1. Introduction

The interference contrast device T serves for the qualitative representation of phase differences in microscopic objects in transmitted-light illumination. Its practical range covers above all biology and medicine.

The method is based on the polarised-light principle and therefore requires the use of a polariser and analyser. The device can be used with the following microscopes :

a) The ORTHOLUX®, DIALUX®*), ORTHOPLAN® and DIAVERT® biological microscopes.

b) The ORTHOLUX-POL and ORTHOPLAN-POL polarised-light microscopes

*) For the DIALUX 20 microscope a modified version is available (see Instructions 550-055).

Fig. 1 LEITZ Interference Contrast Device T

1 Interference contrast condenser with polariser, pre-polariser, built-in Wollaston prisms and light ring

2 1.25x revolving nosepiece

3 Focusing telescope

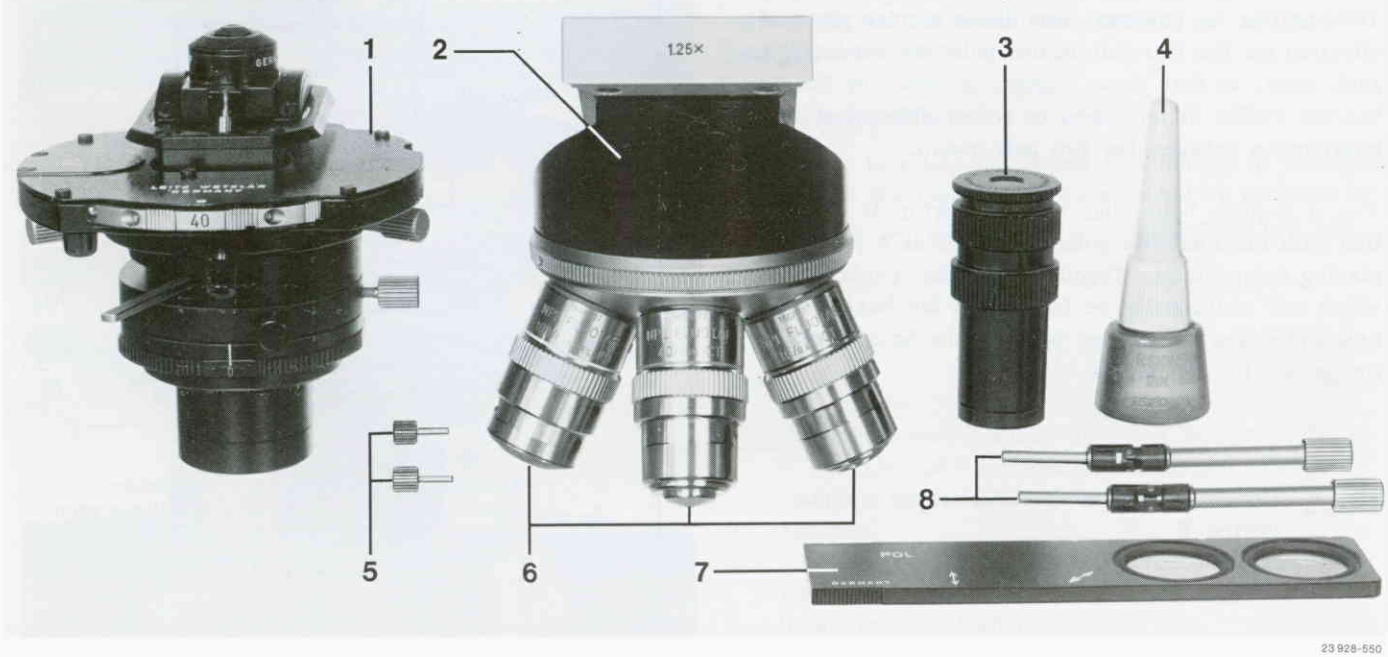
4 LEITZ immersion oil

5 Adjustment key for the interference contrast objectives

6 Interference contrast objectives

7 Analyser

8 Key for centring the light ring (phase contrast)



23 928-550

2. Optical system

The interference contrast device T is based on the principle of dual-beam interference. Unlike the dual-beam interference arrangement according to MACH/ZEHNDER and JAMIN/LEBEDEFF, in which the lateral separation of the sample and reference beam is larger than the extent of the object or at least of the order of the object extent (total image splitting), the interference contrast device T makes use of a beam separation slightly smaller than the resolving power of the objective used (differential image splitting).

The bundles are split and reunited by crystal optical means according to the arrangement, suggested by Smith (1947), of Wollaston prisms in the front and rear focal plane respectively of condenser and objective.

Fig. 2 is a diagrammatic representation of the optical system of the interference contrast device. The rays linearly polarised by the polariser P are split by the Wollaston prism into two parts polarised vertically to each other, passing through different areas of the object and parallel to each other; they are reunited by the Wollaston prism W_2. Two images of the object are therefore formed in the intermediate image plane at a slight lateral displacement.

The analyser A, however, establishes a joint plane of vibration for the two part beams polarised vertically to each other, so that phase changes produced by the object become visible as brightness or colour differences through interference between the two part beams.

The $\lambda/4$-plate below the Wollaston prism W_1 in conjunction with the rotatable polariser P acts as a phase displacing compensator. Together with the λ-plate (Fig. 2), which can additionally be inserted in the beam, the brightness and colour differences between the background of the image and the object can be varied.

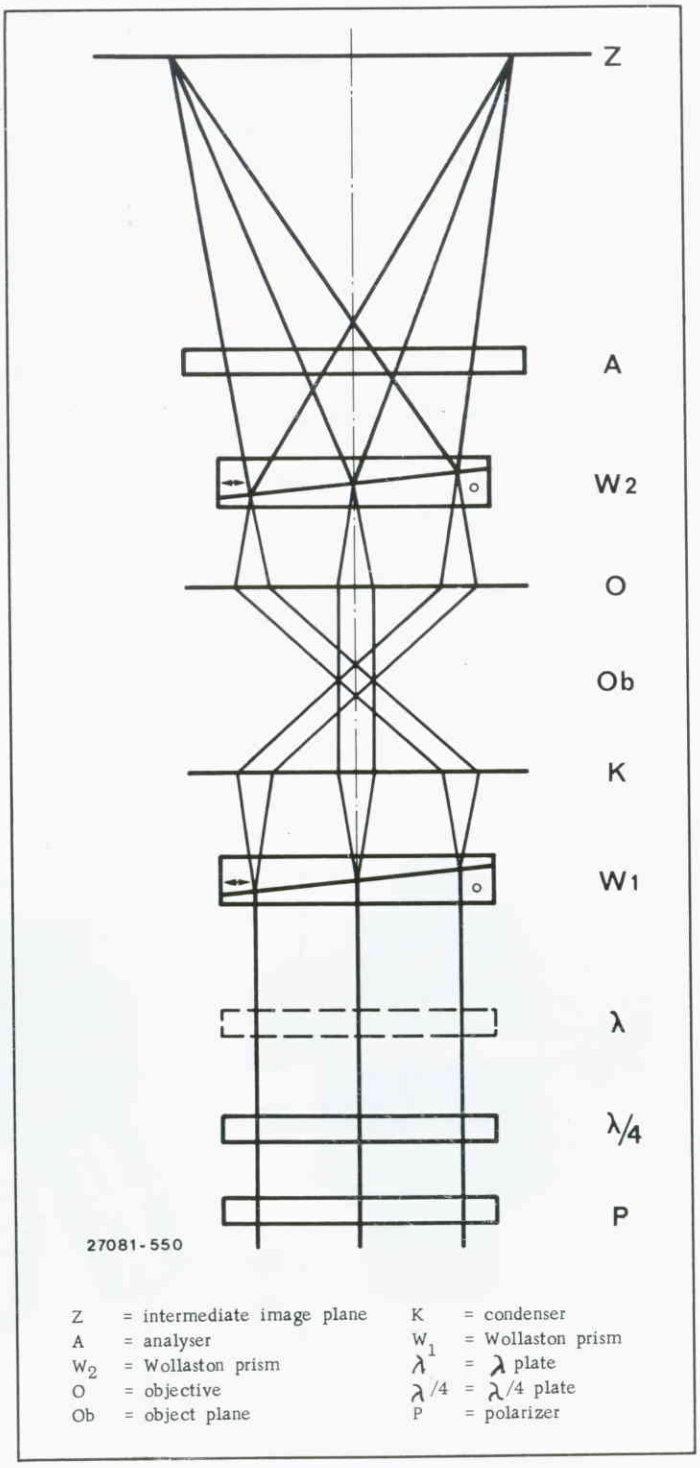
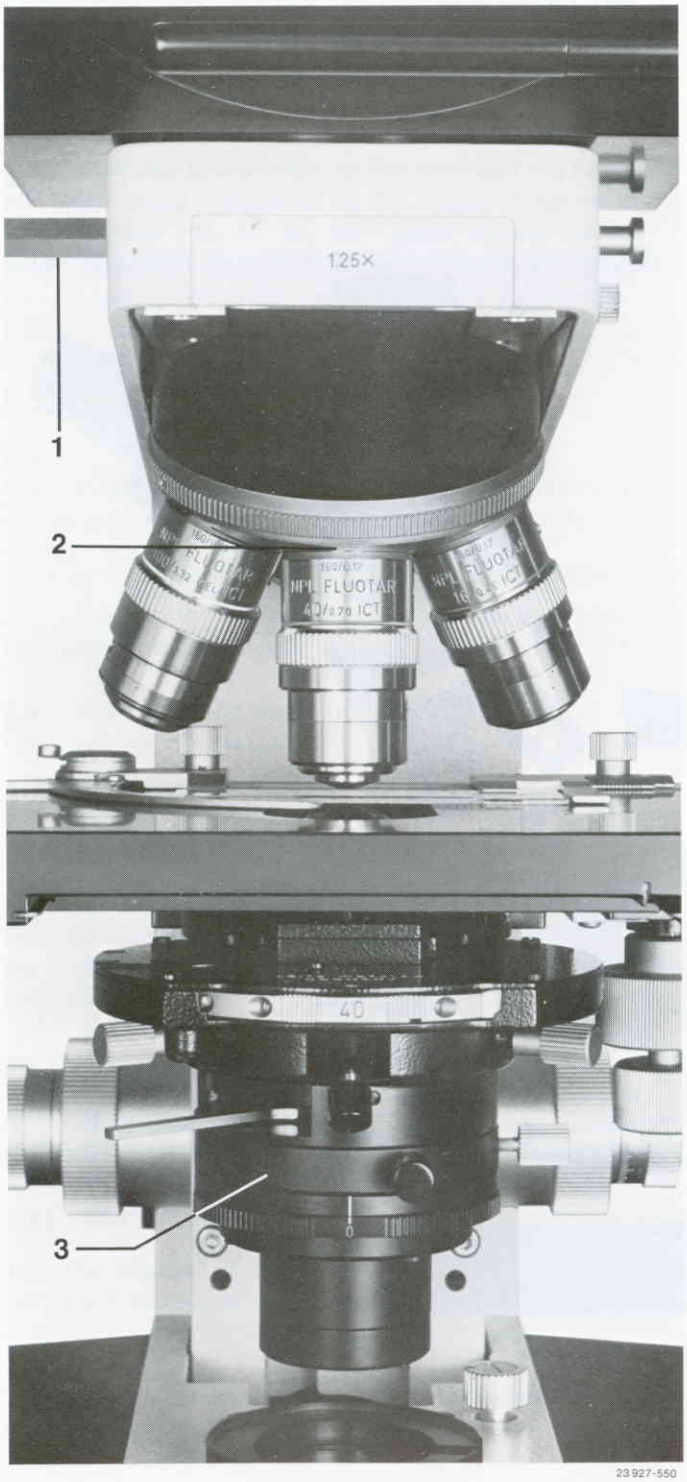


Fig. 2 Optical system of the interference contrast device T



The interference contrast device T consists of the components shown in Fig. 3, which are inserted in place of the corresponding normal outfit of the microscope.

The exchange is carried out as follows :

3.1 Revolving nosepiece

Remove the revolving nosepiece with the standard objectives from the stand (this differs with the various microscopes : consult the relevant instructions).

Attach the revolving nosepiece with orientated objectives (3.2) to the stand. Check centration of the light source.

3.2 Condenser

Lower the standard condenser of the microscope and pull it out of the guide.

Insert the interference contrast condenser (3.3) into the condenser guide. Turn in the condenser lens (4.4) and adjust the condenser upwards.

3.3 Analyser

In biological stands a filter slide (3.1) with filter polariser must be inserted in the tube slot of the microscope.

With polarising microscopes the built-in analyser must be turned into the beam and, if rotatable, set at the 90° position.

3.4 Rotating stage

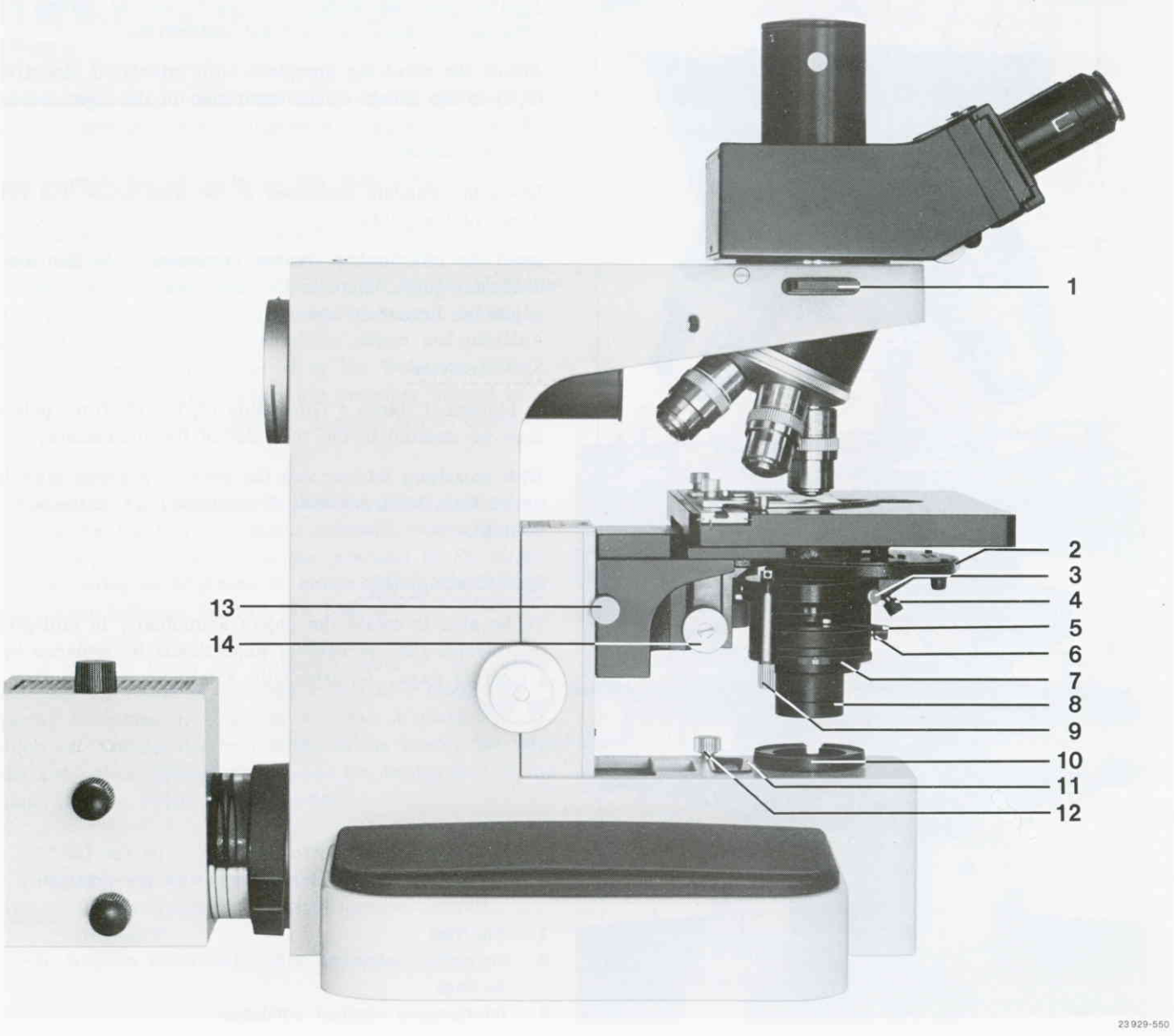
To be able to rotate the object azimuthally in biological microscopes the mechanical stage should be replaced by a rotating stage. An attachable rotating stage can also be used.

Fig. 3 Interference contrast device T on the LEITZ ORTHOPLAN microscope (with pre-polariser)

1 Analyser
2 Revolving nosepiece with interference contrast objectives
3 Interference contrast condenser

Fig. 4

1 Analyser
2 Knurled ring for setting the Wollaston prisms
3 Condenser centring screws
4 Lever for swinging the condenser top out
5 Aperture diaphragm
6 λ -plate
7 Polariser
8 Pre-polariser
9 Centring key, can be detached for the centration of the light ring (phase contrast)
10 Dust glass - can also be used as a filter support
11 Field diaphragm
12 Hinged lens (is turned out only when the 1:1 objective is used)
13 Object stage clamping screw
14 Knurled screw for the vertical adjustment of the condenser



4.1 Place the specimen on the microscope stage.

4.2 Turn the required objective into the beam.

ATTENTION: The interference objectives are oriented as they are screwed into the revolving nosepiece. Avoid if possible unscrewing the objectives. Reorientation of the oriented objectives, see following page.

4.3 Turn the knurled disc of the condenser (4.2) until the figure which corresponds to the magnification of the objective used (16, 25, 40, 100) points towards the observer. For the L20/0.32 ICT and L32/0.40 ICT objectives a special Wollaston prism is used in conjunction with the condenser top 0.60 L 11 P.

4.4 Focus the object.

4.5 Close the field diaphragm (4.11).

4.6 Form a sharp image both of the field diaphragm and of the object by raising or lowering the condenser (4.14).

4.7 Centre the field diaphragm in the field of view by means of the condenser centring screws (4.3).

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

4.9 Close the aperture diaphragm (4.5) so that two thirds of the full objective aperture is transmitted.

(The front lens of the condenser (4.4) in conjunction with the interference objectives as well as the lens (4.12) in the foot of the stand must always be turned into the optical path).

For objects that require a greater depth of field the aperture diaphragm can also be narrowed. It must, however, be borne in mind that resolution decreases with decreasing aperture.

4.10 Set the analyser (4.1) at position ↕

Turn the polariser (4.7) slightly to the right or left until the object appears at the required contrast (relief-like image).

This produces a slight unevenness in the illumination for physical and optical reasons and can be ignored in microscope investigation.

4.11 For additional colour contrast move the λ -plate into the beam by pulling the bottom knob on the condenser (4.6).

4.12 For normal brightfield observation move the analyser into the position ↙↗ (with pol-microscopes in position 45°) and move the knurled disc (4.2) on the condenser into position "H" = brightfield.

4.13 For phase contrast observations screw the appropriate phase contrast objectives into the revolving nosepiece.

For the NPL-FLUOTAR 10/0.30 PHACO I and NPL-FLUOTAR 16/0.45 PHACO 1 objectives an annular diaphragm 1 is already in position 1 of the condenser. If PHACO objectives of higher magnification are to be used, the corresponding annular diaphragms must be inserted in position II.

Centration of the light rings see Instructions Phase Contrast No. 513-084.

4.14 For orientating observation in polarised light:

Use brightfield, phase contrast, or pol objectives in conjunction with the position "H" of the condenser. Use analyser position ↕, polariser at position "0".

This is required only if the objectives had been supplied separately from the interference contrast device T (for instance NPL-FLUOTAR 25/0.55 ICT) or had been unscrewed from the revolving nosepiece. The corresponding Wollaston prisms must be installed at Wetzlar. Already adjusted objectives and revolving nosepieces must be marked with a pencil or adhesive strip so that the objectives can be screwed in at the same orientation. Use the objectives in the order of ascending magnification in the revolving nosepiece (clockwise assembly in ascending order of magnification). If necessary unscrew all objectives from the revolving nosepiece except the one to be adjusted. Turn in analyser (4.1) (↕), set the polariser (4.7) at 0. Turn out the λ -plate (4.6).
Form a sharp image of the object.
Set up Koehler's illumination (see instructions for the microscope).

ATTENTION : Use the condenser top Chr. 0.90 P and L-ICT condenser 0.60 L 11 (P) for the NPL-FLUOTAR ICT objectives.

Turn in the empty aperture on the revolving nosepiece.
Find an empty area in the object.
Set the position of the rotating turret corresponding to the ICT objectives.
Turn out the condenser top.
Open the aperture diaphragm.

Fig. 5

23 466-550 R

Fig. 6

23 467-550 R

Replace one eyepiece with the focusing telescope and, by adjusting the top, focus on the objective pupil (circular bright area); a curved dark band running obliquely through the field of view must now be visible.

Make a note of the direction of this curved band or record it on a sheet of paper (in the majority of cases this band runs roughly from bottom left to top right).

Set position "H" (= brightfield) of the revolving turret, turn ICT objective into the optical path.

Check whether the objective has been screwed into the revolving nosepiece as far as it will go.

Insert the Allen key in the clamping screw for the objective rotation and slightly slacken it (Fig. 5).

Rotate the front part of the objective until an almost straight dark band runs diagonally through the field of view (in the same direction as previously observed on the Wollaston prism on the condenser side) (the aperture diaphragm of the condenser must be open for this purpose).

Turn in the condenser top.

Turn the Wollaston prism corresponding to the objective into the optical path by means of the revolving turret. The field visible through the focusing telescope must now show a relatively uniform grey tone. If a diagonal band is again present, the front part of the objective must be rotated through 180° (it is advisable to use the "H" position of the condenser temporarily to rotate the objective into the correct position indicated by the most contrasty diagonal position of the band).

The most accurate objective orientation exists when the empty field of view appears darkest (polarisers exactly crossed).

Secure the front part of the objective with the Allen key (Fig. 5).

Insert both Allen keys on the front part of the objective in the opposite adjustment screws (Fig. 6).

Replace the focusing telescope with the eyepiece.

Alternately turn the polariser slightly to the left and right. If during a left turn the field of view shows a different hue to that at an equal turn to the right, the Wollaston prism built into the objective must be adjusted by alternate release of one clamping screw and tightening of the other (Fig. 6) until approximately the same hue change in the object field is observed with left and right turn of the polariser.

Fix the Wollaston prism with even, slight tightening of the screws (Fig. 6).

Remove the Allen key from the objective.

Retighten the upper clamping screw (cf. Fig. 5).

Unscrew the objective from the revolving nosepiece (mark the threaded bore with an adhesive strip or pencil), because the objective must always be screwed into the same hole at the same orientation without fail).

Adjust the other objectives.

All adjusted interference contrast objectives as well as the other objectives must now be screwed into the revolving nosepiece.

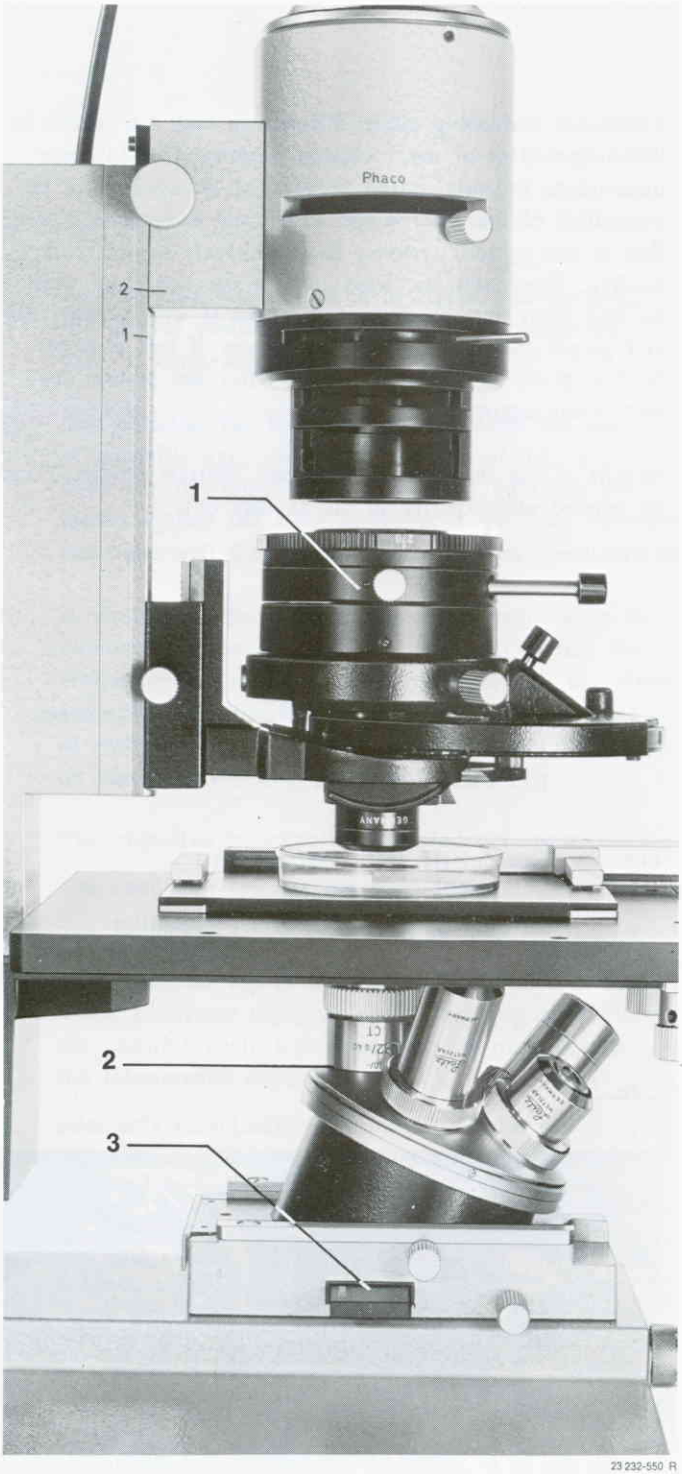


Fig. 7 Interference contrast device T on the LEITZ DIAVERT inverted microscope

1 Interference contrast condenser with condenser top 0.60 L 11 P

2 L 32/0.40 ICT objective

3 Analyser

5. Directions for the preparation and maintenance of the instrument

Additional disturbing phase differences may be caused by inhomogeneities of the inclusion medium (for instance incomplete mixture during preparation or ageing) or by impurities of the microscope slides and coverglasses used. Dirt in the optical system, too, adversely affects image quality. Especially the front lens of the condenser must be free from dust. It is best to clean it with a fine, dry, and grease-free sable brush or a piece of soft chamois leather glued to a wooden stick. After use protect the instrument against dust.

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

Fig. 8 Epithelial cells of the human buccal mucosa
- interference contrast -
NPL FLUOTAR 40/0.70 ICT objective

Fig. 9 Opercularia spec. (infusorium)
- interference contrast -
NPL FLUOTAR 40/0.70 ICT objective

6. Directions for the interpretation of the interference contrast images

The interference contrast device T produces a relief-like presentation of the object, roughly as if it were obliquely illuminated. The relief, however, is not real, but purely optical. The "heights" and "depths" in the image correspond to the different optical path lengths in the object (phase displacement of the wave fronts), which are caused by differences in thickness and/or differences in the refractive index.

In the simplest case the object has a uniform refractive index, when the optical relief seen is at the same time the geometrical relief, for instance that of glass splinters embedded in a fluid. But as a rule the relief observed is superelevated compared with the true relief. It does not correspond with the surface relief, but with the thickness profile.

In some cases the object is of uniform thickness, but its refractive index is not the same everywhere. Here the observed image corresponds to the distribution of the refractive index, for instance with Schlieren in a coverglass.

In most cases the object exhibits differences in the refractive index and in thickness, for instance in a cell. The optical relief produced by means of interference contrast is therefore cause by both factors.

Fig. 8

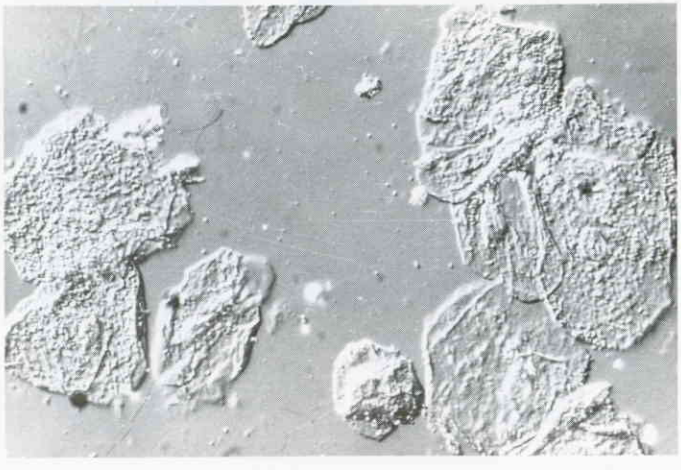


Fig. 9