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SIMPLIFIED DIFFERENTIAL COUNTING OF PARTICLES IN LIGHT MICROSCOPY

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ABSTRACT

A simplification of the differential counting of particles can be obtained by double counting the particles in the same slice: firstly, through the whole slice thickness and secondly, in the optical section only. This method was empirically tested on the epithelial nuclei of the adult male mouse, namely on five step slices of one thyroid lobe. It showed that the results obtained by simplified differential counting and the original one were of the same class size. The economy index was higher with the modified method. But, the economy of the simplified method is still greater because there is no need to prepare two differently thick section series. On this basis the simplified differential counting of particles in light microscopy can be recommended as an alternative method with higher economy.

Keywords: Depth of focus, differential particle counting, light microscopy, numerical density, slice thickness, thyroid gland.

INTRODUCTION

Bok and van Erp Taalman Kip (1940) proposed the differential counting of particles in two differently thick slices. This idea was used in practice by Ebbesson and Tang (1965) and Loud et al. (1978). The advantage of the differential counting of particles over classical methods lies in the fact that only the slice thicknesses must be known: not the shape, average diameter, particle size distribution, or height of the lost caps. Recently we proposed an improved method for particle counting in light microscopy by counting their profiles in the optical section only, that is in the objective depth of focus; for example, at objective magnification x100 the depth of focus is 1.250 µm (Haug, 1955).

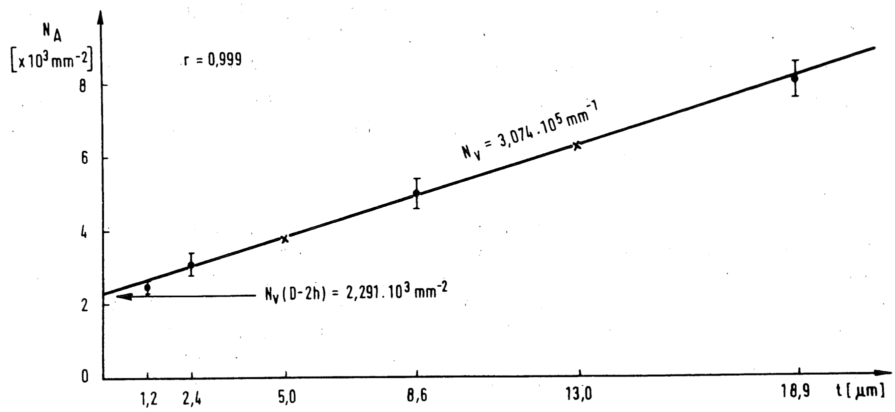
MATERIAL AND METHODS

Further improvement of the differential counting of particles can be obtained by double counting the particle profiles in the same slice: firstly, through the whole slice thickness and secondly, in the optical section only. With this the efficiency of the method is improved and the expected accuracy is satisfactory. We propose the use of the following formula:

$$N_V = \frac{N_{At} - N_{Ao}}{t - o}, \qquad (1)$$

where N_{At} is the number of profiles in the whole slice thickness t, N_{Ao} the number of profiles in the optical section through this slice, t the thickness of the slice and o the depth of focus of a certain objective.

This method was empirically tested on the epithelial nuclei of the adult male mouse, namely on five step slices of one thyroid lobe of the following thicknesses, calculated for the depth of focus and/or estimated by the microscrew: 1.250 µm, 2.380 µm, 8.580 µm and 18.860 µm. The values for N_A were used for calculations of N_V by the formulas according to: Floderus (1944), modified Floderus where (D - 2h) was estimated by the Loud et al. method (1978), our method (Pajer and Kališnik, 1984), Ebbesson and Tang (1965) for all possible different combinations, the modified Ebbesson and Tang method where the depth of focus was taken for the thinnest slice and Loud et al. (1978). The results of the last method were used as a reference value (graph 1). The economy index for all methods was also calculated.

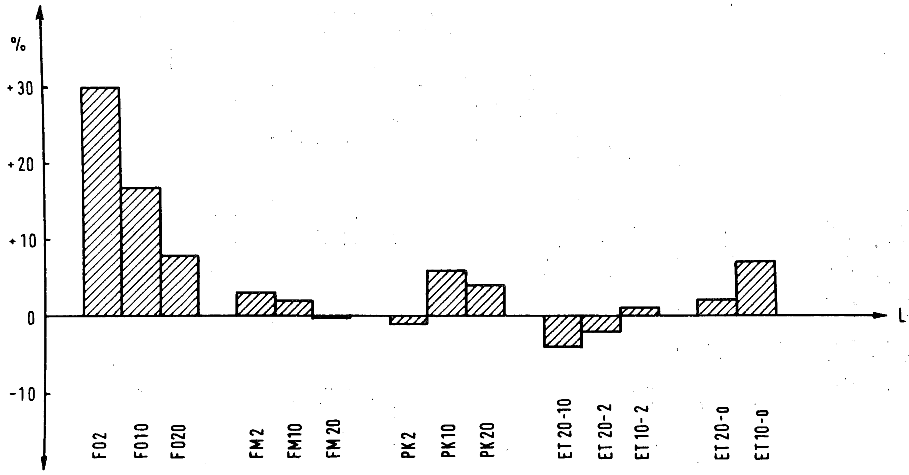


Graph 1. Regression line between numerical areal density ($N_A \pm 2SE$) of the thyroid gland epithelial nuclei and different slice thicknesses (t). The slope of the line is equal to the reference numerical density (N_V). The intersection of the regresion line with the ordinate axis at t=0 equals (N_V(D - 2h)), where D is the diameter of particles and h the lost cap height; r is the correlation coefficient.

RESULTS

Results are presented in table 1. The values for the numerical density (N_{Vn}) for different methods have been calculated from empirically obtained values for N_A. Besides, the modified numerical densities (N_{Vn}') have been calculated according to the corresponding equations where N_A values from the regression line were used.

Empirical testing showed that the results obtained by simplified differential counting and the original Ebbesson and Tang method were of the same class size (relative deviations from the reference value with the original Ebbesson and Tang method were between -0.02 and +0.01, with the modified method between +0.02 and +0.07) (graph 2).

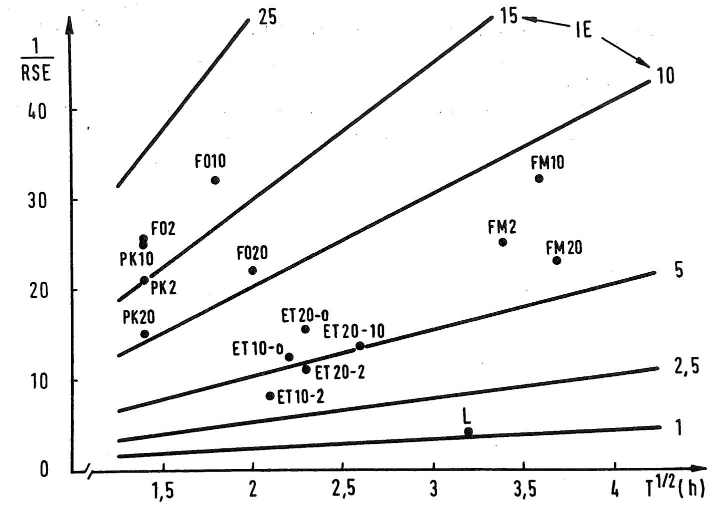


Graph 2. Relative deviations of the mouse thyroid gland epithelial nuclei numerical densities for different methods from the reference value of the Loud et al. method (L)

F02 F010 F020	original method of Floderus for three differently thick slices (2.380 µm, 8.580 µm, 18.860 µm)
FM2 FM10 FM20	modified method of Floderus with values for (D - 2h) obtained according to the Loud et al. method
PK2 PK10 PK20	method of Pajer and Kališnik with counting in one sharp optical section with the depth of focus 1.250 µm
ET20-10 ET20-2 ET10-2	method of Ebbesson and Tang for all possible combinations of differences for differently thick slices
ET20-o ET10-o	simplified method of Ebbesson and Tang with counting in two differently thick slices and in their optical section

Table 1. Numerical density (N_{Vn}) of the mouse thyroid gland epithelial nuclei, relative standard error (RSE), economy index (IE), relative deviation according to the results of the Loud et al. method ((X-L)/L), modified numerical density from theoretical N_A (N_{Vn}') and corresponding relative deviation ((X'-L)/L) according to different methods

Reference	Method	Section thickness or difference (μm)	N_{Vn} (X) ($\times 10^5 mm^{-3}$)	RSE	IE	$\frac{X-L}{L}$	N_{Vn}' (X') ($\times 10^5 mm^{-3}$)	$\frac{X'-L}{L}$
FLODERUS 1944	F02	2.380	4.011	0.039	18.718	+0.305	3.864	+0.257
	F010	8.580	3.593	0.031	17.669	+0.169	3.515	+0.143
	F020	18.860	3.325	0.045	11.168	+0.082	3.328	+0.083
FLODERUS MODIFIED	FM2	2.380	3.175	0.040	7.309	+0.033	3.075	+0.0003
	FM10	8.580	3.132	0.031	9.003	+0.019	3.074	0.000
	FM20	18.860	3.065	0.043	6.182	-0.003	3.074	0.000
PAJER-KALIŠNIK 1984	PK2	1.250	3.041	0.047	15.146	-0.011		
	PK10	1.250	3.245	0.040	17.315	+0.056	3.355	+0.091
	PK20	1.250	3.185	0.065	11.065	+0.036		
EBBESSON-TANG 1965	ET20-10	10.280	2.962	0.074	5.176	-0.036	3.074	0.000
	ET20-2	16.480	3.011	0.089	4.830	-0.020	3.074	0.000
	ET10-2	6.200	3.103	0.119	3.880	+0.009	3.074	0.000
EBBESSON-TANG MODIFIED	ET20-o	17.610	3.146	0.064	6.744	+0.023	3.074	0.000
	ET10-o	7.330	3.300	0.080	5.691	+0.073	3.074	0.000
LOUD ET AL. 1978	L	...	3.074	0.265	1.186	0.000	3.074	0.000



Graph 3. Relation between the reversed value of the relative standard error (1/RSE), time used for counting in hours ($T^{1/2}$) and economy index (IE) for the estimation of numerical densities according to different methods

But the economy index was higher with the modified method (with the original method it was between 3.9 and 5.2, with the modified between 5.7 and 6.7) (graph 3). The economy of our method is still greater because there is no need to prepare two differently thick section series.

CONCLUSION

On this basis we may recommend the simplified differential counting of particles in light microscopy as an alternative method with higher economy.

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