Romuald Pawluczyk *

Holographic Interference Microscope

An experimental model of a holographic microinterferometer was developed and constructed. The optical system used in the present paper allows to continuousky change the intensity ratio of the object and reference beam in a very wide range, which greatly facilitates the recording of high quality holograms, as well as obtaining high contrast interference images.

Depending on the type of the hologram employed, the device can be regarded as a normal two-beam interferometer, or the laterally or angularly shearing interferometer. In the case of changing objects, the interference in the real time, or the double exposure method employing studies can be performed. The microinterferometer can also be used for interferometric comparison of the object investigated with the reference object.

Besides, the optical arrangement allows for employing the device as a normal microscope, or a polarizing microscope with laser light illumination.

I. Introduction

The early works of Denis Gabor [1-3] comprising the basis of holography were aimed at improving the resolution of the electron microscope. Next, there was also a trend to employ the principles of holography to the X-ray microscopy [4]. As there were no coherent light sources available, experimental results were not very encouraging, and consequently no researches in the field of optical holography were carried out in that period. Such a situation existed until the advent of lasers and the design of holographic systems with a side reference beam [5-7] which enabled to imagine great possibilities offered by holography in visible light. Among them the most important are microscopy and microinterferometry. A number of papers [8-18] devoted to the above mentioned problems were published. We have checked the usefulness of one holographic microscopic device, namely the mixed lens-holographic system. We have chosen this system, because it offers the possibility of obtaining a higher resolution than lensless holographic systems. Further, we decided to use one of the two possible arrangements — convergent object beam [13, 15], because it was possible to employ standard microscopic elements, and moreover no changes between recording and reconstruction were needed in such a system. This arrangement could also be adapted for interferometric studies.

II. Description of the microscope optical system

Figure 1 shows the optical scheme of the constructed device. The device, generally, comprises the optical parts of a standard microscope, namely condenser *K*, objective *Ob*, and eyepiece *Ok*. These elements together with a number of auxiliary elements comprise the optical system for recording and reconstruction of holograms, and for holographic interference studies.

As is shown in Figure 1, the laser light beam *WL* is filtered and expanded by means of a collimating lens *KL*, characterized by magnification of 6.25x.

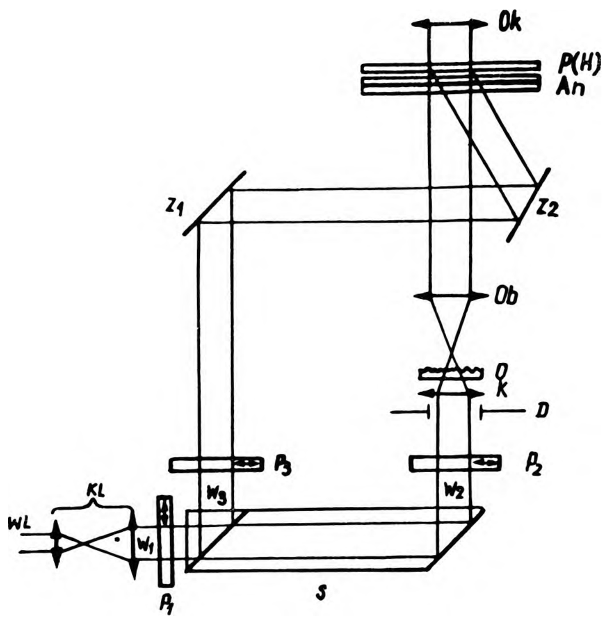


Fig. 1. The optical system of the holographic microscope: *WL* — laser beam, *KL* — collimating lens, P_1, P_2, P_3 — halfwave plates, *S* — polarizing interference beam-splitter, Z_1, Z_2 — auxiliary mirrors, W_2, W_3 — object and reference beam, respectively, *D* — iris diaphragm, *K* — condenser, *O* — object, *Ob* — objective, *An* — analysing polarizer, $P(H)$ — high resolution photographic plate or the hologram, *Ok* — eyepiece

After passing the halfwave plate P_1, the beam W_1 is divided by a polarizing-interference beam-splitter *S* into two beams W_2, W_3 polarized in two mutually perpendicular directions. When the beam W_1 is polarized, the intensity ratio of those two beams

*) Physical Optics Department Central Optical Laboratory, Warszawa, ul. Kamionkowska 18, Poland.

depends on the polarization direction of the beam W_1 which can be changed by rotation of a halfwave plate P_1. Consequently the intensity ratio can be changed. The beam-splitter S divides beam W_1 into two beams changing their direction to entering further parts of the holographic system.

A halfwave plate P_2 placed in beam W_2 is used for fixing the polarization of this beam in the desired direction when the system is used for the polarization studies. The light passing through the halfwave plate falls on the iris diaphragm D, and the condenser K, which focuses it on the investigated object O. The light diffracted by the object is transformed by the microscope optical system comprising an objective Ob and an eyepiece Ok. The described part of the system can be used as a normal microscope with the laser illumination.

If the light illuminating object O is linearly polarized, then the direction of this polarization can be changed by rotation of the halfwave plate P_2, on the other hand if an analizing polarizer An is placed after the objective, then the described system can be used as a polarizing microscope with the monochromatic illumination.

Holographic recording in the above described microscope system is obtained by means of the known side reference beam arrangement. As a reference beam, beam W_3 is used, which after reflecting on auxiliary mirrors Z_1 and Z_2 combines with beam W_2. The two beams are coherent, so if additionally the direction of polarization of beam W_3 is adjusted with the direction of polarization of beam W_2 by means of the halfwave plate P_3 in the area of overlapping of the two beams, the interference occurs. The interference pattern is recorded on the high resolution photographic plate $P(H)$. In the case of polarization studies, or if the presence of an additional polarizer in the system is not spoiling the observed pattern, the direction of polarization of both beams can be additionally adjusted by such a polarizer. For avoiding unnecessary losses in the last case a polarizing prism should be used.

The adapting of the microscope for a cooperation with the holographic system with a convergent object beam allows employing several methods of studies. Different possibilities of using the above described system will be outlined below.

III. Direct object observations and polarization studies

As already mentioned, the described microscope can be used as a normal microscope with laser illumination in transmitted light. However, it was observed, that with coherent light illumination the resolution of the microscope becomes lower than in the case of light sources normally used. Moreover, because of this well-known detrimental diffraction phenomena, the observed image is strongly perturbed. These detrimental phenomena seriously limit the possibilities of employing the described device as a normal microscope with laser illumination. Figure 2 shows the specimen of bone tissue as seen with coherent laser illumination.

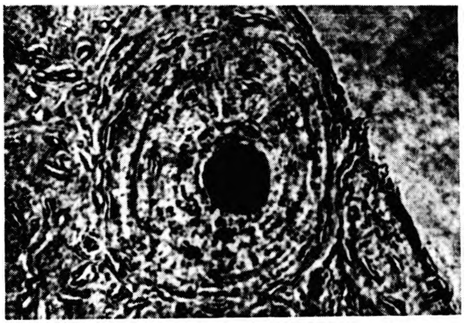
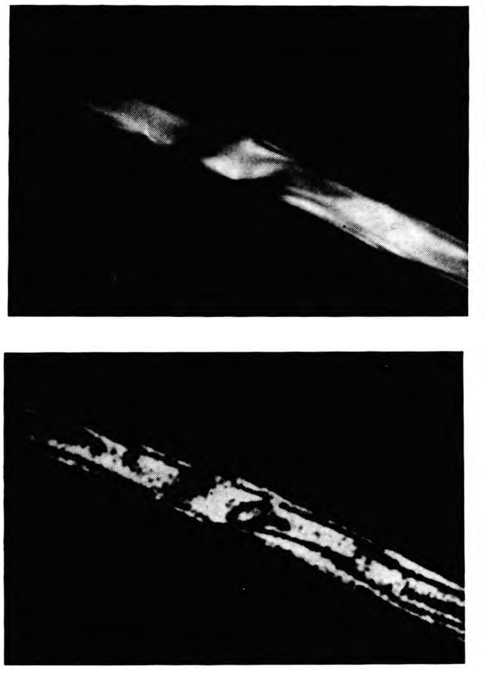


Fig. 2. Cross-section of the bone tissue as seen in coherent laser light



a)

b)

Fig. 3. Conoscopic picture of a birefringent filament: a — in white light, b — in monochromatic laser light

The system promises greater possibilities when employed as a polarizing microscope with the laser illumination. The polarizing microscope comprises an analizing polarizer An. Such a microscope can be used for studies of anizotropic media characterized by large dispersion of the refraction coefficient and large birefringence. When observed in white light such objects present polarization images of poor contrast and are distorted, which makes their proper interpretation very difficult. Applying of the monochromatic laser light in such a case allows us to obtain more distinct and demonstrative pictures (Fig. 3 a, b).

IV. Recording and reconstruction of holograms

For the sake of simplicity we can assume that the recording of a hologram is a photographic recording of interference pattern produced by two combined beams: object beam and reference beam. In our system, the said beams are W_2 and W_3 respectively (Fig. 1). As shown in Figure 1, both beams overlap in the area between the objective *Ob* and the eyepiece *Ok*. For recording the hologram it is necessary that the beams should interfere, so they should be coherent and have the same direction of polarization. Moreover, for obtaining a high quality hologram it is necessary for the two beams to maintain the optimum ratio of the intensity. The device is designed for studies of transparent objects with different optical characteristics, so the intensity ratio should be optimized for every specimen.

As said above, for optimization of the intensity ratio of the beams W_2 and W_3, the halfwave plate P_1, and polarization-interference beam-splitter S are used. At the output of this part of the optical system two light beams polarized in mutually perpendicular directions are obtained. The direction of the object beam polarization can be changed by means of the halfwave plate P_1. For adjusting the polarization of the object beam to that of the reference beam, the halfwave plate P_3 is used. For the polarization studies the polarization direction of both beams may be additionally adjusted by means of a polarizer An. In this way the halfwave plates P_1, P_2 and P_3 (and additionally the polarizer A_n in the case of polarization studies) allow obtaining of optimal conditions for hologram recording. The interference pattern generated in the area where both the light beams combine with each other is recorded on photographic plates Agfa-Gevaert 10E70, 10E75, 8E70 and 8E75 and processed according to producers specification.

For reconstruction the hologram is placed in the same device in the position where it was recorded. The reference beam is now used as a reconstructing beam. The reconstructed image, as well as the real image can be observed by means of the eyepiece *Ok*. If one employs the same system for recording and reconstruction, the two features are obtained, which are of essential importance in the case of using the device for holographical-interferometric studies:

a) the holographic process in which the reconstructing beam is identical with the reference beam is aberration free,

b) simultaneous observation of the image reconstructed from the hologram and of the real image is possible.

The first one of above mentioned features is demonstrated in Fig. 4, showing the microphotograph of the same object as in Fig. 2, recorded and reconstructed from a hologram.

If a microscopic tube of changeable length is used in the reconstruction, the defocussing of the optical

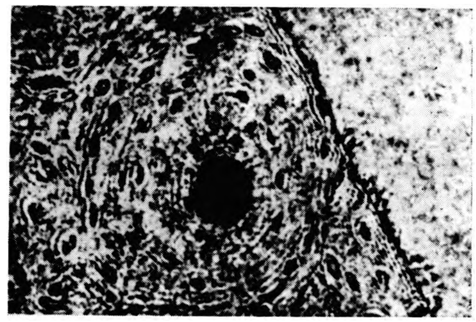


Fig. 4. Image of the same specimen as in Fig. 2 reconstructed from the hologram recorded in the described device

system and in this way the observation of different parts of the object, placed at different distances from the objective, is possible. However, the microscopic objectives are characterized by a small depth of focus and, moreover the longitudinal magnification changes with a square of lateral magnification, so consequently the possibilities of defocusing are relatively small (about 0.01 mm for 40× objective and 1 mm for 5× objective).

Thanks to accurate imaging and possibilities of observation of different parts of the object, lying at different depths, the described arrangement of the microscopeholographic system offers greater possibilities and in some cases replaces usual photographing. The superiority of holography over traditional photography can be easily seen in case of specimens, for which a full study in every respect is not possible because of the short life-time of the specimen. Thanks to the great accuracy of imaging the hologram seemingly prolongs this time enabling the performance of the necessary measurements in the time most convenient for the observer.

V. Holographic-interference studies

In a general case the interferometric studies can be simply treated as a comparing of two coherent light waves. The comparison is carried out by way of combining the two waves and causing their interference. The obtained interference patterns enable to find the differences between these waves. The basic problem arising in connection with all interferometric studies lies in generating the reference wave and combining it with the object wave. For this purpose very expensive and complicated devices are constructed, for example comprising special mirrors etc. Holography enables the recording of the reference wave on a hologram in place of constructing the device for generating reference waves whenever necessary. The recorded waves can be reconstructed and compared each time with an object wave. As opposed to the normal interferometers, in which the wave under study can be compared only with that of exactly defined shape, holographic interferometers enable us to compare any waves; it is only necessary for one of them to be recorded on a hologram.

The described device can also be used as a holographic microinterferometer, by means of which it is possible to realize several types of interference. In the most simple way the two-beam interference

a)

b)

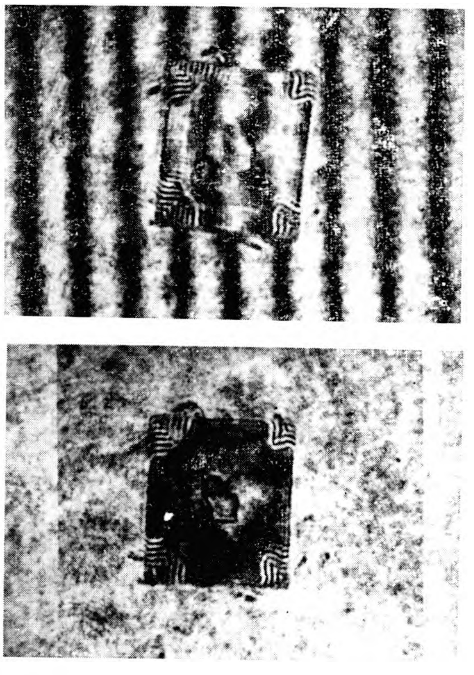


Fig. 5. Interference images obtained as a result of interference of wave W_2 (Fig. 1) with wave reconstructed from a hologram, a — interference in fringe field obtained by transversal movement of the condenser, b — uniform field — hologram and condenser were not moved since the time of hologram recording

in both the uniform and fringe fields can be realized. For this purpose an image of the light wave passing through the optical system of a microscope with a clear object glass on the stage is recorded on the hologram. After processing, the hologram is placed in the position where it was recorded. In the field of view of the eyepiece the fringe pattern will be seen, in which the fringe density changes with the transversal motion of the hologram or the condenser (Fig. 5a). In the case of a certain position of the hologram the field of uniform brightness will be seen in the eyepiece (Fig. 5b), which corresponds to the interference in the uniform field in normal interferometers. The fringe position or the brightness of the uniform field of holographic interferometer depends on the phase difference between the object and the reference beams. This difference can be varied by a transversal motion of the hologram or the microscopic condenser, which corresponds to a small inclination of one mirror in the usual interferometer.

a)

b)

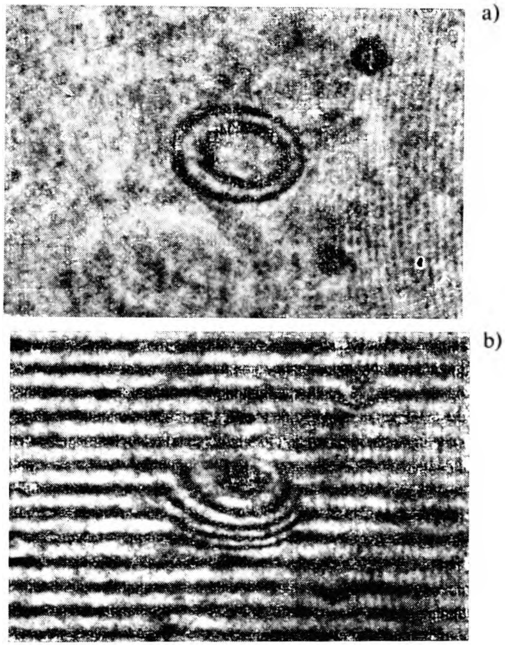


Fig. 6. Interference images of a drop of balsam: a — in uniform field, b — in fringe field

In the case when since the time of recording of the hologram, the specimen and the optical system remained unchanged, then after a geometrically exact placing of the hologram in the position where it was recorded, a maximally darkened uniform interference field is obtained. If now the clear object glass is replaced by an identical object glass carying a specimen on it, the introduced changes of the optical path in the object beam will cause changes in the image — interference fringes will occur, which represent lines of equal optical path (Fig. 6a). Transversal movement of the condenser or the hologram will cause a mutual inclination of interfering waves, which corresponds to the measurements in the fringe interference field (Fig. 6b).

In the case of fast changing objects, the occurring changes can cause the above mentioned method not to be applicable, because each measurement will correspond to another state of the object. In such a case it is convenient to record the object wave on the hologram, and afterwards (when it is needed) to compare it with the wave shaped by the optical system with a clear object glass on the stage. Both waves are then reversed. It means that the wave reconstructed from the hologram is now the investigated wave, and the wave shaped by the optical system — the reference wave.

The hologram carrying the image of a microobject also enables us to carry out investigations of the changes taking place in the object. For this purpose image of the specimen reconstructed from the hologram is compared with the real image of the object. If during the time, which had passed after the recording of the hologram the object remained unchanged, then after placing the hologram in the position where it was recorded, the observed image will be uniform. Otherwise in areas where the change of the optical path is equal to an odd number or half-waves of the used radiation, bright interference fringes will occur. The phase changes occurring in the investigated specimen can be found and measured in this way.

By means of a hologram carrying the image of the object shearing interferometry may be applied [19]. In a general case this method can be understood as an interferometric comparison of two displaced images of the same object. Depending on the way of the displacing (shearing) employed, the following types of shearing interferometry can be distinguished:

a) lateral shearing (both images are split in the direction perpendicular to the optical axis of the system);

b) angular shearing (both images are mutually rotated by a small angle);

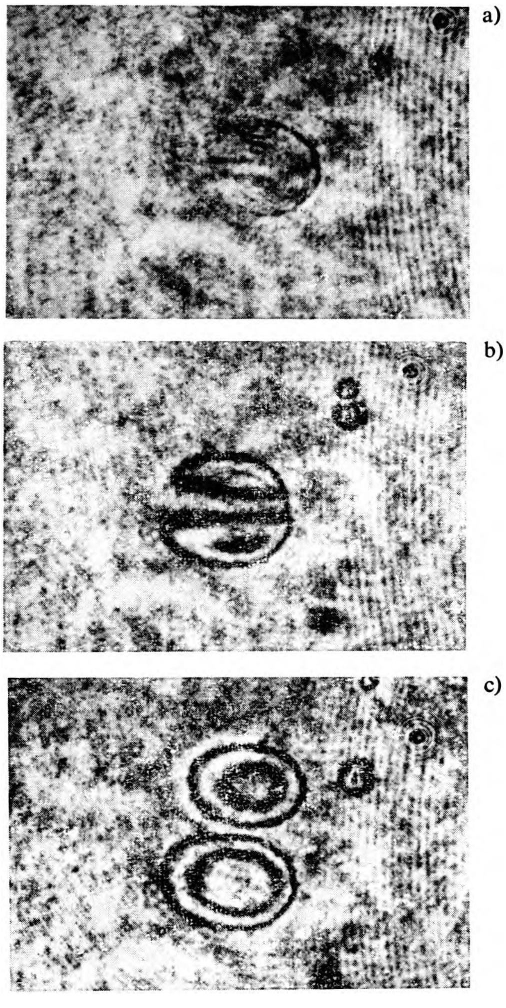
c) radial shearing (the dimensions of one image are somewhat larger than the other);

d) longitudinal shearing (one image is generated a little further from the observer than the other).

Additionally, if the wave fronts corresponding to both the objects are parallel, then interference in the uniform field is obtained, and if they are inclined, a fringe pattern occurs. If the magnitude of shearing is comparable with the resolution limit of the used optical system, then the term "differential interferometry" is used. In this case, the generated interference image is related to the gradient of optical path changes in the investigated object.

The holographic microscope allows us to obtain in a simple way the interference with lateral and angular shearing of any magnitude, in a uniform as well as fringe field. The direction and density of the obtained fringes are independent of the direction and magnitude of shearing. Figs 8 a-d show examples of different types of shearing.

As already mentioned, in order to obtain the shearing interferometry of different types, the image of the studied object is recorded on a hologram. After being processed the hologram is placed in exactly the same position where it was recorded. In this case an unsheared image of the object in



a)

b)

c)

Fig. 7. Shearing interference images of a drop of balsam in uniform field: a — differential shearing, b — small shearing, c — large shearing

uniform interference field is obtained (Fig. 7a). After moving the specimen on a microscopic stage with X-Y motion, suitably sheared interference image in a uniform field is obtained (Fig. 7 a-c). If additionally the condenser is slightly decentered, interference fringes will occur in the field of view (Fig. 8 a-d). The density as well as the direction of the fringes depend on the direction and magnitude of the condenser decentration. Lateral shearing of the image can also be obtained by means of the transverse movement of the hologram. However, in this case the interference fringes will always occur and their direction and density are related to the direction and magnitude of the image shearing.

Angular shearing in the uniform field (Fig. 9a) is obtained when the hologram is placed exactly in

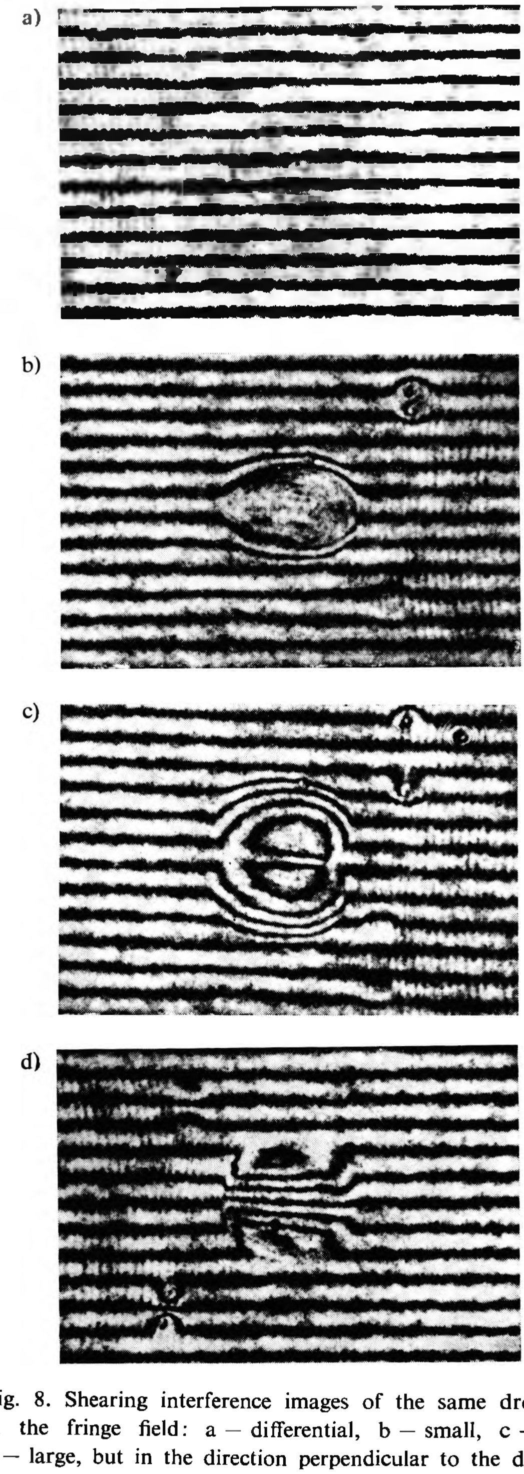


Fig. 8. Shearing interference images of the same drop, but in the fringe field: a — differential, b — small, c — large, d — large, but in the direction perpendicular to the direction shown in Fig. 7

the position where it was recorded and the investigated specimen is rotated on a rotatable microscopic stage around the optical axis of the microscope. Again, in this case decentration of the condenser causes the interference fringes to appear (Fig. 9b).

Thanks to a great freedom of changing the position of the hologram, the specimen and the condenser, combined shearing can also be obtained.

Naturally, it is unnecessary that the image of an investigated specimen should be recorded on a hologram. Any object can be recorded on the hologram — in particular a reference object. Replacing the reference object with the object under study, and placing the

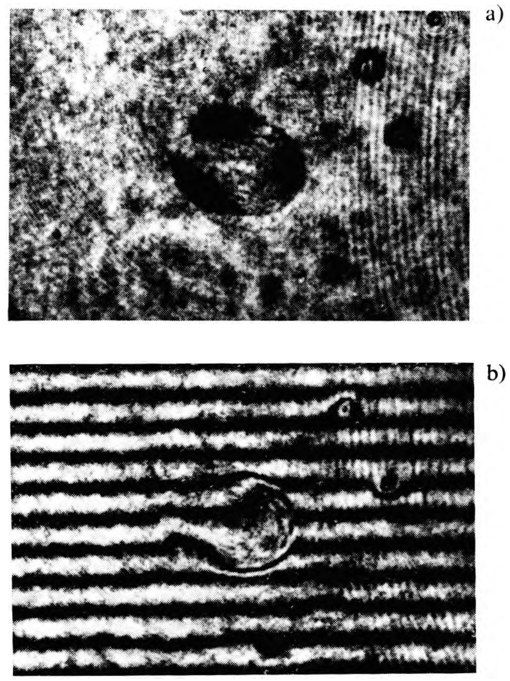


Fig. 9. The same drop as in the previous figures, but with angular shearing in uniform field: a — angular shear of 30° in fringe field

hologram in the system allows to obtain the interference of the two waves, one shaped by the reference object, and the other shaped by the investigated object. The generated interference image allows us to determine the differences between these objects, and in this way to eliminate objects differing too much from the reference object (Fig. 10). Such a device can be used for quality testing and the elimination of faulty, small, transparent industrial elements.

The described principles of the two-beam interference realization consisting in comparison of really existing object image with the reference wave, or the image of the same object, can be used in the case of changing or short living specimens, when the life-time of the studied object states is much shorter than the time of the hologram processing. Sometimes it is desirable to record the interference image together with the investigated object on the same hologram. In such cases the double exposure method is employed, in which two waves are recorded on the same hologram: the wave corresponding to the investigated object and the reference wave (in the case of shearing interferometry in place of the reference wave, the wave corresponding to another state of the object is recorded).

This method is particularly convenient for studies of optical path changes in fast changing objects. In

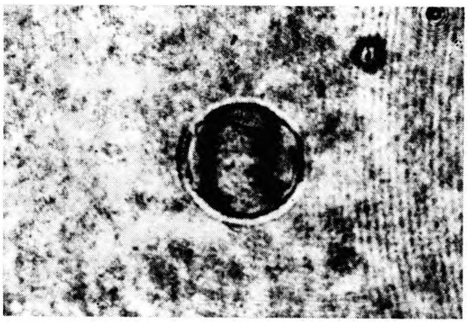


Fig. 10. Interference comparison of two different drops of balsam

this case the same photographic plate is doubly exposed to two different states of the studied object. In the reconstruction the interference image of the changes is obtained in a uniform interference field (Fig. 11). If between the two exposures the condenser

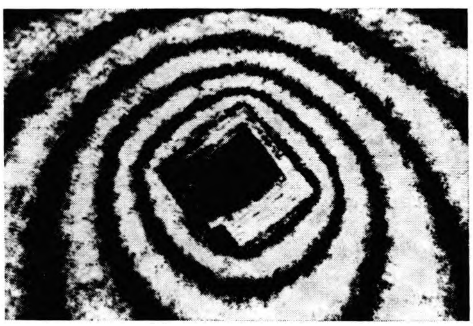


Fig. 11. Interference image of salt crystal growth obtained by the double exposure method

is slightly decentered, the fringe field will be obtained.

Interference with a plane reference wave in the uniform field is obtained by replacing the specimen before the second exposure with a clear object glass, and in the fringe field by means of an additional decentration of the condenser, or a small movement of the hologram.

Different types of shearing interferometric images are obtained by means of recording the two suitably sheared images of the same object on one hologram (transversal movement, rotation) and a suitable decentration of the condenser. The interference images obtained in the reconstruction procedure do not differ from the images obtained by interference of the wave reconstructed from the hologram and the wave shaped by the really existing object (Fig. 7-9). Simultaneous application of the polarizing elements readily existing in the microscope together with the interferometric possibilities of the system, gives rise to some further methods of investigation, which will not be outlined here.

The presented scope of experimental results points out to the possibilities of realization of various experimental techniques by means of one holographic device. Wide possibilities of its employment in different fields of science and technology seem to be possible. At the moment the wide use of the described holographic microscope is retarded by the problem of detrimental interference and diffraction phenomena caused by the high degree of coherence of the employed laser light. This is the main difficulty retarding the development of the holographic microscopic technique.

VI. Acknowledgements

The author's thanks are due to all his coleagues from the Physical Optics Department, for fruitfull discussions and remarks in the course of construction of the described device, and particularly to Mr A. Kuc for his help in recording the holograms and microphotographs. Besides I would like to express my gratitude to my colleagues from the Quantum Electronics Department of Adam Mickiewicz University in Poznań, and especially to Mr Z. Błaszczak for preparing excellent laser tubes which enabled the carrying out of this work.

Le microscope interférentiel et holographique

On a préparé et mis au point un modèle expérimental d'un microinterféromètre holografique. Le système optique appliqué rend possible les changements d'intensités du faisceau-objet par rapport au faisceau de repère d'une facon continue dans une large étendue, ce qui facilite à son tour la mise au point des hologrammes d'une très haute qualité et permet d'obtenir les images interférentielles à un haut degré de contraste.

En fonction du type d'hologramme utilisé, le système peut remplir le rôle d'un interféromètre ordinaire à deux ra yons, fonctionnant sur la lumière transitive, ou bien d'un interféromètre à dédoublement transversal et angulaire du front d'onde. Au cas des objects variables on peut faire des recherches interférométriques au temps réel, ainsi que des recherches à l'aide du procédé de double exposition. On peut également utiliser le microinterféromètre pour comparer l'objet donné au test-objet.

En plus, la facon de mise au point du dispositif rend possible son application comme microscope ordinaire ou microscope polarisant fonctionnant à la base de la lumière laser.

Интерференционно-голографический микроскоп

Разработана и изготовлена модель голографического микроинтерферометра. Применённая оптическая система даёт возможность изменять отношение интенсивности предметного пучка и пучка отнесения.

Непрерывным способом в очень широком диапазоне, что значительно облегчает изготовление голограмм высокого качества, а также позволяет получать высококонтрастные интерференционные изображения.

В зависимости от типа используемой голограммы система может выполнять роль обыкновенного двулучевого интерферометра, работающего в проходящем свете или также интерферометра с поперечным и угловым раздвоением фронта волны. В случае переменных объектов можно производить интерферометрические исследования в реальном времени, а также исследования с применением двойного метода экспозиции. Микроинтерферометр может быть использован также для интерферометрических сравнений данного объекта с объектом-образцом.

Кроме того, конструкционное решение прибора даёт возможность использовать его как обыкновенный микроскоп или поляризационный микроскоп, работающий в лазерном свете.

References

[1] Gabor D., *A New Microscope Principle*, Nature, **161**, No. 4098, p. 777-779, 1948.

[2] Gabor D., *Microscopy by Reconstructed Wave-Fronts I*, Proc. Roy. Soc. **A197**, No, 1051, p. 454-487, 1949.

[3] Gabor D., *Microscopy by Reconstructed Wave-Fronts II*, Proc. Phys. Soc. **B64**, No. 378, p. 449-469, 1951.

[4] Baez A. V., *A Study in Diffraction Microscopy with Special Reference to Y-Rays*, J. Opt. Soc. Am. **42**, No. 10, p. 756-762, 1952.

[5] Leith E. N., Upatnieks J., *Reconstructed Wavefronts and Communications Theory*, J. Opt. Soc. Am. **52**, No. 10, p. 1123-1130, 1962.

[6] Leith E. N., Upatnieks J., *Wavefront Reconstruction with Continuous Tone Objects*, J. Opt. Soc. Am. **53**, No. 11, p. 1377-1381, 1963.

[7] Leith E. N., Upatnieks J., *Wavefronts Reconstruction with Diffused Illumination and Three-Dimensional Objects*, J. Opt. Soc. Am. **54**, No. 11, p. 1245-1301, 1964.

[8] Leith E. N., Upatnieks J., *Microscopy by Wavefront Reconstruction*, J. Opt. Soc. Am. **55**, No. 5, p. 569-570, 1965.

[9] Leith E. N., Upatnieks J., Haines E. A., *Microscopy by Wavefront Reconstruction*, J. Opt. Soc. Am., **55**, No. 8, p. 981-986, 1965.

[10] Knox C., *Holographic Microscopy as a Technique for Recording Dynamic Microscope Subjects*, Science, **153**, No. 3739, p. 989-990, 1966.

[11] Ellis J. W., *Holomicrography, Transformation of Image during Reconstruction a Pasteriori*, Science, **154**, No. 3753, p. 1195-1197, 1966.

[12] Toth L., Colins S. A. *Jr*, *Reconstruction of Three-Dimensional Microscopic Sample Using Holographic Techniques*, Appl. Phys. Letters, **13**, No. 1, p. 7-9, 1968.

[13] Daszkiewicz M., Pawluczyk R., Pluta M., *A Microholographic System Enabling to Obtain Holograms with No. Pseudoscopic Real Image*, Acta Phys. Polonica, 36, No. 1 (7), p. 27-31, 1969.

[14] Gabor D., Goss W. P., *Interference Microscope with Total Wavefront Reconstruction*, J. Opt. Soc. Am., **56**, No. 7, p. 849-858, 1966.

[15] Magill P. J., Wilson A. D., *Applications of a Holographic Interference Microscope*, J. of Applied Phys. **39**, No. 10, p. 4718-4725, 1969.

[16] Snow K., Vanderwarker R., *An Application of Holography to Interference Microscopy*, Appl. Optics, **7**, No. 3, p. 549-554.

(17] Van Ligten R. F., *Holographic Microcsopy for the Determination of Failure Mechanisms in Monolite Circuits*, American Optical Corporation, 1969.

[18] Anderson W. L., *Carrier Suppression and Restoration in Hologram Microscopy*, J. Opt. Soc. Am., **59**, No. 2, p. 224-226, 1969.

[19] Bryngdall O., *Shearing Interferometry by wavefront Reconstruction*, J. Opt. Soc. Am., **58**, No. 7, p. 865-871, 1968.

Received, September 16, 1971