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Complex Networks, Fractals and Topology Trends for Oxidative Activity of DNA in Cells for Populations of Fluorescing Neutrophils in Medical Diagnostics

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Abstract

A novel nonlinear statistical method of immunofluorescence data analysis is presented. The data of DNA fluorescence due to oxidative activity in neutrophils nuclei of peripheral blood is analyzed. Histograms of photon counts statistics are generated using flow cytometry method. The histograms represent the distributions of fluorescence flash frequency as functions of intensity for large populations $10^4-10^5$ of fluorescing cells. We have shown that these experiments present 3D-correlations of oxidative activity of DNA for full chromosomes set in cells with spatial resolution of measurements is about few nanometers in the flow direction the jet of blood. Detailed analysis showed that large-scale correlations in oxidative activity of DNA in cells are described as networks of small-worlds (complex systems with logarithmic scaling) with self own small-world networks for given donor at given time for all states of health. We observed changes in fractal networks of oxidative activity of DNA in neutrophils in vivo and during medical treatments for classification and diagnostics of pathologies for wide spectra of diseases. Our approach based on analysis of changes topology of networks (fractal dimension) at variation the scales of networks. We produce the general estimation of health status of a given donor in a form of yes/no of answers (healthy/sick) in the dependence on the sign of plus/minus in the trends change of fractal dimensions due to decreasing the scale of nets. We had noted the increasing biodiversity of neutrophils and stochastic (Brownian) character of intercellular correlations of different neutrophils in the blood of healthy donor. In the blood of sick people we observed the deterministic cell-cell correlations of neutrophils and decreasing their biodiversity.

1. Introduction

We present a nonlinear statistical analysis of immunofluorescence data for DNA oxidative activity in populations of neutrophils. Immunofluorescence histograms are obtained using flow cytometry method

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DNA oxidative activity, 3D DNA correlations in cells, immunofluorescence networks, intercellular nets
It is shown that DNA fluorescence is triggered by biochemical reactions of respiratory oxidative burst[2,3]. Flow cytometry histograms typically represent a fluorescence flashes frequency as the functions of fluorescence intensity for populations of thousands of cells. Immune system interconnected with different networks of diverse populations of neutrophils in the human body such as networks of metabolic, geographical, ecological, information, genetics, migration and other processes in the blood. DNA in neutrophils’ nuclei hasn’t uniform homogeneous fluorescence. The fragments of nuclear and mitochondrial DNA with oxidants determine the basic place for localization of the fluorescent dye, its distributions, intensity and statistics of fluorescence. These fragments may belong to the coding and non-coding portions of DNA. The heterogeneous fluorescence of chromosomes reflects genetic special, individual features and immune response to the pathogenic actions.

Spatial resolution of the instrument in flow cytometry may be very high [4]. This fact is little known and poorly used. Resolution and sensibility in flow cytometry measurement allows registering very small heterogeneities of neutrophils, with dimensions of the order of nanometers and smaller sizes, inaccessible of optical microscopy. Registration of fluorescence with flash duration $\sim 10^{-9}$ s in the jet of blood flowing through the laser beam with the velocity $\sim 1$ m/s provides measurement with spatial scales $\sim 10^{-9}$ m in the flow direction [4]. Synchronization and registration of short fronts $\sim 10^{-12}$ s of fluorescence pulses gives an increasing of sensitivity and extending of lower limit of spatial measurements in the flow direction.

Registration of very small heterogeneities allows detecting new peculiarities of DNA and new peculiarities of neutrophils in to compare with observations in the optical microscope. Moreover we observe in vivo full DNA set and all fluorescing fragments with oxidative activity of DNA into neutrophils nuclei. Therefore we observe the structural peculiarities of oxidative activity of DNA in living cells, with full set of chromosomes, for all chromosomal and inter-chromosomal correlations.

In flow cytometry we observe a big collection $10^4-10^5$ of fluorescing cells. Each cell has the chaotic Brownian motions and chaotic rotations during jet flow of blood through the laser beam. Therefore in histograms we observe the statistical results of 2-D projections of fluorescing cells at the photomultiplier for real 3D- distributions of DNA fluorescence in neutrophil populations. Thus we have statistics for 3D-correlations of DNA in living cells with rather high spatial resolution in the flow direction. As well known real 3D-inner life of genome in various cells [5,6] is differ from linear bioinformatics’ description and other linear extrapolations. We are focused on the interpretation and use of practical information in immunofluorescence histograms for different people in real life for medical diagnostics.

Irregularity and strong roughness of immunofluorescence distributions and fractals in fluorescence networks closely connected with expression of genes, epigenetics, chromosomal and inter-chromosomal correlations in real cells. Nature and structure of these networks and correlations usually are unknown. We analyzed frequencies of flashes, information and Shannon entropies and their fractal properties in order to determine a sequence of statistical criteria for classification of immunofluorescence in diagnostics [4]. It gives new approaches to analyze, control and classify various pathologies for wide spectra of diseases [4]. New correlations and conclusions gives a coherent analysis of modern multi-channel experiments without artificial smoothing of the results, without many and varied direct and hidden assumptions and manifold statistical hypothesis. On this way we constantly meet and observe a wide range unsolved physics-mathematical and biological problems. Hierarchy of complexity, patterns, fractals and topology of oxidation' networks and other fundamental large-scale correlations for real DNA in cells bad fit into the framework of standard schemes for DNA in textbooks, but often contain much more valuable and meaningful information than standards or their simple complementation. We investigate many experimental data and tens of diseases in vivo and in medical treatment for not solitary isolated theoretical DNA but full set of chromosomes in living cells, with all nonlinearities of chromosomal and inter-chromosomal correlations. Large-scale correlations for distributions of fluorescence' flashes real DNA in cells differ from those that we would like by abnormal fractal dimensions and substantially non-Gaussian statistics [4]. These natural peculiarities of immunofluorescence often are accompanied by the statistical instabilities of local intensity distributions.
[4,7,8] that ensure insolvency and inapplicability of standard methods of data analysis, when there isn’t a lawful basis for their applicability. Here statistical instabilities of local intensity distributions mean that the average value of intensity is smaller than dispersion, and the dispersion is smaller than asymmetry and other statistical moments of intensity fluctuations [4,7,8]. The exponential growth of central moments of fluorescence intensity reflects the clear sign of turbulence [8]. We need to develop a sequence of new nonlinear statistical methods to data analysis of immunofluorescence. Some of first steps in this direction [4,7,8] show that different types of bistabilities [8] and switching of wavelet spectra of immunofluorescence noise [7] can provide robust and statistically stable types classification for different peculiarities of histograms in diagnostics. Next steps in this direction include the classifications of topological structure of correlations for oxidative activity of DNA in cells.

We have classified the features and types of irregularity and of brokenness for fluorescence histograms as very important components of properties for large classes of non-smoothed correlations in networks of oxidative metabolism of DNA in cells, which can't be smoothed; standard smoothing eliminates, destroys and removes fractal networks and correlations, leads to changes of real statistics, blurs and distorts many aspects of reality. Here we analyze and classify different types of complex and fractal networks, fractal manifolds in DNA-fluorescence, their topological peculiarities and trends for diagnostics of health conditions in vivo and during medical treatment. We exploit these results for the objective estimation of health status including hidden diseases of given patient in the given time. Oxidative metabolism of DNA in neutrophil populations includes presence of intercellular correlations. We will describe and will discuss differences for intercellular correlations in neutrophil populations for healthy and unhealthy people.

2. Experiments

Experimental methods and procedures are described in [1-4,7,8]. Experiments based on respiratory burst or oxidizing explosion. The volume of peripheral blood is $V = (1 \ldots 2)$ ml. We used hydroethidine addition with concentration 150 $\mu$g/ml for fluorescence initiation. Small concentration 100 ng /ml additives of phorbol myristate acetate (PMA) to blood samples ensure the intensive staining of the cell nuclei of polymorph nuclear leukocytes. At the beginning hydroethidine is transformed in ethidium bromide as the results of chemical oxidative reactions in the blood cells. Ethidium bromide binds with fragments of nuclear DNA and has strong red fluorescence excited by TEM$_{00}$ mode radiation Argon laser light at 488 nm wavelengths. Intercalation of ethidium bromide in chromosome is determined by oxidative activity fragments of DNA. The rate of measurements is about $(1-2)10^4$ cells per min. Mean time of the measurement of one model is about 2 min. This empirically selected regime is self-consistent with noises level of various nature and gives statistically stable and reproducible results. The inaccuracy and reproducibility for preparations and measurements procedures usually compose $\approx 2\%$. This inaccuracy and reproducibility level corresponds to unavoidable and irremovable noises and errors both physical and biological nature.

Three typical examples of the initial cytometric histograms are shown in Fig.1.
Let us compare the brokenness for three typical histograms in Fig.1, using the Hurst exponent H. Results are shown in Figs. 1c. Hurst exponent $H$ is determined by means of regression equation

$$\ln(R/S) = H \cdot \ln(I) + \text{const}$$  \hspace{1cm} (1)

Where $R/S$ is rescaled range ($R=S$), $R$ is range or maximal deviation of $P(I)$ from local mean level, $S$ is standard deviation of $P(I)$. Hurst index $H$ for frequency of flashes $P(I)$ corresponds to fractal (Hausdorff) dimension $D$ if

$$D = 2 - H$$  \hspace{1cm} (2)

In particular Hurst exponent $H$ indicates persistent or correlated ($H>1/2$) and anti-persistent or uncorrelated ($H<1/2$) behavior of trend of irregularity in distributions of $P(I)$. Persistent behavior is observed in for asthma, when $H(r=256)=0.5435$. Anti-persistent behaviors are observed for healthy person with $H(R=256)=0.1971$ and for oncology with $H(R=256)=0.4018$. Correspondence fractal dimensions are $1.4<D_H<1.8$. We have different Hurst exponent for different three groups the states of health. Good health corresponds to anti-persistent behavior of $H<1/2$ for any range number $r$. Unhealthy people corresponds to persistent behavior of $H>1/2$ for range number $r > 64$. From this point of view the dominant of anti-persistent immune behavior for healthy people gives richer spectra responses of immunity than more poor reaction of unhealthy people; this feature of immunity interconnected with Shannon-Weaver biodiversity of neutrophils [4] (see also Figs.6).

3. Networks of small worlds for DNA in fluorescing neutrophils

In order to provide more detailed quantitative description of fluorescence correlations need to define structural regularities and pattern changes in the irregularity and strong roughness of histograms. Changes in the irregularity and strong roughness of fluorescence's distributions with variations of rank of histograms reflect very important information about hierarchy and architecture of networks with different scales that permit us to clarify topology and main structural interconnections.

Let us consider some fractal peculiarities of immunofluorescence networks. Different analogies of fractal networks such as bronchial tree, structure of oncology tumor, arterials tree, etc with networks and distributions of immunofluorescence are described in [1]; the varieties of histograms of different nature are the similar to the histograms for fluorescence of neutrophils in Figs. 1a,b. Range of histogram $r$ interconnected with the selection of multistage clusters in networks with structure of bronchial tree; here range $r$ coincides with the number of columns in a histogram or with the number of channels for measurements of fluorescence intensity at given maximal value of dimensionless intensity $I$. We don’t know the structure, origin and details of immunofluorescence networks, but some features and peculiarities of these networks are reflected in the irregularity and brokenness of histograms. Variations of range $r$ (i.e. rank of histogram $r$) or variations the scale in fluorescence networks when $r=1$ provide the changes in irregularity and brokenness of histograms. The quantitative measure for the irregularity and brokenness in histograms may serve a Hurst index $H$. 
Consider an undirected network, and let us define $d$ as the mean geodesic (i.e., shortest) distance between pairs of vertex or nodes in a network of fluorescence flashes. The certain number $N$ of synchronized nodes-flashes in networks of DNA fluorescence in cells are characterized by the intensity $I \sim N$, where $N$ defines a common number of correlated nodes in network, if every node in fluorescence network has the approximately identical fragment of oxidative activity of DNA with approximately identical quantity of fluorescing dye. More detailed determination of correlated nodes $N$ in the clusters of fluorescence networks of DNA in cells now is unknown. The correlation length $d$ depends on the network topology. Random networks with a given degree distribution may be the networks of “small worlds” [10]. Small world behavior is typically characterized by logarithmic scaling for path length tends $d \sim \ln N$ [10]. On the other hand the expression of $d \sim N^{1/D}$ defines a linear size of $D$-dimensional lattice or the size of a fractal cluster $d \sim N^{1/D}$. Therefore estimation of fractal dimension $D$ in the “small worlds” fluorescence networks is $D(N) \sim \ln N / \ln \ln N$. Standard definition of fractal dimension [9]

$$D = \lim_{d \to 0} \left( \frac{\ln(N(d))}{\ln(d)} \right)$$

leads to the same correlation for $D(N) \sim \ln N / \ln \ln N$ in small worlds network. We use the experimental data in immunofluorescence histograms to define Hurst index $H$ and fractal dimension $D$ according to eqns (1), (2). The transformation of small world networks due to reduction of range $r = I \sim N$ leads to expression \{lnr/(2-H)\} \~ {lnlnr} in the full according with the experimental data in Fig.2.

![Fig.2 Dependence of Hurst index $H(r)$ on range $r$ in the small-world networks; initial histograms see in Fig.1a](image)

We see that linear growth rate of function \{lnr / D(r)\} \~ {lnlnr} in Fig.2 depends on the state of health. The lowest growth rate in the dependence of \{lnr/D\} on \{lnlnr\} corresponds to a healthy person. The highest growth rate in the dependence of \{lnr/D\} on \{lnlnr\} corresponds to inflammatory diseases. Thus, we shown that the experimental results presented in cytometric histograms interrelated with the structural and fractal peculiarities of immunofluorescence networks. These networks have the universal character of small worlds and vary with changes the states of health. Any healthy and unhealthy donor has self own “small-world” for oxidative metabolism of DNA in cells at given time. The stratification of the growth rate in the linear dependence of \{lnr/D\} on \{lnlnr\} permits to define the state of health in diagnostics.

Let us consider changeability of networks of oxidative activity of DNA in vivo and during medical treatment. Variations of fluorescence distributions and stability of small-worlds networks for healthy donor and donor with oncology at different time shown in Figs.3,4.
Fig. 3 Dependence of normalized spontaneous fluorescence flashes frequency $P(I)$ on intensity $I$ (a) and for more clearest only central part of histogram (b), for one, invariably healthy, donor at different times. Rhombus 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 $N_0=40109$, analysis time is 03 June, before 11 July, histogram range $r=256$. Fig. 3c Dependence of Hurst index $H(r)$ on range $r$ in the small-world networks; initial histograms see in Fig. 3a.

Fig. 4 Dependence of normalized spontaneous fluorescence flashes frequency $P(I)$ on intensity $I$ (a) and for more clearest only central part of histogram (b) for one donor with oncology disease; symbol rhombus relates to analysis date 05 November, one year $N_0=43752$; symbol ring corresponds to 15 December, this year, after treatment of hepatitis B, $N_0=26265$; patient was infected hepatitis B during treatment of main oncology disease; Fig. 4c Dependence of Hurst index $H(r)$ on range $r$ in the small-world networks before and after hepatitis treatment; initial histograms see in Fig. 4a.

We observe variations of distributions for frequencies of flashes $P(I)$ in Figs. 3a,3b but networks of oxidative activity of DNA in neutrophils are very stable, as it’s shown in Fig.3c. It means stability of immunity and the states of health for given healthy donor during one year. The same picture of changeability we observe in Figs.4 (oncology with hepatitis B) without dependence on medical treatment.

**4. General state of health from the point of view of neutrophil populations**

Previously we found and identified the empirical rule (the rule of thumb) for classifying the General State of Health in the form of answer Yes/No or healthy/sick for all donors [4] from the point of view of populations of fluorescing neutrophils in the donor blood. This response includes the presence of all kinds of health and illness, including the hidden diseases. This classification is based on the analysis of the structural correlations that are defined by the sign of plus/minus of trends of topology (fractal dimension) at variations of networks scale. Examples of such classifications are shown in Figs. 5.
Fig. 5 Dependence of Hurst index $H(r)$ on double logarithm of range $r$ in the networks of DNA fluorescence with different scales; (a) comparison of different states of health (asthma, good health, oncology), initial histograms see in Fig.1a; (b) variations of fractal dimensions in the networks of DNA fluorescence with different scales, for one, invariably healthy, donor at different times, initial histograms see in Fig.3a; (c) variations of fractal dimensions in the networks of DNA fluorescence with different scales, for complex disease-oncology and hepatitis B, before and after hepatitis treatment, initial histograms see in Fig.4a.

Negative trends in the dependencies of $H(r)$ on $r$ in Figs.5 correspond to good health. Positive trends in the dependencies of $H(r)$ on $r$ in Figs.5 correspond to no good health and all kinds of diseases. Variations of fractal dimensions in the networks of DNA fluorescence with different scales in Fig.5b for one and the same, invariably healthy, donor at different times at constantly negative trends of $H(lnlnr)$ reflect good stability of immune system and positive states of health for given donor. Slow variations of fractal dimensions in the networks of DNA fluorescence with different scales in Fig.5c for one and the same oncology patient before and after hepatitis treatment at constantly positive trends of $H(lnlnr)$ reflect poor health and the presence of unknown or incurable diseases or bad treatment. Fractals have more dynamic changes in time than very conservative networks of small-worlds (see Figs.3c, 5b and 4c. 5c).

5. Intercellular correlations in neutrophil populations

The role of intercellular correlations is now little known but these correlations are present in real experiments. Intercellular correlations associated with the most rare and most large-scale fluorescence flashes, which are synchronized and give most intensive glow. In the intercellular interactions dominate large-scale networks at low values of $r=4$ and 8. In this cases fractal dimension $D = (2 – H)$ in networks of neutrophils fluorescence for a healthy person is much lower than for the unhealthy patient (see Figs.1c, 2, 3c, 4c, 5). For sick people with different types of diseases fractal dimension of most large-scale networks $(r = 4)$ is close to the deterministic values of $D = 2, H = 0$. In this case we have non-random or deterministic communications in populations of neutrophils and certain, but as yet unknown, interaction between cells at different diseases such as oncology, bronchial asthma, hepatitis etc. For populations of neutrophils in the blood of healthy human the correlations between different neutrophils also exist and are characterized by small-world networks with a fractal dimension close to $D=1.5$ as for Brownian motion that provide additional stability of immune system of healthy people.

Next important characteristic for intercellular interactions is defined by a Shannon-Weaver biodiversity of cells [4]. Shannon-Weaver biodiversity $E$, interconnected with distributions of Shannon information entropy $S(P(I)) = -P(I)\ln(P(I))$ in histograms of fluorescence [4]; sum of $S(P(I))$ for all $I$ defines the Shannon-Weaver biodiversity $E$ of neutrophils in the blood of given donor at given time [4]. Biodiversity of neutrophils in the blood of healthy human remains stable over time in networks of small-worlds for the different scales of fluorescence networks, as can be seen in Fig.6b Biodiversity of neutrophils in the blood of a cancer patient, with acquired during the treatment of oncology addition of hepatitis B, depends on medical interventions, as can be seen in Fig.6c. Here the treatment of inflammation associated with the treatment of hepatitis B leads to the stratification in the dependence of
Shannon entropy $E$ on ln $r$. Hepatitis a marked decrease of neutrophils’ biodiversity (the lower line in Fig.6c). The fractal dimension of large-scale ($r = 4$) of fluorescence network in this case is close to $D = 2$, $H= 0$ (see the bottom point in Fig.5c). Thus we observe large-scale deterministic unknown to us network with interconnections of unknown origin for intercellular interactions in populations of peripheral blood neutrophils for patient with hepatitis and oncology. The same decreasing of biodiversity of neutrophils we observe for any inflammations (asthma, lower line, Fig.6a). Thus bad health corresponds to decreasing of cells biodiversity and weaker immune response just as sad species extinction for bad ecology.

![Fig.6](image)

**Fig.6** Dependences of Shannon-Weaver biodiversity $E(r)$ of immunofluorescence on logarithm of range $r$; (a) initial histograms see in Fig.1a; (b) initial histograms see in Fig.3a; (c) initial histograms see in Fig.4a;

### 6. Conclusions

The strong roughness and irregularity of cytofluorescence distributions, non-Gaussian statistics and corresponding fractal networks of immunofluorescence are more important than the characteristics of averages $<I>$, $<(I-<I>)^2>$ and so on combined with smoothing and other mysterious statistical assumptions instead of health diagnostics.

We had show that main correlations in oxidative activity of DNA in neutrophils belong to networks of small-worlds i.e. to the complex networks with logarithmic scaling just as manifold of complex systems of different origin. Networks of small worlds for oxidative activity of DNA in cells give not only good approximation to the reality but also reflect the best protective properties of the immune system such as efficiency, flexibility and a close relationship correlations of different scales from gene to intercellular nets. These correlations ensure stability of the good health and oncology as it can be seen in unchangeable trends of fractal dimensions in Fig.5b and in Fig.5c. Each state of health has self own small-world networks at given time as in Fig.2. Trends of topology and fractal variation in DNA oxidations are more dynamic and changeable in time for given donor than conservative networks of small-worlds (see Figs.3c, 5b and 4c, 5c).

Structural change in oxidative networks due to range reduction for histograms of multi-channel measurements is a new characteristic for cytofluorescence distributions. For example, diagnostics of health or illness are depend on sign of trend for changes of Hurst index with reduction of histogram's range, as it is shown in Figs.5. Therefore, we have the clear general criterion of health/illness in a form of yes/no answers. Positive and negative drift in the changes of hierarchy the scales of fractal correlations define the architecture and topology of networks for oxidative metabolism of DNA in cells and health status.

Knowledge and awareness of neutrophils for each other and the general state of health (the environment in host blood) in large populations of blood cells far exceed our current knowledge on cell-cell interactions. Intercellular interactions are determined by low-range histograms of fluorescence when intracellular processes in different cells are synchronized as small-scale noise in the networks of small-scale fluorescence. Neutrophils living in the blood of healthy people have random intercellular correlations, as in Brownian motion (fractal dimension $D = 1.5$) and are characterized by high biodiversity than neutrophils in the blood of sick people, as shown in Figs.6. Neutrophils in the blood of
sick people are almost deterministic relations (D=2) of unknown nature, which stimulate the degradation of the community of cells and weakening of immunity, as well as clear information or Shannon-Weaver index about bad environment in Figs.6.

These results are important not only for general estimation of health statuses and early diagnostics of diseases because of wide prevalence of oxidative abnormality as the reason of various illnesses and aging.

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References