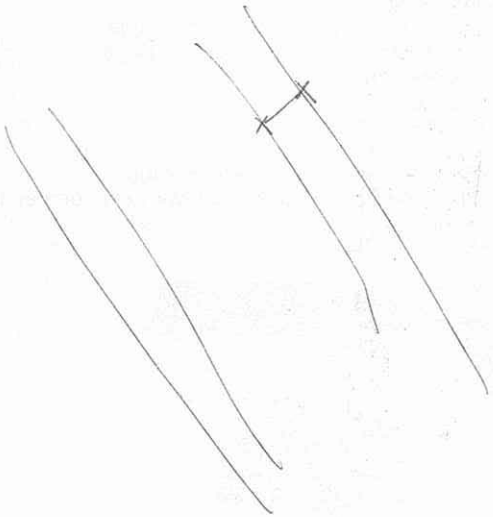
Interference Contrast T for the LEITZ ARISTOPLAN

Instructions



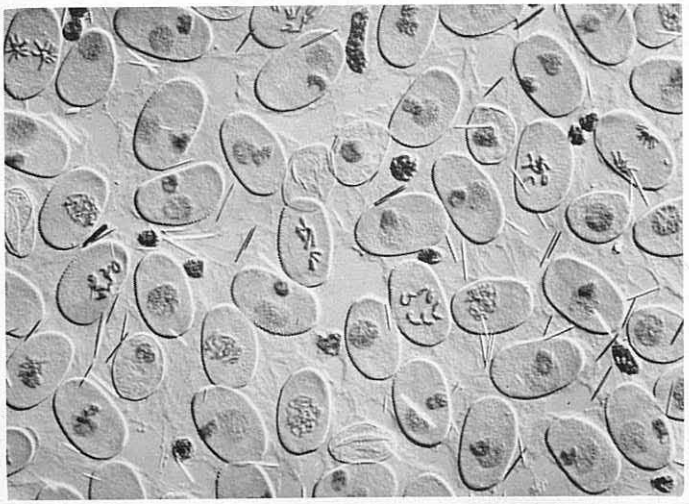
Ernst Leitz Wetzlar GmbH

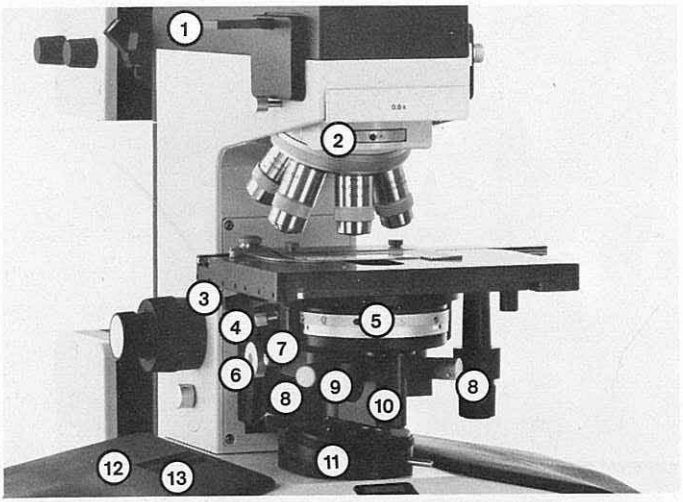
1. Principle and applications

Fig. 1 Interference contrast components mounted on the ARISTOPLAN

1 ICT analyzer
2 Objective prism turret
3 Stage rotation clamp
4 Centering screws for phase contrast light rings
5 Turret plate for Wollaston prisms and light rings
6 Condenser height control
7 Condenser clamp
8 Condenser centering screws
9 Lambda compensator
10 Supplementary lens, joined to condenser top
11 Polariser, can be rotated or swung out (with $\lambda/4$ compensator)
12 Aperture diaphragm
13 Field diaphragm

Pollen, Tradescantia in interference contrast T





In interference contrast, each illumination light wave is split into two. Each wave pair passes through two close-lying points on the specimen. If the two points have different thicknesses or different refractive indexes, one wave will move ahead of the other; a phase difference is created which can be made visible using polarization.

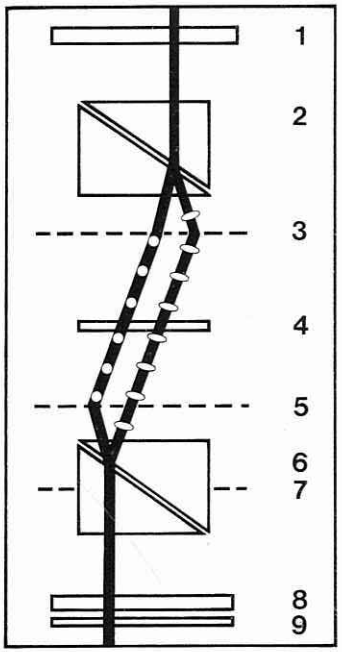
Transmitted light interference contrast (ICT) is used primarily for the study of non-stained specimens, a 3-dimensional impression being created. Unlike phase contrast, the image is halo-free. Additional color contrast can be produced by using a lambda compensator.

The light waves are split by means of Wollaston prisms (2.6) built into the interference contrast condenser. A Wollaston prism consists of two birefringent crystal prisms cemented together. The resolution of the objective is insufficient to detect the splitting, so that twin images are not visible.

Corresponding Wollaston prisms (2.2), located above the objective, bring the split light waves back together. Polarized light is required to make the phase difference visible. This is produced by the rotatable polarizer (2.9) in the microscope base and the analyzer (2.1) underneath the observation tube. The light transmitted by the polarizer is linearly polarized, i. e. it vibrates in a single direction.

Fig. 2 Principle of interference contrast T

1 Analyzer
2 Wollaston prism (objective)
3 Objective
4 Specimen
5 Condenser
6 Wollaston prism (condenser)
7 Lambda compensator
8 Quarter wave compensator
9 Polarizer



In the Wollaston prism, each light wave from the lamp is split into two waves, with the same intensity but vibrating at 90° to one another. If the two waves pass through specimen points with the same thickness and refractive index, they remain in phase (3b, d, f). The objective Wollaston prism brings the two waves back together. Interference is, however, not possible until the analyzer has aligned the vibration directions of the two waves.

If the polarizer and analyzer are parallel to each other, the intensities of in phase waves add together, resulting in a brightfield type image. In practice, however, crossed polarizers are used (or, rather, nearly crossed polarizers). With exactly crossed polarizers, in-phase waves (thickness and refractive index of both specimen points the same) produce darkness as the intensities of the two waves cancel each other out. If the refractive indices or thicknesses are different, however, gray tones are seen, the brightness of which increases with the phase difference.

This darkfield-type setting is, however, rarely used in practice. The pseudo relief image characteristic of interference contrast is produced by rotating the polarizer as follows:

A birefringent compensator plate is located above the polarizer. When the polarizer is rotated, this plate causes a phase difference of up to $\pm$ 1/4 wavelength (quarter-wave). This phase difference is superimposed on that caused by the specimen features.

On specimen points with the same thickness and refractive index (3h, k, m), the phase difference created by the plate remains unaltered. These points appear gray (3H, K, M), with the intensity depending on how much the polarizer has been rotated away from the zero position.

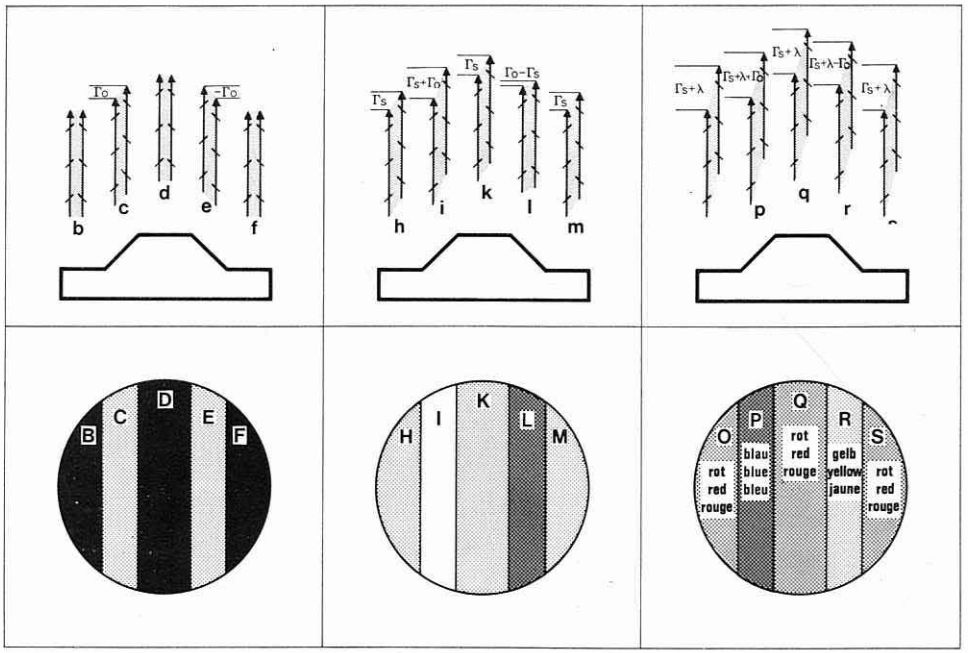
Points where the thickness is changing, however, cause an addition (3i) or subtraction (3l) of the phase differences, and the points appear paler (3l) or darker (3L) respectively. This gives the impression that the specimen has been illuminated from the top left (Fig. 3).

An additional birefringent plate can be inserted into the condenser to supply color contrast. The lambda compensator produces the interference color red of the first order in polarized light. Added or subtracted phase differences give blue or yellow respectively (3P, R).

Fig. 3 Creation of a pseudo-relief image

Upper row: section through the specimen with phase differences Γ_O in the specimen and Γ_S set in the condenser.

Lower row: Brightness distribution in the image

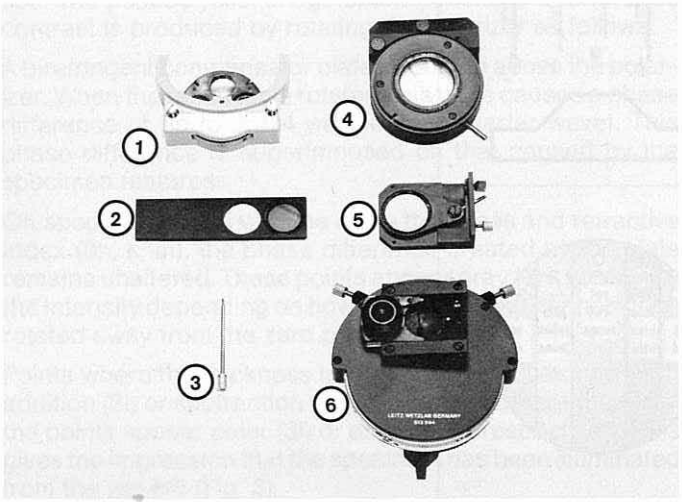


2. Mounting

The components shown in figure 4 are necessary for interference contrast T. If the set is supplied complete from the factory, the Wollaston prisms are already mounted and adjusted. The steps marked * below are thus not necessary.

Fig. 4 Interference contrast T components

1 Objective prism turret
2 Analyzer
3 Centering key for Wollaston prisms and phase rings
4 Polarizer
5 Lambda compensator
6 Universal condenser with turret plate



2.1 ICT analyzer

Remove the dust protection slide from the filter slot (1.1). Insert the analyzer (4.2) into the slot to the ↕ position.

2.2 Objective nosepiece

Loosen the nosepiece clamp screw on the side of the microscope, and pull the nosepiece out to the front. Fit the nosepiece with the ICT turret plate as follows*:
Unscrew the cover and replace with the objective prism turret (4.1). Screw in the objectives.

Important: Use PL FLUOTAR objectives for ICT. Information on the suitability of other objectives for ICT can be given on request.

2.3 UKO condenser

Raise the stage to the upper stop by means of the coarse focus control. Lower the condenser mount using control (1.6), then pull out the condenser to the front. Turn the condenser clamp screw (1.7) so that the mark is to the front. Mount the Wollaston prisms in the condenser as follows*: Pull the turret (5.1) out from the condenser after loosening the screw (5.2). Remove the four screws (6.1) from the underside of the turret, and lift off the cover (6.2).

Press the Wollaston prisms against the spring pressure into the three pentagonal mounts (7.1). Make sure that they are inserted in the correct order, corresponding to that of the objectives but in the **reverse** order, as the turret is mounted in the condenser with the upper side down.

Referring to figure 7, insert the long centering key through the hole (7.2) and turn it until each prism is approximately in the center of the mount. Take care to avoid making fingermarks on the glass surfaces! Stick the appropriate plastic label (7.3) with magnification marking on the opposite edge of the turret, taking into account the fact that the turret will be inserted into the condenser upside-down.

Fig. 5 Removing the condenser turret
1 Turret
2 Fixing screw

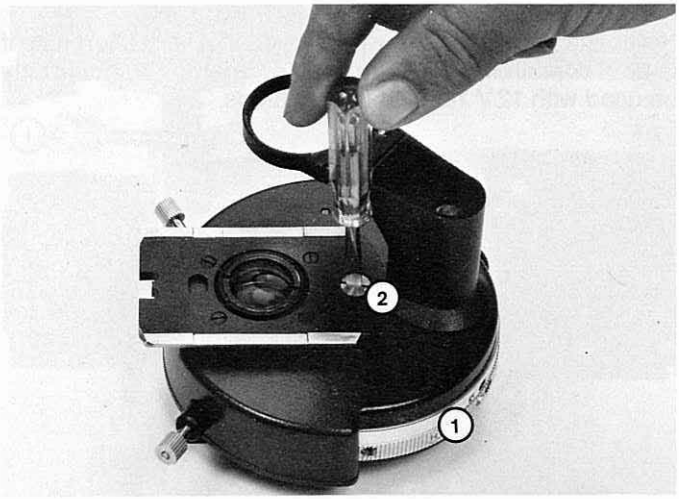
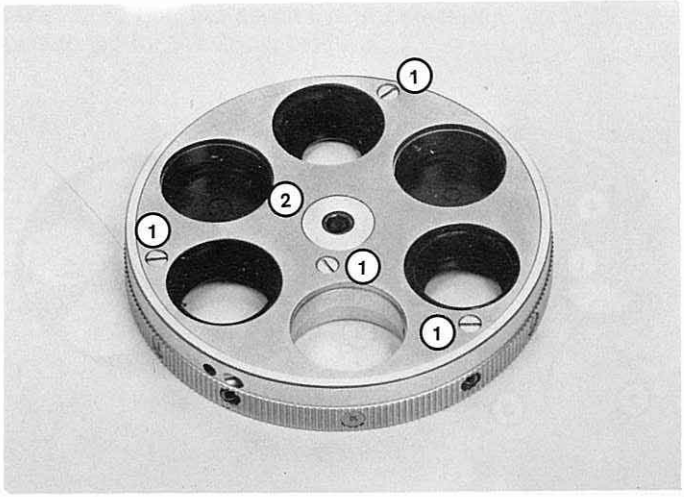
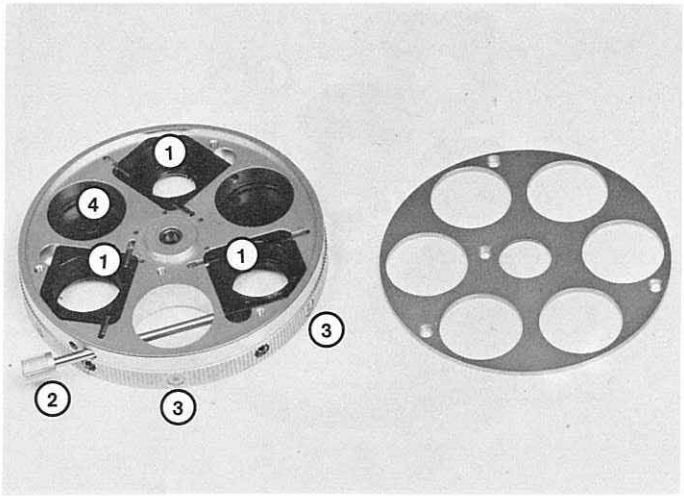


Fig. 6 Condenser turret
1 Cover fixing screw
2 Cover



Replace the cover on the turret and tighten the four screws. Insert the phase contrast ring stops as described in the microscope instruction manual. Replace the turret in the condenser and tighten the fixing screw gently.

Fig. 7 Turret after removal of cover (right)
1 Wollaston prisms
2 Wollaston prism centering key
3 Magnification labels
4 Phase contrast ring mount



If required, use an ACHR 0.90 S 1.1 top instead of a long working distance condenser top.
Insert the condenser as far as possible into its mount and tighten the clamp screw. Raise the condenser to its upper stop using the height control (1.6).

Lower the stage by means of the coarse focus control and insert the objective nosepiece into its slot as far as possible. Tighten the clamp screw. Raise the stage with the coarse focus.

2.4 Lambda compensator

Clamp the lambda compensator (4.5) on the right of the condenser mount (1.9) with the two screws.

2.5 ICT polarizer

Remove the collar from the dust glass in the microscope base. Place the polarizer (4.4) on the glass so that the pin engages in the slot. Move the clamp lever on the rear to the left (= clamped).

Note: The polarizer can be destroyed in a very short time if used in combination with gas discharge lamps. It should only be used with 12 V 100 W halogen lamps.

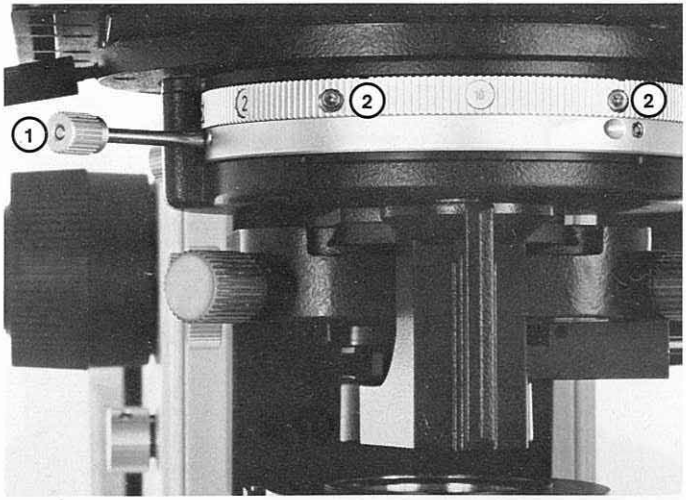
3. Adjustment*)

If the components are ordered as a set, they are already adjusted when they leave the factory. The following adjustment is thus unnecessary.

First cross the polarizers:

Push the analyzer to position (↕).
Turn the objective prism turret (1.2) to position H (brightfield).
Rotate the condenser turret to position H (1.5)
Remove the specimen.
Disengage the lambda compensator (1.9, 4.5) (lever should face the front)

Fig. 8 Centering the Wollaston prisms (condenser)
1 Centering key
2 Centering screw for phase ring



Rotate the polarizer (1.11) to the zero position, i. e. to complete extinction.
Select an ICT objective and the corresponding position of the condenser turret. Place a specimen on the stage and focus on it.

Unless using the PL FLUOTAR 10/0.30 objective (when the condenser top must be out of the light path), engage the condenser top in the light path.

Set Koehler illumination (see microscope instruction manual).

Rotate the polarizer alternately to the left and to the right. If the color changes of the specimen background are different, insert the long centering key into the left side of the turret (Fig. 8) and turn until the color change or gray tone is the same for rotation of the polarizer to the left and right.

For the 40:1 objective (first position 25/40 objective prism for 25:1 objective) the centering key should be inserted into the front hole of the ICT objective prism turret (Fig. 9) and rotated until the color change is the same for left and right rotation. Make sure that the prism in the condenser turret remains positioned for the 25:1 objective.

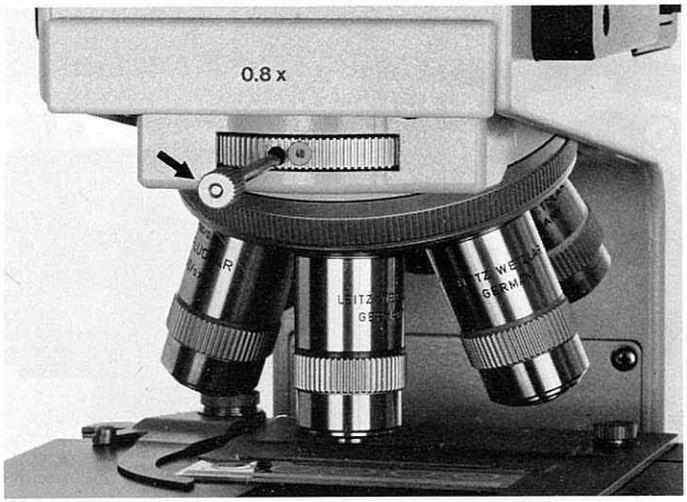
4. Operation

4.1 Interference contrast T

Select an objective. **Caution:** not all objectives are suitable for interference contrast.
Set the condenser and objective prism turrets to the corresponding position.
Slide the ICT analyzer (4.1, 1.1) to position ↕.
Set Koehler illumination.
Engage the condenser top in the light path (disengage for PL FLUOTAR 10/0.30 objective).
Adjust the polarizer (1.11) and aperture diaphragm (1.12) to provide the best contrast.
Engage the lambda compensator (1.9) if color contrast is desired.
It is recommended that specimens with strong parallel structures be rotated (rotate the stage) until the best results are obtained.

4.2 Brightfield

Pull the analyzer out to the left (clicks into place) and rotate both the turrets to position H (= brightfield).



4.3 Phase contrast

See microscope instruction manual, the objective prism turret must be in position H.

4.4 Polarized light

Set the condenser turret to position H. Disengage the lambda compensator (1.9). Slide the analyzer (1.1) to position ↕. Rotate the polarizer (1.13) until an empty field of the specimen appears at its darkest.

5. Possible causes of poor image quality

Sample preparation

a) Embedding medium, specimen slide (petri dish) or specimen (e. g. crystals, fibers) are of birefringent material. The phase difference created by birefringence affects the interference contrast. In some cases rotating the specimen can help.
b) The specimen is extremely thin or thick
c) The specimen slide or coverglass is too thick or the coverglass is missing (apart from the PL FLUOTAR 10/0.30 objective which can be used with or without a coverglass)
d) The refractive index difference between specimen and embedding medium is too small (this is often the case when specimens without coverglasses are studied using an immersion objective)
e) Inhomogeneous embedding medium

Equipment faults

a) Polarizer not in place or too far from the crossed position (analyzer in 45° position instead of ↕)
b) Polarizer damaged due to use of strong lamp or absence of heat filter in lamphousing. The polarizer can be checked by holding it up to a light source or window, whereby a damaged polarizer exhibits clearly uneven coloration.
c) Wollaston prisms wrongly mounted in the condenser. This can be tested by combining a Wollaston prism with all available ICT objectives and noting the interference effect
d) Condenser or objective Wollaston prism incorrectly aligned (see section 3)
e) Condenser top in wrong position
f) Incorrect condenser top or unsuitable objectives
g) Koehler illumination not set correctly
h) Aperture diaphragm closed too far
i) Dirty optics or polarizer
j) For specimens with parallel structures: wrong orientation (rotate stage to correct problem)
k) Objectives or condenser strained due to damage

Ernst Leitz Wetzlar GmbH

D-6330 Wetzlar, Tel. (0 64 41) 29-0, Telex 483 849 leiz d

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