ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/257863743

Peculiarities of cytometrical methods of DNA content determination in the nucleus

Article *in* Cell and Tissue Biology · May 2012

DOI: 10.1134/S1990519X12030091

CITATIONS
2

READS
25

6 authors, including:

Andrei V. Chernyshev
Russian Academy of Sciences
82 PUBLICATIONS **958** CITATIONS
SEE PROFILE

Irina Palchikova
Russian Academy of Sciences
41 PUBLICATIONS **114** CITATIONS
SEE PROFILE

Valeri Maltsev
Russian Academy of Sciences
148 PUBLICATIONS **1,885** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

http://cyto.kinetics.nsc.ru/proposal_rus.html View project

Characterization of red blood cells by scanning flow cytometer View project

All content following this page was uploaded by Andrei V. Chernyshev on 16 October 2014.

The user has requested enhancement of the downloaded file.

ISSN 1990-519X, Cell and Tissue Biology, 2012, Vol. 6, No. 3, pp. 302–308. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © Yu.G. Pichugin, K.A. Semiyanov, A.V. Chernyshev, I.G. Palchikova, L.V. Omelyanchyuk, V.P. Maltsev, 2012, published in Tsitologiya, 2012, Vol. 54, No. 2, pp. 185–190.

Peculiarities of Cytometrical Methods of DNA Content Determination in the Nucleus

Yu. G. Pichugin[b, c], K. A. Semiyanov[d], A. V. Chernyshev[b, c], I. G. Palchikova[c, e], L. V. Omelyanchyuk[a, c, *], and V. P. Maltsev[b, c]

[a] *Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*

[b] *Institute of Chemical Kinetics and Combustion, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*

[c] *Novosibirsk University, Novosibirsk, Russia*

[d] *Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*

[e] *Technological Design Institute of Scientific Instrument Engineering, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*

**e-mail: ome@mcb.nsc.ru*

Received May 27, 2011

Abstract—This work has proposed a new theoretical approach to analysis of histograms of DNA content, which are obtained by the method of flow cytometry, in cells of *Drosophila melanogaster* imaginal discs. The precision of measurements of the DNA amount in G_1 and G_2(M) phases has been shown to be limited by precision of instrument tuning of zero of the flow cytometer. Use of the calculative zero of the flow cytometer and of dividing cells as standards of the DNA content is able to increase severalfold the precision of the DNA measurements in nuclei of the species. Comparative analysis of errors of various methods of measurement of the DNA content in cell nuclei is also performed. For methods of flow fluorescent cytometry, confocal scanning, and cytophotometry of the Feulgen-stained nuclei, it has been shown that, at present, the mean square errors of the DNA content measurements are within the interval of values considered acceptable for biological studies ($0.02 < CV < 0.06$).

Keywords: fluorescent flow cytometry, DNA content, cell cycle.

DOI: 10.1134/S1990519X12030091

INTRODUCTION

Cell nucleus DNA content determination is a widespread method of cytological studies the results of which are important for genome studies and practical medicine. The following approaches to nucleus DNA content determination are known: fluorescent flow cytometry, cytophotometry of fuchsin-stained nuclei, and confocal fluorescent scanning cytometry. The instrumental basis and preparation of cell material for these methods differ essentially. For flow cytometry, methods of tissue destruction with subsequent nuclei staining by fluorochrome are used. For fluorescent scanning cytometry, fixed and fluorochrome-stained whole organs are used. In the cytophotometry method, squash preparations fixed in acetic acid (or in other fixatives) of fixed organ preparations are used. The precision of measurements of the DNA amount depends on the procedure of preparation of samples, accuracy of use of instruments, and methods of experimental data mathematical treatment.

The method of fluorescent flow cytometry consists in passing a stream of fluid containing cells with stained nuclei (or nuclei proper) through a laser beam with subsequent detection of fluorescence from each cell. The flow cytometry data allow studying of cell cycle phases ratios (de la Cruz and Edgar, 2008). The cell cycle phases G_1 and G_2(M) are revealed on DNA histograms as peaks. The ratio of fluorescence levels of cell nuclei corresponding to the G_1 and G_2(M) peaks (upon use of external calibration) provides diploid and tetraploid DNA content for the studied organism. The most widespread approach to quantitative determination of the cell fraction in the phase S (DNA synthesis) is approximation of the interpeak interval of a DNA histogram by the rectangular model (Stal and Baldetorp, 1998).

Another method of nuclear DNA content determination is cytophotometry of Feulgen-stained nuclei. Cytophotometrical methods of DNA content determination have been considered before in a monograph by Agroskin and Papayan (1977); they include scanning, two- and polywave, photoemulsional, and integral methods. The most precise currently is the method of scanning in monochromatic light with use of a Vickers M8 densitometer (Rasch et al., 2004). Also known is the approach based on analysis of digital

images in monochromatic light had been developed for the medicine (Puech and Giroud, 1999). We have developed and are regularly using an integral cytophotometrical method based on analysis of digital images obtained with the aid of digital cameras (Omelyanchuk et al., 2010; Semeshin et al., 2011) with a widened dynamic range. Integration of the fluorescence intensities' values of stained nuclei on the confocal microscopy optical sections also can be used for DNA content determination. Data of such experiments for cell nuclei of Drosophila imaginal discs have been found in our works (Lebedeva et al., 2010, 2011).

In this work, a new approach is presented for analysis of histograms of the DNA content in cells of *Drosophila melanogaster* imaginal discs, and a comparative analysis of peculiarities of various methods of DNA content measurement is performed.

MATERIALS AND METHODS

Drosophila melanogaster cells of the wing and eye imaginal discs of the third instar larvae of Hikone A/W strain were isolated in Hanks' solution, dissociated by trypsin, stained with propidium iodide, and analyzed by flow cytometry according to Vindelov et al. (1983) and de la Cruz and Edgar (2008). CaliBriteTM3 microspheres with a medium size of 6 μm (Becton Dickinson, United States) were used as control. The cell distribution was analyzed on a FACSAria device (Becton Dickinson, United States). The experimental distributions were formed by fluorescence signals from polymer microspheres and stained nuclei.

Experimental histograms were treated by construction of the approximating function F(1). We used the known rectangle model (Stal, Baldetorp, 1998) with a slight improvement as the basis of approximation of the S phase data. The function of cell distribution according to the DNA amount consists of two δ-functions describing cells in G_1 and G_2(M) phases and of function rect composed of two Heaviside functions. The probability of finding a cell in a certain cell cycle phase (G_2(M), G_1, or S), under the condition that the probability of finding a cell in any phase is equal to 1, is proportional to the time T of the cell passage through the corresponding cell-cycle phase. The widening function describes the decorrelation between the DNA content and the measured intensity of fluorescence. This function contributes, for instance, to the nonstoichiometry of the dye binding to DNA, the nonideal optical scheme, etc. The approximating function F(1) represents the convolution of the DNA amount distribution and the widening function approximated by the normal distribution with dispersion σ^2:

$$F(I) = \int e^{-\frac{(I - I' + I_b)^2}{2(\sigma(I_b - I'))^2}} \times \left(\delta(I_{G1} - I' + I_b) + \frac{T_{G2}}{T_{G1}} \delta(2I_{G1} - I' + I_b) + \frac{T_S}{T_{G1}} H(I' - I_b - I_{G1}) H(-I' + I_b + 2I_{G1}) \right) dI',$$

where $\delta(x)$, $H(x)$ are the delta-function and Heaviside step function, respectively. In this expression, the following designations are used: I_{G1} is the fluorescence intensity corresponding to the mean value for cells in G_1 and T_{G2}/T_{G1} is the ratio of durations of phases G_2(M) and G_1, analogously to T_S/T_{G1} being the ratio of durations of phases S and G_1 and I_b being the shift of zero of the cytometer or intensity of the background signal (external illumination, residual irradiation at the excitation wavelength, balancing of the amplification tract, etc.). The sought parameters I_{G1}, σ, I_b, T_{G2}/T_{G1}, and T_S/T_{G1} (the two latter being characteristics of the cell cycle) were determined from comparison of experimental distributions and the distributions calculated with use of the model of global optimization in the DIRECT program (Jones et al., 1993) with determination of the errors of parameters calculated in the course of optimization (Strokotov et al., 2009).

RESULTS

The experimental distribution of fluorescence signals of CaliBriteTM3 microspheres, as expected, corresponds well to the normal distribution (not presented, since such distributions can be found in passports of cytometers). This justifies the above theoretical suggestion made for the widening function. We measured the distributions of the fluorescence intensities of the larval wing and eye imaginal discs cells. In doing this, we would like to test our DNA distribution model on cells at the stationary growth phase (the ratios of the cycle phases in this case remain constant). The fluorescence intensity distribution for the wing disc cells is presented in Fig. 1. The approximating distribution function was used to determine the cell cycle characteristics. The calculated maximal likelihood function with respect to the experimental distribution is presented in Fig. 1. The parameters of the function are presented in Table 1. Optimization parameters are given in the first column of this table. DIRECT was used for global optimization of parameters. In the second column, optimal values of the parameters are presented. In the third column, the mean square parameter deviations calculated by DIRECT are exposed, and in the fourth column the mean square deviation divided on the parameter value (coefficient of variation (CV)). A similar procedure

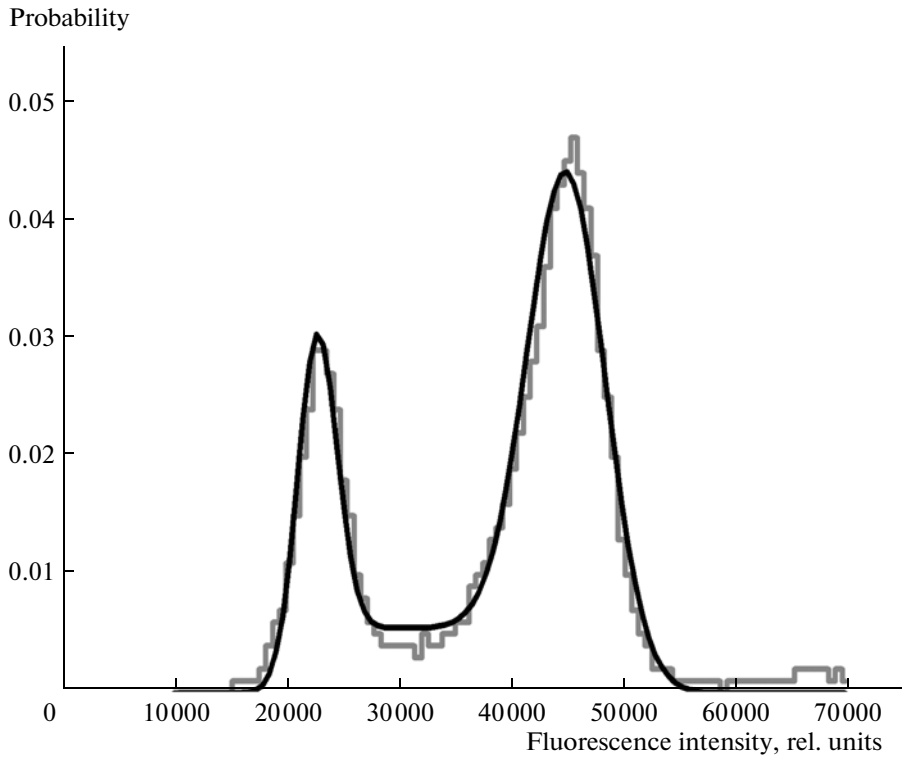

Fig. 1. Histograms of the DNA content for cells of the wing imaginal disc (gray curve) and its theoretical approximation (black curve).

was used in treating results of measurement of distribution of fluorescence intensity for eye disc cells. The experimental distribution and the function of maximal likelihood for these cells are presented in Fig. 2. The parameters of the approximating function are presented in Table 2. It should be noted that the coordinates of the fluorescence peaks corresponding to G_1 and G_2(M) cell phases on graphs of Figs. 1 and 2 are different. This is due to differences in the dye concentrations used for the eye and wing discs.

The standard deviation of the widening function (that characterizes the width of both peaks) can be used as the measure of precision of fluorescence intensity determination (proportional to the DNA amount) from a single cell. For a reliability level of 95%, the relative dispersion will exceed twice the mean square deviation σ of the widening function related to the mean value; i.e., $0.077 \times 2 = 0.54$ and $0.096 \times 2 = 0.192$ (Table 1).

Table 1. Parameters of the cell cycle model obtained from treatment of the distribution function for fluorescence intensity for cells of wing and ocular imaginal discs

Parameter	Wing disc			Eye disc		
	value	MSD[a]	MSD related to the value of the parameter	value	MSD	MSD related to the value of the parameter
The mean intensity of fluorescence of G_1 cells, I_{G1}	22640	50	0.002	29330	140	0.005
The MSD function of widening σ related to the mean intensity	0.0766	0.0015	0.019	0.096	0.002	0.021
T_{G2}/T_{G1}	3.0	0.1	0.03	0.73	0.03	0.035
T_S/T_{G1}	1.0	0.1	0.10	0.25	0.05	0.18
Shift of zero I_b	–540	60	0.11	760	30	0.04

Note: MSD—mean square deviation; MSD[a]—determinations of values of parameters indicated in the first column. Obtained by results of operation of DIRECT.

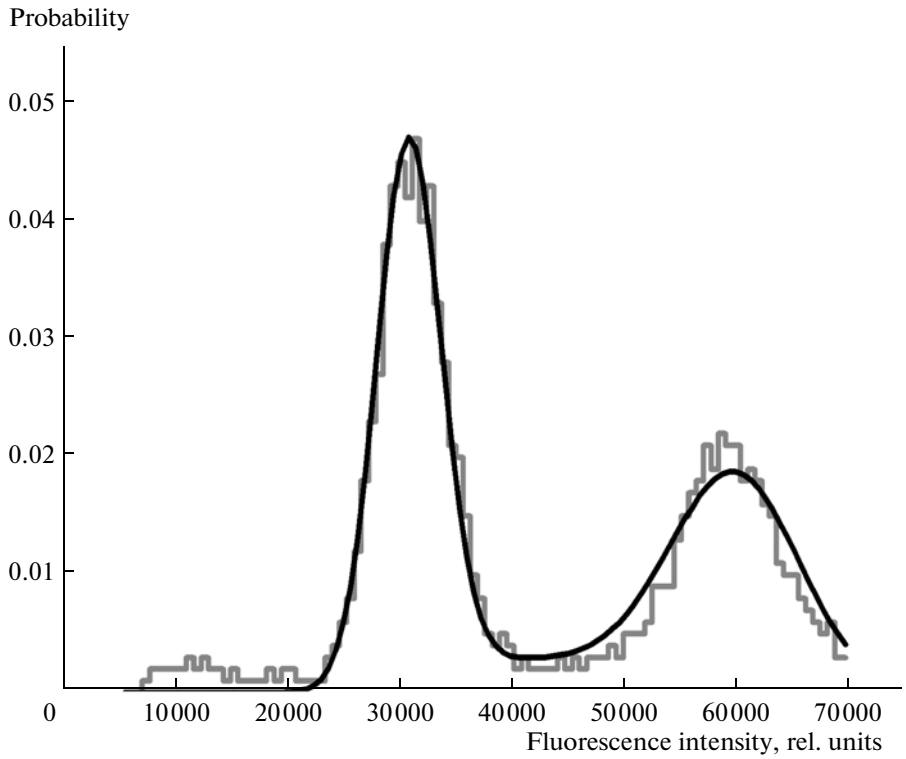

Fig. 2. Histograms of DNA content for cells of the eye imaginal disc (gray curve) and its theoretical approximation (black curve).

The internal structure of the DIRECT optimization algorithm (Strokotov et al., 2009) allows determining not only the value of the model optimized parameters, but also the confidence intervals for these values. The precision of determination of the G_1 peak position (the mean square deviation related to the mean value) determined by the algorithm DIRECT can be used as a measure of precision of all the other mean value of model parameters (fluorescence intensity, DNA amount). For the cells of wing and eye imaginal discs, this value turns out to be equal to 50/22640 = 0.002 and 40/29330 = 0.005, respectively (Table 1). The error of the mean value is inversely proportional to the square root of the sample volume. Since the sample volume is very high for the flow cytometer, the precision of the mean value of fluorescence intensity also is high.

An essential role should be noted of the parameter "shift of zero" I_b in determination of the error of the mean fluorescence intensity value. Whereas the calculated error of the mean value amounts to 50 units (Table 1), the shift of zero has the tenfold higher value (540). The parameter introduction allowed improving the approximation of the experimental data by the

Table 2. Precision of densitometrical measurements for blood cells obtained with the aid of various instruments

Cells	DNA, pg	Coefficient of variation		
		DIALUX+ Canon EOS 500D	Axoivert-200+ AxioCam MRc	DM-4000 Leica+ DFC420
Erythrocytes of *Gallus domesticus* (hen)	1.25	0.047	0.088	0.026
Erythrocytes of *Danio rerio* (fish)	1.68–1.80	0.0475	0.14	0.06
Leukocytes of *Homo sapiens* (human)	3.50	0.054	0.086	0.065
Erythrocytes of *Rana arvalis* (frog)	4.65–7.17	0.072	0.17	0.087

function F(1). The mean square deviation of the value I_b was equal to 0.02%. Consideration of the "zero shift" (that was introduced here) is able to increase the precision of determination of the nucleus DNA content by 2.4% as compared with the case in which such a procedure was not carried out.

The level of the mean cell fluorescence intensity error can be compared with the precision of the test measurements on Beckton Dickinson cytometers (reported in the cytometer passport). The variation coefficients are from 2 to 9%, depending on the test samples. For example, the measurements of the binding of the various markers to lymphocytes by FACSCanto and FACSCalibur cytometers (see Reference Manual Table E-1) show a difference between these measurements of the value of 2%. This value is an estimation of the precision of the cytometer measurements and, in our opinion, is determined by such factors as tuning of the instrument to zero and the level of autofluorescence.

DISCUSSION

There are many ways to prepare the samples for flow cytometry. There are approaches in which the analyzed cells are stained supravitally, as well as ones in which the tissue is fragmented up to the level of cells or even of nuclei (de la Cruz and Edgar, 2008). Since the proteolytic procedure of the tissue destruction to the level of individual nuclei (Vindelov et al., 1983) is considered the most favorable in terms of stoichiometry of binding of dye to DNA (Darzynkiewicz et al., 2010), we have chosen it for model experiments on determination of the precision of measurements achieved in flow cytometry. The procedure of blood cell preparations for densitometry of the Feulgen-stained nuclei was elaborated in our earlier works (Omelyanchuk et al., 2010; Semeshin et al., 2011). The critical phase of preparations is DNA hydrolysis, which, in our experiments, amounted to 30 min. By the present time, we have experimentally checked the optimum of this choice (which is recommended by many authors) and have shown that the maximum of the hydrolysis curve for the cell types used in the present work (as well as for many others) is indeed achieved under these conditions.

Quantitative interpretation of flow cytometry data needs the approximating function describing cell cycle phases' cell distribution and the peak widening due to peculiarities of recording of fluorescence signals from individual particles (Dean, 1991). There are approaches known in which the S phase is modulated by square spline or by a series of rectangles "widened" with aid of Gaussian with optimized parameters (Dean, 1991). Instead, we modeled the S phase using the rectangular model well recommended in medical practice (Stal, Baldetorp, 1998). The proposed model is described by the formula presented in Materials and Methods.

The approximating function F(I) for the experimental data can be interpreted in two ways. First, it describes the possible locations of the maxima values of the sample of experimental data, the height of the "shelf" between maxima, and the shape of the curves describing falls. The normal distribution characterizes the widening introduced both by the instrument and by the procedure of preparation of the samples. The use of mathematical formulation for the approximating function in the form of a convolution of a single widening function in the entire range of measurement corresponds to the theory of linear systems. Second, it is possible to interpret the components of the approximating function from the positions of the cell-cycle: δ-functions indicate the location of fluorescence intensity of the cell in G_1 and G_2(M) phases; the cell of the S phase is located within the limits of the function rect. The amplitude characteristics T_{G2}/T_{G1} and T_S/T_{G1} characterize the duration of the cell-cycle phases. Otherwise, the second interpretation coincides with the first one.

From the data of general genetics and genomics, it can be concluded that a genome size with a high degree of precision is the constant and the variation of the DNA content from cell to cell can be excluded from factors that can affect the widening function. Unlike the Feulgen staining, dying of DNA with fluorochromes is not stoichiometric; therefore, it is quite probable that the source of widening in this case may be due to the different spectrum of the chromatin states (at the different cell-cycle stages), which may differ by the fluorochrome binding level. Our measurements for calibration microspheres have shown that the ratio of the mean square deviation of the widening function and the mean microsphere distribution is $\sigma_1 = 0.05 \pm 0.001$. It is known that, whereas the statistical dispersion of instrument recordings is the result of the action of two random factors with the mean square deviations σ_1 and σ_2, the mean squar $\sigma = (\sigma_1^2 + \sigma_2^2)^{1/2}$ (Agroskin and Papayan, 1977, formula II.7). By suggesting that the microspheres used do not have essential dispersion for the fluorescent stain level, while their distribution for the fluorescence level characterizes only the error of instrument, on the basis of the data of Table 1, it is possible to calculate dispersion σ^2 connected with nonstoichiometry in our experiments. For the wing imaginal disc, $\sigma = 0.058$, while, for the eye disc, $\sigma = 0.081$. Thus, the widening, which is probably connected with the nonstoichiometry of the stain binding to DNA, can exceed the error value of the instrument itself. It also cannot be ruled out that the nonstoichiometry of the fluorochrome binding to DNA can be reflected in the ratio of DNA content in G_1 and G_2(M) cells (Darzynkiewicz et al., 2010).

In our earlier works (Omelyanchuk et al., 2010; Semeshin et al., 2011), using the densitometrical method, we carried out measurements of the DNA amounts in nuclei of nondividing blood cells of various

organisms (erythrocytes of *Gallus domesticus, Danio rerio*, and *Rana arvalis* and lymphocytes of *Homo sapiens*; Table 2). It can be seen that the variation coefficient in measurements of the DNA content in these experiments characterizes the precision of operation of various digital cameras rather well and allows choosing equipment providing sufficient precision (CV < 6%) (Darzynkiewicz et al., 2010). The minimal experimental dispersion was obtained for *Gallus domesticus* with the use of a DM-4000 microscope (Leica) and a DFC420 digital camera and are equal to 0.026.

Densitometrical measurements of the Feulgen-stained nuclei on a Vickers M8 scanning densitometer (Rasch and Wyngaard, 2001; Rasch et al., 2004, 2008) are a commonly accepted method of determination of DNA content. The authors use SEM (the standard error of the mean) related to the DNA content value as the measure reflecting variation of the DNA content in cells of one species. According to this criterion, the most precise measurements on this instrument (the criterion values of 0.07) were obtained for nuclei of adult individuals *Mesocyclops edax* (Table 1; Rasch et al., 2008), where the DNA content, SEM, and number of measurements N amounted to 28.6, 0.219, and 25, respectively. Since the variation in our experiments was estimated as the mean square deviation from the mean:

$$ST = \sqrt{\frac{\sum (x - x_0)^2}{n - 1}},$$

the used mean square deviation in the considered experiment of these authors is equal to $\mathrm{SEM}\sqrt{n}$; i.e., $0.219 \times 5 = 1.095$, while the relative error, hence, is $1.095/28.6 = 0.038$ (3.8%). Thus, use of a Vickers M8 scanner does not provide essential advantages with respect to the precision of measurements, as an even higher precision (2.6%) can be achieved in our experiments.

Earlier, we developed a method of determination of the amount of DNA in cell nuclei on the basis of summation of the fluorescence signal of the fluorochrome-stained nuclei in confocal microscopy optical sections (Lebedeva et al., 2010). The results of measurements of the DNA content in metaphase and anaphase chromosome groups of imaginal disc cells are given in Lebedeva et al., 2010, Fig. 1. We showed that the variation coefficient both for metaphases and for anaphase does not exceed 10% of the value of DNA content. In another work (Lebedeva et al., 2011), we also measured the DNA content in metaphase and anaphase groups of Drosophila imaginal discs with the use of confocal scanning. In this experiment, a special selection was carried out of the measured anaphasic and metaphasic groups for low background fluorescence (in our method, the background fluorescence value is subtracted from levels of the chromosome signal). This allowed obtaining the variation coefficients of DNA content for metaphase groups (4%) and for anaphase groups (2%).

The precision of the stained cell fluorescence level determination in a flow cytometer, according to the passport data of such instruments, is about 2%. The best precision achieved in our densitometrical experiments was equal to 2.6%. This corresponds well with the practice. Our minimal obtained error of DNA content of the cell nuclei of Drosophila imaginal discs also is about 2%. Thus, the error values of the DNA content measurements by different standard methods are close.

At the same time, the data presented here show that, under optimal conditions, the error of fluorescent flow cytometry measurements may be essentially lower than errors of other methods. Such precision of flow cytometry owes to the large number of the cells that can be used for measurements, as well as to a new way of recording of the instrument zero. At the same time, the flow cytometry method is not optimal in cases in which the amount of cell material is not high (biopsy or study of animals of small size), as well as in cases in which identification of studied cells is achieved more easily with the aid of cytological screening than with the aid of specific antibodies (cells of the fetal line or polyploid cells of Diptera salivary glands).

Our analysis has shown that the greatest contribution to the error of DNA content determination in flow cytometry is made by the "shift of zero" detected in our experiments. Use of the method is able to reduce errors in the database (http://www.genome-size.com) that provides biological standards in measurements of the size of genomes. As follows from the presented data, the most precise determination of the DNA content can be achieved when using dividing cells, i.e., in cases in which there are two peaks on distribution of cells by fluorescence level that allow determining the "shift of zero" value.

ACNOWLEDGMENTS

This work was supported by the Integrational Projects of the Siberian Branch of the Russian Academy of Sciences (projects 37 and 7); the Russian Foundation for Basic Research (project no. 09-08-00651-a), and federal targeted programs of the Ministry of Education and Science (projects nos. R422, R2497, R1039, and 14.740.11.0729); the Presidium of the Russian Academy of Sciences (project no. 2009-27-15); and the President of the Russian Federation ("Scientific Schools," project no. 5387.2010.4).

REFERENCES

Agroskin, L.S. and Papayan, T.V., *Tsitofotometriya* (Cytophotometry), Leningrad: Nauka, 1977.

Darzynkiewicz, Z., Halicka, H.D., and Zhao, H., Analysis of Cellular DNA Content by Flow and Laser Scanning

Cytometry, *Adv. Exp. Med. Biol.*, 2010, vol. 676, pp. 137–147.

De la Cruz, A.F. and Edgar, B.A., Flow Cytometric Analysis of Drosophila Cells, *Methods Mol. Biol.,* 2008, vol. 420, pp. 373–389.

Dean, P.N., Data Processing, in *Flow Cytometry and Sorting*, New York: Wiley-Liss, 1991, pp. 415–444.

Jones, D.R., Pertunnen, C.D., and Stuckman, B.E., Lipschitzian Optimization without the Lipschitz Constant, *J. Optimiz. Theor. Applic.*, 1993, vol. 79, pp. 157–181.

Lebedeva, L.I., Akhmametyeva, E.M., and Omelyanchuk, L.V., Dynamics of the Spatial Organization of the Chromosome Set in Cells of *Drosophila melanogaste* Imaginal Disks Normally and under the Action of the Tumor-Inducing Mutation *Merlin, Russ. J. Genet.*, 2010, vol. 46, no. 2, pp. 157–163.

Lebedeva, L.I., Dubatolova, T.D., and Omelyanchuk, L.V., Fluorometric Identification of Chromatin-Packing Level beyond Resolution of Light Microscopy, *Cell. Tiss. Biol.*, 2011, vol. 5, no. 1, pp. 98–102.

Omelyanchuk, L.V., Semeshin, V.F., Alekseeva, A.L., Pal'chikova, I.G., and Zhimulev, I.F., Integral Method for Measuring the Quantity of Cellular DNA Content by Digital Microphotography, *Cell. Tiss. Biol.*, 2010, vol. 4, no. 3, pp. 305–308.

Puech, M. and Giroud, F., Standardisation of DNA Quantitation by Image Analysis: Quality Control of Instrumentation, *Cytometry*, 1999, vol. 36, pp. 11–17.

Rasch, E.M. and Wyngaard, G.A., Evidence for Endoreduplication: Germ Cell DNA Levels Prior to Chromatin Diminution in *Mesocyclops edax*, *J. Histochem. Cytochem.*, 2001, vol. 49, pp. 795–796.

Rasch, E.M., Lee, C.E., and Wyngaard, G.A., DNA-Feulgen Cytophotometric Determination of Genome Size for the Freshwater-Invading Copepod *Eurytemora affinis*, *Genome*, 2004, vol. 47, pp. 559–564.

Rasch, E.M., Wyngaard, G.A., and Connelly, B.A., Heterochromatin Endoreduplication Prior to Gametogenesis and Chromatin Diminution during Early Embryogenesis in *Mesocyclops edax* (Copepoda: Crustacea), *J. Morph.*, 2008, vol. 269, pp. 387–397.

Semeshin, V.F., Omelyanchuk, L.V., Alekseeva, A.L., Ivankina, E.A., Sheveleva, N.G., and Zhimulev, I.F., DNA Content in Nuclei of *Cyclops kolensis* and *Cyclops insignis* (Crustacea, Copepoda), *Cell. Tiss. Biol.*, 2011, vol. 5, no. 3, pp. 300–304.

Stal, O. and Baldetorp, B., S-Phase Fraction Assessed by a Variant of the Rectangular Model Adapted to the Flow-Cytometric DNA Histogram Profile, *Cytometry*, 1998, vol. 33, pp. 487–491.

Strokotov, D.I., Yurkin, M.A., Gilev, K.V., van, Bockstaele, D.R., Hoekstra, A.G., Rubtsov, N.B., and Maltsev, V.P., Is there a Difference between T- and B-Lymphocyte Morphology?, *J. Biomed. Opt.*, 2009, vol. 14, p. 064036.

Vindelov, L.L., Christensen, I.J., and Nissen, N.I., A Detergent-Tripsin Method for the Preparation of Nuclei for Cytometric DNA Analysis, *Cytometry*, 1983, vol. 3, pp. 323–327.

View publication stats