

Advances in

**OPTICAL and ELECTRON
MICROSCOPY**

Volume 9

Edited by

R. BARER

AND

V. E. COSSLETT

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Preface

Methods for the microscopic examination of matter continue to expand in both their variety and their complexity. Before we have had time to evaluate fully the scanning optical and the photo-electron microscopes, the scanning electron-acoustic instrument is with us. The Auger micro-analytical method has developed a visual display system and it may not be long before laser-excited "destructive analysis" does likewise. It is a teasing question as to how far these techniques should be covered in this series. They all involve beams of light or electrons in one way or another. We can no longer appeal to the simple idea that a microscope includes lenses as essential components of an imaging system.

The solution must be to await substantial advances in a subject, in its performance and applications, before including it, and at the same time to increase the frequency of publication, which we hope to do. To assist in this task we now have two Assistant Editors and an Editorial Board; their names are given opposite the title-page.

Articles for future volumes have already been commissioned on the following subjects:

- Auger Microscopy
- High Resolution Electron Microscopy
- Field Ion Microscope
- Calculation of Structure Images in Electron Microscopy
- Photographic Emulsions for Electron Microscopy
- The Early History of the Electron Microscope
- Holographic Microscopy
- Contact Microscopy
- Acoustic Microscopy
- Scanning Optical Microscopy

Other subjects are under discussion. Suggestions are invited for topics (and authors) in any relevant fields of microscopy.

The present volume continues the established pattern of three articles in each division of microscopy, optical and electron. In the latter, a full account is given of electronic image recording in the conventional EM, as distinct from the scanning instrument. The theoretical limitations as well as the present state of instrumentation are presented in detail by Herrmann and Krahl, from a laboratory with great experience in the subject. The inclusion of energy loss spectroscopy might have been considered a marginal decision a

few years ago. Now it is an essential component of the analytical electron microscope in so many branches of materials science, and in addition, techniques for forming filtered images have been devised. The article by Colliex, one of the earliest and most active workers in the field, provides an authoritative and up-to-date review of it. The third article, by Hines, considers the special problems of high resolution electron microscopy. It is particularly valuable since it is concerned with those problems encountered in the running of a normal high grade instrument, not with one custom-built for the highest possible resolution. The title could equally well have been "how to get the best performance from your EM".

Optical articles cover a wide range of topics. Laser microanalysis, reviewed by Lieselotte Moenke, a pioneer in the field, is now a widely accepted and valuable technique. The development of binocular image-shearing microscopes is described by F. H. Smith. Such instruments have achieved a striking success particularly in the microchip industry. Finally, Rosen reviews some of the image analyser systems now available commercially. He gives a broad account of some basic problems which should perhaps correct some of the over-enthusiastic claims in some manufacturer's brochures.

As happened on a previous occasion, circumstances outside the control of the editors have caused some delay in the publication of particular articles and resulted in the reversal of the two divisions of our subject matter.

May 1984

R. BARER.
V. E. COSSLETT.

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Electronic Image Recording in Conventional Electron Microscopy

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LIST OF SYMBOLS

- a local extension of single-electron signal (in TV lines)
- $A = \frac{f}{e \text{ 12 MHz}}$
- B subtracted constant level in digital storage

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c	speed of light
$C = \frac{\Delta q}{q}$	image contrast
d	linear dimension of an image detail
$D_s = S_{\max} / \sqrt{\Delta S^2}$	dynamic range of analog storage
DQE	detection quantum efficiency
e	elementary charge
E	electron energy in first-generation intensifiers
E_0	energy of primary electrons
E_{BS}	energy of backscattered electrons
E_F	mean formation energy per charge carrier
ΔE	absorbed electron energy in first converter stage
f	processed image field at intensifier input
f'	partial area of processed image field
F	F -number of light optical systems
g	gain of system scintillator—light optics—photocathode
g_P	photon gain of intensifiers
g_T	target gain in camera tubes
g_v	quantum yield of converter stage v
G	spectral matching factor scintillator—photocathode
h	Planck constant
I	signal current of camera tube
I_m	maximum signal current of camera tube
$\overline{I_N^2}$	mean noise square of video readout and amplification process
j	current density at image converter input
j_0	current density in object plane of the electron microscope
j_m	overmodulation limit of current density
\bar{k}	mean exposure of a detector array
\bar{l}	mean counting response of a detector array
L	word length of pixel storage capacity
m	temporal extension of single electron signal (half frames)
M	magnification
$n_e = j/e$	pulse density rate of electrons

n_0	refractive index of scintillator
n_1, n_2	refractive index of fibre core resp. case
n_B	background pulse density rate
$\overline{n_E^2}$	electron noise equivalent of target readout process
n_{\min}	minimum detectable single electron signal on the target
n_{PE}	number of photoelectrons
n_P	number of photons
n_r	pixel number per unit cell
n_T	number of charge carriers representing the single primary electron on the target
N	number of electrons
N_B	number of background counts
N_D	pixel number per unit cell
N_e	number of processed unit cells
N_m	counting limit of digital storage
N_p	linear pixel number of digital storage
NA	numerical aperture
$p(n)$ $v(n)$	probability distribution of stochastic variable n
$P(z)$ $V(z)$	generating functions of distributions $p(n), v(n)$
q	charge density
q_{\max}	maximum charge density corresponding to upper exposure limit of a storage
s	size of single-electron signal
s_B	size of background signal
s_E	single-electron video signal
$s(\lambda)$	spectral response of camera tubes
S	signal
S_{\max}	upper exposure limit of analog storage
S_N	video noise contribution to the output signal referred to the target
SNR	signal-to-noise ratio
SNR _m	maximum signal-to-noise ratio

$\overline{\Delta S^2}$	mean squared noise of analog storage
t	total accumulation time
t'	total scanning time of a partial area f'
Δt	total readout time of single electron signal
T	transmission factor of light optical systems
U	converter voltage of camera tubes
x^2	number of resolved image elements in digital storage
Z	atomic number
α_0	collected half angle in the scintillator
α_1	acceptance half angle of light optical system
α_i	illumination aperture
β	gun brightness
γ	slope of a recorder
δ_p	pixel size at image converter input
ε	energy conversion coefficient of cathodoluminescence
η	fraction of utilized electrons
η_B	fraction of counted background signals
η_{BS}	backscattering coefficient
η_L	collection efficiency of light optical system
$\eta_{PC}(\lambda)$	quantum yield of photocathode
$\eta_T(\lambda)$	quantum yield of camera tube
κ	matching factor between charge density q and signal S in analog storage systems
λ	wave length of light
ν	frequency of light
τ	frame time
Ω	total collected solid angle in the scintillator

I. INTRODUCTION

The conventional transmission electron microscope as well as various other imaging electron beam devices produce information on the specimen as a two-dimensional electron current density distribution. In order to observe, record and evaluate this electron image, it is converted by means of sensors into a suitable form and conserved in a storage medium (Fig. 1) which not

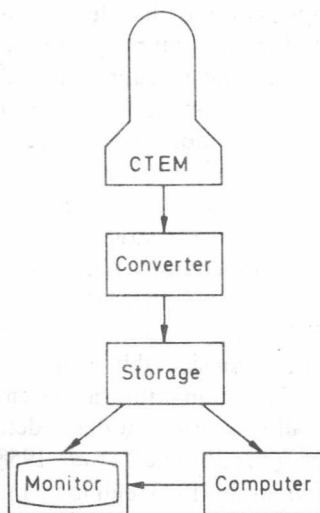


FIG. 1. Scheme of information processing.

only holds the information but performs statistical averaging. The photoplate combines both functions. From the beginning of electron microscopy, the photoplate has been the favoured medium for registration, and it can hardly be surpassed as to number of image elements and simplicity of handling. A serious disadvantage, the long processing time, has led to the development of more sophisticated devices which carry out the recording in "real time". Partition of their function into "information conversion" and "information storage" steps allows both a high degree of flexibility and immediate "on-line" image processing (Fig. 1). Here, electronic image recording systems operating with TV techniques offer good prerequisites within the number of image elements given by TV standards; the connected frame stores can operate analogously and digitally.

For some time, TV image intensifiers have been simply an aid to observation of low-intensity electron images, for which various reasons are responsible. The final current density of an electron microscope is connected with the current density j_0 in the object plane via the relation

$$j = \frac{j_0}{M^2} \quad (1)$$

where M is the electron optical magnification. In the bright-field mode, the current density j_0 is, according to

$$j_0 = \pi \beta \alpha_i^2 \quad (2)$$

given by the gun brightness β and the illumination aperture α_i . Current densities below 1 pA cm^{-2} in the final plane are therefore encountered, for example, when microscopy is done with approximately coherent illumination ($\alpha_i < 10^{-4}$) with thermionic beam sources ($\beta = 4 \times 10^5 \text{ A cm}^{-1} \text{ sr}^{-1}$ at 100 keV electron energy). Even more unfavourable is the situation in the darkfield mode or when imaging with energy filters if microscopy is done exclusively with elastically or inelastically scattered electrons. Often the thermal sensitivity of the specimen restricts the permissible object current density. But also in several other instruments or procedures, the yield of imaging electrons is limited for different reasons (e.g. photoemission microscopy, reflection microscopy with diffracted beams). Since the resolving power of the eye decreases considerably with decreasing image brightness (Schober, 1970), image observation with the systems screen-eye or screen-light optics-eye does not allow a sufficient detail detectability at low current density. Image intensifier tubes (Nirikoff *et al.*, 1958) or low-light-level TV techniques (Herrmann and Krah, 1973) eliminate this restriction by offering to the eye the image at a higher brightness level.

In order to compete with photographic recording, however, such systems would have to be able to integrate the signal, because at current densities below 10 pA cm^{-2} the electron statistics is already distinctly visible. Indeed, the storage time is, by means of long-persistence monitors, prolonged up to about 2 s compared to the usually decisive storage time of the eye (0.2 s), thus obtaining a certain averaging of the statistic fluctuations. A fully satisfying recording system is, however, only achieved if the storage time of the accumulator can be chosen arbitrarily and if its standby-time for a subsequent—not necessarily visual—evaluation is sufficiently long. The production of such electronic recording systems is under way and some are already in operation. Their aim is not only to improve the observation of the final image in order to adjust the microscope exactly for subsequent photographic recording but also to permit the computational on-line image processing, which has, up to now, mostly been carried out off-line by digitizing the photoplate transparency via a scanning photometer.

The importance of electronic recording systems and the requirements for these come to light in an application area which reveals the present limitations of high resolution electron microscopy. Radiation sensitivity, particularly of molecular-biological specimens, prevents their imaging in an undamaged state. Damage-free imaging is only possible, if at all, by minimum-exposure technique (Williams and Fisher, 1970) and by cryo-electron microscopy (Knapek and Dubochet, 1980) with low-dose images, where preirradiation is avoided and a compromise made between image quality and damage probability. Corresponding electronic control of the specimen irradiation is provided in modern electron microscopes. Since an