Gabor Diffraction Microscopy : the Hologram as a Generalized Zone-Plate

SINCE the publication of the papers of Gabor on diffraction microscopy[1,2], work has been going on in this Laboratory on the effect of varying the source-hologram distance and the hologram size on the position of the reconstituted image. It proves possible to represent the information obtained in a very simple way by the use of an instructive analogy.

It should be noted that a zone plate is a particular case of an artificially constructed hologram, corresponding to the introduction of a second coherent point source into the diverging beam from the first point source. A zone plate, moreover, when suitably arranged in front of a point source, duly reconstitutes the second point source, together with the symmetrically placed point on the far side of the original source. This follows from the fact that the zone plate has both a positive and a negative primary focal-length.

Pursuing this analogy, we may regard a hologram as a generalized zone plate, which transforms a point source into an image resembling the object used to generate the hologram. It is found that if the distance from the source to the hologram is regarded as the object distance (u), that from the hologram to the reconstituted image being taken as the image distance (v), they are related by the simple lens formula :

$$\frac{1}{v} \pm \frac{1}{u} = \frac{1}{f},$$

the positive or negative sign being taken according to the sign convention used. Hence, it is useful and proper to regard the hologram as having a focal-length f. In common with the zone plate, it acts as both a positive and a negative lens. This accounts for the double image formed, and also explains the observation that, if Gabor's condition that the object must be near the source compared with the hologram is disregarded, the two images are *not* formed symmetrically with respect to the source ; but their positions agree exactly with the results of operating on the wave-front from the source with a positive and a negative lens of equal numerical power.

It is found that the effective focal-length of the hologram varies with the wave-length, λ, and with a parameter L, representing the linear scale on which the hologram positive is reproduced from its negative, by the following relationship

$$f \propto \frac{L^2}{\lambda},$$

a result well known to hold for a zone plate[3].

It is a necessary corollary of the existence of the two focal-lengths that the hologram can act as a convex lens, and can thus produce its own reconstruction without the use of the auxiliary lens recommended by Gabor. Since the hologram is essentially a positive photographic reproduction of the Fresnel diffraction pattern of the object in diverging light, and the use of a point source and a hologram alone can only produce a Fresnel diffraction pattern of the hologram, it follows that a two-stage Fresnel diffraction operation can reconstruct an image of the original object. In this way the inverse relationships in the first diffraction operation are re-inverted to give a semblance of the original object, in a way reminiscent of the Abbé theory of microscopic vision and the standard Fourier technique in X-ray crystallography.

There is one point at which the analogy with a zone plate appears to break down. The zone plate not only has a primary focus, but also a number of higher-order foci corresponding to powers which are odd integral multiples of the primary power. This, it seems, arises from the practice of constructing zone plates with abrupt changes from black to white at a sharp boundary, analogous to a square-topped wave. As is well known, a square-topped wave has a large number of Fourier coefficients, and thus the sharper the boundaries in a zone plate, the greater the number of orders obtained. The hologram, on the other hand, produced by a continuous tone photographic process, does not have sharp alternations, but ones more closely conforming to sinusoidal variations ; thus we expect the greater part of the diffracted energy to be concentrated in the first-order patterns. The use of an altogether unusual degree of photographic contrast might give rise to higher order effects ; but none has been so far observed.

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[1] *Nature*, **161**, 777 (1948).
[2] *Proc. Roy. Soc.*, A (July 7, 1949).
[3] See, for example, Jenkins and White, "Fundamentals of Physical Optics", 183 (McGraw-Hill).

Occurrence of 5-Methyl-Cytosine in Nucleic Acids

THE presence in a nucleic acid of the pyrimidine 5-methyl-cytosine was first reported in 1925 by Johnson and Coghill[1], who claimed its discovery among the hydrolysis products of tuberculinic acid. As their identification, however, was based solely on the optical properties of the crystalline picrate, the correctness of this report has been subject to speculation ; yet until the recent application of paper chromatography to nucleic acid analysis, there has been no published attempt to confirm their finding. Recently, using a chromatographic method, Vischer, Zamenhof and Chargaff[2] have estimated the purines and pyrimidines in deoxypentose nucleic acid from avian tubercle bacilli, and could find no trace of methyl-cytosine. Hotchkiss[3], however, has noted in hydrolysed thymus nucleic acid a small amount of a substance the chromatographic behaviour and ultra-violet absorption spectrum of which are compatible with its being 5-methyl-cytosine.

Taking advantage of the sensitive photographic technique of Markham and Smith[4] for the detection of ultra-violet-absorbing substances on paper chromatograms, I have now been able to demonstrate and estimate 5-methyl-cytosine in several animal and plant deoxyribonucleic acids, but not in certain viral and bacterial nucleic acids, including that of tubercle bacilli.

The bases liberated by hydrolysis of nucleic acids with formic acid (175° C. for 30 min.) are separated on one-dimensional paper chromatograms using as the solvent an aqueous solution containing 65 per cent *iso*-propanol and 2·0 *N* hydrochloric acid. In this system, guanine, adenine, cytosine and thymine are separated in that order, and it was noted first in chromatograms of nucleic acid from herring sperm that a faint additional spot is visible just beyond cytosine, which was also present when hydrochloric acid was used for hydrolysis. A sample of this sub-