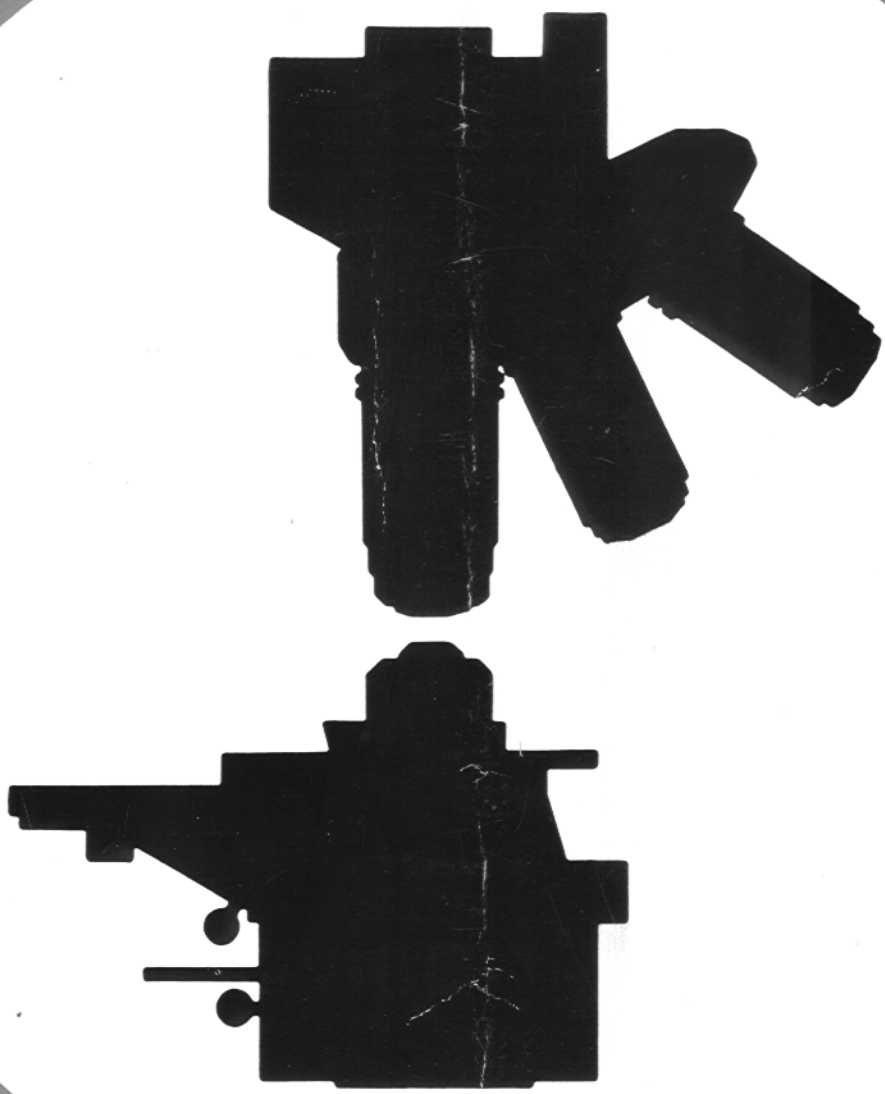
Interference Contrast Device T

Instructions



550-37/Engl. R

1. Introduction

The interference contrast device T serves for the quantitative representation of phase differences in microscopic objects in transmitted-light illumination. The range of applications covers mainly biology and medicine.

The method is based on the principle of polarized light and therefore on the use of polarizers and analysers.

The device is supplied in two versions :

a) For the biological microscopes LABORLUX, ORTHOLUX, DIALUX, ORTHOPLAN and Panphot.

b) For the polarizing microscopes LABORLUX-Pol, DIALUX-Pol, ORTHOLUX-Pol, Panphot-Pol and ORTHOPLAN-Pol.

To allow the orientation of line-shaped objects so that the detail of interest appears at optimum contrast, a rotating object stage or an attachable rotating stage is recommended with the biological stand.

The interference contrast device T is based on the princ - iple of two beam interference. In contrast to the two beam interference arrangements according to Mach/Zehnder and Jamin/Lebedeff, where the lateral separation between sample- and reference-beam is larger than or at least the same as the object size (total image separation), in the present device the beam separation has been chosen a little smaller than the resolving power of the objectives used in the microscope (differential image separation).

The splitting and recombination of the beams is carried out with optical crystal aids, according to the arrangement of Wollaston prisms in the front and rear focal plane of condenser and object recommended by Smith (1947).

Fig. 1 is a diagrammatic representation of the optical design of the interference contrast device. The rays linearly polarized by the polarizer P are split into two components polarized vertically to each other by the Wollaston prism ; they pass through the object parallel to each other in different places, and are recombined by the Wollaston prism W_2. In the intermediate plane two images of the object are thus produced, at a slight lateral displacement to each other.

The analyser A, however, causes the 2 part-beams polarized vertically to each other to vibrate in a common plane , so that changes in the phase caused by the object become visible through interference of the 2 part beams as brightness or colour differences.

The λ /4 plate situated below the Wollaston prism W_1 acts as a phase-changing compensator in conjunction with the rotating polarizer P. By means of the λ -plate (Fig. 1), which can also be inserted in the beam path, the brightness and colour differences between the background and the object can be varied.

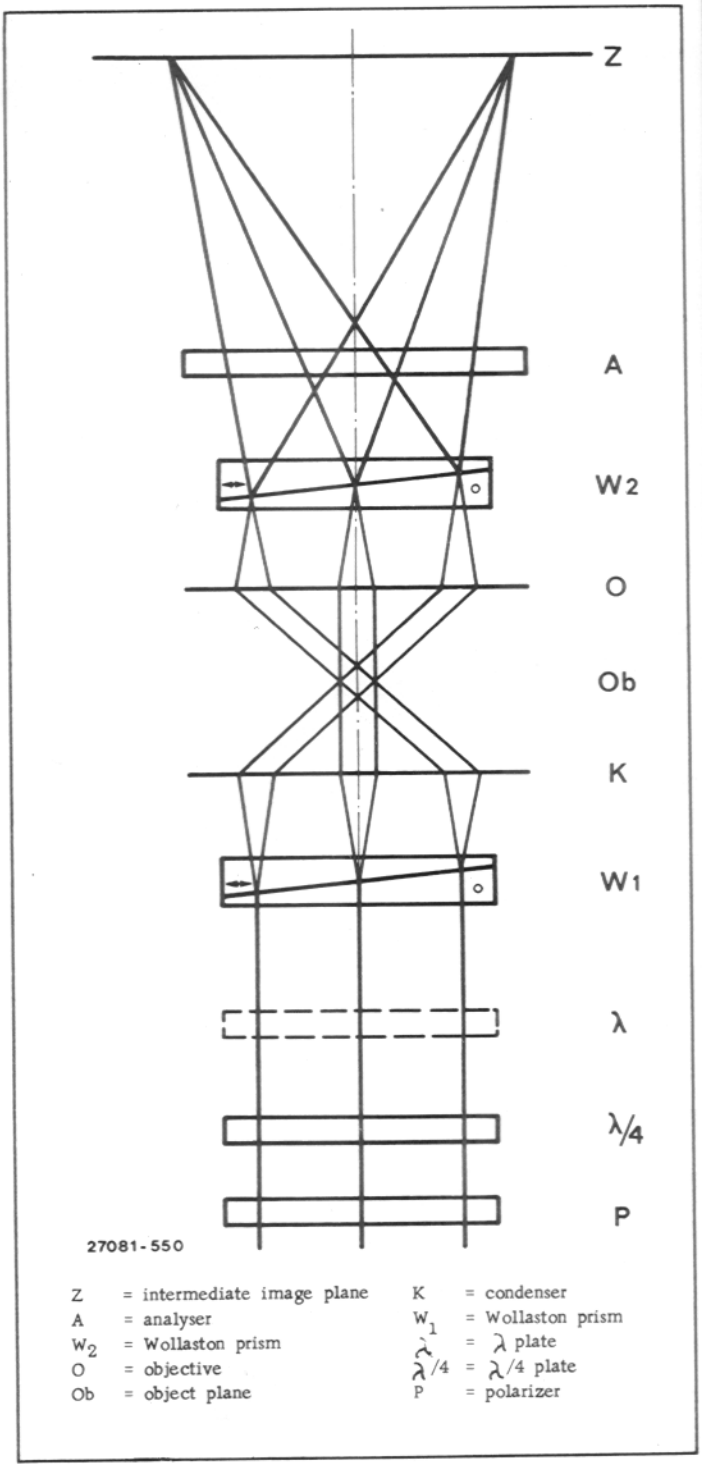
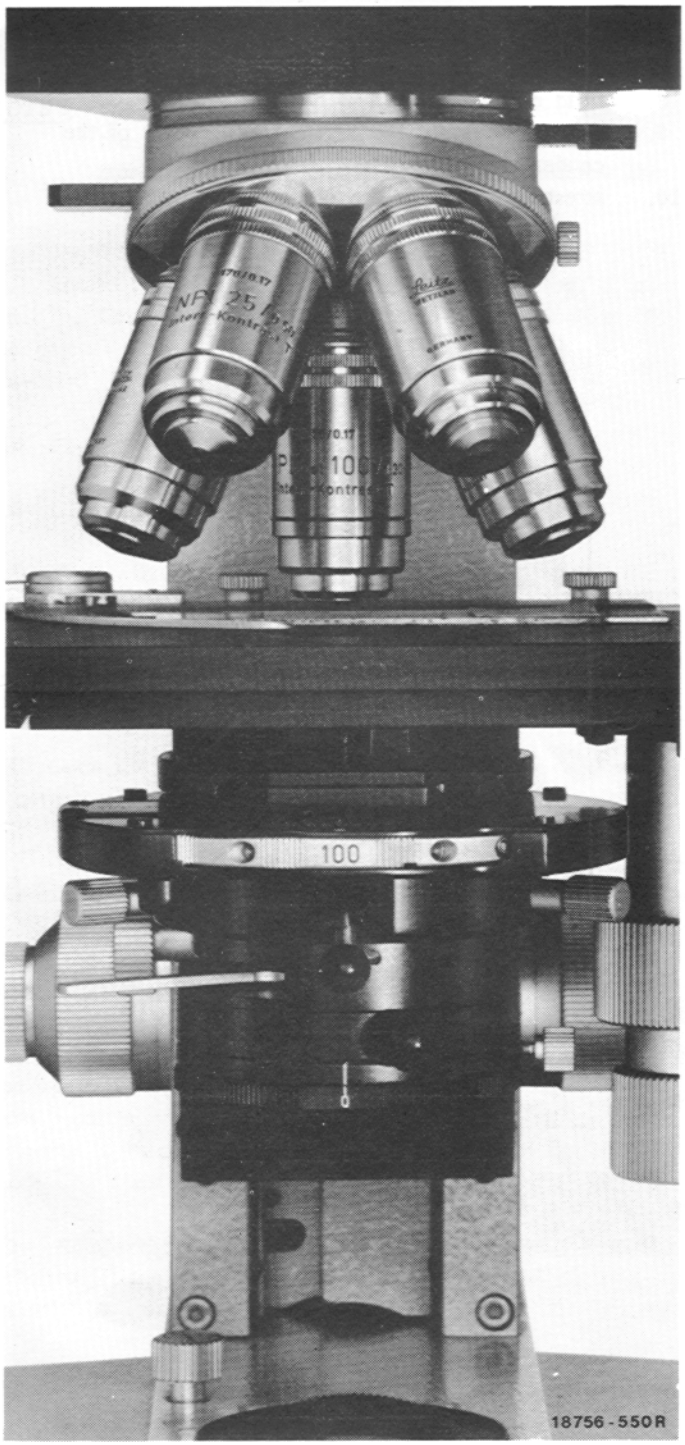


Fig. 1 Optical diagram of the interference contrast device T



The interference contrast device T consists of the components shown in Fig. 2, which replace the corresponding standard equipment of the microscope.

Replacement must be carried out as follows :

3.1 Revolving nosepiece

Remove the revolving nosepiece with standard objectives from the stand (this operation differs with the various microscope stands ; adhere to the relevant insturctions).

Mount the revolving nosepiece with permanently fixed objectives (Fig. 2) on the stand. Check centration of the light source.

3.2 Condenser

Lower the standard condenser of the microscope and pull it out towards the front.

Insert the interference contrast condenser (Fig. 2) into the condenser guide. Swing in the condenser top (Fig. 3.4) and raise the condenser.

3.3 Analyser

A filter slide with filter polarizer must be inserted in the tube slot of biological microscopes (Fig. 3.2).

With polarizing microscopes the built-in analyser must be turned into the beam and set at position 0.

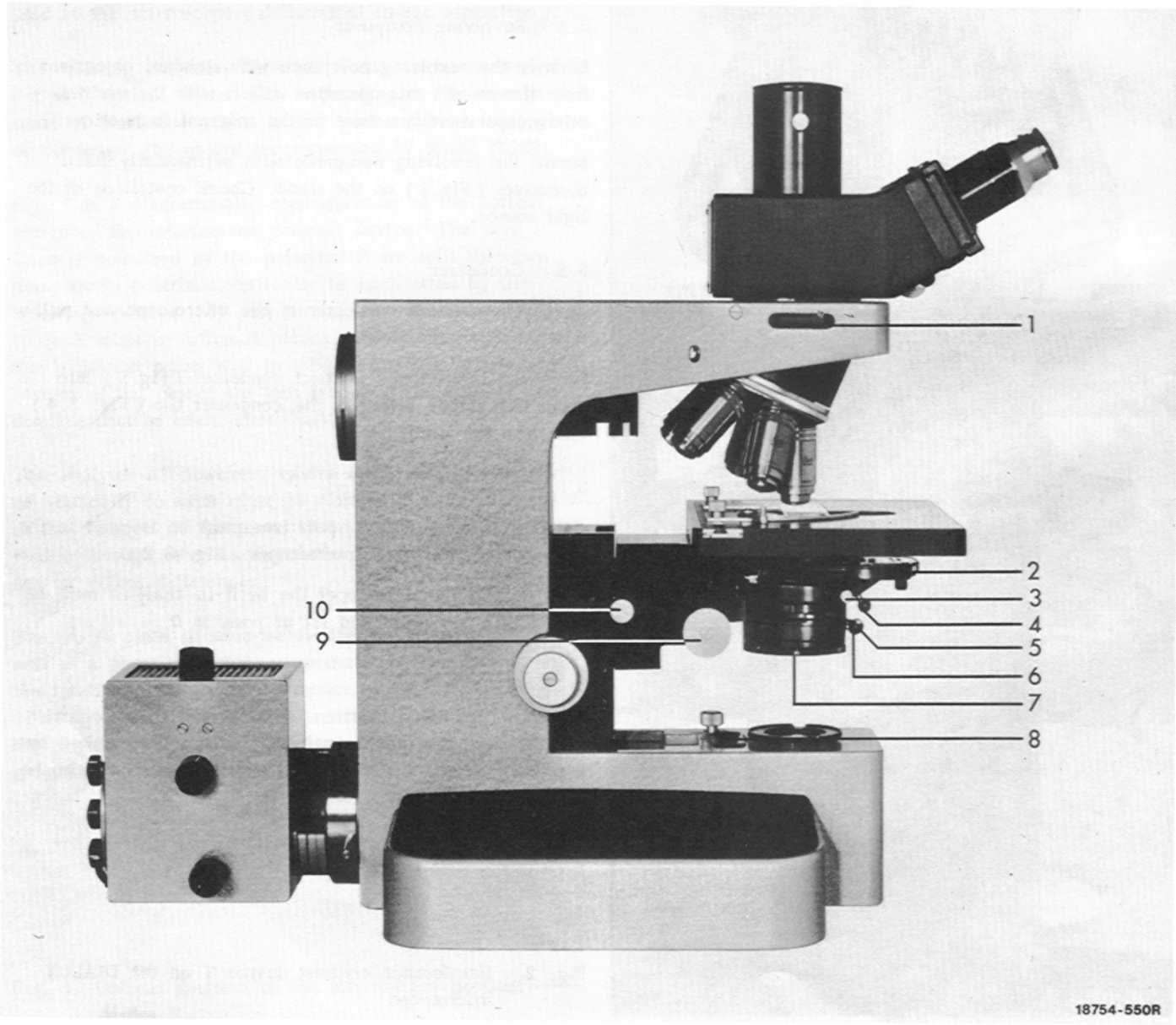
3.4 Rotating stage

To allow azimuthal rotation of the object in biological microscopes, the mechanical stage should be replaced by a rotating stage. An attachable rotating stage can also be used.

Fig. 2 Interference contrast device T on the DIALUX microscope

Fig. 3

1. Analyser
2. knurled ring for rotating the annular stops
3. condenser centring screws
4. lever for turning in the condenser top
5. λ -plate
6. aperture diaphragm
7. polarizer
8. field diaphragm
9. knurled knob for the vertical adjustment of the condenser
10. arresting screw for the object stage



4) Adjustment and operation

4.1 Place the specimen on the microscope stage.

4.2 Turn the desired objective into the beam.

NOTE : The interference objectives are cemented into the revolving nosepiece at their correct orientation. No attempt should be made to unscrew the objectives.

4.3 Rotate the knurled ring of the condenser (Fig. 3.2) until the figure which corresponds to the magnification of the objective used (25, 40 or 100) points towards the observer.

4.4 Focus the specimen.

4.5 Close the field diaphragm (Fig. 3.8)

4.6 Vertically adjust the condenser (Fig. 3.9) until the field diaphragm, too, is in focus.

4.7 Centre the field diaphragm to the field of view by means of the centring screws of the condenser (Fig. 3.3).

4.8 Open the field diaphragm so that the field of view is completely illuminated.

4.9 Close the aperture diaphragm (Fig. 3.6) so that 2/3 of the full objective aperture is open.

(With the interference objectives the front lens of the condenser (Fig. 3.4) must always be turned into the beam).

The aperture diaphragm can be slightly closed for specimens requiring a greater depth of field. It must, however, be borne in mind that the resolving power decreases with decreasing aperture.

4.10 Rotate the polarizer (Fig. 3.7) slightly to the right or left until the specimen appears at the desired contrast (relief-like image).

The slightly uneven illumination thus produced is based on the laws of optical physics and without significance in the microscopic investigation.

4.11 For additional colour introduce the λ-plate into the beam by pulling the bottom-most knob on the condenser (Fig. 3.6).

4.12 For ordinary brightfield observation rotate polarizer or analyser (with polarizing microscope) through 45° from the crossed position and turn the knurled ring (Fig. 3.2) on the condenser into position "H" = brightfield.

4.13 For phase contrast observation screw the required phase contrast objectives into the revolving nosepiece.

For the NPl 10/0.25 and NPl 16/0.40 Phaco objectives a suitable annular stop is already in position I of the condenser. If Phaco objectives of higher magnification are used corresponding annular stops must be inserted in position II.

5) Hints for the preparation of specimens and for the care of the instrument

Additional disturbing phase differences may be caused by unevenness of the embedding medium (e.g. incomplete mixture during preparation or aging) or impurities on the microscope slides and coverglasses used. Impurities in the optical system, too, adversely affect the image quality. Particularly the condenser front lens must be free from dust. It is best to clean it with a fine grease-free brush or a piece of soft leather glued on to a little wooden stick. Dirt that resists removal should be treated with eylene. Alcohol and other solvents must not be used on any account, since they may dissolve the cement of the objectives.

After use protect the instrument from dust.

Repairs of the sensitive interference contrast device T can be carried out expertly only in the factory.

6) Hints for the interpretation of interference contrast images

The interference contrast device T produces a relief-like representation of the specimen, as if under oblique illumination. The relief, however, is not real, but purely optical. The heights and depths in the picture correspond to the differential path lengths in the specimen (phase displacement of the wave front), which are caused by thickness differences and/or differences in refractive index

In the simplest case the object has a uniform refractive index. Here the optical relief seen also represents the geometrical relief, e.g. of glass splinters embedded in a liquid. The relief seen, however, is as a rule super-elevated compared with the real relief. It does not correspond to the surface relief but to the thickness profile.

In a different case the object is uniformly thick but its refractive index is not everywhere the same. Here the image observed corresponds to the distribution of the refractive index, e.g. in schlieren in a coverglass.

In most cases the specimen e.g. a cell, exhibits both refractive-index and thickness differences. The optical relief produced by means of interference contrast is therefore the outcome of both factors.

Interference Devices for Transmitted Light

The interference contrast method serves for the qualitative demonstration of phase structures.

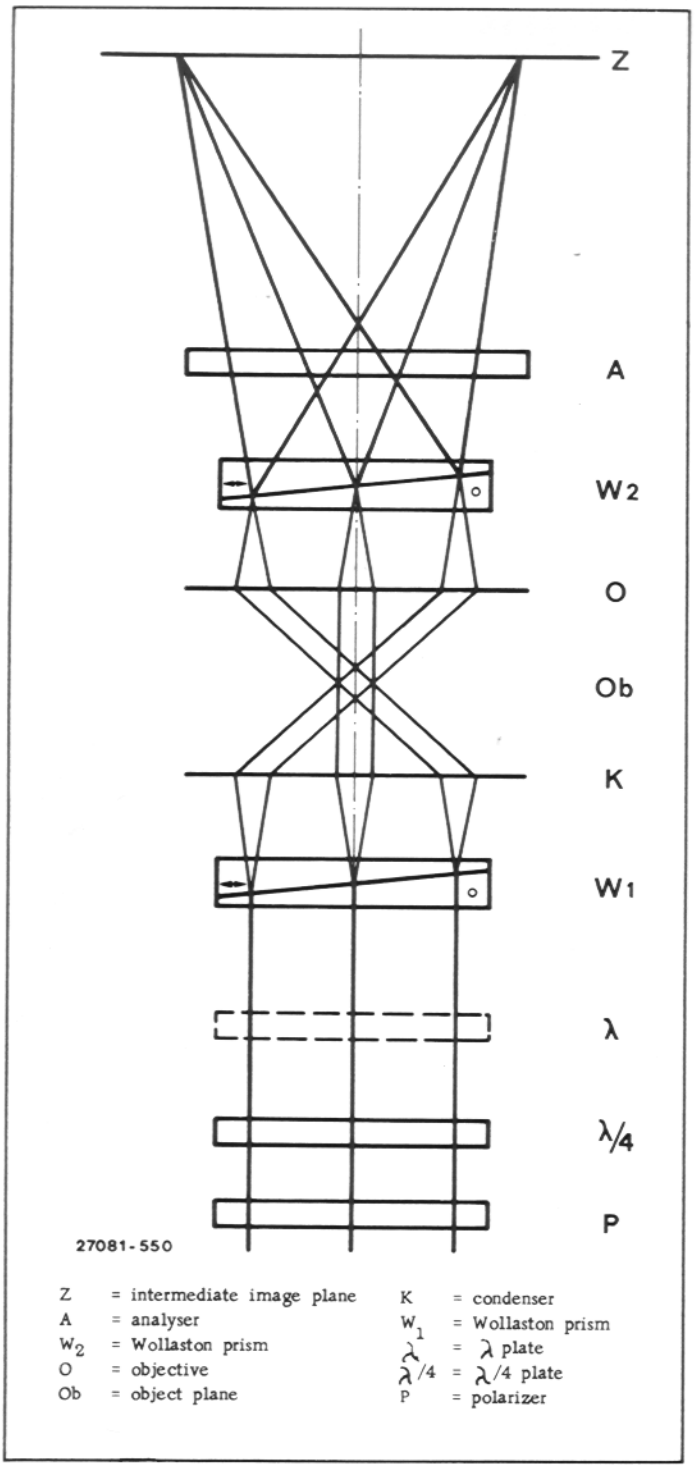
In the microscope the objects and especially their fine structure details appear in relief-like contrast. But stained specimens, too, can be observed in interference contrast to great advantage.

The current main field of application is biology. Here especially those object structures can be shown which cannot be recognized sufficiently clearly with the conventional methods of microscopy (e. g. fibrous elements, flagellae, cilia, etc.).

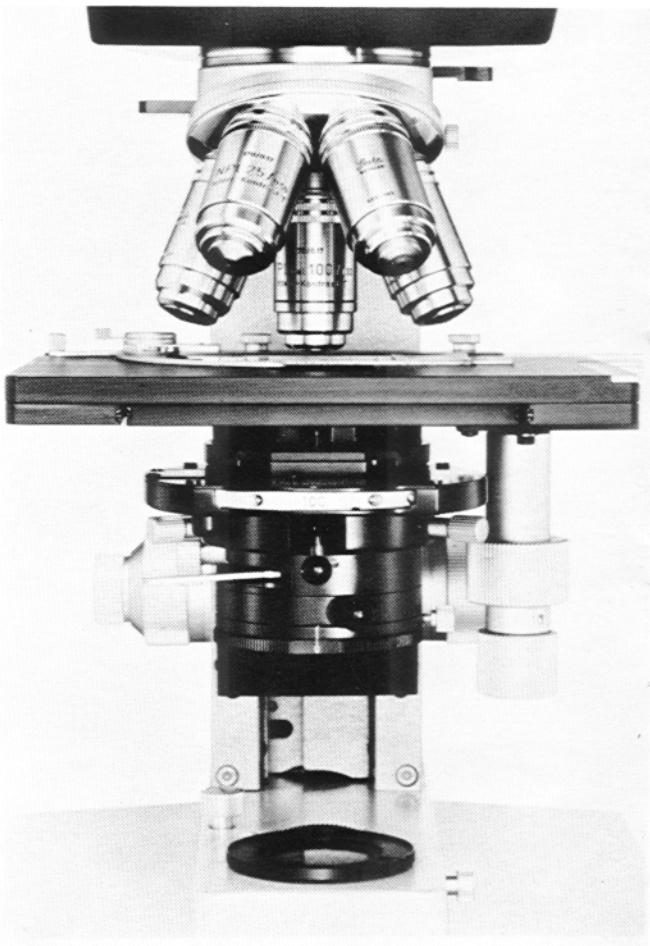
The surface of non-biological transparent objects (e. g. glass or plastics) can also be three-dimensionally rendered by interference contrast. This provides information on their properties.

The method therefore complements phase contrast microscopy and the quantitative interferometric methods.

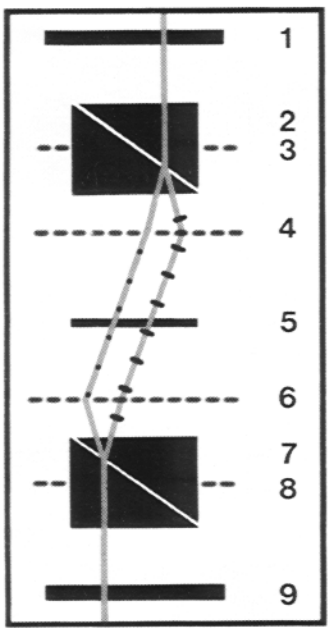
Interference contrast device T for transmitted light



Z = intermediate image plane
A = analyser
W_2 = Wollaston prism
O = objective
Ob = object plane
K = condenser
W_1 = Wollaston prism
λ = λ plate
$\lambda/4$ = $\lambda/4$ plate
P = polarizer



Principles and Function



To keep the background of the image homogeneous, the cross-section of the bundle of rays must be made very narrow, i. e. parallel light must be used. Parallel light, i. e. a very small condenser aperture, however, is not welcomed by the microscopist, because this reduces resolving power considerably. In practice, therefore, an arrangement according to Fig. A is used, in which the condenser aperture is not reduced and the ability of the part rays to interfere nevertheless ensured.

The natural light emitted by the light source is linearly polarized by the polarizer 9. From there it passes to the Wollaston prism 7, which is arranged diagonally to the polarizer. In this position the linearly polarized ray is split into two part-rays vertically polarized to each other and of the same intensity, which diverge from each other. Since the point of divergence is situated in the focal plane 8 of the condenser 6, the angle split in the Wollaston prism becomes lateral in the object space. The part-rays therefore pass through the object 5 at two different points where their phase, too, is differently affected. The magnitude of the split has been chosen so that it is below the resolving power of the microscope. No visible double image is therefore produced in the microscope. The objective 4 recombines both part-rays in the rear focal plane 3. This is where the second Wollaston prism* 2 is situated, which recombines the two part-ray bundles in space. They now pass through the analyser 1 and, brought to a common vibration direction, can now interfere. The interference color or intensity in each point of the image field depends on the phase difference of the two part-ray bundles and thereby on the thickness and refractive index of the two object points.

Function of λ - and λ -/4-plates

The λ /4-plate situated below the Wollaston prism, in combination with the rotating polarizer P, acts as a phase-advancing compensator. Together with the λ -plate which can additionally be inserted in the beam, brightness and color differences between surrounding field and object can be varied.

SMITH INTERFERENCE CONTRAST SYSTEM T

consisting of:

Interference contrast condenser T, with aperture diaphragm, centering mount and interchange carrier, rotating polarizer indexed at 90 degree intervals, revolving disc with three beam splitting prisms for the special objectives 25/0.50, 40/0.65, and oil 100/1.30. The condenser has in one position, an additional light ring for the objectives Phaco NPL 10/0.25 and Phaco NPL 16/0.40 (filter analyser included)

Quintuple centering revolving nosepiece, with slide changer and objective:

25/0.50, interference contrast T with built-in Wollaston prism

40/0.65, interference contrast T with built-in Wollaston prism

Oil 100/1.30, interference contrast T with built-in Wollaston prism

553 209 Complete equipment for LABOLUX . . . . . .
553 266 Complete equipment for DIALUX . . . . . .
553 210 Complete equipment for ORTHOLUX and PANPHOT . . . . . .
553 265 Complete equipment for ORTHOLUX II . . . . . .
553 211 Complete equipment for ORTHOPLAN . . . . . .
553 306 Complete equipment for DIAVERT . . . . . .
553 206 Complete equipment for LABOLUX-POL and DIALUX-POL . . . . . .
553 207 Complete equipment for ORTHOLUX-POL and PANPHOT-POL . . . . . .
553 271 Complete equipment for ORTHOLUX-II-Pol MK and BK . . . . . .
553 208 Complete equipment for ORTHOPLAN-POL . . . . . .

SMITH INTERFERENCE CONTRAST SYSTEM-T WITH PRE-POLARIZER (required when light intensity light sources are to be used)

553 276 Complete equipment for DIALUX . . . . . .
553 275 Complete equipment for ORTHOLUX II . . . . . .
553 273 Complete equipment for ORTHOPLAN . . . . . .

553 274 Complete equipment for ORTHOLUX-II-Pol MK and BK . . . . . .
553 272 Complete equipment for ORTHOPLAN-POL . . . . . .

SMITH INTERFERENCE CONTRAST SYSTEM T

consisting of:

Interference contrast condenser T, with aperture diaphragm, centering mount and interchange carrier, rotating polarizer indexed at 90 degree intervals, revolving disc with three beam splitting prisms for the special objectives 25/0.50, 40/0.65, and oil 100/1.30. The condenser has in one position, an additional light ring for the objectives Phaco NPL 10/0.25 and Phaco NPL 16/0.40 (filter analyser included)

Quintuple centering revolving nosepiece, with slide changer and objective:

25/0.50, interference contrast T with built-in Wollaston prism

40/0.65, interference contrast T with built-in Wollaston prism

Oil 100/1.30, interference contrast T with built-in Wollaston prism

553 209	Complete equipment for LABOLUX	$2,843.00
553 266	Complete equipment for DIALUX	$2,800.00
553 210	Complete equipment for ORTHOLUX and PANPHOT	$2,871.00
553 265	Complete equipment for ORTHOLUX II	$2,956.00
553 211	Complete equipment for ORTHOPLAN	$2,949.00
553 206	Complete equipment for LABOLUX-POL and DIALUX-POL	$2,841.00
553 207	Complete equipment for ORTHOLUX-POL and PANPHOT-POL	$2,921.00
553 271	Complete equipment for ORTHOLUX-II-Pol MK and BK	$2,957.00
553 208	Complete equipment for ORTHOPLAN-POL	$2,887.00

SMITH INTERFERENCE CONTRAST SYSTEM-T WITH PRE-POLARIZER (required when light intensity light sources are to be used)

553 276	Complete equipment for DIALUX	$2,920.00
553 275	Complete equipment for ORTHOLUX II	$3,086.00
553 273	Complete equipment for ORTHOPLAN	$3,071.00
553 274	Complete equipment for ORTHOLUX-II-Pol MK and BK	$3,087.00
553 272	Complete equipment for ORTHOPLAN-POL	$3,073.00

(Prices as of 1972)