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STABILITY, TILTING, COUP, DELAY AND SWITCHING FOR DISTRIBUTIONS OF NEUTROPHILS FLUORESCENCE IN MEDICAL DIAGNOSTICS

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Abstract. Communication contains the immunology data treatment. New nonlinear methods of immunofluorescence statistical analysis of peripheral blood neutrophils have been developed. We used technology of respiratory burst reaction of DNA fluorescence in the neutrophils cells nuclei due to oxidative activity. The histograms of photon count statistics of the radiant neutrophils populations' in flow cytometry experiments are considered. Distributions of the fluorescence flashes frequency as functions of the fluorescence intensity are analyzed. Statistic peculiarities of histograms set allow divide all histograms into three classes. The classification is based on three different types of smoothing and long-range scale averaged the immunofluorescence distributions with maximum, minimum and monotonic change. First histograms group belongs to healthy donors. Two other groups belong to donors with inflammatory and autoimmune diseases. Some of the illnesses are not diagnosed by standards biochemical methods. Dynamics of a change in the immunofluorescence distributions of healthy and sick people in the process of medical treatment are examined; we observed the right and the contrary transitions or coup of the averaged distributions in histograms. Peculiarities of immunofluorescence for women in pregnant period are classified. Health or illness criteria are connected with statistics features of immunofluorescence histograms. Neutrophils populations' fluorescence presents the sensitive clear indicator of health status.

1. Introduction

Flow cytometry is one of the main methods of modern analytical biology [1, 2]. The method is based on registrations of rather big collections of photon count statistics for fluorescence cells and DNA. This technological process is performed at rates of thousands of cells per second. There are many applications of flow cytometry to immunology, cell cycle kinetics, cell kinetics, genetics, molecular biology, microbiology, parasitology, bioterrorism, biological oceanology, husbandry and others.

We used flow cytometry for immunology. In these experiments collective spectra of gene regulation, gene expression and their correlations are observed in the living population cells in free life in natural conditions without especial adaptations. Unfortunately we do not know how to extract information from these experiments. Non-Gaussian fast growth of central moments for fluorescence intensity fluctuations [3-6] causes strong difficulties for strict, precise, quantitative interpretations of the experimental results. The statistical instabilities of local immunofluorescence intensity distributions provide the main reason of difficulties. Average value of intensity is smallest than dispersion, asymmetry and others higher statistical moments of intensity fluctuations. We observe the universality of strong exponential growth for

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central moments of immunofluorescence intensity fluctuations in any fluorescence conditions and various cells [3-6]. Rapid exponential increase of the statistical moments forms the basic question about the methods of the correct description of very informative neutrophils fluorescence distributions. Now we present examples for one of the opportunity approaches, which interconnected with simplest description of averaged immunofluorescence distributions. Other nonlinear approaches to classification of immunofluorescence for health statuses diagnostics were discussed in the [3, 5, 8, 9].

Distributions of the fluorescence flashes frequency as functions of the fluorescence intensity are analyzed. The large-scale averaging of the initial histograms gives the simplification and smoothing of statistical flashes distribution for healthy and unhealthy donors. The statistical signs of the qualities differences of averaged distributions allow divide all available immunofluorescence histograms into three large groups. First group corresponds to the autoimmune or oncology diseases. Second group corresponds to the inflammatory diseases. Third group corresponds to the healthy donors. The histograms of immunofluorescence of the pregnant women have their specific character and special features of classification, which are examined below.

The inflammatory processes of different nature and the concealed infections must be discovered as early as possible, for the timely treatment. The part of these inflammations is not diagnosed by standard methods and, therefore, immunofluorescence occupies special position between traditional medical observations and registration by neutrophils of the clinical reality.

2. Experiences

The cytometrical histograms of the blood immunofluorescence in Fig.1 contain unique information about the neutrophils' populations, which reflect the types and dynamics of pathology processes. These histograms are changeable. Their special features depend on health status. Detailed comparison of histograms in Fig. 1 and their disagreements represent the indicators of disease, frequently outwardly, or according to the data of other analyses of full health of the patient. Differences of histograms have diverse and statistical nature and, at present, they are not described by the clear qualitative and quantitative criteria, to determination of which is oriented this work.

Preliminary experiments have shown the usage opportunities of proposed approach for the solution of various medical problems. We observed the inflammatory processes after different surgical interventions, appearance and flow of autoimmune processes with the post infection complications, neurological and heart complications after diphtheria, chronic inflammation with rheumatoid arthritis, inflammatory reaction with bronchial asthma, course of inflammatory events with the myocardial infarctions, inflammations with system lupus erythematosis, hepatitis, peritonitis, purulent appendicitis, pneumonias, cardiovascular, oncology and other diseases, connected with the oxidants or other reasons for the disturbance of oxidizing metabolism.

The spectrum of medical applications, possibly, is considerably wider, because of the wide prevalence of oxidative abnormality as the reason of various illnesses and the aging. Now we represent some examples of health statuses registration for diagnostics of diseases and monitoring of patient conditions in medical treatment.

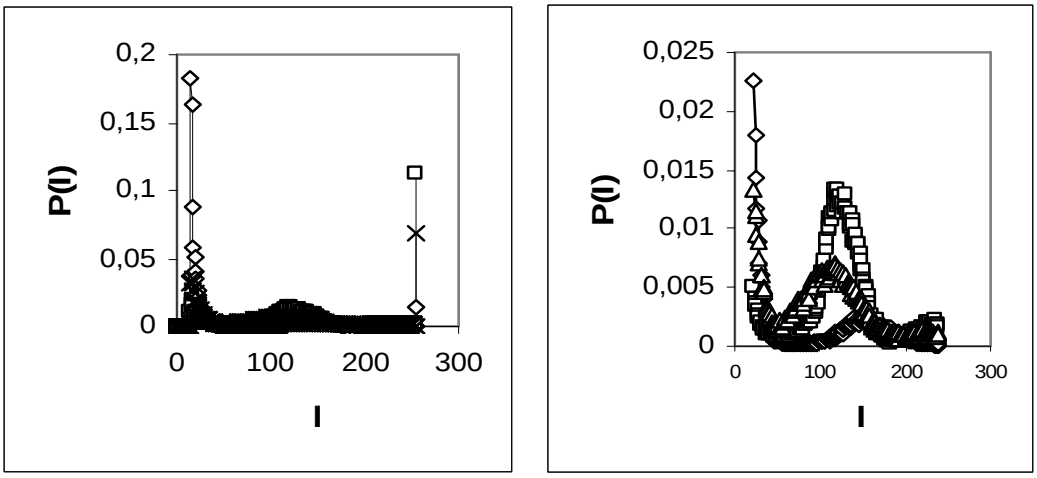

Fig. 1. Dependence of normalized spontaneous fluorescence flashes number P (I) on their intensity I (left) and for more clearest only central part of histogram (right). The area under the final histograms of P (I) normalized to unit; rhomb points correspond to bronchial asthma. Total number of flashes is N_0 = 76623; quadrate points correspond to the healthy donor. Common number of flashes is N_0 = 40109 ; triangle points correspond to the oncology disease. Common number of flashes is N_0 = 40752 .

3. Experiments

We present the experimental data treatment of highly sensitive quantitative method for registration the inflammatory reactions of organism founded on collective features of cytofluorescence. The method based on the flow cytometrical measurement of the capability of the peripheral blood neutrophils for the reaction of respiratory burst or oxidizing explosion [4, 7]. The volume of peripheral blood is V = (1 ... 2) ml. We used hydroethidine addition with concentration 150 μg /ml for fluorescence initiation. Hydroethidine easily penetrates into cell nuclei. Through intracellular oxidation by oxygen radicals, hydroethidine is converted into ethidium bromide. Ethidium bromide binds with fragments of nuclear DNA and has strong red fluorescence excited by TEM_{00} mode radiation of Argon laser light at 488 nm wavelengths. Fluorescence is registered by flow cytometry technique [1]. The fluorescence is proportional to the ability of neutrophils to produce the active forms of oxygen.

Detail description of biochemical and other procedures are given in [3, 4]. Results are represented in the form the histograms of immunofluorescence in the variables the number flash-intensity of flashes for 256 channels of intensity measurements. The average number of flashes is $N \sim 10^4 \ldots 10^5$, in the range of intensities I from 10….14 to 256 dimensionless units, which correspond to the numbers of the channels l of intensity measurements. Low boundary of intensities range usually constitutes 12 and in the general case is variable. The fluorescence with intensity less than lowest boundary is not considered. Lowest boundary of intensities limit is determined by the stochastic interception of background noise. The background noise is cut off. Detail description is indicated in [3, 8].

Three typical examples of histograms are shown in Fig. 1. DNA of neutrophils nuclei absorbs a dye. The heterogeneous fluorescence of all chromosomes in the cells reflects simultaneously the genetic special, individual features and immune response to the pathogenic actions due to oxidative activity of DNA. These features are illustrated by histograms individualities in Fig. 1. We observe the increase of common number of flashes N_0 for transition from a healthy donor to a donor with autoimmune and to donors with inflammation diseases. Total number of flashes N_0 has shown only one of essential signs of diseases.

4. Exponencial increase the moments of intensity fluctuations

We use the centered random variables and their centered moments for all procedures on any step. This approach is necessarily for the exception of the uncontrollable and systematic errors, instabilities of algorithmic procedures and corresponding drift of averages. Let us consider the relative deflections of fluorescence flashes number from their average level

$$n = (P_l - < P >) = < P > \left(\frac{N}{< N >} - 1 \right), \qquad (1)$$

where $P_l = N(l)/N_0$ is the probability distribution density of the flashes number, l is the number of channels, $l = 1,2,\ldots 256$; N_0 is the total number of flashes; $< P > = (I_{max} - I_{min})^{-1}$ is the mean probability value; $N = N(l)$ is the number of flashes with the assigned intensity $I = l$, for the dimensionless intensity I coincides with the number of channels l; $< N > = N_0 \, x < P >$ is the average value of flashes number; symbol <…> denotes statistical average of the fluorescence fluctuations for all 256 channels of intensity measurement. The mean probability value $< P > = (I_{max} - I_{min})^{-1}$ is equal to 0.03906 for 256 channels of intensity measurement.

The relative deflection of the intensities I from the average value of intensity level $< I >$ for channel l, where $l = 1,2 \ldots 256$ is

$$i(l) = \frac{P_l \cdot l}{< I >} - 1 \ , \qquad \text{at that} \quad < I > = \sum_{l=1}^{256} P_l \cdot l \qquad (2)$$

The central moment $< i^k >$ of order of k for the fluctuation $i(l)$ is designated as $M(i,k)$, i.e.

$$< i^k > = \sum_{l=1}^{256} P_l \cdot i^k = M(i,k) \qquad (3)$$

Let us note the universality of exponential growth for the moments $M(i,k)$ with $k > 2$ (Fig. 2). The same universal distributions are observed for any types of fluorescence for any cells such as neutrophils, lymphocytes and erythrocytes with very high authenticity, with correlation index of $R^2 > 0.9$. The indices of exponential curves are different for various groups of diseases. The indices change for one and the same patient in the process and development of disease and its treatment. Thus is possible both diagnostics and monitoring of disease with the exponentially high sensitivity. In Fig. 2 the ratio of upper (quadrate) to lower (rhomb) distribution of $M(i,k)_{upper} / M(i,k)_{lower}$ composes the value $e^{17.4}$ for $k = 16$. This example characterizes the reproducible and systematic difference between criteria of health and illness.

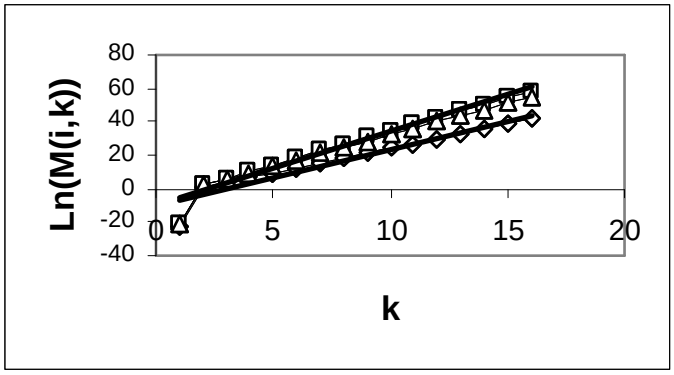

Fig. 2. Dependence of logarithmic distributions of intensity fluctuations for central moments of order of k. Data in Fig. 2 correspond to Fig. 1 data: rhomb points correspond to bronchial asthma; quadrate points correspond to the healthy donor; triangle points correspond to the oncology disease.

5. Discription by means of averaged histograms

The cytometrical histograms of immunofluorescence contain unique information about the neutrophils populations, which reflect the types and dynamics of the pathology processes. The deviations, connected with the inflammatory reaction for unhealthy donors, are visible, as it is shown in Fig. 1. Unfortunately, these differences have a statistical nature. Differences between the averaged histograms for healthy, inflammatory and autoimmune reaction are more noticeable.

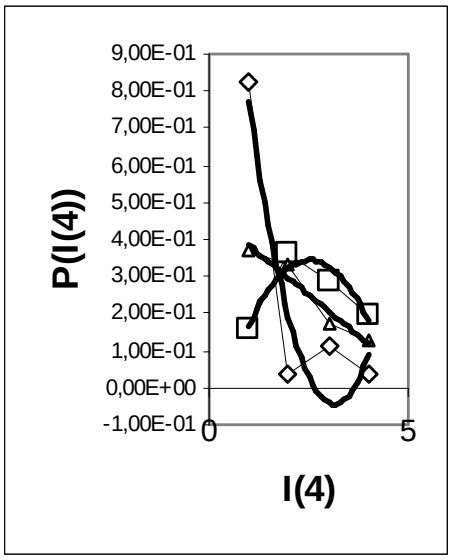

Fig. 3. Parabolic approximations of averaged probability density distributions P(64) are presented as the functions of smoothing fluorescence intensity I (4) [6, 8, 9]. The area under the final histograms normalized to unit; rhomb points correspond to bronchial asthma; quadrate points correspond to a healthy donor; triangle points correspond to an oncology disease.

Let us consider averaged histograms [6, 8, and 9]. Let us give the brief illustration of three different immunofluorescence groups division based on simplest criteria related to the averaged histograms for the initial distributions in Fig. 1 [6, 8, and 9]. We observe illustration of simpler the immunofluorescence diagnostics of health statuses in Fig. 3. Averaged scale of I is determined by value $I_{in}^{1/4}$ of the initial fluorescence intensity I with 256 channels of measurements. Averaged probability density distributions $P(64)$ correspond to smoothing long-range scale $I_{in}^{1/4}$ for 4 channels of long-range scale intensity. The solid lines in Fig. 3 are smoothing parabolic approximation of $P(64)$ upon the initial distributions in Fig. 1 [6, 8, 9] for smoothing fluorescence intensity $I(4)$.

Larger value of flashes number N_0 indicates higher oxidant activity DNA, which is associated with different diseases. Quality criterion of health state is described by the character of curve of the averaged statistical distributions in Fig. 3 [6, 8, 9]. Concave parabola (repeller) in Fig. 3 relates to the averaged histogram of donor with inflammations [6, 8, 9]; in our case it is asthma. Convex parabola (attractor) in Fig. 3 relates to the averaged histogram of healthy donor [6, 8, 9]. We observe qualitative alteration of type of probability distribution. Transition from a healthy to an unhealthy human is observed when the ratios of min/max upset. This upsetting corresponds to transcritical bifurcation in averaged intensity space [6, 8, 9].The statistical attractor appearance in Fig. 3 for a healthy human is differing from the statistical repeller distribution in Fig. 3, which is typical for inflammations. The intermediate case of neutral stability is typical for the autoimmune and oncology diseases as in Fig .3. Corresponding to Fig.

3 distributions of central moments for intensity fluctuations are shown in Fig. 2. Here we used the statistical determination of the attractor as the more probable state, when the distribution function has a maximum, as in Fig. 3 [6, 8, 9]**.**

Change of a stability position of $I_{extr}^{1/4}$ or transcritical bifurcation corresponds to transition from health (attractor) to unhealthy (repeller) [6, 8, 9]. Bifurcation describes the healthy-unhealthy transition and vice versa. Bifurcation is accompanied by a lag time.

Other approaches to the immunofluorescence histograms classification [3, 6, 8, 9] ensure the same three types of the histograms division in dependence on health statuses; such as inflammations or oncology (autoimmune) diseases and a healthy human.

6. Fluorescence changeability of helthy donor states

Let us examine three examples of changeability of immunofluorescence histograms for a healthy donor. Noticeable variations of initial histograms in Figs. 4a and b give practically identical final quadratic approximations in Fig. 4c. Thus it indicates the stability, with insignificant and unessential changes, i.e. the health status of the observed healthy donor.

In Fig. 5 the ratio of upper (quadrate) to lower (rhomb) distribution of $M(i,k)_{upper} / M(i,k)_{lower}$ composes the value $e^{7.6272} = 2953.3$ for $k = 16$. This example characterizes changeability, the reproducible and systematic differences between registrations of health status conditions for immunoflorescence histograms of a good health man at different time.

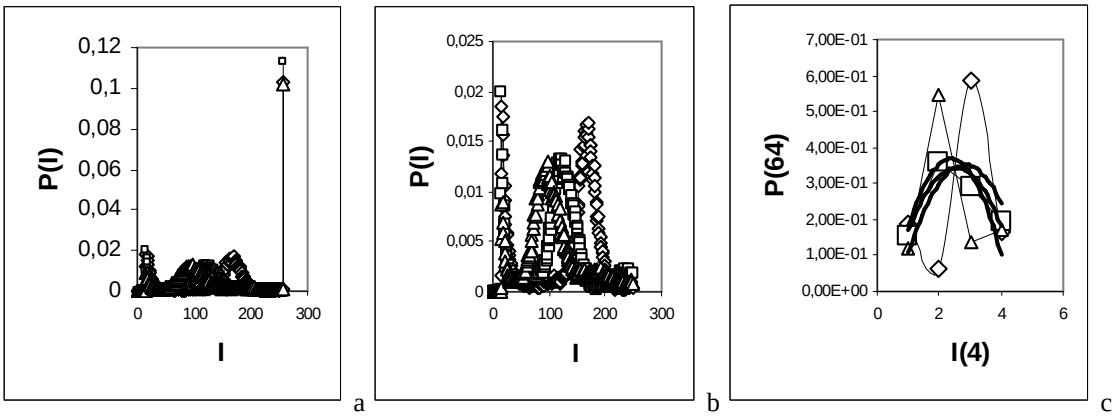

Fig. 4. Immunofluorescence histograms: initial (a), only central part of histograms (b) and averaged parabolic approximations of initial histograms (c) for one, invariably healthy, donor at different times. Rhomb points correspond to the total flashes number $N_0 = 30832$, analysis time is 19 July (first year); triangle points correspond to the total flashes number $N_0 = 38758$, analysis time is 11 July (next year); square points correspond to the total flashes number $N_0 = 40109$, the date of analysis is 03 June, before 11 July. Last examples (for square symbol) are used here and elsewhere in [4, 5, 6, 8, 9] as the model of immunofluorescence histogram for a healthy donor.

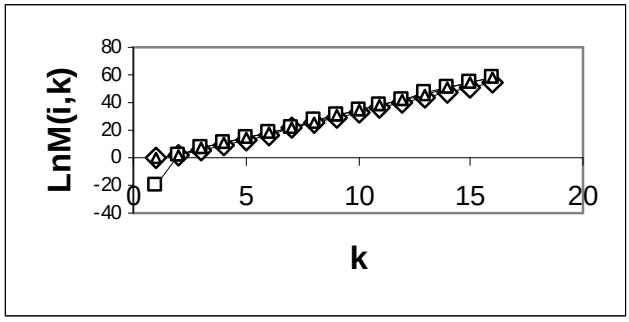

Fig. 5. Dependence of logarithmic distributions of intensity fluctuations for central moments of order of k. Data in Fig. 5 correspond to Fig.1 data: rhomb points correspond to 19 July; quadrate points correspond to 03 June next year; triangle points correspond to 11 July, after 03 June.

7. Fluorescence changeability of oncology in medical treatment

Let us examine two examples the changeability of immunofluorescence histograms for oncology due to medical treatment (Fig. 6). Detail description of this example contains in [8]. In Fig. 6d the ratio of upper (quadrate) to lower (triangle) distribution of $M(i,k)_{upper} / M(i,k)_{lower}$ composes the value $e^{7.6272} = 3.6 \cdot 10^8$ for $k = 16$. This example characterizes the reproducible and systematic differences between the health status registrations by immunoflorescence histograms for oncology disease before and after medical treatment.

8. Fluorescence changeability of pregnant women

Let us examine two examples the changeability of immunofluorescence histograms for pregnant women (Fig. 7). It is worth noting that in Fig. 7d the ratio of upper (quadrate) to lower (triangle) distribution of $M(i,k)_{upper} / M(i,k)_{lower}$ composes the value $e^{46.2688} = 1.24 \cdot 10^{20}$ for $k = 16$. This example characterizes the systematic differences between the health status registrations by immunoflorescence histograms for unhealthy (rhomb) and healthy (triangle) pregnant women.

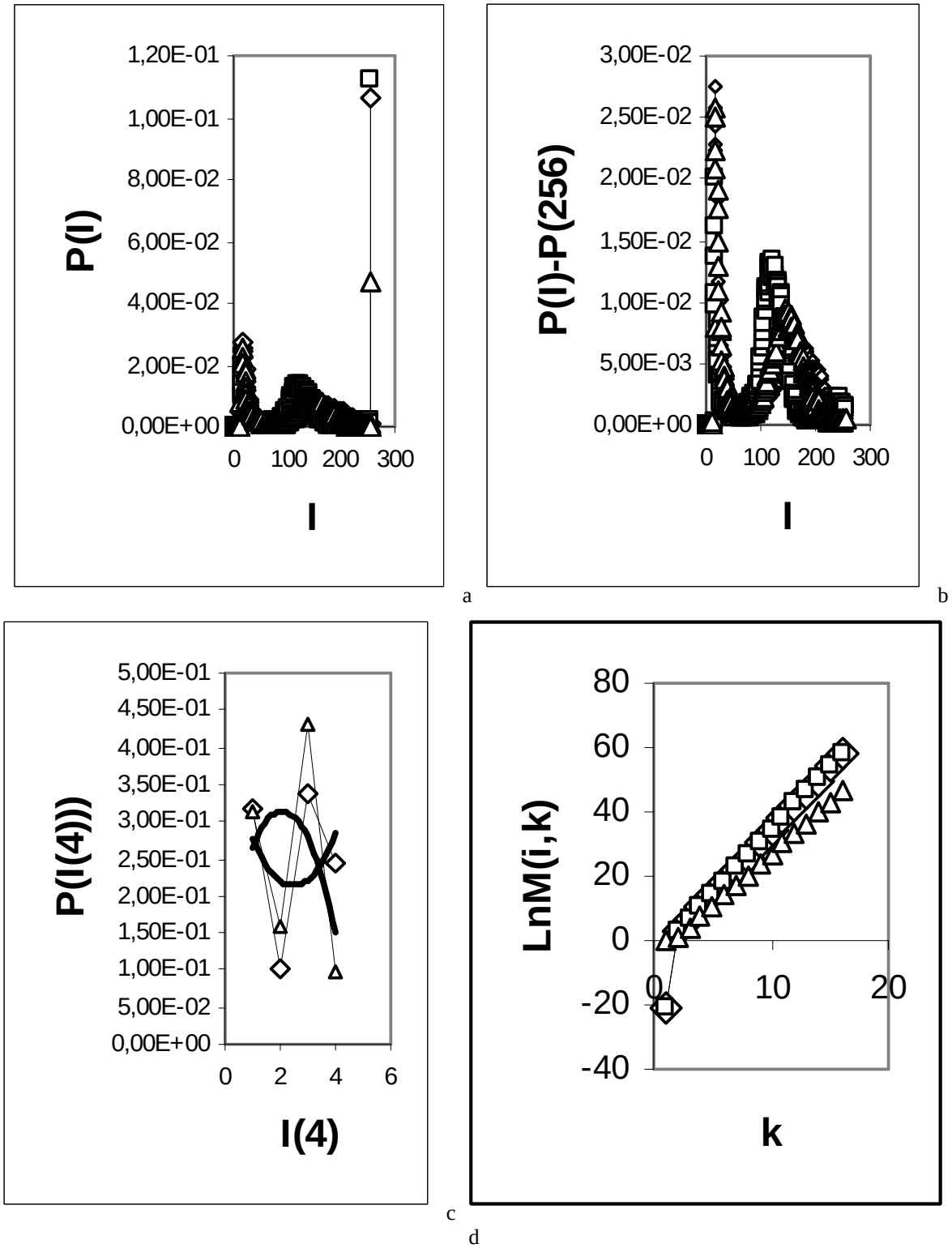

Fig. 6. Immunofluorescence histograms: initial (a), only central part of histograms (b) and averaged parabolic approximations of initial histograms (c). Rhomb points correspond to oncology, the total flash number $N_0 = 46000$, 21 March; triangle points correspond to oncology after positive medical treatment, total flash number $N_0 = 45142$, 11 July; square points correspond to a healthy man in Fig. 1, the total flash number $N_0 = 40109$; (d) Dependence of logarithmic distributions of intensity fluctuations for central moments of order of k.

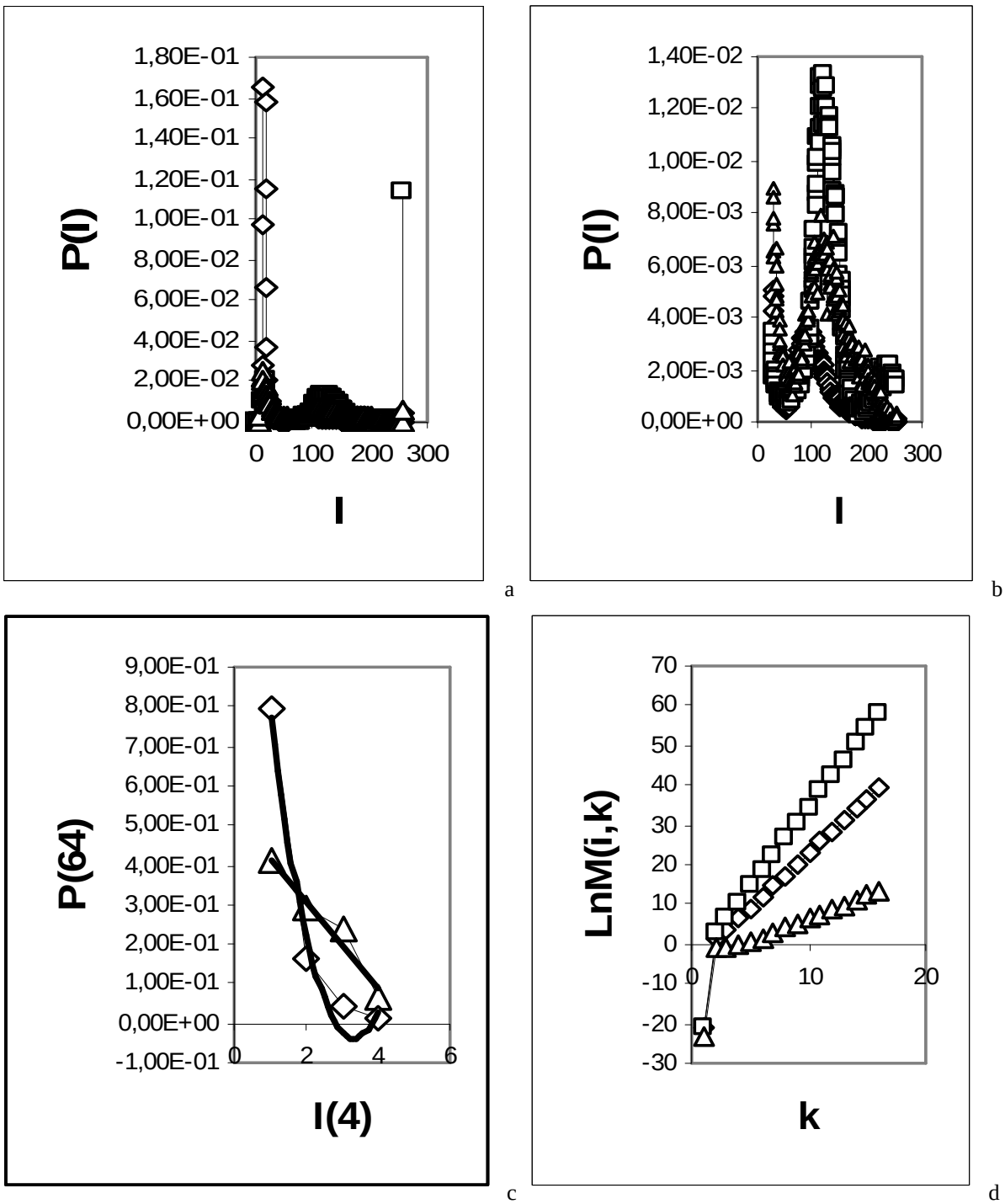

Fig. 7. Immunofluorescence histograms: initial (a), only central part of histograms (b) and averaged parabolic approximations of initial histograms (c). Rhomb points correspond to the first pregnant woman, the total flash number $N_0 = 104405$; triangle points correspond to the second pregnant woman, the total flash number $N_0 = 17500$; square points correspond to a healthy man in Fig. 1, the total flash number $N_0 = 40109$; (d) Dependence of logarithmic distributions of intensity fluctuations for central moments of order of k .

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