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RECENT PROGRESS

OPTICAL DIFFRACTION APPLIED TO ELECTRON MICROSCOPY
A MODEL OF DIFFRACTOMETER AND IMAGE PROCESSING UNIT

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The analysis of images obtained in the electron microscope is made mostly through subjective mechanisms, using the observer's experience to recognize morphologic patterns which will allow the identification of the structures.

The development of techniques allowing a quantitative evaluation of certain parameters (e.g. the volume of cell components, quantitative autoradiography, electronprobe microanalysis, etc.) made an important contribution toward the interpretation of electron micrographs. Amongst those techniques optical diffraction, using the coherent light properties of diffraction and interference, are of paramount importance.

Coherent light is light which remains equal to itself along time and space. In terms of colour, this means that coherent light is emitted by exactly the same transition between the atomic states of an atom. This effect is achieved in an environment which is called a laser.

Laser light, because it is coherent, recognizes itself even when it crosses different stretches of space or when the light which is emitted now meets the light which was emitted a few minutes before or which will be emitted a few minutes later.

When this coherent light crosses the image registered on a film it sees opaque and transparent zones. The transmission from the transparent zone is done in all forward directions and for each direction the light ray has a different experience or a different tale to tell. Now, when it meets light coming from other transparent zones, the result of the meeting can be anything from total accordance to complete opposition. If the tales the two rays have to tell each other are opposite, as the two rays are of the same kind, they annihilate each other; if, on the other hand, they fully agree, they add up and reinforce the amount of light in that zone (Fig. 1 — Schema A). This property of coherent light rays to add up or to cancel each other is the principle of diffraction and interference and constitutes the basic physical phenomena giving rise to the image formation on an optical machine (Preston Jr. 1972).

Optical diffraction has been used to calculate, from the electron micrographs, some of the physical properties of the periodic structures (dimensions of the elementary cell, mass of the molecular unit) where the usual techniques of transmission electron microscopy would be able to give only a rough model for the type of arrangement of the periodicities.

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In biological research, optical diffraction as well as image processing has been employed mainly in the analysis of paracrystalline structures and other periodical arrangements such as viral structures (Berger 1969; Markham 1970; Johansen 1970; Horne and Markham 1973).

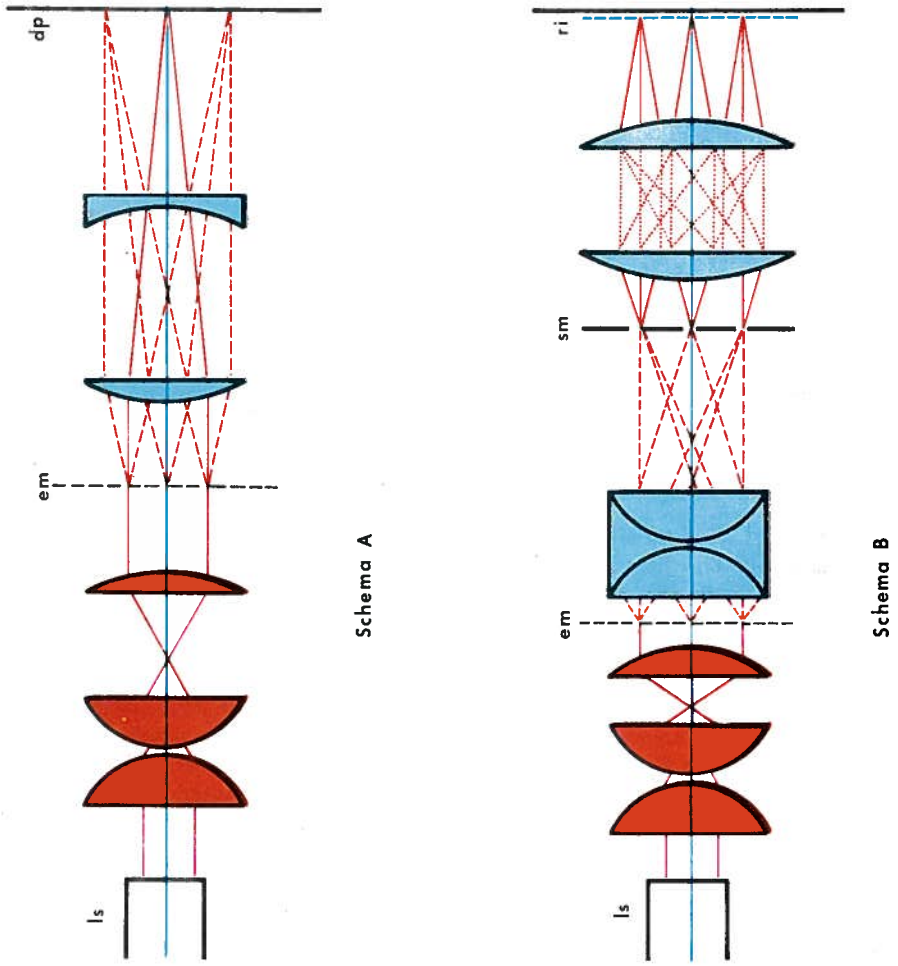

Fig. 1. Schema A — *Orange-painted lenses make up the collimator, providing a parallel light beam. This beam is diffracted on the electronmicrograph (em) and the large optical diffractometer constant is attained with a lens system made up of a positive and a negative lens having a long equivalent focal distance. Light source (ls); diffraction pattern (dp)*

Schema B — *By changing the blue-painted lenses of Schema A to those of Schema B an image processing device can be readily built. Light source (ls); electronmicrograph (em); spatial mask (sm) at the diffraction pattern plane; reconstructed image (ri)*

To study these types of biological periodic structures a machine able to perform optical diffraction analysis capable of processing electron micrographs, was built having the following characteristics (Fig. 2a and 2b):

(a) Light source — an O.E.M. helium-neon laser with 2 mW output power;

(b) Diffractometer — with a lenght of 3 meters and an equivalent focal lenght of 10 meters; the optical diffractometer constant is 1.58×10^{-1} mm^{-1} nm^{-1};

(c) Set-up — the optics are mounted on aluminium injected supports and the collimating lenses are designed to provide a 5 mm diameter parallel beam. This is the area of analysis by optical diffraction and for the image processing of the micrograph.

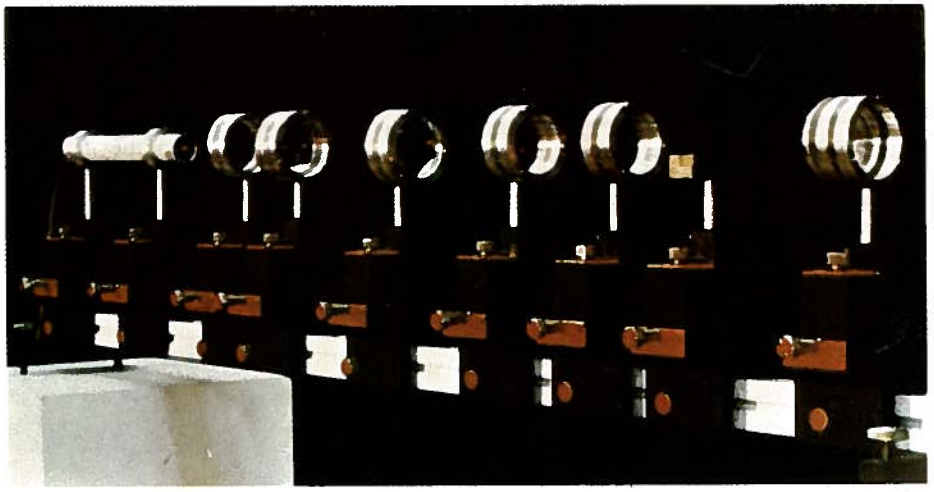

Fig. 2 a — *The diffractometer and image processing unit*

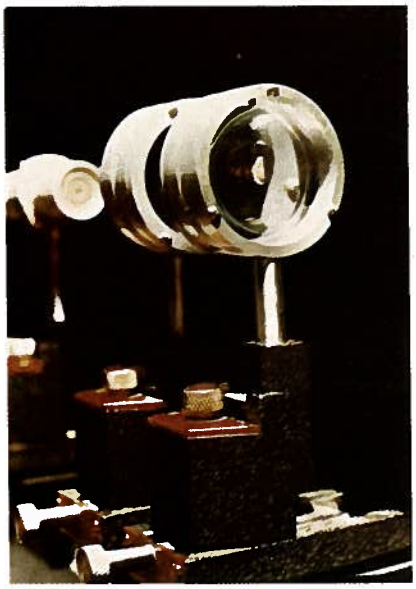

Fig. 2 b — *Close-up of the light source and the collimating lenses*

Results of the application of optical diffraction to the analysis of a periodic structure are shown in Figs. 3 and 4. In Fig. 3 a hexagonal honneycomb pattern is observed whereas in Fig. 4 parallel dense lines are separated by light bands of similar width. While these two morphological patterns are quite distinct they correspond to views from different angles of the same crystalline structure. This hypothesis could be stated by a direct electron microscopic observation, but the confirmation can be obtained through optical diffraction of random sections of the periodical structure.

By using a goniometric stage, the electronmicrographs of controled-tilted single sections of paracrystalline structures contain all the information required to determine its elementary cell parameters.

The shape and the dimensions of the paracrystalline elementary component (unit cell) allow an estimation of the molecular weight of the substance which constitutes the structure. These physical properties of the molecule can be helpful for the identification of the substance.

The task of optical diffraction is easy when the periodic structures appear clearly on the electron micrographs as in Fig. 3. However in most instances the diffraction pattern is blurred either by errors of focusing or by artifacts produced during the processing of materials for the electron microscopic observation as it can be seen in negative stained viral components (Figs. 5 a and 5 b).

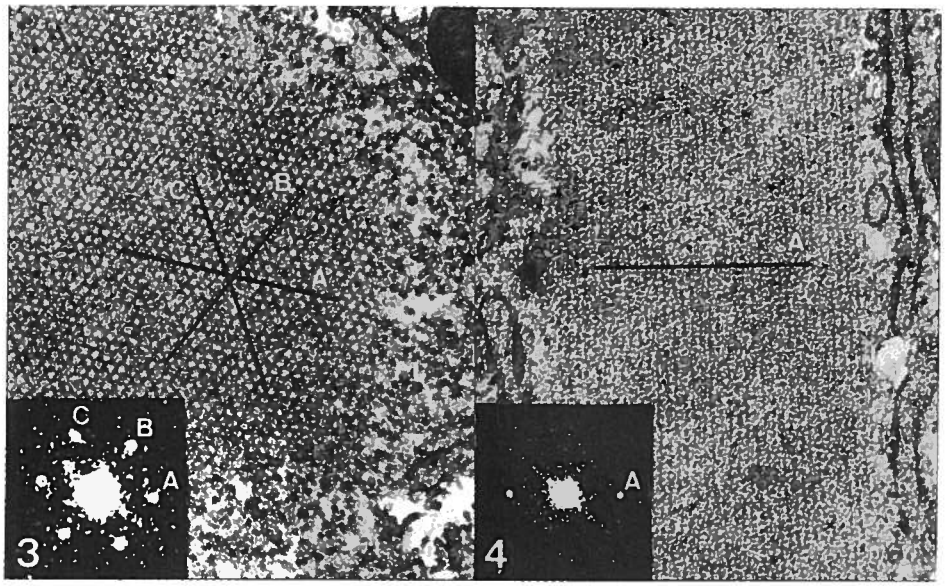

Fig. 3 — *Tubulin paracrystal induced in HeLa culture cells by incubation with 1 μg/ml of vinblastine during 24 hours (× 45,000). Notice the hexagonal honneycomb pattern. The diffraction pattern of the central area of the paracrystal shows 3 pairs of bright spots (A.B.C) around a very bright central spot. The distance between each of the symmetrical bright spots and the center defines (through the product by the constant of the diffractometer) the periodicities. The angles between the lines, defined by the symmetrical points, correspond to the angles between periodicities. The lines in the image correspond to the directions defined by each pair of bright spots*

Fig. 4 — *The same material and magnification as in Fig. 3. Rows of dense dots giving an overall aspects of vertical parallel lines are seen. The diffraction pattern shows a pair of bright spots (A) corresponding to the periodicity along the line A of the electronmicrograph*

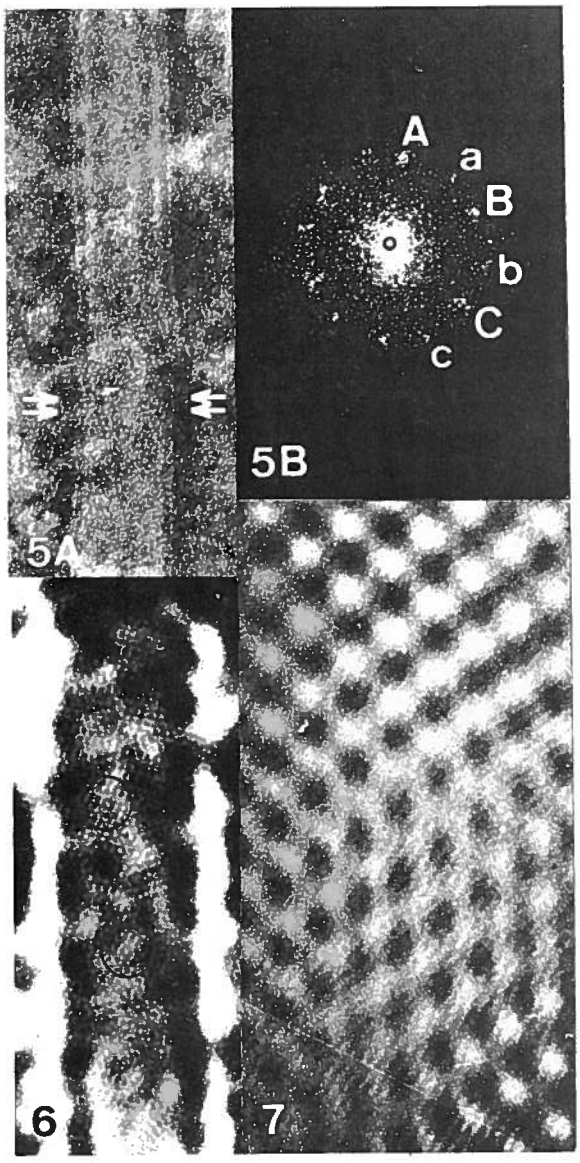

Fig. 5 a — *Cylindrical form of the African swine fever virus negatively stained with 2 % PTK. pH 7.0 (× 105 000). A periodical arrangement can only be suspected in some parts of the image (arrows)*

Fig. 5 b — *Diffraction pattern of the Fig. 5 a. A regular pattern of 6 pairs of symmetrical spots. just appearing above a spread of light speckle, are seen. The angle* AOB *is almost identical to* aOb *(~ 120°)*

Fig. 6 — *Spatial filtering of the diffraction pattern of the viral cylinder. The image is obtained by adding up the light rays coming from the 12 brighter spots and from part of the central light spot. A clear pattern is readily seen on the reconstructed image (circles)*

Fig. 7 — *The diffraction pattern of the cylinder correspond to the superposition of the two faces of the viral structure: the* A, B, *and* C *pairs of points corresponding to one face and* a, b *and* c *corresponding to the other face. Adding up only light coming from 3 pairs of bright spots taken alternatively the arrangement of the periodical structures of just one face is then obtained*

The absence of a clear identification of bright spots in the diffraction patterns prevents the desirable definition of the layout of the repetitive elements in the structure. But by changing some of the lenses, the diffractometer can clean the unwanted information from the electron micrograph through a method called image processing or spatial filtering.

Image processing consists (Fig. 1 — Schema B) in the recreation of the image in the micrograph allowing only a few bright spots (those corresponding to the periodic structure) of the diffraction pattern to add up in the plane of the image, thus eliminating light arising from unwanted frequences (corresponding to structures other than those being studied)

In the case of Fig. 5a, the image obtained by the adding up of the 12 bright symmetrical points (Fig. 5b) gives rise to the reconstructed image shown in Fig. 6. Comparing the two images it is found that in the latter an enhancement of the periodic arrangement became apparent due to the elimination of the non-periodic information and to the higher contrast given by the optical system.

In Fig. 7 a further spatial filtering was performed: the image was obtained by adding up the contribution coming from just one face of the viral capsids.

The large field of applications of the diffractometry in biological research makes this equipment, used either as a diffractommeter or as a spatial filtering device, suitable as a complementary aid for electron microscopic work.

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