HOW THIN SHOULD A SECTION BE?

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In considering the electron microscopic study of sections of tissue as a practical approach to the study of cell structure, the cytologist is faced with a number of possible decisions with respect to the way in which he will prepare his material. Not the least of these is the problem of how *thin* the section of tissue should be to serve his purpose.

A useful section of a sample must satisfy the following demands: (*a*) it must provide the observer with a preparation which is sufficiently transparent (one which has a sufficiently low electron-scattering "power") to be viewed by transmitted electrons; (*b*) it must provide a sample which is sufficiently limited in thickness to permit structural analysis in the other two dimensions without the confusion of structural overlap; and (*c*) it must be thin enough to permit the optical system to resolve the structure to be studied.

Because cutting involves bond rupture and plastic flow, the surface layers of a section are badly deformed. The thinner the section, the larger the fraction of its volume which has been permanently distorted. A more accurate picture of the distribution of structures within a tissue can therefore be expected from thick rather than thin sections, *provided the three requirements mentioned above are satisfied.* It almost goes without saying that a thicker section with a given percentage of deformation is easier to cut and handle than a thinner one.

What, then, are the considerations which set the upper limit of "usable" section thickness?

A section with a density of approximately 1 gm. per cc. and 0.2 micron thickness will transmit from 1 to 10 per cent of the incident electrons in the voltage range of 50 to 100 kv. with the usual range of objective numerical apertures. (See Gettner and Ornstein, 1.) This usually constitutes sufficient "transparency" for study.

The problem of overlap will, of course, vary from structure to structure, but often it will be found that the overlap in a section of 0.2 micron thickness will not lead to confusion in analysis.

Let us then examine the dependence of resolution on section thickness. Electrons can lose energy in passing through a specimen. A loss in energy involves a change in the associated de Broglie wave length of the electron. Since electron microscopes have monochromatic lenses, *i. e.*, they have no correc-

tions for chromatic aberration, *this change in wave length can set the limit of the resolution obtainable for a particular thickness of section.* The chromatic limit of resolution, l_c, is described by the following equation (2):

$$l_c = \frac{K\Delta V f \alpha}{V},$$

in which K is a constant nearly equal to one, ΔV is the change in voltage of an electron (in this case, on passing through a specimen), V is the accelerating voltage of the microscope gun, f is the focal length of the objective lens, and α is the numerical aperture semi-angle. If ΔV is taken as the most prob-

TABLE I

Objective lens	K*	f in *cm.*	V in *kv.*	α in radians $\times 10^{-3}$	$d \simeq \frac{10^{-3}V^2 l_c}{Kf\alpha\rho}$ in angstroms for l_c = 20 A
1. RCA EMU-2	0.85	0.28	50	9 (50 micron aperture in center of lens)	200
2. RCA long focus (6)	0.9	1.2	50	2 (50 micron aperture)	200
3. RCA EMU-3	0.85	0.39	50	6.4 (50 micron aperture in rear focal plane)	200
			100	" "	800
4. Philips EM100 6 mm. bore	0.85	0.48	50	10 (50 micron aperture)	100
			100	" " " "	400
5. Philips EM100A & 100B	0.85	0.17	50	10 (20 micron aperture)	300
			100	" " " "	1200
6. Siemens ELMISKOP I	0.85	0.27	50	9.3 (50 micron aperture in center of lens)	200
			100	" "	800

* Computed from Liebmann (5).

able loss in voltage suffered by an electron on passing through a section of thickness, d, angstroms and density, ρ, in grams per cubic centimeter, then (2),

$$V \simeq \frac{10^3 d\rho}{V},$$

and

$$d \simeq \frac{10^{-3}V^2 l_c}{Kf\alpha\rho},$$

in which l_c and f are expressed in angstroms and V in volts.

This equation assumes "opaque" specimen detail on the objective side of the section. Differential sublimation of the embedding medium and part of the specimen in the electron beam, as well as differential extraction of specimen substance during or after fixation, aids resolution by providing contrast which may approach the "opacity" assumed in the derivation of the resolution formula. "Stoichiometric staining" (as the histochemist understands it), with "electron stains," does not seem to hold any promise as a means of increasing contrast (1, 3).

Sublimation of the methacrylate of the section will usually reduce the "effective thickness" of the section in the microscope to about one-half its original thickness. This can be easily demonstrated by observing the interference colors of mounted sections before and after exposure to the electron beam (4) as well as by observing the change in transmittance of the section as the beam intensity is increased.

Table I gives a listing of the properties of a few electron microscope objectives and the maximum thicknesses of the sum of the section and its supporting film which could permit 20 angstroms resolution for material of density, $\rho = 1.2$ gm. per cc. under several different operating conditions. It is assumed that the axial astigmatism has been reduced to 0.2 micron or less for lenses 1, 3, 4, 5, and 6, and to less than 1.0 micron for lens 2.

It thus becomes evident that for instruments with short focal length lenses and accelerating voltages of 100 kv., high resolution is attainable even on sections which are *originally* as thick as 0.2 micron. (See also Plate 94.)

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