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Laser Fourier Morphometry on Cell Surface with Micro-spheres Bound by Means of Antigen-antibody Reaction to Specific Sites Assisted by Computer Graphics

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ABSTRACT

Laser fourier morphometry was developed to detect the spacing of specific sites on cell surface and their changes by stimulating. Micro-spheres of the same radius and of high refractivity, coated with antibody, were marked on a spacing of IgG sites on human lymphocytes. Laser beam was then diffracted by these distributed markers. The diffraction pattern of the marker spheres was a set of concentric circular fringes at the optical axis, as that of a single sphere. Interfered by phase difference of diffracted light of each pair of marker spheres, the sets of coaxial interference fringes were superimposed on the diffraction pattern of the sphere. Then, the distance between a pair of specific sites can be determined from the density of the interference fringes, and also, the direction of orientation of the pair of sites can be figured out from the common axis of interference fringes, assisted by computer graphics.

Introduction

We present a new type of morphometry to detect the spacing of specific sites on cell surface and their changes by stimulating.

The spacing and its changes are interesting as morphological information. Micro-spheres of the same radius and of high refractivity, coated with antibody, were marked on specific sites of the cell surface. Laser beam was diffracted by the distributed marker spheres. The Fourier transform of the distribution was obtained from the diffraction pattern. Wherever the living cell may move, the transform of deformation is separated from that of translation and rotation (Ishizaka '81 & '83), to be the product of stimulation function with the response function (Ishizaka '82). The diffraction pattern of sphere is immobile against any translation and invariant from any rotation. The distance between distributed marker spheres of the same size was correspondent to the density of interference fringes on the diffraction pattern. Since the distribution of living sites is complicated in the higher background noise, the morphometry was assisted by computer graphics (CG).

Laser Fourier morphometry assisted by CG

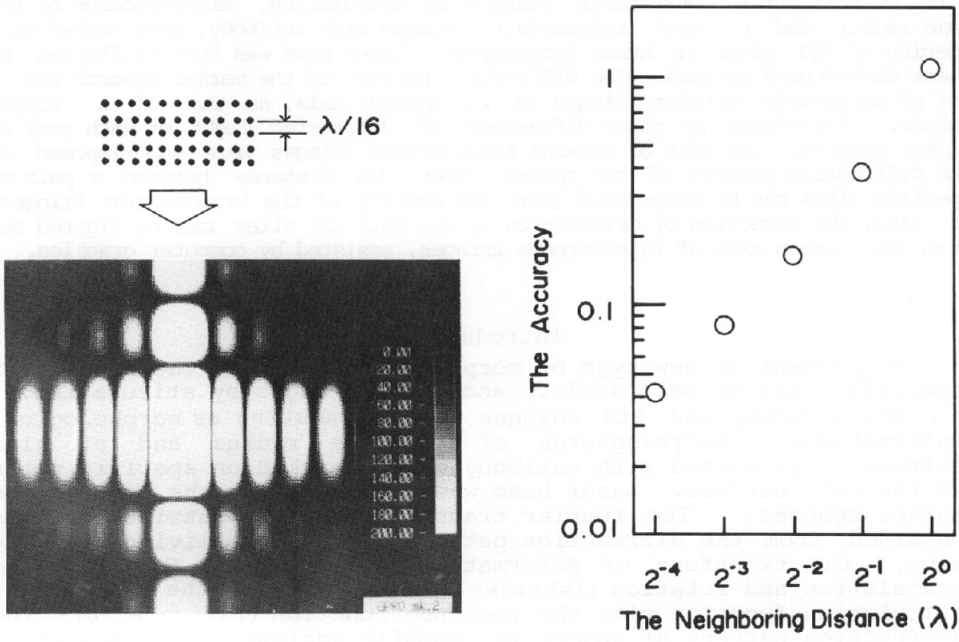
We designed models analogous to three dimensional objects $f(\vec{x})$. The model was a set of minimum numer of scattering points $\{ \vec{x}_i \mid f(\vec{x}_i) < 0 \}$, each of which was nearer than the wave length λ of the laser light from its neighbours.

The laser plane wave $\vec{k}_0$ was diffracted by these scattering points and the phase difference δ of Fraunhofer diffraction wave $\vec{k}$ from the scattering points $\vec{x}_i$ and $\vec{x}_j$, was calculated.

$$\delta_{ij} = (\vec{k} - \vec{k}_0) (\vec{x}_i - \vec{x}_j) \equiv \vec{K} (\vec{x}_i - \vec{x}_j)$$

Diffraction intensity is proportional to the sum of the phase factors $\Sigma \Sigma \cos \delta_{ij}$, when each scattering point is equally due to diffract. The relative intensity was expressed by ten steps of density in each 133 × 133 pixel.

The accuracy of CG was examined from the analytical results on a rectangular slit. The model on a $10\lambda \times 4\lambda$ rectangular slit was a set of scattering points, each of which was $\lambda / 16$ apart from its neighbours. The diffraction pattern of the model was graphically simulated, as shown in Figure 1a. The difference in diffraction intensity between the CG and the analytical calculation was divided by the analytical intensity, to obtain the accuracy, as shown in Figure 1b. The accuracy is dependent upon the distance between scattering points. Higher accuracy of CG on the rectangular slit was obtained as the distance from the neighbours was closer to $\lambda /16$.



a. b.

Figure 1. Simulation of Diffraction Pattern on Rectangular Slit.
a. The diffraction pattern over diffraction angle 30° of a set of scattering points with the neighbouring distance of $\lambda/16$ simulating a 10λ x 4λ rectangular slit.
b. Accuracy of simulation of diffraction pattern. abscissa: The neighbouring distance per unit wavelength of a set of scattering points. ordinate: The accuracy is indicated by the ratio of the difference between the simulated and the analytical intensity.

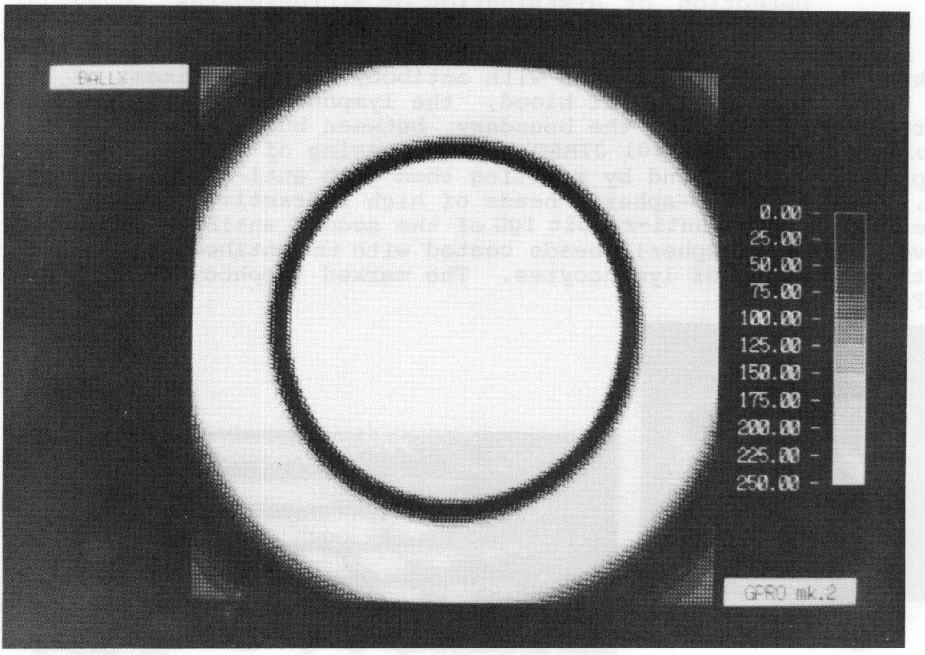


Figure 2. Simulation of Diffraction Pattern of a Sphere.
The diffraction pattern of a set of scattering points with neighbouring distances $\lambda/16$ simulating a sphere with diameter of 5λ.

The same simulation to a sphere of 5λ in diameter set at the origin was run, as shown in Figure 2, and compared with its analytical results.

$$| F(\vec{K}) |^2 = | \iiint f(\vec{x}) \exp i\vec{K}\vec{x}\, d\vec{x} |^2$$

The diffraction patterns are a set of concentric circles, and the densities of the circles were proportional to the radius of the sphere.

The diffraction pattern of arbitral spacing of spheres of the same size was graphically computed easily. Whatever number of spheres there are, the diffraction fringes were the same as of a single sphere. The distance between every pair of spheres is exhibited by the density of the interference fringes on the diffraction pattern. The direction of orientation of the pair of spheres is parallel to the axis of common interference fringes.

$$\begin{aligned} & | \iiint \Sigma f(\vec{x} - \vec{x}_i) \exp i\vec{K}\vec{x}\, d\vec{x} |^2 \\ = & | F(\vec{K}) |^2 \Sigma\Sigma \cos \vec{K} (\vec{x}_i - \vec{x}_j) \\ = & | F(K) |^2 \Sigma\Sigma \cos \delta_{ij} \end{aligned}$$

As mentioned above, the Laser Fourier Morphometry was assisted by CG of a set of scattering points, according to accuracy to the need.

Detection of Distribution of Micro-spheres
Coated with Antibody Reacted to its Specific Sites.

A spacing of IgG sites on human lymphocytes was detected by marking micro-spheres coated with antibody on their sites.

From human peripheral blood, the lymphocytes were centripetrally separated at the boundary between buffer solution and ficoll (s.d. 1.077±0.001 JIMRO). The spacing of IgG sites on the lymphocytes were found by reacting them with anti-human IgG rabbit IgG. The 5μm micro-spheric beads of high refractive polystylene were coated with anti-rabbit IgG of the second antibody mentioned above. The micro-spheric beads coated with the antibody were marked on the IgG sites of lymphocytes. The marked lymphocytes are shown in Figure 3a.

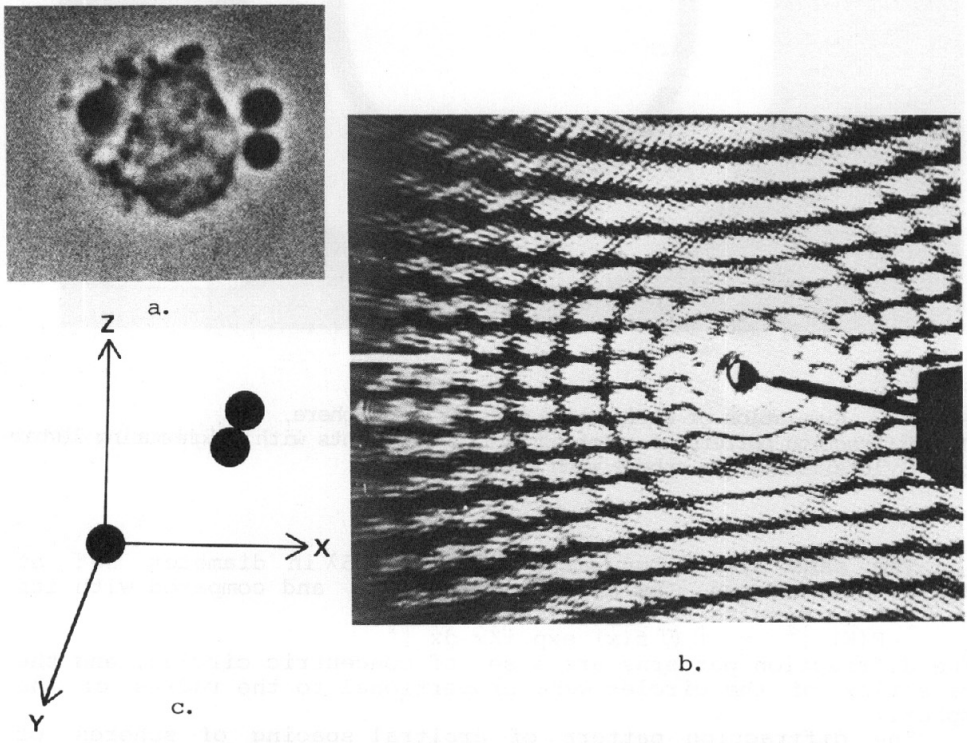


Figure 3. Diffraction Pattern of Lymphocyte Marked with Micro-spheres
a. A Lymphocyte marked with micro-sphere
Three marker micro-spheres bound to a peripheral lymphocyte seen under the microscope.
b. Diffraction Pattern with Interference Fringes
The coaxial diffraction pattern, which originates from a single sphere is accompanied by three sets of interference fringes. From these interference fringes, the distance between two spheres and its orientation can be obtained. And finally, we can figure out that one sphere lies 2 x 10 μm apart, at an angle of 5 x 10 degrees from the sphere on the Z-axis, and that the third sphere lies in contact with the second sphere on the XY - plane and parallel to the Y-axis.
c. The spacing of the spheres indicating the IgG sites of a lymphocyte.

The distribution of markers was detected by Laser Fourier Transformation assisted by CG. Irradiated laser light was diffracted on the distributed micro-spheres on the lymphocytes. The diffraction pattern of the micro-spheres was a set of concentric circular fringes at the optical axis, as shown in Figure 3b. i.e. the diffraction pattern of a single sphere. Some sets of coaxial interference fringes were superimposed on the diffraction pattern of a sphere, as shown in Figure 3b. which are the interferences with all pairs of marker spheres on the cell surface. The distance between a pair of marker spheres can be determined from the density of the interference fringes, and also, the direction of orientation of a pair of marker micro-spheres can be figured out from the parallel axis to the common axis of interference fringes.

Thus, the spacing of the specific sites on the cell surface can be figured out by means of laser Fourier transformation of the distribution of marker micro-spheres coated with antibodies reacting to the sites. The Laser Fourier morphometry can be, generally, applied on nonperiodic phenomena, to detect the distribution of distance among specific sites and their orientations.

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