Diatoms (Pleurosigma angulatum), LEITZ interference microscope for transmitted light, pair of objectives 20x/0.35, monochromatic light 546 mμ, with ORTHOMAT® microscope camera

LEITZ Interference Microscope

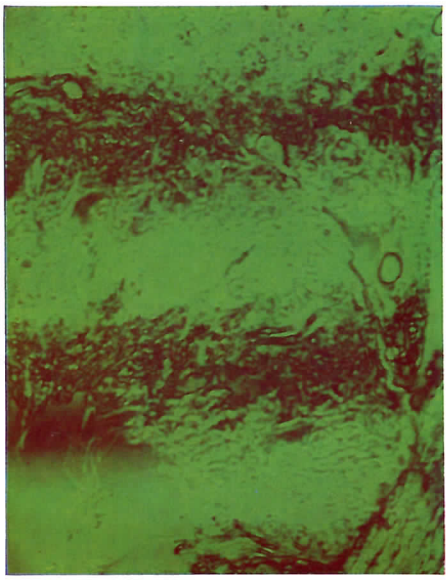
for Transmitted Light

ERNST LEITZ GMBH WETZLAR

52 - 23 / Engl.

Interference Microscope

Fig. 1 a



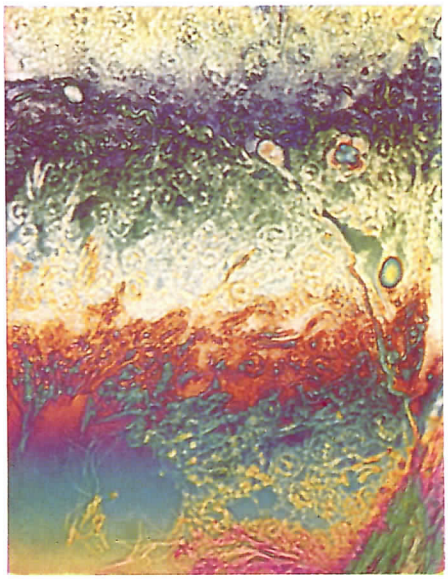


Fig. 1 b

Transverse sections through the testicle of a rat.
Broad interference fringe setting.
a) in monochromatic,
b) in white light.
The coordination of the fringe running through the object with the corresponding fringe in the empty surrounding field (bottom left) can be based on the colour of the fringe; the measurement is carried out in monochromatic light. For reasons of layout the illustrations have been rotated through 90°.

The new LEITZ interference microscope is an instrument which combines the extraordinary precision of the interferometer with the features of a large research microscope. It has the following advantages:

Double microscope with wide (62 mm) separation of object and control beams. Complete independence of object size and distribution during examination, even inside micro-cells.
No obstruction of the control beam by the object. No ghost- or double images.
Free choice of the reference point within the field of view for any measurement. Continuous variation of the fringe width and complete freedom of interference fringe orientation to suit object and nature of the problem, hence high measuring accuracy and superior adaptation to the object. In-phase field of view as a limiting case of continuous fringe variation for objects with little phase difference.
Built-in polarizing device for the orientation of anisotropic objects.

Leitz
WETZLAR

Introduction to the interference microscope

If living preparations or thin unstained biological sections are observed by the methods of classical microscopy, the images will appear almost empty and structureless. The cell and the cell nucleus have practically the same light transmittance within the visible range of the spectrum, so that it is impossible to observe differences in brightness and colour. Nevertheless, an "image" of the object is hidden in the light transmitted by the preparation. Light waves passing through the optically denser cell nucleus will be retarded in their phase compared with the waves passing through the surrounding field. However, neither the human eye nor the photographic plate are suitable to record these changes. Both register differences of intensity and wave length, i.e. variations of brightness and colour only, but ignore wave trains of different phases. If the micro-structure of an object is optically orientated, the polarizing microscope can convert the phase differences of the polarized waves into brightness differences. Differences in refractive index or thickness are thus determined in birefringent substances. The only method of differentiating the optically empty object in isotropic substances was for a long time confined to the various staining techniques. However, fixing, tanning, and staining interfere with the morphological structure of the object no matter how gentle the treatment.

The second method obviates interference with the object with its well-known disadvantages. Here, by interfering with the beam path of the microscope the phase structure of the object is translated into an amplitude (brightness) structure; the phase, which is very sensitive to changes in the optical path, is thereby used as a source of information. The only means to achieve this is that of interference. In principle, the phase contrast method (Zernicke), too, is based on this conception. The phase contrast microscope is also a special interference microscope, but its information is more qualitative, and does not easily lend itself to quantitative interpretation. However, where pure visual observation is to be replaced by measurement, modern research will take advantage of the interference microscope.

Physicians and biologists whose objects are almost invariably transpartent and appear almost structureless will in the majority of cases choose the transmitted light interference microscope, with which the product of refractive index and thickness of living or fixed isotropic or anisotropic subjects can be determined directly. Where the refractive index is known the thickness can be immediately calculated. In addition it is possible to find the dry weight of the substances composing the cell indirectly, since in the living cell this is proportional to the refractive index. For further details please consult "Observations and Measurements with the LEITZ Interference Microscope".

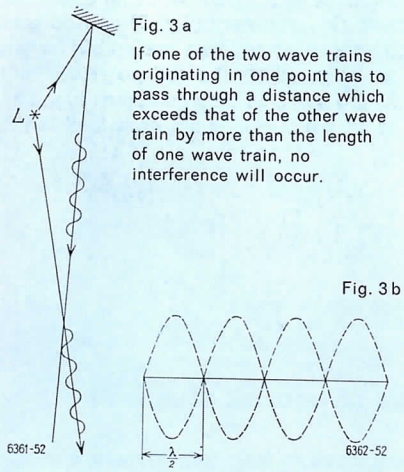


Fig. 3 a

If one of the two wave trains originating in one point has to pass through a distance which exceeds that of the other wave train by more than the length of one wave train, no interference will occur.

Fig. 3 b

Fig. 3 c

Interference of two waves of the same frequency and amplitude at various phase differences:
3 b Phase difference
Extinction takes place
3 c Phase difference
Reinforcement takes place

Introduction to interference

By interference we describe the undisturbed superposition of two or more waves. If two wave crests coincide (phase difference of the two waves 0,1 λ, 2 λ, etc.) the oscillations (amplitudes) will be added to each other; since these have a direct bearing on the intensity, this will be correspondingly stronger. Conversely, the amplitudes will be subtracted from each other if a wave crest and a wave trough coincide (phase difference of the two waves $\lambda/2$, 3 $\lambda/2$, etc.). Intermediate values of phase difference produce correspondingly reduced increases or decreases in amplitude (see Figs. 3 b and 3 c).

Interference and coherence

Light interferences will occur only if the interfering wave trains are emitted simultaneously by the same point of a light source, i.e. if they are coherent. It is therefore not possible to produce observable interferences, i.e. a system of alternate bright and dark bands, by means of two light sources. It is true that these, too, cause interference, but this changes so rapidly in time and space that it remains unnoticed. The reason for this must be sought in the nature of the origin of light. Every atom emits light only when an electron carries out a quantum "jump" into an orbit of lower energy. This emission lasts for a very short time, and the occurrence of another emission is completely indeterminate and random.

The same applies to all the other atoms contributing to the light emission, so that the phase differences between the individual atoms change extremely quickly in time. This results in interferences of correspondingly rapid variation, which are therefore impossible to observe. Their observation will become possible only if constant phase relations exist between the interfering waves; this is the case only with coherent wave trains, i.e. wave trains originating from the same elementary emission process.

If it is borne in mind that the wave trains sent out by the atoms during the time of emission have a certain limited length (coherence length) only, it will be obvious that coherence cannot be the sole factor. If, for instance, of two wave trains obtained by splitting a beam one has a path length n.s. from the split to the reunion exceeding the coherence length, no interference at all will be produced. From this follows the second condition for the production of interference phenomena. The optical path difference $I' = n_1 \cdot s_1 \cdot n_2 \cdot s_2$ must not exceed the coherence length*) (n = refractive index, s = geometrical path length).

*) Since the coherence length I is related to the number N of the observable interference fringes through the equation $I = N \lambda$, the coherence length can be determined by this formula. In the case of very homogeneous spectral lines (with $N = 2 \cdot 10^6$), a coherence length of about 1 m is found for $\lambda = 500$ m μ. This relationship is no longer valid for wider spectral ranges $\Delta \lambda$ — strictly speaking even monochromatic light occupies a spectral range of finite width — if an attempt is made to proove, for instance, that relatively few interference fringes only can be observed in the interference microscope if white light is used. However, a different approach will show that it is possible to establish a formal relationship between N and $\Delta \lambda$, so that only a finite number of interference fringes depending on $\Delta \lambda$ can be observed. A detailed discussion of this problem must be left to textbooks of physics.

However, in practice we have to deal with light sources of definite dimensions rather than point sources, consisting of a large number of luminous points. If for a certain point in space the interferences of all light points of the source have approximately the same phase difference, interference effects will be observed here. If, e.g., all phase differences range from 1.9 to 2.1 λ, there will be brightness, with values from 1.4 to 1.6 λ darkness. If, on the other hand, all possible phase differences are distributed in a point in space, our eye will register merely a mean brightness value.

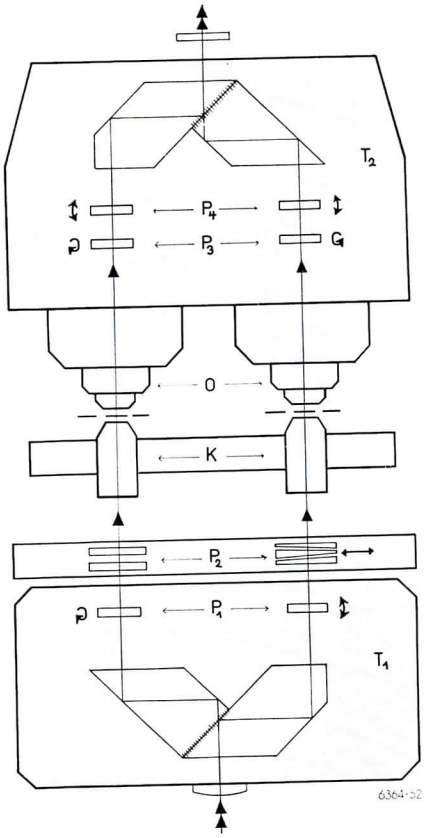


Fig. 4
Ray diagramme of the transmitted-light interference microscope.

The interference microscope is an instrument combining a microscope and an interferometer in such a way that the magnified image of the object appears to be superposed by interferences.
The new LEITZ interference microscope is a micro-MACH-ZEHNDER interferometer. The light emitted by the light source is split into two coherent beams (object and control beam) by a beam splitter, and reunited after a certain path length by means of a second beam splitter. Between the two beam splitters, the object and control beams pass through two identical microscopes (Fig. 4). Special prisms of high optical quality and long base are used as beam splitters; at a base length of 62mm the two beams are widely separated. The user therefore enjoys all the advantages of a **double microscope:**

Size and distribution of the objects to be investigated are practically unlimited.
In this design the control beam is unobstructed.
Ghost- and double images which, e.g. in shearing interferometers, disturb the interference with possibly impaired measuring accuracy cannot occur.

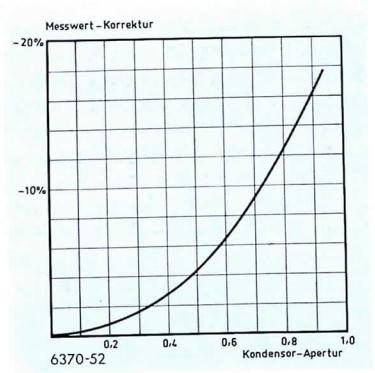


Fig. 5
Dependence of the measuring accuracy on the condenser aperture
(after Ingelstam and Johansson, 1958).

The condenser aperture is 0.5. Larger apertures reduce the contrast of the interferences, smaller apertures reduce the lateral resolving power. The condenser aperture of the LEITZ interference microscope represents a sensible compromise — in agreement with experiments on the effect of the condenser aperture on the measuring accuracy (Fig. 5) carried out by Ingelstam and Johansson. They have found a small condenser aperture preferable for exact phase measurements, since here no corrections are required for the oblique rays. For the image formation objectives of N.A.s. as high as 1.36 are used, as they guarantee high image contrast and good lateral resolution. The various structural elements are described in detail in the following paragraph.

Design and function of the various structural elements

Stand

The extreme demands on the stand for rigidity and freedom from torsion make a compact design of the interference microscope imperative. A base plate forming an integral part of the microscope contains the controls for the electric lamps as well as the setting buttons for the field and aperture diaphragms. The bilateral, coaxially mounted drive buttons for the coarse and fine movements of the microscope stage are arranged on the stand. The stage itself has no mechanical connection with the interferometer part.

Microscope stage

The microscope stage consists of a rotating stage with mechanical movement for the preparation to be measured, and of a simple stage for the control specimen. Since the optical path length must be compensated with extreme accuracy in the interference microscope, it is necessary at the outset to restrict the differences between the object- and the control beam. Hence, the optical path differences of microscope slide, cover glass, etc. should, if possible, approximate zero. In the simplest case, the path in glass can be compensated by the introduction of a so-called empty specimen. However, the basic equipment provides a rotating wedge for the purpose of compensation with dry objectives. In consists of a wedge-shaped, circular glass disc, which can be rotated eccentrically to the beam path. When oil immersion objectives are used, the height of the column of oil, too must be compensated, and instead of the rotating wedge a control object slide is introduced. The top of the stage on the control side is vertically adjustable, so that the height of the oil column can be accurately allowed for.

Fig. 6
Interchange of the objectives
in the interference microscope.

Lower and upper prism housing

The interferometer part proper consists of the lower and the upper prism housing, rigidly connected by two steel columns (Fig. 6), and the optical system. The lower prism housing contains the beam splitting prism, which splits the light coming from the light source into two part-wave-trains, and two plate compensators. The path differences of the two part-ray-pencils can be compensated with one of these compensators. The beams are centred if the images of the field of view diaphragms of the measuring and of the control beam coincide. If the beams are out of centre these images are made coincident by means of the condenser centring mechanism (or with the objective centring mechanism, see below). The more accurate the superposition of the two diaphragm images, the greater the contrast between the interference bands and the surrounding field. The final adjustment of the contrast is carried out with the aid of an additional rotating plate compensator, which constitutes an extremely sensitive means of centration. By the tilting of plate compensators in the upper prism housing the aperture diaphragm images can be adjusted in the same way in order to orientate the interference fringes in the field of view of the microscope or to vary their widht (for further details please consult LEITZ Mitteilungen II/3).

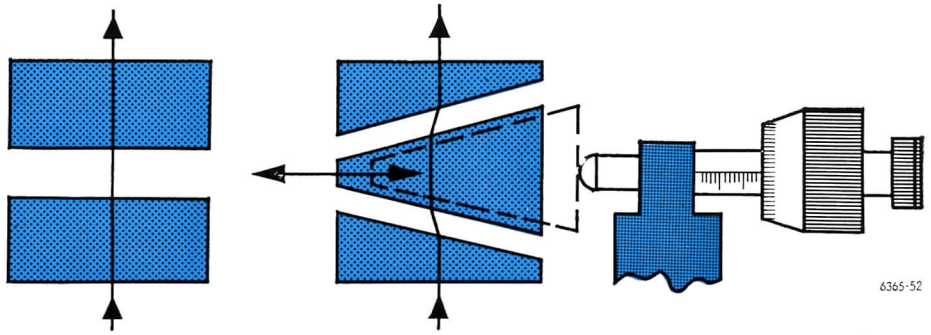


Fig. 7
Wedge compensator.
On the right in the control beam the movable glass wedge. On the left in the measuring beam plane-parallel plates for the compensation of the differences in the optical path through glass.

The lower prism housing also contains a wedge compensator for the determination of phase differences during visual measurements (Fig. 7). The amount of the displacement is indicated on a graduated measuring drum with great accuracy of about 1/90 wave length. Even higher accuracy can be obtained with the aid of microdensitometry, where an accuracy of 1/200 λ can be reached under favourable conditions.

The upper prism housing contains two pairs of plate compensators. In practice the two lower compensators are used to tilt the interference fringes, while the upper pair of plates permits the continuous variation of the interference band width up to the extreme case of infinity band width, when the interference fringe will occupy the entire field of view of the microscope. Such a setting is also called interference contrast.

After the two part beams have been reunited in the upper prism block the light enters the photo-tube, which can be equipped either with the ORTHOMAT® microscope camera or another photo-micrographic camera.

Optical equipment

As a double microscope the LEITZ interference microscope has an objective and a condenser each for the two coherent beams. The condensers of which the right-hand one can be centred, are rigidly connected with the microscope stage. In order to meet the condition of optically identical path lengths accurately the objectives are mounted in pairs on a common slide. One of them is equipped with a centring and focusing mechanism. Four pairs of objectives are available:
Achromatic pair of objectives 20x/0.35/∞/0
Fluorite pair of objectives Fl 50x/0.85/∞/0.17 for preparations under 0.17mm cover glasses
Fluorite pair of objectives Fl 50x/0.85/∞/0 for preparations without cover glass
Fluorite pair of objectives Fl Oil 100x/1.36/∞/0.

Illuminating device

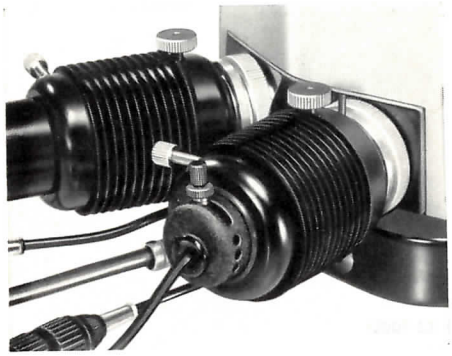


Fig. 8
Illuminating device.
On the left mercury lamp,
on the right low-voltage lamp.

Two light sources are available for the interference microscope, both to be attached to the back of the stand (Fig. 8).
1) 6 v 30 W low-voltage lamp for white light
2) Q 100 mercury burner (D. C.) for monochromatic light.

Built-in filters are used for the filtering-out of the green (546 m μ) and the blue (436 m μ) lines of the mercury light; they are operated from the base plate, where the brightness of the low voltage lamp, too, is controlled. If necessary, additional filters can be inserted between lamp housing and stand. With thick objects and large fringe displacement it is sometimes difficult to identify the corresponding interference fringes during the measurement. In this case the monochromatic light of the mercury lamp can be superposed by the polychromatic light of the low-voltage lamp (mixed light). After the zero-order fringes have been determined the displaced bands can be easily coordinated.

Polarizing equipment

In birefringent substances any phase differences depend on the direction of the light oscillations. Hence, the polarizing outfit built into the interference microscope can be used for measurements of anisotropic objects. The polarizer is mounted on a slide and fitted to the lower part of the microscope; the rotating analyser is situated in the tube.

Photomicrographic attachments

Fig. 9 Interference microscope with NORMKAM.

Fig. 10 Interference microscope with LEICA.

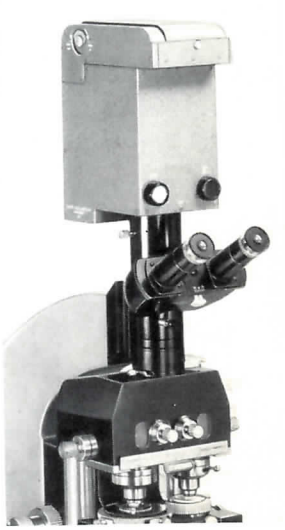


Fig. 11 Interference microscope with ORTHOMAT.

Photomicrographic practice frequently calls for the instant recording of phenomena as they occur. However, in the interference microscope the photographic record of the interference image, called interferogramme, represents more than mere documentary evidence. The measurement or densitometry of the fringes in the interferogramme is considerably more precise than direct measurement in the microscope. If rapid changes occur in the specimen during the study of dynamic events, direct measurement is in any case impossible. Here, the combination of our interference microscope with the fully automatic ORTHOMAT microscope camera has proved of excellent value in the photomicrography of interference pictures. Pressing a single button sets in motion all the functions which in the past were the responsibility of the microscopist. Exposure is automatic, so that faulty exposures are practically impossible. Further details will be found in our List 54—18.
Our NORMKAM plate camera is designed for the 6 x 9 cm (1—20 / 6—20) size; the LEICA is suitable for serial records on 35mm film, and is used on the ARISTOPHOT. Our List 54—8 contains a detailed description.

Fig. 12 a

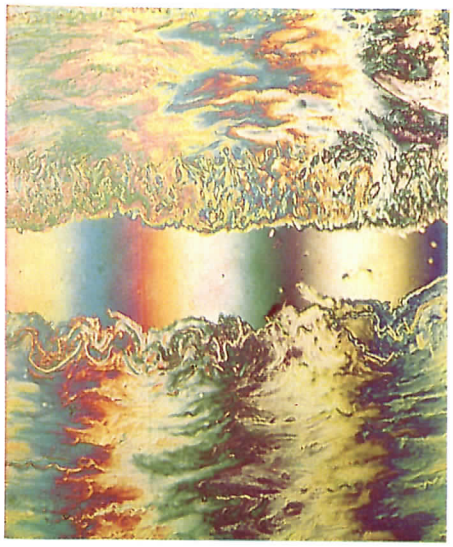


Fig. 12 b

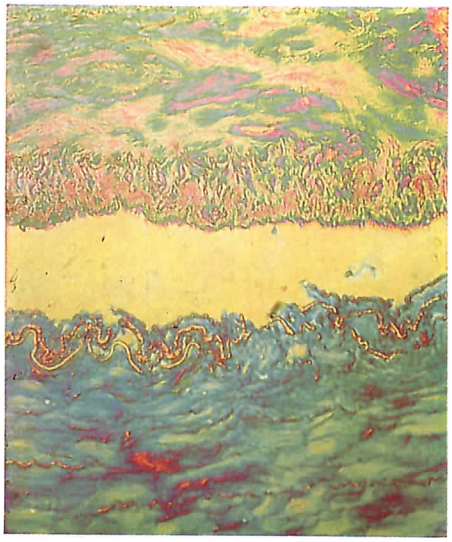
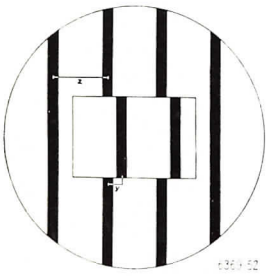


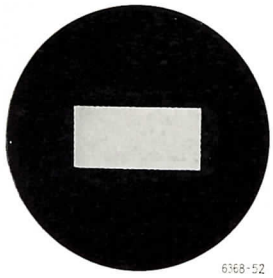
Fig. 12 c

Interference picture (white light) of the walls (interna and media) of two physiologically different arteries of a bull (Preparation by Prof. G. Conti, Institut d'Histologie, University of Fribourg, Switzerland).

Combined picture making use of the double-microscope principle: The top preparation lies on the control side; the bottom preparation on the measuring side of the microscope stage. a) narrow-fringe field, b) broad interference fringe, c) homogeneous field (interference contrast). Phase difference, bottom preparation between $\lambda/4$ and $\lambda/2$, top preparation approx 1 λ.

Fig. 13
Displacement of interference fringe by a homogenous object.





Phase object in homogenous field. Fringe width infinite.

In white light, the bright, empty field of the interference microscope contains a number of parallel, coloured, evenly spaced interference fringes; in monochromatic light a system of parallel, equidistant dark fringes appears on the bright background whose colour corresponds with that of the light. It is possible to adapt this interference system optimally to the specific tasks of measurement and observation in the LEITZ interference microscope.

It is possible

1. to vary the number and width of the fringes continuously up to infinite fringe width (homogeneous field; increase of measuring accuracy)
2. to align the direction of the interference fringes in the field as required
3. to change the position of the fringes or the colour and brightness of the homogeneous field by means of a wedge compensator which can be moved in the control beam.

An object in the measuring beam of the interference microscope displaces the phase of the wave passing through it according to its thickness and refractive index. This phase displacement is visible in the interference fringe field as a displacement of the interference fringes and in the homogeneous field as a change in the colour or in the brightness at the site of the image. Analogously to the phase contrast the homogeneous field is also called interference contrast. In interference contrast, the reproduction of object structures is similar to that by phase contrast, but without the halo which sometimes occurs with the latter method.

A qualitative observation of the object in the interference band field supplies evidence of its shape or surface structure. Typical examples are red blood corpuscles or objects with stepped surfaces (mica cleavage).

Interference contrast makes it possible to demonstrate certain morphological details such as cell nucleus, nucleolus, chromosomes, cell inclusions and cytoplasmic constituents chromatically or with brightness contrast. Dynamic events occurring in the cell, involving changes in the density of the living substance, such as growth, cell division, etc., or crystallization processes in non-biological preparations can be visually followed by observing changes in colour or contrast and recorded by serial photomicrographs.

However, the most important task of interference microscopy is not the qualitative examination, but the quantitative determination of states, and changes in the state, of substances. Since in the interference fringe field the distance between two neighbouring dark interference fringes corresponds to one light wave length (one phase difference), the phase difference caused by the object and visible in the interference microscope as fringe displacement or contrast change can be accurately determined with the aid of the calibrated wedge compensator. As a result, either the thickness or the refractive index of the object can be found, since the phase difference depends on the object thickness and the difference between the refractive index of the object and that of the surrounding field. When the mass of a fixed or a living preparation is determined under the most varied experimental conditions the value of the phase difference serves as a basis for the calculation of the dry weight of the protoplasmic cell constituents and the "wet" weight of the cell. These histochemical determinations can be carried out with the greatest precision by means of the interference microscope, without the need for upsetting the vital processes in the cell.

Fig. 14 a

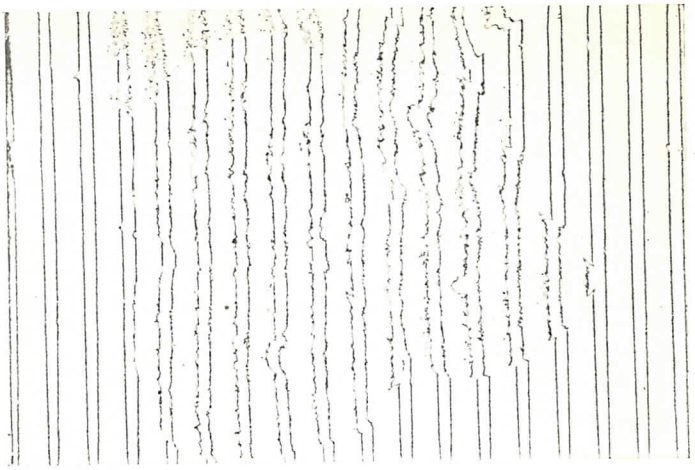


Fig. 14 b

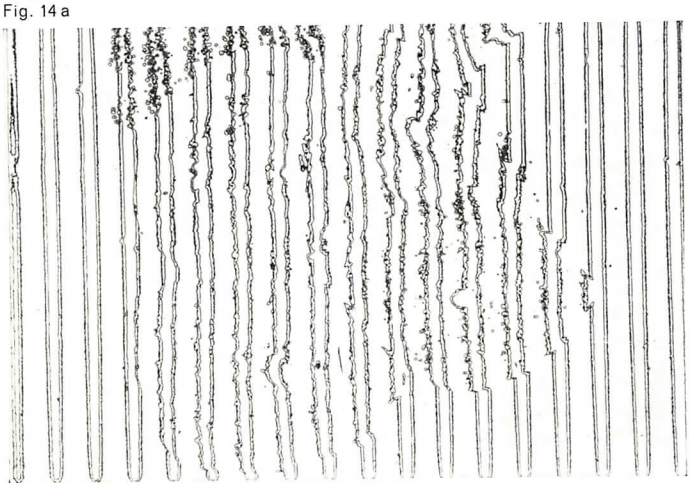


Fig. 14 c

Fig. 14
Epithelial cells of the human buccal mucosa
(pair of objectives 50x/0.85/0.17; ORTHOMAT microscope camera).
a) normal photomicrograph of the interference picture
b) 1st order equidensites, and
c) 2nd order equidensites, produced from the negative of the normal interference picture a).

Our production programme includes:

Microscopes for every purpose
for general investigations
Metallography, mineralogy, ore microscopy
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Phase contrast equipment
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Monochromators
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Surface testing instruments
LEICA® miniature camera
with accessories for scientific
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Miniature projectors, school projectors
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8 mm LEICINA® cine camera
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