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FRACTAL MICROSCOPE FOR DIRECT VIRUS-CELL INTERACTION MONITORING

The fractal type of natural science laws' understanding has become the leading one in biomedical research, especially in the last two decades. It takes start from the entropy based proof of the necessity of living cell to divide in order to survive. The Fourier transform of the sensitive cell substrate was formed through laser diffraction patterning and used as the reference data. It was shown that each specimen could be marked with fractal dimension D which could be changed only in the process of non-equilibrium fractal growth and could serve as laboratory research identifier. It was shown experimentally that the viral particles attacking the sensitive substrate changes the D value at every stage of virus-cell interaction. All the mentioned features of the proposed fractal approach to the problem of virus-cell interaction makes it to be attractive and useful laboratory/clinical express-tool for the purposes of antiviral research, antiviral drug testing and clinical research.

BACKGROUND

Many natural objects, including most objects studied in pathology [1], have complex structural characteristics and the complexity of their structures, for example the degree of branching of vessels or the irregularity of a tumor boundary, remains at a constant level over a wide range of magnification. The fractal geometry (or coherent geometry) overcomes the limitations of Euclidean geometry for the complex dynamic systems and gives the fractal dimension D as an index of the space-filling properties. The critical parameter in the life of the cell, which makes the cell to divide, is the surface-to-volume ratio and it remains in adoptable range only for the fractal objects [2]. Fractal concepts have also been successfully incorporated into models of biological processes, including epithelial cell growth, blood vessel growth and viral infections [3].

HYPOTHESIS AND SCOPE OF THE PRESENT WORK

Taking into account the results of previous experimental investigations in the field, we may suppose that practically every stage of virus-cell interaction could be described in a numerical way using the fractal approach and corresponding instrumentation.

We would like to demonstrate starting from the experimental data of the fractal analysis of diffraction patterns of the interacting virus-cell dynamical system that the method could provide the critical linear data of the system as well as the fractal analogs to the widely used viral parameters such as ID_{50} and infectivity.

MATERIALS AND METHODS

We have used the single-mode and intensity stabilized Spectra-Physics He—Ne laser with 5.0 mW output power and wavelength of 0.6328 ± 0.01 microns as a source of coherent radiation. The geometrical magnification of the whole microscope was about 125×.

The samples of the interacting virus-cell dynamic system used in our experiment could be numbered as follows:

#1 — monolayer of Hep-2 cells cultivated for 24 hours.

#2 — monolayer of the same cells cultivated for 48 hours.

#3 — monolayer of Hep-2 cells cultivated for 24 hours with addition of E-aminocaproic acid (E-ACA) (known proteolysis inhibitor) in 10 E-3 M concentration.

#4 — Hep-2 cells monolayer infected with *Herpes simplex virus* (HSV) of US-1 strain (24 hours after infection).

#5 — Hep-2 cells monolayer infected with HSV US-1 (48 hours after infection).

#6 — Hep-2 cells pretreated with E-ACA and infected with HSV US-1 (24 hours after infection).

#7 — Hep-2 cells pretreated with E-ACA and infected with HSV US-1 (48 hours after treatment).

The described samples were washed out with Hanks solution and afterwards fixed with ethanol. The reference group of the samples with the identical prehistory was stained by 0.01% solution of acridin-orange and investigated using luminescent microscope. 500 cells were counted and the percentage of infected cells was defined. The inhibition of HSV reproduction was evaluated through the account of the decrease

of the number of the cell intra-nuclear inclusions.

The diffraction patterns (DP) of the samples generated due to the cell structural changes resulted in the corresponding difference in local optical density of the samples were registered with VZM Color LabVideo system which provided the resolution better than 200 lines/mm. All the DP corresponding to the numbered samples were processed using the ImageFractPro software package elaborated in our group. As the result of DP processing the values of fractal dimension D were obtained during not more than few seconds of computer work which could be successfully compared to hours of microscopic view counting.

EXPERIMENTAL RESULTS

The fractal dimension $\boldsymbol{D}$, according to [1—4], could be obtained in the case when the $\log N$ *vs* $\log R$ has the linear part or parts in certain range of target radii R. N is the number of spots included in the circle of this same R. N here is the number of bright spots on the DP encircled into the radius R.

The *DP* fractograms (bi-logarithmic plots) for samples *NN 1—3* are presented at Fig. 1.

Main informative part of fractogram lies between $\log R = 0.85$ and $\log R = 2.2$.

It could be seen also that the doubling of the cultivation time of Hep-2 leads to the small decrease of D_{c2}. The D_p, *vice versa*, increases from sample N 1 to N 2. It is known that the density of cells increase in time due to their divison.

The addition of E-ACA causes the significant increase of $\boldsymbol{D}_{c2}$ and even more profound decrease of $\boldsymbol{D}_p$. It is known also that the proteolysis inhibitor E-ACA could increase the rigidity of cell membranes and the packaging tightness could decrease, as a result of E-ACA addition.

The result of HSV infection on the fractal dimension $\boldsymbol{D}$ is shown at Fig. 2. This fractogram demonstrates that the central part of the fractal cluster of infected cells is almost of the same density practically independent of the cells' cultivation time. But, the peripherical part of it has obtained the significant increase of the fractal dimension $\boldsymbol{D}_p$.

We could state, in general, that E-ACA addition has a significant stabilizing action on the process of virus-cell interaction.

The minimal size value R_{min} of the cluster in each case could be directly found from fractograms as the intersection point of the extrapolation of the corresponding linearized part with the horizontal coordinate axis at the level when $\log N = 0$ (or the number of particles equals to 1. This value should be divided by the device's magnification factor, which in our case was about 125×. The fractograms' review shows us that the minimal cluster size could be considered as the sensitive parameter indicating the changes of cell structure during virus-cell interaction.

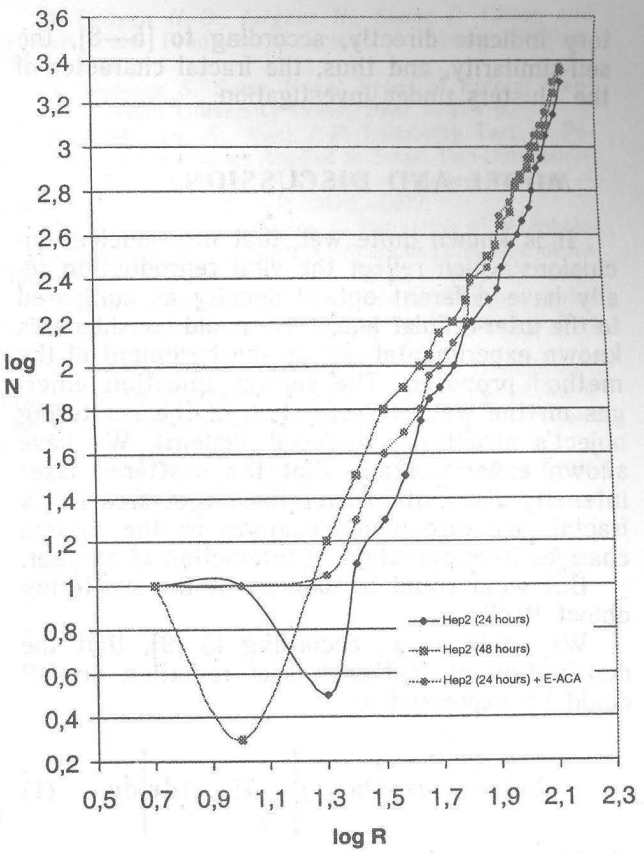

Fig. 1. Fractograms of sensitive cells

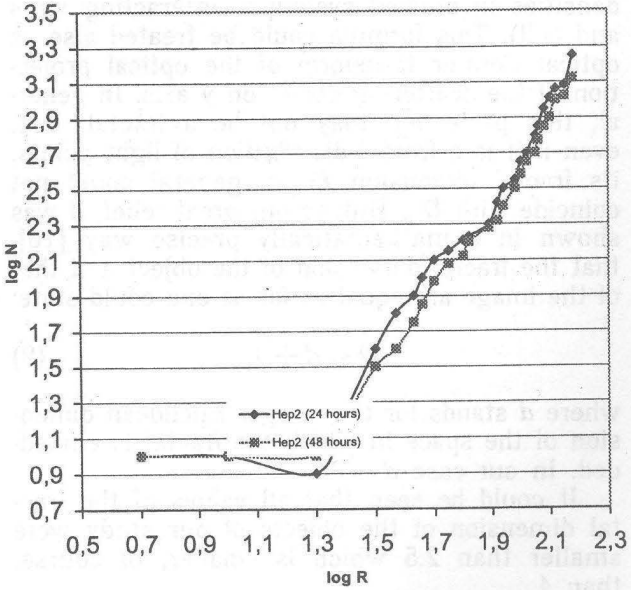

Fig.2. Fractograms of the infected cells

One could see that R_{min} about triples in the period of tissue culture cultivation. Addition of E-ACA makes the R_{min} to be about two times smaller than the starting value for Hep-2 (24 h). E-ACA treatment and addition to the infected cells return the R_{min} practically to the starting values for non-infected cells.

The linearized parts found on the fractograms of the virus-cell interaction with various pre-his-

tory indicate directly, according to [5—8], the self-similarity, and thus, the fractal character of the clusters under investigation.

MODEL AND DISCUSSION

It is known quite well that intra-nuclear inclusions which reflect the viral reproduction really have different optical density as compared to the inter-cellular liquid. We could consider this known experimental fact as the basement of the method proposed. The serious question emerges on the way of restoration of the scattering object's structure (virus-cell system). We have shown experimentally that the scattered laser intensity distribution over the target area has a fractal structure which changes as the system changes from one stage of interaction to another.

But what could be said about the scattering object itself?

We could state, according to [9], that the distribution of scattered laser radiation on DP could be expressed as:

$$I(q) = \int_{-\infty}^{+\infty} \exp(2\pi i q y) \left[\int_{-\infty}^{+\infty} T(x, y)\, dx \right] dy, \quad (1)$$

where $T(x, y)$ denotes the coefficient of optical transmission of the object under investigation (or individual distribution of masses and optical densities in studied system of interacting virus and cell). This formula could be treated also as optical Fourier transform of the optical projection of the scattering object on y axis. In general, this projection may not be a fractal. But, even if it is a fractal distribution of light points, its fractal dimension D_{im} in general could not coincide with D_{ob}. But, to our great relief, it was shown in a mathematically precise way [10], that the fractal dimension of the object and that of the image are equal as far as one could state:

$$D < d + 1 \quad (2)$$

where d stands for the integer Euclidean dimension of the space in which the fractal is embedded. In our case $d = 3$.

It could be seen that all values of the fractal dimension of the objects of our study were smaller than 2.5 which is smaller, of course, than 4.

So, we could consider the obtained values of the images as the real fractal dimensions of the virus-cell dynamic system.

It was proven earlier [9], that the optical Fourier transform of the fractal object could be expressed in analytical way as:

$$I_n(q) = S(q)F(q), \quad (3)$$

where $S(q)$ is the structural factor and $F(q)$ is the form-factor. The physical meaning of the form-factor $F(q)$ is the intensity of light diffracted on the elementary unit of the fractal object. For the simplest case of line portion of width ε, $F(q)$ obtains the following form:

$$F(q) = \left(\frac{\sin \pi q \varepsilon}{\pi q \varepsilon} \right)^2. \quad (4)$$

This value turns to zero (or the elementary diffractive scattering vanishes) if $q = 1/\varepsilon$.

So, we could establish the minimal size of the fractal element which is imaged in a form:

$$\varepsilon_{min} = \frac{1}{q_{max}} = \frac{\lambda}{2\pi}. \quad (5)$$

We have evaluated this minimal elementary size previously substituting for wave vector q the wave length of He—Ne laser which is equal to 0,6328 mkm divided by 2π.

The result is the same or $\varepsilon_{min} = 0{,}1$ mkm. It should be noted that according to the present knowledge in the field, the *Herpes simplex virus* capsid and many other viral particles are of almost same size.

The structural factor $S(q)$ describes how the fractal cluster is composed of elementary units.

It was shown as well in [9] that:

$$\langle S(q) \rangle \sim q^{-D} \quad (6)$$

or

$$D = -\frac{\log \langle S(q) \rangle}{\log q}. \quad (7)$$

So, we would like to consider the fractal approach to the problem of virus-cell interaction could as the comprehensive physically based method in any biomedical application including antiviral research.

CONCLUSIONS

As the result of the theoretical discussion of the real experiment we would like to state the following:

1. The possibility of the fractal approach application to the problem of virus-cell interaction is comprehensively based on the platform of modern coherent optics.

2. The use of the proposed approach has a lot of benefits as compared with the standard techniques, especially due to its better and simpler way of the quantitative, objective and express improvement of virus-cell in-line interaction monitoring.

3. The fractal approach could be used widely with the purpose of virus-cell interaction every stage details' evaluation and will allow, in perspective, the perpetual dynamic monitoring of the processes on the molecular level of self-organization.

4. The proposed fractal approach is mainly applicable in laboratory and clinical antiviral research as well as in drug design and testing process due to its attractive abilities of high

sensitivity, express character and numerical way of data processing.

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