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A technique for interpretation of high resolution electron micrographs

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The electron microscope supplies information about an object in the form of a photomicrograph. With appropriate enlargement, the micrograph provides the ultimate opportunity to obtain detailed information about the specimen under investigation. It represents the last link in a long chain of manipulations of the specimen.

To date biologists have relied on their eyes to interpret electron micrographs. Since interpretation is the last step towards communication, it is important to retrieve as much information as possible from our micrographs.

Recently, a new system for data analysis of photographic images has been developed (see Acknowledgments). The Image Quantizer is an instrument that can automatically quantize density values into small finite increments and record these data in a permanent contour map (to quantize is: to express in multiples of a definite quantity). Since the human eye cannot screen densities quantitatively, the Image Quantizer is a useful tool to eliminate background from photomicrographs in order to reveal their main information. By choosing appropriate density differences read by the machine, interfering background can be eliminated to bring out the true information stored in the micrograph. An added convenience is a derivative print-out in which the machine produces an image in which density changes in the negative are represented as contrasting shades of grey.

The nuclear pore complex was studied in nuclei of fixed mesophyll cells, in order to ascertain its possible role in the transport of fully assembled virus particles to and from the nuclei of virus-infected plant cells (de Zoeten and Gaard, 1968).

Fig. 1A shows a tangentially sectioned nuclear pore of *Petunia hybrida*. A faint impression of a regular structure in the pore annulus was obtained. The picture was subjected to Markham's rotational contrast enhancement technique (Markham et al., 1963) (Fig. 1B), and eight-fold symmetry could be resolved (de Zoeten and Gaard, 1968).

Enlarged continuous tone negatives of Fig. 1B were then quantized at two different settings of the machine. The density changes in the negative are recorded in a permanent contour map. In Fig. 1C and D, significant details stored but obscured in the original negative were extracted by eliminating background interference by changing the ΔD (density increment) from 0.095 in Fig. 1C to 0.16 in Fig. 1D. The result was a clearer picture of the annular symmetry. A derivative print-out of Fig. 1B at ΔD of 0.002 created a three-dimensional picture of the nuclear pore complex (Fig. 1E).

Fig. 1. A: Tangential view of a nuclear pore in the nulcear membrane of a plant mesophyll cell (× 600,000).
B: Contrast enhanced nuclear pore of a plant mesophyll cell; note 8-fold symmetry in the annulus. Center symmetry is a turning artifact (× 600,000).
C and D: Quantized tracings of Fig. 1B. Information is analyzed in terms of varying density contours. Note the clarity of the 8-fold symmetry, especially in Fig. 1D ($\triangle D = 0.095$ and 0.16 respectively).
E: A derivative print out of Fig. 1A creates a three-dimensional impression of the nuclear pore complex (× 600,000).

Fig. 1. A: Tangentiale coupe van een porie in de nucleaire membraan in een mesofylcel (600.000 ×).
B: Fig. 1A werd onderworpen aan contrastverhoging en nu werd acht-voudige symmetrie zichtbaar in de annulus van de porie (600.000 ×).
C en D: Een quantitatieve vertaling van Fig. 1B. De dichtheid van het negatief is nu omgezet in dichtheidscontouren. De acht-voudige symmetrie is nu beter zichtbaar, speciaal in Fig. 1D ($\triangle D = 0{,}095$ in Fig. 1C en 0,16 in Fig. 1D).
E: Een afdruk afgeleid van de dichtheidstraceringen in de figuren C en D. Aldus wordt een drie-dimensionale impressie van de porie verkregen (600.000 ×).

Since the original negative subjected to contrast enhancement showed more contrast on one side of the annulus, we must assume that the section through the pore was not precisely tangential.
The density difference was eliminated by our contrast enhancement and, therefore, an artifact was essentially created. However, the Image Quantizer has given us an exceptionally clear interpretation and visualization of structure not known before.
The technique of image quantitation used here for image analysis offers good perspectives for better micrograph interpretation and is a valuable tool in the study of high resolution electron micrographs.
The study of host–parasite interfaces is often carried out at high initial magnification, and it is here that we visualize the application of image quantitation to the analysis of quantitative differences between these membrane-like structures.

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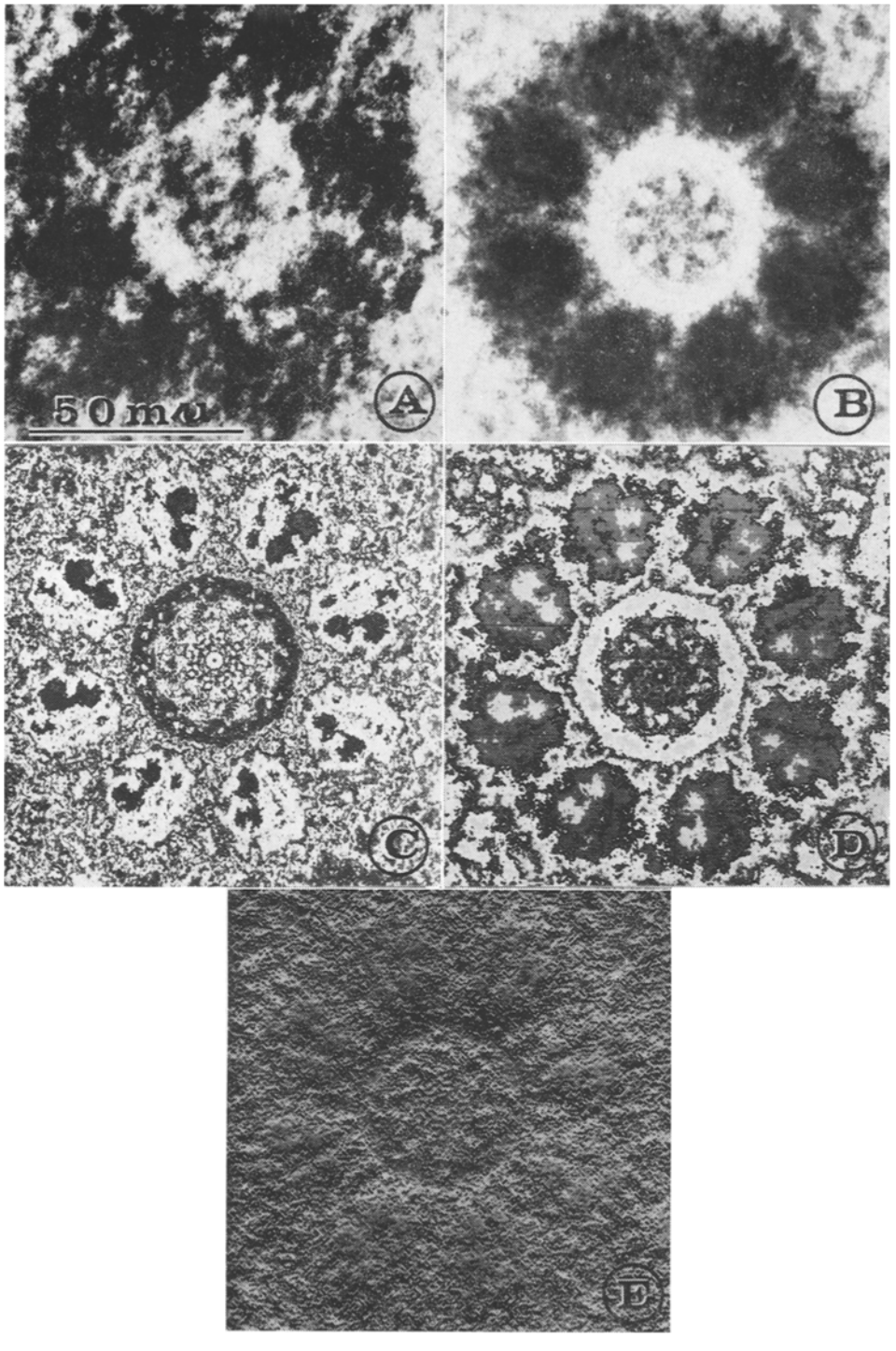
Samenvatting

Een techniek ter interpretatie van foto's gemaakt met een elektronenmicroscoop met sterk oplossend vermogen

Een instrument voor quantitatieve analyse van elektronenmicroscopische foto's wordt besproken.


50 mμ
A
B
C
D
E

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