Nikon

Biological Microscopes OPTIPHOT LABOPHOT

PHASE CONTRAST EQUIPMENT "Ph"

INSTRUCTIONS

NIPPON KOGAKU K.K.

The Phase Contrast Equipment "Ph" permits us to perform darkfield microscopy (10× ~ 40×) as well as phase contrast microscopy using the Biological Microscope OPTIPHOT or LABOPHOT.

CAUTIONS

1 Avoid sharp knocks!

Handle the microscope gently, taking care to avoid sharp knocks.

2 Place for using

Avoid the use of the microscope in a dusty place, where it is subject to vibrations or exposed to high temperatures, moisture or direct sunlight.

3 Dirt on the lens

Do not leave dust, dirt of finger marks on the lens surfaces.
They will prevent you from clear observation of the specimen image.

4 In lighting the lamp

Some parts of the lamp housing may take a high temperature while the lamp is being lighted, so don't touch the lamp housing and don't bring inflammable substances such as gasoline, thinner and alcohol near to the lamp housing.

CARE AND MAINTENANCE

1 Cleaning the lenses

To clean the lens surfaces, remove dust using a soft hair brush or gauze. Only for removing finger marks or grease, should soft cotton cloth, lens tissue or gauze lightly moistened with absolute alcohol (ethanol or methanol) be used.
For cleaning the objectives and immersion oil use only xylene. For cleaning the other lens surfaces, use absolute alcohol.

2 Cleaning the painted surfaces

Avoid the use of any organic solvent (for example, thinner, ether, alcohol, xylene etc.) for cleaning the painted surfaces and plastic parts of the instrument.

3 Never attempt to dismantle!

Never attempt to dismantle the instrument so as to avoid the possibility of impairing the operational efficiency and accuracy.

4 When not in use

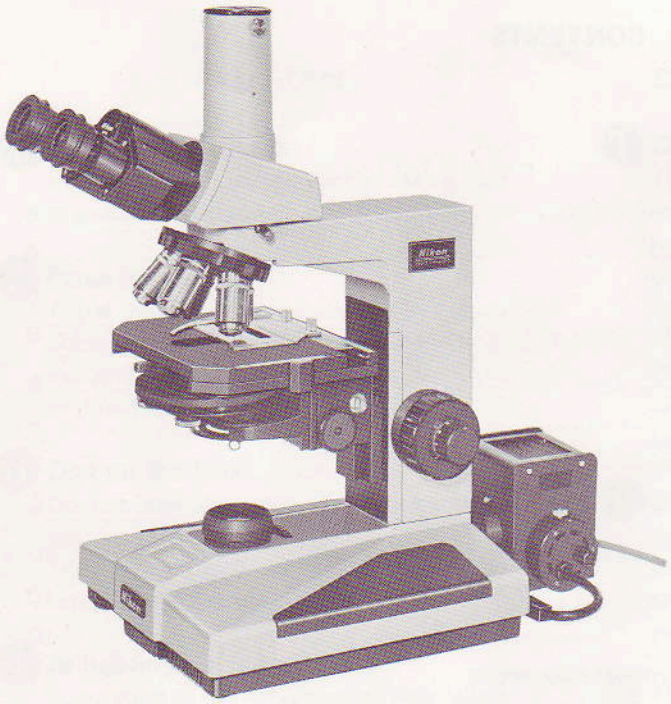
When not in use, cover the instrument with the accessory vinyl cover, and store it in a place free from moisture and fungus.
It is especially recommended that the objectives and eyepieces be kept in an airtight container containing desiccant.

5 Periodical checking

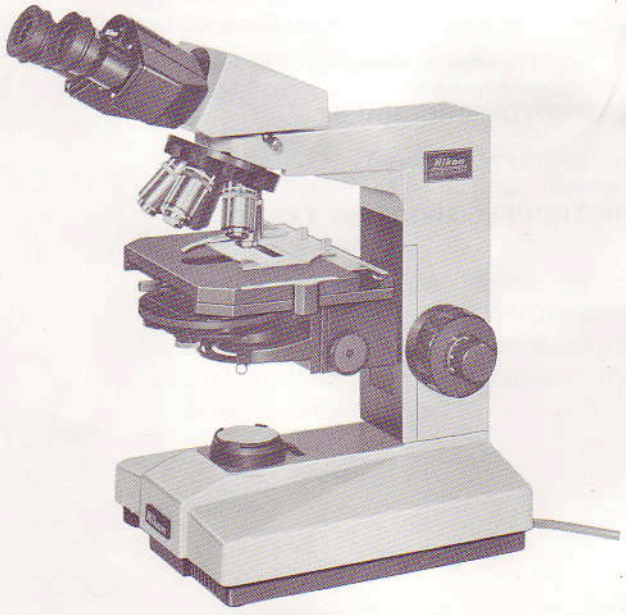
To maintain the performance of the instrument, we recommend the customers to check the instrument periodically by our qualified service personnel. (For details, contact our dealers.)

CONTENTS

I . NOMENCLATURE



PHASE CONTRAST EQUIPMENT "Ph"
mounted on the OPTIPHOT microscope stand



PHASE CONTRAST EQUIPMENT "Ph"
mounted on the LABOPHOT microscope stand

Phase Contrast Objectives

Marking DL, DM or BM on the outer barrel of objective indicates the types of contrast; "Dark contrast for general use", "Dark contrast for low phase difference" or "Bright contrast", respectively. Ph 1 ~ Ph 4 are the markings on the objectives, with which that of the condenser annular diaphragm is to be matched.

CF Plan DL or CF DL

100× 40× 20× 10×

CF Plan DM

100× 40×

CF Plan BM

100× 40× 20× 10×

Phase Contrast Turret Condenser

Magnification indication plate

Markings [Ph 1] ~ [Ph 4] indicate the types of annular diaphragms which are respectively used for phase contrast objectives 10× ~ 100×. Turn and set the turret to the same marking as that on the objective being used.
[DF] indicates the position of darkfield ring which is commonly used for 10× ~ 40× objectives.
[O] indicates the empty hole for brightfield microscopy.

Aperture diaphragm knob

For adjusting the opening of brightfield aperture

Turret

For changing-over the condenser annular diaphragm according to the magnifying power of objective being used.

Circular dovetail

Clamp screws

After centering the phase contrast annular diaphragm, clamp each screw of the centering knobs.

Centering knobs

For centering the turret as a whole

Centering Telescope

Holding the milled ring, rotate its eyepiece to focus on the phase plate in the objective.

Filters

Green interference filter

Heat absorbing filter

Fig. 1

II. ASSEMBLY

For assembling the Phase Contrast Equipment "Ph" to the Nikon Biological Microscope OPTIPHOT or LABOPHOT, refer also to the instruction manual supplied for each microscope.

1. Attaching the Objectives

1) Remove the ordinary brightfield objectives, if attached, from the revolving nosepiece.
2) Attach the phase contrast objectives to the revolving nosepiece in such positions that, when the nosepiece is revolved clockwise viewed from above, the Ph number advances. (Fig. 2)

[Note] The darkfield ring to be used commonly for the 10× ~ 40× objectives is incorporated in the position [DF] on the turret.
If the ordinary brightfield objective 10×, 20× or 40× is attached to the nosepiece, this objective will facilitate finding out the target on the specimen. Select suitable objectives from among the Nikon Phase Contrast Objectives as listed in Table 1.

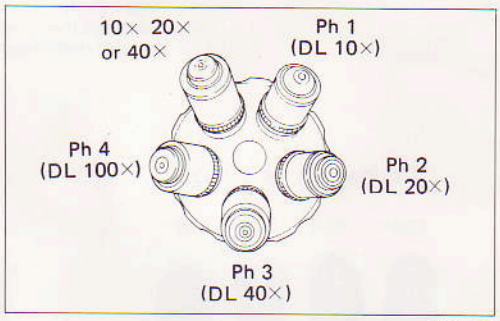


Fig. 2

2. Centering the Light Source

It is important to perform exact centering of the light source for phase contrast microscopy, because this method is liable to yield darker images on account of stronger absorption of illuminating light, compared with in case of

Table 1. Phase Contrast Objectives

Type		Magnification	Numerical aperture (N.A.)	Working distance (mm)	Focusing distance (mm)	Coverglass thickness (mm)	Remarks
Achromat	Dry	CF DL 10×	0.25	5.6	16.6	——	DL phase contrast for general use
		CF DL 20×	0.40	2.23	8.8	0.17	DL phase contrast for general use
		CF DL 40×	0.65	0.53	4.4	0.17	DL phase contrast for general use Provided with safety device
	Oil	CF DL 100×	1.25	0.14	1.8	0.17	DL phase contrast for general use Provided with safety device
Plan Achromat	Dry	CF Plan DL 10× BM 10×	0.25	7.1	16.7	——	DL phase contrast for general use and BM for reversal contrast Commonly used for ultra wide viewfield
		CF Plan DL 20× BM 20×	0.40	1.4	8.4	0.17	DL phase contrast for general use and BM for reversal contrast Commonly used for ultra wide viewfield
		CF Plan DL 40× DM 40× BM 40×	0.65	0.48	4.1	0.17	DL phase contrast for general use DM for low phase difference and BM for reversal contrast Provided with safety device Commonly used for ultra wide viewfield
	Oil	CF Plan DL 100× DM100× BM 100×	1.25	0.20	1.8	0.17	DL phase contrast for general use DM for low phase difference and BM for reversal contrast Provided with safety device Commonly used for ultra wide viewfield

brightfield microscopy.

- **For the OPTIPHOT microscope**

1) Connect the power source cord to the socket.
2) Put the ND2 and ND16 filters into the filter receptacle.
3) Turn the brightness control dial to switch ON and adjust the voltage to 6 on the indicator.
4) Place the specimen on the stage, and focus on the specimen using 10× objective. In this case, open the condenser aperture and field diaphragms to the largest extent.
5) Roughly center the condenser lens using 10×objective, following the procedures given on P. 8 - 2).
6) Put the lamp centering tool on the field lens and onto the tool place a ND filter taken out of the filter receptacle. (Fig. 3)

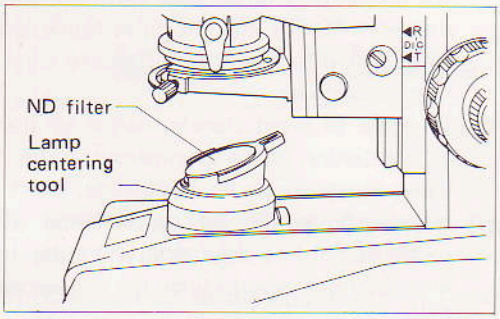


Fig. 3

7) Stop down the condenser aperture diaphragm, release the lamp housing clamp screw, and move the lamp housing back and forth (Fig. 4), until a sharp image of the lamp filament appears on the aperture diaphragm surface, which can be seen by the reflection from the ND filter.

Note: When using the achromat/aplanat condenser, a filament image (reddish violet) by the reflection from the lens surface is sometimes taken for a filament image (bluish white) appeared on the aperture diaphragm surface. In this case, stop down the field diaphragm, and the reddish violet-colored filament image will disappear and the bluish white-colored image is easy to see.

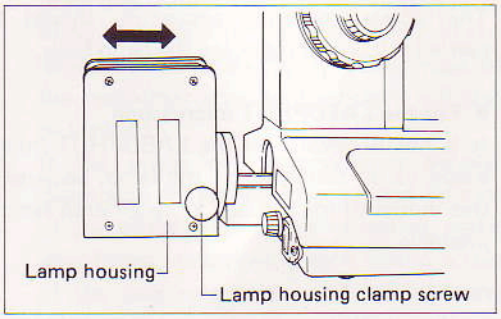


Fig. 4

8) Release the socket sleeve clamp screw (Fig. 5). Turning the lamp lateral centering screw and vertical centering ring, bring the filament image as shown in Fig. 6.

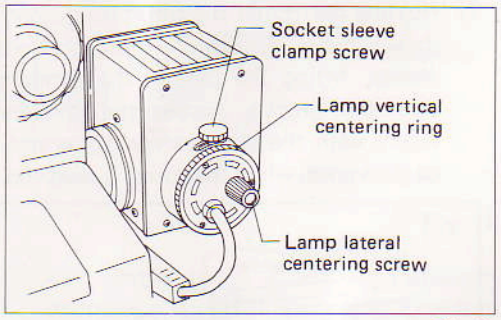


Fig. 5

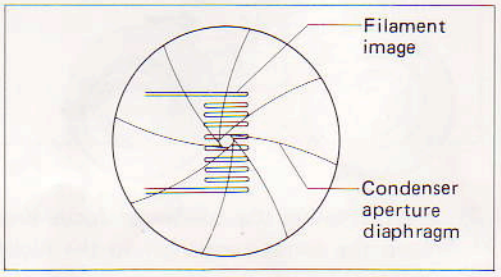


Fig. 6

9) As shown in Fig. 7, put the diffuser, with its matte surface faced toward the microscope stand, into the filter receptacle which is the closest to the microscope stand.

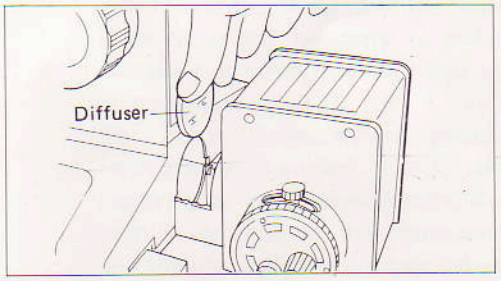


Fig. 7

The above centering procedure should be carried out, when replacing the lamp bulb.

- **For the LABOPHOT microscope**

It is not necessary for the LABOPHOT microscope to do centering of the lamp, because in this model of microscope a precentered lamp is available.

3. Attaching the Turret Condenser

1) Manipulating the condenser focus knob, move the condenser carrier to the lowest limit, to remove the ordinary brightfield condenser, beforehand.
2) Holding horizontal the turret condenser, as shown in Fig. 8, push it into the condenser carrier, fitting the pin on the condenser into the groove. Fasten the condenser firmly with the clamp screw so as not to be slackened, when the turret is revolved.

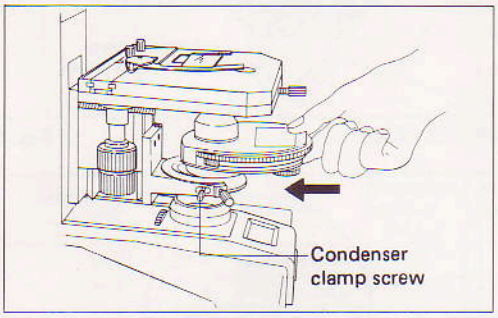


Fig. 8

3) Then, rotating the condenser focus knob, move the turret condenser to the highest limit.

III. PREPARATION

1. Filter

- **For the OPTIPHOT microscope**

1) Insert the NCB 10 filter into the filter receptacle the same as in the case of making ordinary brightfield observation. (Fig. 9)

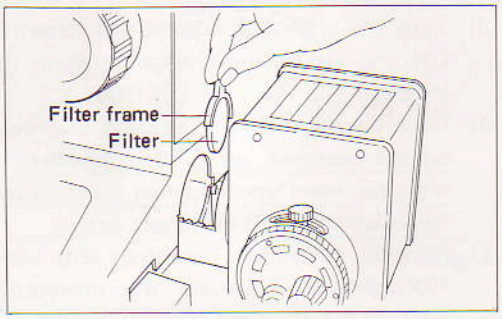


Fig. 9

2) For monochromatic observation, insert the green interference filter (with the outer diameter 45mm) into the filter frame, and place this into the filter receptacle on the microscope base.
3) In order to avoid transformation or transubstantiation of the specimens or death of living organisms as far as possible, insert a heat absorbing filter with the outer diameter 45mm into the frame, and place this into the filter receptacle on the microscope base.

- **For the LABOPHOT microscope**

When using this model, place the daylight filter, green interference filter and heat absorbing filter without the frame on the field lens on the microscope base.

2. Centering the Turret Condenser

1) Turn the brightness control dial (including power switch) to ON to light the lamp. Set the voltage to 7 on the indicator for the OPTIPHOT microscope and 5 for the LABOPHOT microscope.
2) Place the specimen on the stage.
3) Revolve the condenser turret to the position [0] and the objective Ph 1 (10×) into the optical path.
4) Focus on specimen.
5) Manipulate the field diaphragm control ring on the microscope base to close down the diaphragm to its smallest limit.

the diaphragm to its smallest limit.

6) Rotate the condenser focus knob to move the condenser vertically so that a sharp image of the field diaphragm is formed on the specimen surface.
7) Bring the field diaphragm image to the center of the field of view by means of the condenser centering screws. (Fig. 10 - 1)
8) Change over to the objective Ph 3 (40X), and adjust the field diaphragm so that the image of the diaphragm is about the same as that of the field of view, as shown in Fig. 10 - 2 . If not centered, use the condenser centering screws again.

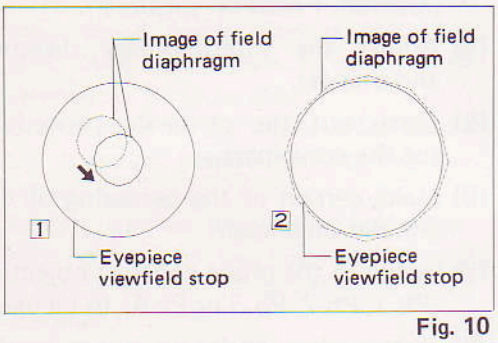


Fig. 10

3. Centering the Phase Contrast Annular Diaphragm

1) Change over to the objective Ph 1 (10X). Set the condenser turret to the position [Ph 1].
2) Manipulate the stage travel knobs, move the glass slide to the position, where no object is seen and yet covered by the cover-glass.
3) Draw out the eyepiece from the eyepiece tube, and in its place insert the centering telescope.
4) Holding the milled part of the centering telescope, rotate its eyepiece (Fig. 11) to bring the image of the phase plate in the objective into sharp focus. (The image of the condenser annular diaphragm will also be visible.)

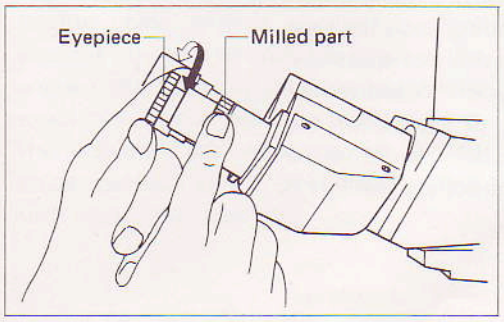


Fig. 11

5) If the image of the condenser annular diaphragm is not found coincided to that of the phase plate in the objective, make adjustment, unscrewing each clamp screw of the centering knobs and manipulating the centering knobs to move the condenser turret as a whole, as shown in Fig. 12. Tighten the clamp screws.

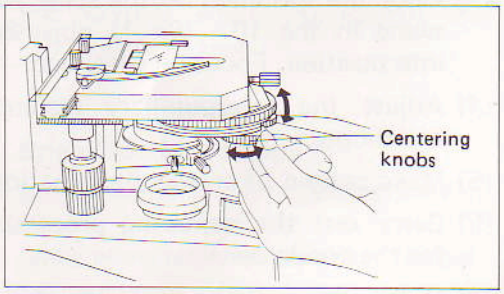


Fig. 12

Such coincidence is to be exactly attained, otherwise a remarkably low image contrast of phase difference would result. (Fig. 13)

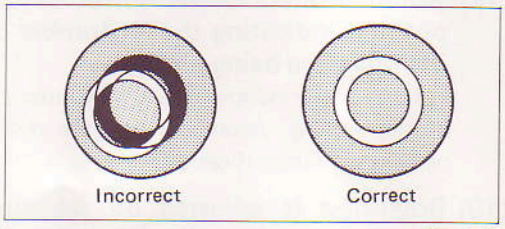


Fig. 13

[Note] All the annular diaphragms in this condenser turret are adjusted beforehand taking the annular diaphragm [Ph 1] as the standard. Therefore, if the centering of the diaphragm [Ph 1] and the phase plate has been done, it is not necessary to make centering at other magnifications in common use.

For strict microscopy or photomicrography, however, check the centering at each magnification. By slightly decentering the diaphragm and the phase plate, the image shows relief with the shadowing effect.

IV. MICROSCOPY

1. Phase Contrast Microscopy

1) Operating procedure

- **For the OPTIPHOT microscope**

(1) Turn the brightness control dial (including power switch) to ON and adjust the voltage to 7 on the indicator.

(2) Remove the lamp centering target and place the filters to be used.

(3) Place the specimen on the stage and swing in the 10× (Ph 1) objective into position. Focus on specimen.

(4) Adjust the interpupillary distance and diopter.

(5) Make certain of correct illumination.

(6) Carry out the centering procedure for the condenser.

(7) Make certain of the centering of the annular diaphragm.

(8) Swing in the phase contrast objective (Ph 1, Ph 2, Ph 3 or Ph 4) to be used.

(9) Rotate the condenser turret to the position indicating the Ph number of the objective being used.
For strict microscopy, check the centering of the annular diaphragm and the phase plate at each magnification.

(10) Brightness is adjusted by selecting ND filters or by changing the lamp voltage to 6 ~ 12.

(11) Adjust the field diaphragm.

- **For the LABOPHOT microscope**

(1) Turn the brightness control dial (including power switch) to ON and set the scale on the dial to 4.

(2) Remove the dust cup and place the daylight filter onto the field lens.

(3) Place the specimen on the stage and swing the 10× (Ph 1) objective into position. Focus on specimen.

(4) Adjust the interpupillary distance and diopter.

(5) Carry out the centering procedure for the condenser.

(6) Make certain of the centering of the annular diaphragm.

(7) Swing in the phase contrast objective (Ph 1, Ph 2, Ph 3 or Ph 4) to be used.

(8) Rotate the condenser turret to the position indicating the Ph number of the objective being used.
For strict microscopy, check the centering of the annular diaphragm and the phase plate at each magnification.

Now, all the preparations have been made for the phase contrast microscopy. Since a different seeing will result depending upon the type of the phase contrast objective and specimen, it is necessary to select a type of objective well matching with the phase difference of the specimen, referring to the description on the capacity of phase contrast objectives (see P. 12).

2) Cautions to be taken in phase contrast microscopy

The phase contrast image being liable to change its appearance according to not only the form, phase difference, etc. of the specimen, but also the adjustment of the annular diaphragm, the capacity of the objective, take the following cautions:

(1) Specimen which scatter light or have a lenticular or prismatic effect in themselves may cause image decentering to the annular diaphragm, thus impairing the image quality.
Especially when observing a living, thick specimen or coarse structure object, or when using a microplate, be careful of its prismatic or lenticular effect.
Displacement of the object image caused by diffraction, however, will not have any influence.

(2) In dark contrast, check the phase difference latitude of the objective for well matching with the phase difference of the specimen.
When preparing a phase contrast specimen, it is possible to adjust its phase difference by changing the thickness, and the refractive index of the mounting medium or cultivation agent. Of course, use the DM objective, for example, when the specimen has too weak contrast for the DL objective.

(3) Specimens stained too deep or overstained, or with a strong bright-and-dark contrast, are not favorable for phase contrast microscopy.
Those faintly stained or decolorized, or ultra-thin sections such as prepared for electron microscopy, etc. are quite suitable.

(4) Centering of the annular diaphragm is important.
In the phase contrast microscopy, it is essential above all to bring the image of the annular diaphragm in the condenser into exact coincidence with the phase plate in the objective, for achieving the desired phase contrast effect. Beforehand, check such coincidence carefully.

2. Darkfield Microscopy

1) Set the condenser turret to the position [DF].
2) Swing in the objective (10× ~ 40×) to be used.

The darkfield microscopy prepared in this way will be utilized as a finder for finding out easily the target on the specimen. Also, it will be serviceable for comparison with the brightfield microscopy.
If a thick glass slide is to be used, move the condenser to the highest limit to increase the darkfield effect, manipulating the condenser focus knob.

3. Brightfield Microscopy

1) Set the condenser turret to the position [0].
2) Swing in the ordinary objective to be used.

The brightfield microscopy prepared in this way will be utilized as a finder for finding out easily the target on the specimen. Furthermore, closing down the aperture diaphragm at this time is recommended for easier viewing.

V. CHARACTERISTICS AND PRINCIPLE

1. Capacity of Phase Contrast Objectives

The phase contrast type microscope, unlike the ordinary brightfield type, offers a different appearance of image depending upon not only the capacity of the phase contrast objective, but also the phase difference of specimen, and its distribution therein.

If the characteristic of the phase plate built in the objective well matches with that of the specimen, the best image will be obtained; if it does not, rather a worse seeing will result than by the ordinary brightfield microscope so that the phase contrast method may cause disappointment to the observer.

We, therefore, will grade the objects with respect their phase differences, for convenience in discriminating the capacities of phase contrast objectives as shown in Table 2.

It can be said that the phase contrast microscopy is excellent in detecting so minute phase difference as ranging from $\lambda/100$ to $\lambda/2$, reaching $2^\circ \sim 3^\circ$ in terms of phase angle. On the contrary, by a larger phase difference, the phase contrast microscopy would impair the optical conditions required for the method, giving rise to inferior results compared with the ordinary brightfield microscopy.

The Nikon Phase Contrast Objectives of two types, dark (positive) and bright (negative) phase contrast, are available.

With the dark contrast type objectives, generally an object of larger phase difference appears darker in the relatively bright background, the same as with the ordinary brightfield objective.

Of these dark contrast objectives, two types DL and DM are distinguished according to the degree of absorption of their built-in phase plates.

On the other hand, the bright contrast type objective, similarly to the general brightfield objective, offers a bright image of the object of larger phase difference in the relatively dark background.

One type of BM is available.

With the dark contrast objectives DL and DM, the darkness of image will increase, as the phase difference of specimen enhances, but beyond a certain limit, conversely, the brightness of image begins to increase, and finally, at a point the brightness reaches the same degree as of the background. Therefore, it may not be simply said that the larger the phase difference, the darker the image is produced. Within this proportional range, the image itself will be seen sharp, but if the range is overrun, the image will begin to light up more than the background, enabling us to observe no more image.

In this way, the dark contrast objective suffers a quantitative restriction in its observing capacity of phase difference. This discernible phase difference allowed for the objective is known as "latitude".

Thus, the larger the latitude, the more suitable the objective is for phase contrast observation, the DL objective belonging to this category. This objective, however, provides insufficient contrast for specimens of less phase difference. For this reason, in this case, it is adequate to use the DM objective with harder tone contrast, having a narrow latitude.

Table 2. Grades of Phase Difference of Object

Phase difference		Division	Example	Phase difference between object and mounting medium Appearance of image by brightfield method
Angle	Wavelength			
$0^\circ \sim 45^\circ$	$0 \sim 1/8\lambda$	Low	Blood corpuscles in balsam	Extremely small difference Hardly visible by brightfield method
$45^\circ \sim 90^\circ$	$1/8\lambda \sim 1/4\lambda$	Middle	Blood corpuscles in glycerin	Small difference Only dust visible with fully closed aperture
$90^\circ \sim 180^\circ$	$1/4\lambda \sim 1/2\lambda$	High	Blood corpuscles in physiological saline solution	Fairly large difference Visible with aperture closed to a certain extent
180° or larger	$1/2\lambda$ or longer	Coarse	Little drops of fat in milk	Remarkably large difference Visible quite distinct with slightly closed aperture

On the other hand, the bright contrast objective can be said to be used easily owing to its wider latitude, but it, offering a darkfield-like image, will be suitable rather for observation and detection of the form of granules and particles, for example, bacteria, etc. and counting the number of particles or parts.

Table 3 summarizes the usage of the above mentioned types of phase contrast objectives.

It is a rule for specimens of smaller phase difference, to use the objective DM of stronger absorption, and for those of larger phase difference, the objective DL of weaker absorption.

If the phase plate is not suited for the specimen type, a remarkable halo may come in sight around the object image (see below), and part of the object will light up, showing no contour. In this case, the use of an objective of lower absorption is advantageous. If only a faint image of low contrast results, it will be necessary to resort to an objective of higher absorption.

By changing-over from the dark to bright contrast type, the contrast will be expected to be reversed; in either case, however, any bright part can be judged thicker and with higher refractive index.

2. Characteristics of Phase Contrast Image

1) Halo

In the phase contrast method, as it uses a phase plate, a peculiar phenomenon such as halo will be added around the phase difference image as a bright or dark ring according to the use of the dark or bright contrast objective, respectively, caused by diffraction of the phase plate.

As a result, minute structures in the halo will disappear.

In general the halo surrounding a minute subject shows up to lesser degree, and the DL objective produces not so remarkable halo compared with the DM objective.

2) Resolving power

The resolution of the phase contrast image can generally be considered the same as that of the ordinary brightfield image, and the reproducibility of fine structures is not reduced, although rather rough structures may not be resolved.

Table 3. Usage of Phase Contrast Objectives

Phase contrast objective		Appearance of image	Contrast		Latitude	Example of application
Dark contrast (Positive)	DL	Similarly to bright field, large phase difference appears dark, therefore, image visible dark in relatively bright background	Suited for observation of minute structures; mostly for micro contrast	Intermediate tone (Wider application field)	Low or intermediate phase difference range and absorption (Stained specimens)	Sporangia of fungi, general raw cells, slightly thicker specimens fungi, stained specimens, eggs of worms, fat cells, crystals, etc.
	DM			Hard tone (Narrower application field)	Transparent object of low phase difference range	Fungi, flagella of protozoa, raw fibrins, fine granules, sections mounted in well-chosen medium, ultra thin sections
Bright contrast (Negative)	BM	Similarly to dark field, quite large phase difference appears bright, therefore, image visible bright in relatively dark background	Suited for detection of forms of minute particles, fibers, granules and calculation; mostly for macro contrast		Almost entire range	Fungi, flagella of protozoa, raw fibrins, fine granules, blood corpuscles to be counted.

3. Principle of Phase Contrast Microscopy

Objects characterized by variations in thickness or refractive index from point to point are called "phase objects". Such objects, if transparent throughout, will not be discernible under the ordinary brightfield microscope.

Suppose a small part of thickness e and refractive index n, as shown in Fig. 14, is contained in a plane-parallel glass of medium M with a different refractive index n', and the light ray (1) passes through the part A and that (2), through an adjacent part M. Then, the optical path difference δ therebetween will be given by $\delta = (n - n')e$. The part A is a phase object distinguished only by such an optical path difference of δ. We assume that δ and the phase difference φ, obviously obtained as $\varphi = 2\pi\delta/\lambda$, are slight, λ being the wavelength of the light being used.

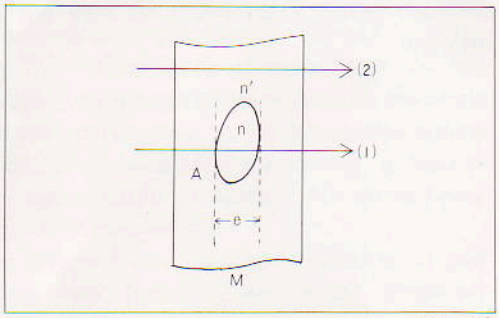


Fig. 14

Now, we will describe that the phase contrast microscope permits us to detect such phase objects, because it can convert the difference of phase to that of amplitude, as follows:

Suppose a transparent object of medium M is illuminated, as shown in Fig. 15, by the parallel rays coming from the point light source S placed on the focal point of the condenser lens C. The light rays (2) passing the medium M will be collected by the objective lens O onto the focal point S' (on the focal plane F), that is, the image of the light source S, and then reach M', the image plane of M.

This light is called "direct waves".

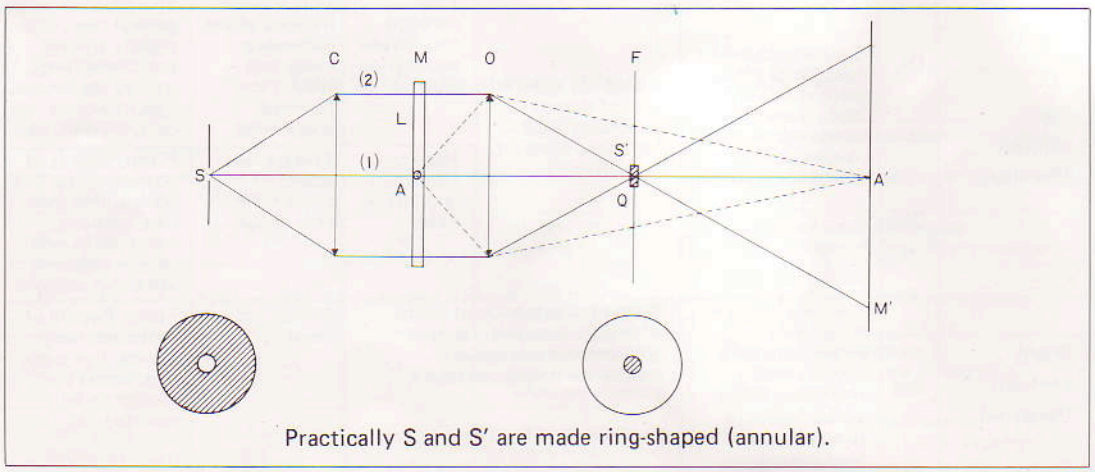
On the other hand, the rays (1) passing through the object A and causing the phase difference φ, will be diffracted in all directions, and converged by the objective O, so as to form the image A'.

This light is called "diffracted waves". The image A' in this way derives from the direct and diffracted light waves, interfered with each other. Let us express the above phenomenon by the Fresnel's diagram (Fig. 16)

Take the original point as O. Draw a vector ON having a length proportional to the amplitude of light wave at the assumed point of the object.

The phase difference φ at the same point is indicated by the angle φ to the X-axis. Since the object is transparent, N exists on the unit circle with its center at O. Only the phase φ changes from point to point.

If all the rays diffracted by A are collected by the objective O, the amplitude at M' will result the same as at M. Fig. 16 shows the amplitude



Practically S and S' are made ring-shaped (annular).

Fig. 15

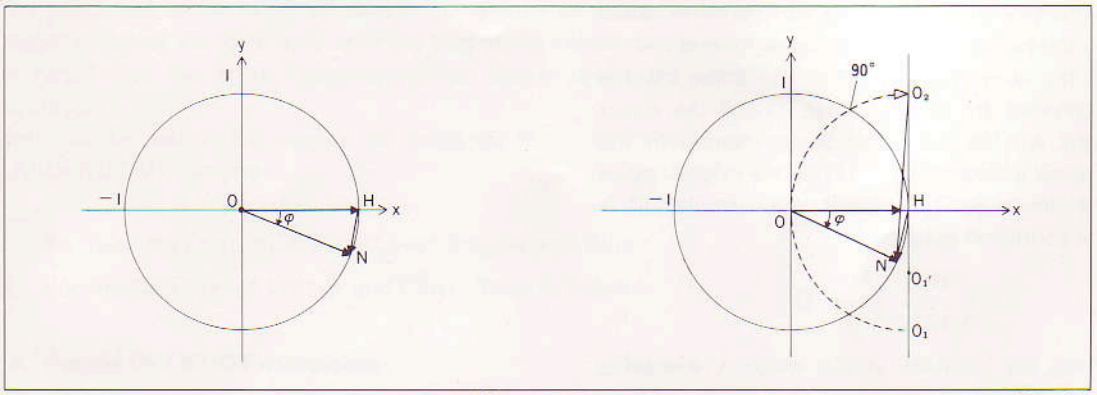


Fig. 16 Fig. 17

and phase of the image at M' simultaneously.

Let us divide the vector ON into two, OH and HN, crossing each other at right angles, as φ is assumed small, and H can be considered a point on the X-axis intersecting the circle. The vibration of light at one point on M' is represented by the resultant obtained by the addition of the vibrations in the directions OH and HN. The amplitude OH being that of the area with no phase shift ($\varphi = 0$) of the object, it corresponds to the one, if no object were present, i.e. that of the direct light. The amplitude HN represents that of the light diffracted at A in Fig. 15.

If there were no object ($\varphi = 0$), the amplitude HN would be zero, and the intensity I of light at the image A' be $I = ON^2 = OH^2 + HN^2$, which is the same as at other points of viewfield, causing no appearance of the object image. Place a small transparent plate Q on S' (Fig. 15)

Then, since the direct light passes Q, but the diffracted light spreads over a wide extent at this position, it can be considered that the plate Q gives actually no effect to the diffracted light.

Let us furnish the plate Q with such an optical thickness as will be mentioned below, so that the vibration of direct light passing Q suffers a retardation of 1/4 wavelength with reference to the vibration of diffracted light not passing Q.

Such retardation in the Fresnel's drawing (Fig. 17) corresponds to the rotation 90° (1/4 wave) of OH around H, whereby the original point O is transferred to the position O_2. In this condition, the amplitude at the image A' will be $O_2H + HN = 1 + \varphi$, and the light intensity $I_1 = 1 + 2\varphi$, φ^2 being neglected. Outside the image A', $\varphi = 0$, therefore, the intensity $I_2 = 1$.

As a result, the image A' will be seen with the contrast γ:

$$\gamma = \frac{I_1 - I_2}{I_2} = 2\varphi \quad \cdots\cdots\cdots\cdots\cdots (1)$$

As above considered, when the object A causes a retardation of φ to the phase of the direct light, in other words, a corresponding elongation of the optical path, the light intensity at A' will be larger as compared with that on the other parts of viewfield; the phase contrast is a "bright (negative) contrast".

On the contrary, place at S' the plate Q which causes an advance of phase as far as 1/4 wavelength to the direct light, instead of giving rise to retardation. Then, the original point will be moved to O_1 and $I_1 = 1 - 2\varphi$.

In this case, the contrast will be 2φ again, but, since the diffracted light coming from A suffers a retardation of φ, compared with the direct light, the intensity at A' will be less than that at the other points of viewfield; the phase contrast is a "dark (positive) contrast".

The plate Q, which, as mentioned heretofore, enables bringing about an advance or retardation between the phases of direct and diffracted light is called a "phase plate".

The minimum discernible contrast for the eye is 0.02. The distinctly recognizable limit of phase difference is given by $2\varphi = 0.02$, corresponding to an optical path difference of about 10 Å. Furthermore, by converting the phase plate into an absorptive one, the detecting

sensitivity of this method will be more raised on the basis of the calculation as follows:
If the absorption factor of the phase plate is expressed by D, the intensity of the direct light will be 1/D; this is represented in the Fresnel's diagram (Fig. 17) by the original point transferred to O_1', a point which should fulfills the condition as below:

$$\left(\frac{HO_1}{HO_1'}\right)^2 = D$$

Then, the intensity at the image A' will be as below:

$$I_1 = \left(\frac{1}{\sqrt{D}} + \varphi\right)^2 \simeq \frac{1}{D}\ (1 + 2\varphi\sqrt{D}\ ),$$

whereas at the other parts of viewfield, $\varphi = 0$ and $I_2 = 1/D$.
Now, the image contrast γ will be

$$\gamma = 2\varphi\sqrt{D} \quad \cdots\cdots\cdots\cdots\cdots\cdots \quad (2)$$

Thus, the previous contrast being increased $\sqrt{D}$ times.
Therefore, in principle, if D = 2500, for example, it will be possible to observe an optical path difference of about 1Å with a contrast equivalent to 0.1. It will be said that the phase contrast microscopy has quite a high detecting sensitivity.

References:

1) M. Françon — Progress in Microscopy (Pergamon, 1961)
2) M. Françon — Diffraction — Coherence in Optics (Pergamon, 1966)

VI. PHOTOMICROGRAPHY

The procedure to be taken for photomicrography of phase contrast images is almost the same as of ordinary brightfield images, but it is to be noted that the image obtained will be darker under the same conditions.
Refer to the instruction manual for Using the Nikon OPTIPHOT or LABOPHOT Microscope.

1. To Raise the Contrast, Use the Green Interference Filter.
2. For the Selection of Voltage and Filter, Refer to Table 4.

- **For the OPTIPHOT microscope** **Table 4**

Film		Voltage	Filter
Color film	Daylight type	9	NCB 10 is to be used
	Tungsten type	8	Remove NCB 10
Monochrome film		Over 6	Remove NCB 10

- **For the LABOPHOT microscope**

Film		Voltage	Filter
Color film	Daylight type	5.5	NCB 10 is to be used
	Tungsten type		Remove NCB 10
Monochrome film		Adequate voltage	Remove NCB 10

3. Dirt on the Coverglass, Objective, Eyepiece May Give Rise to Flare.
   Remove it thoroughly. Closing down the field diaphragm to limit the illuminated field to the photographing area will be effective for reducing flare.

VII. SPECIFICATIONS

1. Turret Condenser

- Numerical aperture : N.A. 1.25
- Turret provided with 6 holes:
  - 1 empty hole with aperture diaphragm for brightfield microscopy
  - 4 phase contrast annular diaphragms for Ph 1, Ph 2, Ph 3 and Ph 4, each (Annular diaphragms to be centered by moving the turret)
  - 1 darkfield annular ring commonly used for 10× ~ 40× objectives

2. Centering Telescope

- Magnification : 5×~ 12×
- Inserted in the observation tube

3. Filters

- Green interference filter (ϕ = 45)
- Heat absorbing filter (ϕ = 45)

4. Phase Contrast Objectives

Table 5. Phase Contrast Objectives

Type		Magnification	Numerical aperture (N.A.)	Working distance (mm)	Focusing distance (mm)	Coverglass thickness (mm)	Remarks
Achromat	Dry	CF DL 10×	0.25	5.6	16.6	——	DL phase contrast for general use
		CF DL 20×	0.40	2.23	8.8	0.17	DL phase contrast for general use
		CF DL 40×	0.65	0.53	4.4	0.17	DL phase contrast for general use Provided with safety device
	Oil	CF DL 100×	1.25	0.14	1.8	0.17	DL phase contrast for general use Provided with safety device
Plan Achromat	Dry	CF Plan DL 10× BM 10×	0.25	7.1	16.7	——	DL phase contrast for general use and BM for reversal contrast Commonly used for ultra wide viewfield
		CF Plan DL 20× BM 20×	0.40	1.4	8.4	0.17	DL phase contrast for general use and BM for reversal contrast Commonly used for ultra wide viewfield
		CF Plan DL 40× DM 40× BM 40×	0.65	0.48	4.1	0.17	DL phase contrast for general use DM for low phase difference and BM for reversal contrast Provided with safety device Commonly used for ultra wide viewfield
	Oil	CF Plan DL 100× DM 100× BM 100×	1.25	0.20	1.8	0.17	DL phase contrast for general use DM for low phase difference and BM for reversal contrast Provided with safety device Commonly used for ultra wide viewfield

VIII. TROUBLE SHOOTING TABLE

Failures	Causes →	Actions
Annular aperture takes incorrect form or does not appear	• Not correct vertical position of condenser	→ Move the condenser vertically to the correct position
	• Dirt, oil, water, fingermarks, etc. on the surface of coverglass, glass slide, objective, condenser	→ Cleaning with absolute alcohol. For cleaning the objective use only xylene
	• Hole in the glass slide gives a lenticular effect	→ Use a regular glass slide with a hole digged through and a coverglass attached on the bottom surface
Impossibility of exact coincidence of annular diaphragm	• Insufficient working distance of condenser	→ Do not use culture bottles of flat säule of thick glass
Low contrast image results	• Insufficient coincidence of annular diaphragm	→ Use the centering telescope for recentering
	• Too large phase difference of specimen	→ Make the specimen thinner or use a mounting medium of refractive index nearer to the specimen

We reserve the right to make such alterations in design as we may consider necessary in the light of experience. For this reason, particulars and illustrations in this handbook may not conform in every detail to models in current production.

Nikon

NIPPON KOGAKU K.K.

Fuji Bldg., 2-3, 3 chome, Marunouchi,
Chiyoda-ku, Tokyo 100, Japan
☎03-214-5311
Telex: J22601 (NIKON)

Printed in Japan

(84.8.A)H·E-10S