COMPUTER CONTROLLED ON-LINE DIFFRACTOMETRY OF MICROGRAPHS

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ABSTRACT

The method of computerised light optical diffractometry for micrographs is demonstrated.

Keywords: optical diffraction, quantitative power spectrum.

INTRODUCTION

The Fourier analysis with the LFO (Light Fourier Operator) allows to analyse image structures of whatever origin in the spatial frequency domain. So crystal structures, random distributions with overlayered periodics, metal alloys [Eickhorst et al., 1985], arrangements of biological molecules [Klug, 1971, Lipson, 1972, Luciano et al., 1989], the correction of an electron microscope [Thon, 1966, Wilbrandt et al. 1987] or the quality of images, can be measured. An introduction is given in [Boseck et al., 1979, Lipson, 1972]. All these informations are contained in the Fourier power spectrum. Scanning of the pattern can be performed by photographic or optoelectronic and digital methods. This paper describes a computer controlled LFO with digital scanning. Two examples will demonstrate this method.

THEORIE OF TRANSFORMATION

The image is Fourier transformed by a light optical processor and the square of the complex amplitude is registrated. The transformation of the image into the spatial frequency domain can be performed by two ways: digital scanning of the image and calculating the transformation by a computer with the Fast Fourier Transformation algorithm or optical transformation by Fraunhofer diffraction. We prefer the second way as described in [Boseck et al. 1979], but using the possibilty of modern signal processing. The transformation is described by eq. 1.

$$F(u, v) = \iint A(x, y) \cdot \exp\left[-\frac{2 \cdot \pi \cdot i}{\lambda \cdot f} (u \cdot x + v \cdot y)\right] dx dy$$
(1)

u,v	=	spatial frequency	X, V	=	image coordinate
f	=	focal length	λ^{\prime}	=	wave length
A(x,y)	=	transmission function	-		wave rengen

The intensity of the Fourier power spectrum is described by $F(u,v) \cdot F^{*}(u,v)$, where $F^{*}(u,v)$ is complex conjugated to F(u,v).

CONSTRUCTION OF THE LFO

The He-Ne laser beam parallel to the optical axis is reflected with two mirrors and through a spatial mode filter to the system (Fig. 1). The beam from the collimator side of lens L1 is nearly parallel. The object can be moved in x,y,z direction and rotated around the optical axis with a step motor in increments $\geq 0.1^{\circ}$. The zero position is defined by a light barrier and a slit. An additional assembly enables a fast rotation, which is only useful, if the diffraction pattern is symmetrical with respect to rotation [Luciano et al. 1989].



Figure 1. Schematic construction of the LFO.

The vidicon tube for scanning the Fourier power spectrum is placed in the focal plane of lens L2. In the front of the vidicon a slit is used to select the cross section of the Fourier spectrum, which is to be measured. By rotating the object the cross section is changed. To adjust the section the beam may be directed to the ground-glass plate. Via a CCD-camera the image of the ground-glass plate is reproduced on a TV-screen.

The 12 bit A/D converter leads the vidicon signal to a microprocessor controlled multichannel analyzer (HEROS, B&M Spektronik, Munich). This analyzer is linked via an IEEE-488 bus to an IBM compatible computer (XT or AT). By using the 180° symmetry of the Fourier spectrum and shifting the zero beam to the border, it is possible to double the resolution. Only one side starting from the zero beam is scanned by the vidicon. By masking the laser beam with a magnetic controlled shutter the background noise is measured.

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SOFTWARE

All components are controlled by the IBM compatible Computer.



Figure 2. Block diagramm of components connection

The software of the IBM compatible computer (XT/AT) is menue and batchfile orientated. The standard measuring procedure registrates the Fourier spectrum and its background noise with plotting the resulting spectrum on the monitor of the computer. An extended standard measuring procedure uses the 180° symmetrical condition of the Fourier spectrum. The spectrum is registrated two times, the left and right side of the zero beam by rotating the object. This method reduces the influence of noise, phase structure of the object and speckle. Additional it is possible to rotate the object during the measurement. By this 360° integral measurement the influence of noise is reduced.

The software contains several procedures for postprocessing: display of the channel information from the analyzer as spatial frequencies or distances; intensity display as absolute, relative or logarithmic value; calculation of the information content by integrating the area of the peaks; numerical processing by addition, subtraction, normalization, smoothing and correlation of spectra. The smoothing function assigns one channel to the average of the neighbour channels.

The correlation function reduces the influence of noise, speckle and phase structure of the object. Both spectra, before and after the rotation, are not congruent. The maximum correlation coefficient has to be evaluated by shifting before the multiplication and normalization of the spectra. (Eq. 2)

$$K(v) = \frac{\sum X_{i} \cdot Y_{i+v}}{\sqrt{\sum X_{i}^{2}} \cdot \sqrt{\sum Y_{i+v}^{2}}}$$
(2)

v = channel displacement

K(v) = correlation coefficient by displacement of v

 X_i = intensity of spectrum 1 in channel i

 Y_{i+v} = intensity of spectrum 2 in channel i+v

EXPERIMENTS

Two examples are demonstrated. First Fig. 3 shows the electron micrograph of a rat kidney and the steps of spatial frequency measurement.



Figure 3. a) electron micrograph of a rat kidney, 10000x, 80kV, Philips EM 301; b) diffraction pattern; the marked section is investigated; c), d) diffraction pattern of both sides of the zero beam; e) correlation distribution; the optimum for both spectra is given after shifting over 7 channels; f) correlated diffraction pattern, reduced on the dimension of the orignal micrograph.



Figure 4. Power spectrum of a carbon foil, Philips EM420, defocus -400nm, 100KeV.



Figure 5. Cross section through the diffraction pattern (solid line) and calculated graph (dashed), reduced on the dimensions of the carbon foil in the specimen holder.

In Fig. 4 the Fourier power spectrum of a carbon foil, produced from a transmission electron micrograph with LFO, is shown. The concentric rings arise from the modulation effect of the instrument and its phase contrast transfer function on the phase structures of the specimen, while it acts as a wide band noise generator [Thon, 1966]. Equation 3 describes the phase contrast transfer function under idealized conditions and neglecting the unknown electron absorption factor [Hanßen, 1971].

$$PTF(u) = -2 \cdot \sin\left(\frac{\pi}{2} \cdot \left[C_{s} \cdot u^{4} \cdot \lambda^{3} - 2 \cdot \Delta z \cdot u^{2} \cdot \lambda\right]\right)$$
(3)

u = spatial frequency $C_s = spheric aberration$ $\Delta z = average defocus$ $\lambda = wave length$

In Fig. 5 a comparison between diffraction experiment and calculation (square of the PTF function) is made. The graphics show that both results take the same extreme value positions along the frequency axis. The intensities are different, due to the unknown absorption factor. The difference at u=0 is inherent in the principle of LFO, as the electron micrograph acts as an amplitude object during diffraction.

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