Symmetry of STRUCTURE

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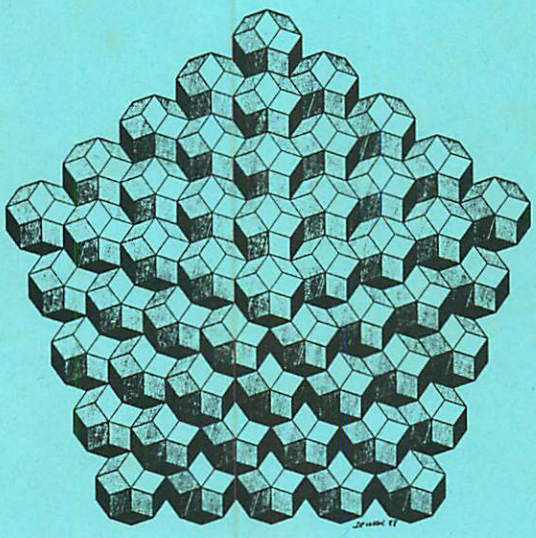
Abstracts

I.



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Interference Fringes on 2D Diffraction Pattern of Radially Symmetric Markers for Determination of its 3D Relative Positions

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INTRODUCTION

Structures are correlated to functions, and structural changes occur in response to stimuli in the cells and tissues. The structure and its change in the cell and tissue should then not only be detected more precisely but also more dynamically to solve bio-mechanisms. Recently, laser confocal scanning microscopes have been used to observe the 3D static structures in the real image. But, to observe the dynamic living structure, its scanning speed is too slow. A higher scanning speed is requested.

It is well known that the diffraction pattern is made on the back focal plane of the objective lens, concurrent to the real image on the back focal plane of the eye piece in the microscope (Zernike, 1946). The information contained in the diffraction light is equivalent to that in the real image. Moreover, the noise in the diffraction light is lower than in the image light.

In contrast to the real image, the diffraction pattern is fixed in spite of any translational movement of the object. Deformation such as the ciliary beat of the protozoa, rotation and spinning of the cell body are exhibited on the diffraction pattern, separate from the translation of the cell(Ishizaka, 1981 and 1982). The diffraction light is mixed with a reference light to make a hologram. The hologram reveals not only light amplitude but also the light phase (Gabor, 1948 & 1949). But, the experimental conditions are delicate. If two similar apertures are placed parallel to each other, in 2D the diffraction pattern is the same as that of each separately, crossed by Young's fringes; the fringes are perpendicular to the separation of the apertures, and have spacings inversely proportional to the distance between them (Lipson, 1972).

To record the 3D light phase, radially symmetric markers of the same size were put on the specific sites of a structure. Analysis of interference fringes on 2D diffraction pattern assisted 3D dynamic morphometry will be reported in this paper.

DIFFRACTION PATTERN OF MICROSPHERES ON CONFOCAL PLANE

To observe the 3D distribution of a certain kind of active sites $\vec{x}_i$ in a cell or tissue, microspheres $f(\vec{x} - \vec{x}_j)$ of the same size and high index of refraction were used as the radially symmetrical markers. As in Fig. 1a, a lymphocyte was marked with microspheres coated by antibody against the active sites. The markers were illuminated with a plane laser wave $\vec{k}_o$. It is well known that incident light is Fourier-transfered by scattering on the markers.

$$F(\Delta\vec{k}) \exp i\Delta\vec{k}\cdot\vec{x}_i = \iiint f(\vec{x} - \vec{x}_i) \exp i\,\Delta\vec{k}\cdot\vec{x}\, d\vec{x} \qquad (\Delta\vec{k} = \vec{k} - \vec{k}_o)$$

The scattered light $\vec{k}$ with phase differences between the microspheres interferes with each other and superimposes upon a diffraction pattern to produce a series of interference fringes.

$$\left| \sum F(\Delta\vec{k}) \exp i\Delta\vec{k}\cdot\vec{x}_i \right|^2 = |F(\Delta\vec{k})|^2 \left(n + 2\sum_{i>j}\sum \cos \Delta\vec{k}\,(\vec{x}_i - \vec{x}_j) \right)$$

Several series of interference fringes $\cos\Delta\vec{k}\,(\vec{x}_i - \vec{x}_j)$ were distinctively produced as shown in Fig.1b.

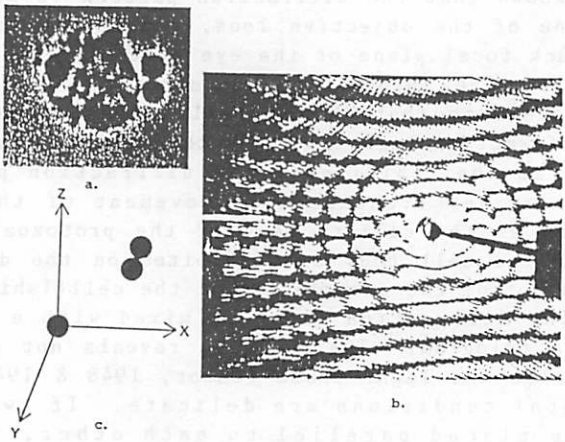

Fig. 1. Diffraction pattern of lymphocytes marked with microspheres. (a)A lymphocyte marked with microspheres. (b)Diffraction pattern with three series of interference fringes. (c)Relative position of the markers.

The position of each series of interference fringes generated on the diffraction pattern is determined by the phase difference,

INTERFERENCE FRINGES ON DIFFRACTION PATTERN

In almost all series of interference fringes

$$\Delta\vec{k}(\vec{x}_i - \vec{x}_j) = \pi m \quad \text{even: Bright, odd: Dark},$$

an eye (sink or source point) was seen, as shown in Fig. 2. The interference fringes are correlated to the relative positions of two microspheres in the three dimensional distribution. The orientation of the two microspheres is represented by the zenith. The direction to the eye from the center of the lens is the zenith. The line to the eye from the optical center of the diffraction plane is the azimuth. The azimuth is perpendicular to the series of interference fringes. The density of the fringes is proportional to the distance between the two microspheres. The 3D distribution of the microspheres could be picturized from the 2D diffraction pattern.

In the case where the zenith is perpendicular to the optical axis, and the eye can not be seen in the view, the azimuth can be determined from the line perpendicular to the interference fringes as shown in Fig. 3.

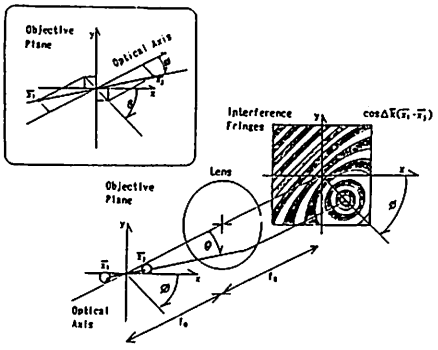

Fig. 2. An eye of the interference fringes.
θ: Zenith angle and ϕ: Azimuth angle which indicates the relative position of two microspheres at $\vec{x}_i$ and $\vec{x}_j$.

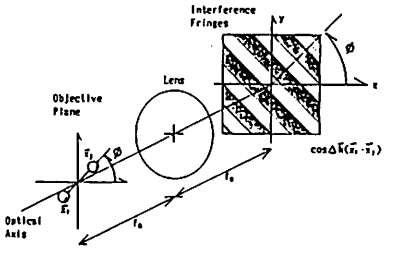

Fig. 3. The case where the zenith is perpendicular to the optical axis and the eye can't be seen. ϕ: Azimuth of the relative positions of two microspheres.

DISCUSSION AND CONCLUSION

Several series of interference fringes were generated on the diffraction pattern by the microspheres immunologically marked on certain active sites in a cell structure. Each combination of two markers generates a series of the fringes from which their relative positions could be determined. The zenith and azimuth between two sites were determined from the eye (sink or source) of the interference fringes and from the line connecting the eye and the optical center on the diffraction pattern, respectively. And the distance between two sites was proportional to the density of the fringes across the azimuth. Thus, from the 2D diffraction pattern, the 3D relative positions of the active sites could easily be determined with the assistance of a computer.

This determination speeds up imaging in confocal scanning microscope. When preliminary scanning catches one of the positions of the active sites in a coarse pixell, the other positions of the sites can be expected and can thus be precisely observed in a shorter time. Concerning the structural changes of the cell, the response time and spatial spectra must be detected to observe the changes to a given spectra applied as stimuli (Ishizaka, 1982; Ishizaka et al., 1983; Ishizaka et al., 1984).

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