount with iris diaphragm and two turrets containing the phase annuli. Each turret disc has three click stop positions, one with a clear aperture and the other two with annuli. The positions are marked with the magnifications of the objectives to which the annuli are matched. The annuli for the immersion objectives are on one turret and those for the dry objectives on the other. Each annulus has individual centring screws operated by special plug-in keys—the adjustment is straightforward and usually needs to be made only initially or when adding a new phase objective. To ensure perfect centring the condenser is always supplied with the unit but when phase contrast is added to a System 70 microscope already fitted with a Universal No 3 condenser, a credit will be allowed on the old condenser depending on its condition.

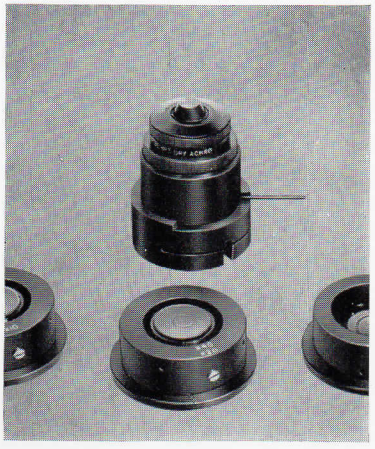


Student phase condenser unit

An inexpensive unit using the same optical system as the Phase 70 unit but having separate plug-in annuli. An iris diaphragm is included for bright field work. The annuli are individually centrable in the plugs.

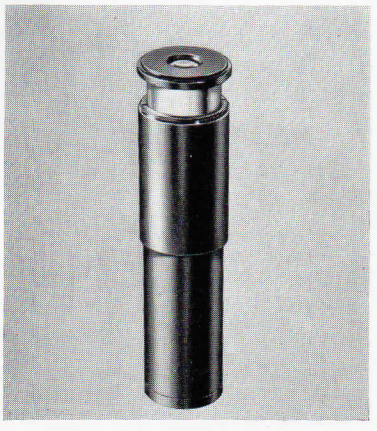
The student phase unit is best used with the Universal No 3 achromatic condenser with which it gives a performance identical to the Phase 70 unit. It is recommended for professional microscope outfits where only one phase objective is required.

The Abbe condenser may be used on inexpensive outfits provided that a small amount of colour in the image is acceptable. Under these conditions it will also be necessary to refocus the substage slightly when changing objectives, in which case the field iris will not always appear to be perfectly focused.



Auxiliary telescope

The telescope may be used to replace an eyepiece for observing the phase plate in order to adjust the illuminating system. The excellent pre-alignment characteristics of the Phase 70 unit and the constancy of adjustment have led some users to consider the telescope unnecessary. When several phase microscopes are installed in one laboratory one telescope may be shared. The telescope is essential for teaching phase microscopy and also when using the long working distance condenser on object slides which may not be flat or of standard thickness. It is also required when adjusting a new phase objective annulus for the first time. The telescope can be used in either monocular or binocular heads.



Long working distance condenser

Either of the phase condenser mounts may be fitted with the LWD condenser as an alternative to the Universal No 3. It is an achromatic condenser with a working distance of about 6mm intended for use with counting chambers or hanging drop preparations. The condenser has to be focused to suit the phase contrast and it cannot always focus the field iris perfectly at the same time owing to the variety of slide thicknesses used. For this reason it is best to have the standard condenser in addition when only part of the work demands the long working distance. The performance of the phase contrast with the LWD condenser is improved if the special etched bulbs are used.

Special etched bulbs

Some authorities consider that the annulus of a phase contrast microscope should not be illuminated with the striations of a lamp filament across it. Ordinary diffuser techniques reduce the light considerably but a specially etched bulb is available for the Köhler illuminating base which eliminates the filament striations while reducing the light by only about 10%. These bulbs are especially recommended for use with the LWD condenser. Etched bulbs are not required with the low voltage illuminating base which uses a diffuse source as standard.

Filters

The excellent colour characteristics of this phase contrast system makes the general use of spectrum limiting filters unnecessary but they are preferred on some occasions.

A green filter will generally improve contrast both visually and for black and white photography on panchromatic material. A yellow filter can provide a very sharp cut-off to limit the blue end of the spectrum for photography on orthochromatic materials which limit the red end by their own sensitivity curve. The pale blue OB8 filter is often preferred for visual work as it reduces any liability to orange and red colour fringing with only slight reduction in intensity.

A heat absorbing filter should be used for wet preparations to reduce drying except with the Hilux 70 illuminating system in which such filters are already incorporated. All the Microsystem 70 bases accept 2″×2″ square filters. The phase condenser mounts do not include holders for round filters. A list of filters is given in the summary of equipment available.

Illuminating bases

The Köhler illuminating base provides adequate illumination for phase contrast at all magnifications with binocular or monocular models. The field iris remains in focus for slides up to 1.2mm thick with the Universal No 3 condenser and enables glare to be reduced to the minimum by appropriately limiting the illuminated field. The Hilux models provide even greater available intensity enabling steep cut filters, photographic beam splitters, etc., to be used in addition.

The low voltage base provides adequate illumination for phase contrast with monocular microscopes or with the binocular with low and medium power objectives.

The mains voltage base is not recommended for phase contrast.

Instructions

Detailed instructions for using the equipment are included in the handbook provided with each microscope.

SUMMARY OF PHASE CONTRAST EQUIPMENT

	code
Phase contrast objectives	
code numbers for negative type given in italics	
x10/0.28 Parachromatic phase contrast objective	1205P *1205P neg*
x20/0.50 Parachromatic phase contrast objective	1216P *1216P neg*
x40/0.65 Parachromatic phase contrast objective	1208P *1208P neg*
x40/0.63 Planpara phase contrast objective	1209P *1209P neg*
x100/1.30 Oil Parachromatic phase contrast objective	1219P *1219P neg*
x50/0.95 Oil Fluorite phase contrast objective	1234P *1234P neg*
x90/1.30 Oil Fluorite phase contrast objective	1236P *1236P neg*
Phase 70 condenser units	
Universal No 3 achromatic condenser; Phase 70 unit with set of rotating annuli; adjusting keys	707/3
Universal No 4 achromatic long working distance condenser; Phase 70 unit with set of rotating annuli; adjusting keys; 6v 30w etched bulb	707/5
Plug-in condenser units	
Universal No 3 achromatic condenser with special iris mount designed to accept plug-in annuli	707/1
Abbe condenser with special iris mount designed to accept plug-in annuli	707/2
Universal No 4 achromatic long working distance condenser with special iris mount designed to accept plug-in annuli	707/4
Plug-in annulus for x10 objective	707/21
Plug-in annulus for x20 or x40 objective	707/22
Plug-in annulus for x50 objective	707/23
Plug-in annulus for x90 or x100 objective	707/24
Optional items	
Auxiliary telescope	707/9
Special 6v 30w etched bulb for Köhler illuminating base	705/25
Chance-Watson filter 2″x 2″ OGR1 Green	53(7)
Chance-Watson filter 2″x 2″ VG5 Pale green	53(18)
Chance-Watson filter 2″x 2″ OY3 Yellow	53(5)
Chance-Watson filter 2″x 2″ OB8 Pale blue	53(9)
Chance-Watson filter 2″x 2″ HA1 Heat absorbing	756

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