An Attempt at X-Ray Phase-Contrast Microscopy

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By use of an X-ray interferometer, X-ray phase-contrast microscopy has been attempted. A phase object inserted on the path of one of a split beam pair causes fringes due to the difference in optical path of these beams. Experimental conditions and procedures are described together with some results on biological and mineralogical specimens, such as bone tissue and granite.

INTRODUCTION

Since X-rays were first recognized as electromagnetic waves, X-ray optical interference phenomena, for instance by use of a slit or a bimirror, were observed by several investigators around 1930.1-4 In most of these experiments, the wavelength of X-rays used was about 10 Å or even longer than that because the required dimensions for a slit system and the like are not prohibitively small. In the case of X-rays with a wavelength of about 1 Å, it was difficult to observe optical interference except when interference within a perfect crystal was observed outside the crystal, not only because the above dimensional requirements becomes prohibitive but also because the coherence length \( \lambda^2/\Delta \lambda \) for characteristic radiation (\( \Delta \lambda \): full width at half maximum) becomes shorter roughly in proportion to wavelength \( \lambda \).

However, the advent of an X-ray interferometer devised by Bonse and Hart in 1965 opened various possibilities for X-ray optical experiments.5,6 One of the promising applications is phase-contrast microscopy or microradiography, as has already been pointed out.7 In the present report, a few preliminary results on biological and mineralogical specimens are described.

EXPERIMENTAL CONSIDERATION

As is well known, the refractive index \( n \) for X-rays is smaller than unity by a small value of about 10^-4 for all materials. Namely, the deviation \( \delta (>0) \) of a refractive index \( n \) from unity can be described as \( \delta = 1 - n = \left( e^2/2m^2 \right) (\lambda^2 N/2\pi) \), where \( e^2/2m^2 \) is the classical electron radius, \( \lambda \) is X-ray wavelength, and \( N \) is the electron number per cubic centimeter of a material examined. Therefore, specimen thickness for causing phase advance by one wavelength is about 10 \( \mu \)m.

The contrast changes according to the phase difference between a pair of split beams. Naturally, the difference between a maximum contrast and its nearest minimum corresponds to the phase difference of \( \pi \). In between these extremes, there is a region where the contrast changes most sensitively depending upon the path difference. If the specimen region of most concern here could be brought to this contrast-sensitive region by inserting a plate made of light material with a uniform and suitable thickness, it would be expected to satisfy the optimum conditions for phase-contrast radiography. When this is done, it might be expected that X-ray phase-contrast microscopy could become superior to the usual X-ray projection microscopy. However, such an experiment has not been possible in the present preliminary work.

As has already been shown, \( K\alpha_1 \) and \( K\alpha_2 \) radiations can be achromatized, if the emulsion of
a photographic plate is set at a position symmetrical to the phase object concerning the analyzer crystal. In this respect, the present micrography is entirely different from the ordinary diffraction topography, where the plate is placed as near the specimen as possible in order to improve the resolution. However, practically speaking, it may be difficult to realize the achromatic condition perfectly. Therefore, in the present experiment, by suppressing the Kaβ reflection with a fine slit as in the Lang topography method, only the Kaα radiation was used. For this purpose, an X-ray generator with an apparent focus size of about 60 μm × 70 μm was used. The vertical spatial resolution, which is about a few microns, was inevitably worse than in the usual topography.

EXPERIMENTAL PROCEDURES

In the present work, an interferometer of L-L-L type—L means the Laue case[40]—shown in Fig. 1 is used. This is cut out from a large, almost perfect, dislocation-free silicon single crystal, so that its 220 reflection may be used. The quality of this interferometer proved to be rather good, because nearly no intrinsic moiré pattern was observed throughout the available area. A phase-contrast micrograph or microradiograph can be obtained by inserting phase material on the path of a beam, for instance between a mirror and an analyzer crystal. However, it should be noted that a wide-area micrograph can be taken by either (a) moving a phase object together with a photographic plate relative to an interferometer, or (b) moving all of these three (an object, a plate, and an interferometer) relative to the incident beam, satisfying the diffraction condition. Naturally, the difference between these methods does not matter much, when the interferometer shows no intrinsic moiré pattern. In the present work, the second method (b) was adopted mainly because the phase object can more easily be supported. Besides, this method allows us to realize the above mentioned achromatization more easily. In fact, each specimen was pasted on a small sheet of film, and the latter was fixed to an interferometer.

Each micrograph was recorded on a nuclear plate of type Fuji MA7A with emulsion 50 μm thick and with a polymethylmethacrylate base, which facilitates monitoring during exposure because of its high transmittivity for X-rays. In all of the following micrographs, the black region corresponds to the part upon which more X-rays fell, the scale marks shown horizontally are nearly applicable to the vertical direction, too, and the MoKaα radiation was always used.

Fig. 1. Experimental arrangement.
EXPERIMENTAL RESULTS

In Fig. 2a, an X-ray phase-contrast micrograph is shown for a part of the body of a butterfly. Many fringes with high contrast due to phase difference can be seen with good spacial resolution.

Figure 2b shows a transmission micrograph of the same specimen taken with the same arrangement as in Fig. 1a except that the reference beam which does not pass through the specimen is suppressed by an absorber. This micrograph shows less contrast due to low absorption of X-rays. A higher-contrast micrograph could have been obtained if a softer radiation such as CuKα or CrKα were used. However, if a thicker specimen is used, such softer radiations will be absorbed. Therefore, the use of soft radiation for a thicker specimen may make fringe visibility worse. Thus X-ray phase-contrast microscopy seems to be helpful in nondestructive investigation of biological material, unless a specimen is too absorbent.

Bone tissues have already been examined by X-ray projection microradiography. According to what has been reported, there are two reasons why information on bone structure is needed: The first reason is that the range of the normal state at various ages must be determined in order to diagnose skeletal diseases, and the second is that certain aspects of bone structure are of great interest in connection with assessment of the local rates of bone tissue renewal.

In the present work, a normal bone has been examined by the X-ray interferometer; the

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(a) ![Micrograph of a butterfly](image1)

(b) ![Transmission micrograph](image2)

**Fig. 2a.** An X-ray phase-contrast micrograph of a part of a common butterfly (3) called the Small White or the Small Cabbage (Pieris rapae crucivora Boisduval).

**b.** An X-ray transmission micrograph of the same specimen taken by cutting off the reference beam. Radiation: MoKα; operating conditions: 50 kV and 2.5 mA; exposure time: (a) 30 hr and (b) 29 hr; field width: 2.6 mm.
specimen is a transverse slice of the cortical bone of the femur of a 24-year-old male. The X-ray phase-contrast micrograph is shown in Fig. 3a. The black dots and lines are probably Haversion canals. Other fine structures found in the X-ray projection microradiograph cannot be recognized in this traverse micrograph, at least at the present stage. However, more details can be obtained by taking phase-contrast section topographs, where spatial resolution can be improved. Figures 3b and 3c show these topographs due to the $D_h$ and $D_0$ waves, respectively, shown in Fig. 1. Because the interferometer used was not sufficient as Borrman crystal (in other words, wave fields other than those with the least absorption penetrate the analyzer crystal to some extent), some undesirable interference pattern not caused by the specimen is seen there. However, various fine structures clearly belonging to the specimen can also be observed.

In the field of mineralogy and petrology, minerals and rocks are often investigated using an optical interference microscope. An X-ray phase-contrast topograph of a granite slice has been obtained as shown in Fig. 4a. The corresponding optical photograph is shown in Fig. 4b. Cracks seen in this photograph intersect with the specimen surfaces at an angle in most cases. However, they cannot be seen in Fig. 4a, probably because phase difference due to the cracks may be too small.
Fig. 4a. An X-ray phase-contrast micrograph of a slice of granite, with thickness about 180 μm.

b. An optical photograph of the same specimen as in (a). The black parts correspond to biotite and grey parts to feldspar group and quartz.

Operating conditions: 50 kV and 2.1 mA; exposure time: 24 hr; field width: 4 mm.

DISCUSSION

If a large and perfect X-ray interferometer is combined with another perfect crystal in an asymmetric double crystal arrangement, the area observable without traverse motion may become much wider.\textsuperscript{13,14} Using this double crystal method, the spacial resolution due to wavelength dispersion of $K\alpha$, radiation will be much improved. For this reason, such a method seems to be promising for observing a wide phase material.

It may be useful to study the three-dimensional distribution of materials with different atomic numbers in a specimen by taking a stereo pair of X-ray phase-contrast micrographs. This can be done, in principle, without chromatic aberration, though the detailed procedures are not described here. Incidentally, at least one point should be noted: when materials with different $n$ values interpenetrate each other, true stereoscopic visualization cannot be expected, because the phase difference is recorded only after it is integrated along each X-ray beam direction.

If X-ray phase-contrast micrographs with higher contrast and higher spacial resolution are to be taken, it is necessary to use an X-ray interferometer with an appropriate absorption (e.g., $\mu t \sim 10$, where $\mu$ is a linear absorption coefficient and $t$ is a total thickness, both of the interferometer) and a suitable compensator, although it is difficult to fulfill these two conditions because longer exposure time is needed.

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REFERENCES

1) Bäcklin, E., Uppsala Univ., Årskrift No. 2 (1928).
3) Larsson, A., Uppsala Univ., Årskrift No. 1 (1929).
4) Linnik, W., Naturwiss., 18, 354 (1930).