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THE PARTICLE NUMBER ESTIMATION AND THE DEPTH OF FOCUS

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ABSTRACT

In the formulas for determining the numerical density and/or absolute number of particles where the slice thickness t is important, the latter can be replaced with the depth of focus corresponding to the objective magnification. In this case the particles should be counted only in one level of a slice and not through its whole thickness.

When we count the particles in light microscopy at one level of a thick slice, but not through the whole slice thickness, we are approaching the counting conditions of the true section. Therefore in the formulas for estimating the numerical density and/or absolute number of particles, where the slice thickness t must be taken into account, the latter can be replaced by the depth of focus DF corresponding to the objective magnification. The higher the magnification of the objective, the greater its numerical aperture NA and the lower its depth of focus. Depth of focus can be calculated by the formula

$$DF = \lambda / (n . NA^2) , \qquad (1)$$

where λ is the wave length of the light (550 nm) and n the refractive index of the intermediate material (air 1, immersion oil 1.516) (Table 1).

Table 1. The numerical aperture (NA), refractive index (n) and depth of focus (DF) of the objectives at different magnifications

objective magnification	NA	n	DF (μm)
3	0.10	1.000	55.00
10	0.25	1.000	8.80
20	0.45	1.000	2.27
40	0.65	1.000	1.30
60	0.75	1.000	0.98
100	1.25	1.516	0.23

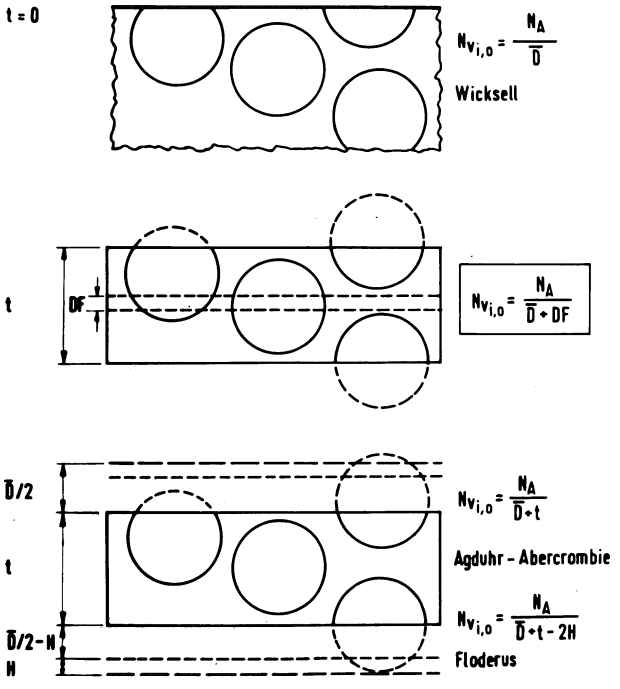

Fig. 1. Schematic presentation of the numerical density $N_{Vi,o}$ of particles with an average diameter $\bar{D}$, according to different methods: DF depth of focus, H lost cap height, N_A numerical areal density of particles, t section or slice thickness

Formulas for determining the particle number in thick slices according to Agduhr-Abercrombie (1941, 1946), Floderus (1944) and Kališnik-Pajer (1983) are modified by substituting DF for t; moreover the correction of the superslice for the lost cap height H is not taken into account, because we are approaching the conditions where Wicksell's formula (1925) is appropriate (Fig. 1).

Thus the numerical density of particles $N_{Vi,o}$ is calculated by the formula

$$N_{Vi,o} = N_A / (\overline{D} + DF) \qquad (2)$$

and their absolute (total) number N_o by the equation

$$N_o = N_i \cdot t_s / (\overline{D} + DF) , \qquad (3)$$

where N_A is the number of particles per test area, $\overline{D}$ their average diameter, N_i the number of particles in all slices of a step series and t_s the thickness of the step.

Values calculated for the average numerical densities of the thyroid gland epithelial nuclei of 4 mice (female, BALB/c strain, 4 months of age) are shown in Table 2.

Table 2. Average numerical densities of the epithelial nuclei of the mouse thyroid gland after consideration of the whole slice thickness (A) or the depth of focus only (B) at objective magnification x100

	$(N_{Vi,o} \pm 1SEM) \times 10^5$ mm^{-3}	
Method	A	B
Agduhr-Abercrombie	4.76 ± 0.24	4.59 ± 0.18
Floderus	4.69 ± 0.18	4.59 ± 0.18
Kališnik-Pajer	4.47 ± 0.26	4.37 ± 0.25

$N_{Vi,o}$ - numerical density, SEM - standard error of mean

The differences between average numerical densities are statistically insignificant.

Two advantages of our modifications for the particle number estimation in thick slices are evident: (1) greater reliability, because two uncertain assumptions (about t and H) are superfluous, and (2) higher counting economy (efficiency).

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