Study of Vibrational Properties of Viral Particles by Computer Modeling

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Abstract

Diseases caused by viral infections are one of the biggest problems for global health, and as methods involved in diagnostics are getting faster and more efficient, methods of their therapy are still need to be stronger. This retrospective study aimed to explore nano biospectroscopy research and technology in the field of virology in order to provide a theoretical and computer modeling support for the techniques used, and to suggest the applying tools that have not been used previously.

Keywords: *virology, spectroscopy, computer modeling, radiation resonance therapy*

1. Introduction

Medical disasters prediction, management and control are one of the main parts medical planning and preparation. The term “disaster medicine” first appeared in the medical lexicon in the post - World War II era. Although coined by former and current military physicians who had served in World War II, the term grew out of a concern for the need to care for military casualties, or nuclear holocaust victim, but out of the need to provide care to the survivors of natural disasters and the not yet distant memory of the 1917-1918 Influenza Pandemic [1]. The term “disaster medicine” would continue to appear sporadically in both the medical and popular press until the 1980s when the first concerted effort to organize a medical response corps for disasters becomes the part of the National Disaster Medical System. Simultaneous with this was the formation of a disaster and Emergency Medicine discussion and study group under the American Medical Association (AMA) in the United States as well as groups in Great Britain, Israel and other countries. Throughout this period, incomplete and faltering medical responses to disaster events and control of different epidemics made it increasingly apparent in the United States of America that federal, state and local emergency management organizations were in need of a mechanism to identify qualified physicians in the face of a global upturn in the rate of medical disasters [2].

Viruses are assembled in the infected host cells of human, animals, or plants. Because of viral breeding the, host cell dies. There are especially viruses which are breeding in the cell of the bacteria. Viruses spread in many different ways. Just as many viruses are very specific as to which host species or tissue they attack, each species of virus relies on a particular propagation way.

The interdisciplinary collaboration (Biomedicine and Biophysics, Physical Research, Information Technologies and Systems) is an engine of strengthen the abilities of researchers in development of new biophysical and biomedical methods and tools. Those works which are based on novel achievements in optical spectrometry, laser and molecular physics as well as information technologies and systems are critically important for study of common properties of nano-scale virus-like particles, and elaboration of basic concepts and new revolutionary method for estimation unique vibration/oscillation properties, determine the unique “fingerprints” of pathogenic micro-organisms, especially viruses.

Farther development of new methods of pathogens treatment is greatly facilitated by an improved understanding of the pathophysiology of epidemic diseases. There is therefore a need to address the current knowledge gaps in disease aetiology in order to support innovation in evidence-based therapy. In this context, a better understanding of the mechanisms that are common to several diseases, in particular of those leading to co-morbidities, constitutes an important challenge. The special attention must be focused on the integration of pre-clinical and clinical studies for the identification of mechanisms common to several diseases. Performing activities should assess and validate the relevance of these common mechanisms and of their biomarkers (where relevant) on the development of disease-specific pathophysiology, as well as their role in the development of co-morbidities in both males and females. The expected impact should provide: A better understanding of disease pathways and / or mechanisms common to a number of diseases; new directions for clinical research for better disease prevention, health promotion, therapy development, and the management of co-morbidities. In this direction the multidisciplinary development of ability to detect rapidly, directly and selectively individual virus particles has the potential to significantly impact healthcare, since it could enable diagnosis at the earliest stages of replication within a host's system. Simultaneous acquisition of the vibrational and electronic fingerprints of molecular systems of biological interest, at the interface between liquid media, or at the air/solid, air/liquid interfaces is difficult to achieve with conventional linear optical spectroscopy due to their rather poor sensitivity to the low number of molecules or their maladjustment to water environment (infrared absorption). It relies on inelastic scattering of monochromatic light, usually from a laser in the visible, near infrared, or near ultraviolet range. The laser light interacts with molecular vibrations, phonons or other excitations in the system, resulting in the energy of the laser photons being shifted up or down. The shift in energy gives information about the vibrational modes in the system. Infrared spectroscopy yields similar, but complementary, information. Spontaneous scattering is typically very weak, and as a result the main difficulty of this kind of spectroscopy is separating the weak non-elastically scattered light from the intense Rayleigh scattered laser light.

Current paper presents some information about studies carried out in the last decade using spectroscopic methods as a research tool in the field of virology. Spectroscopic analyses are sensitive to variations in the biochemical composition of the sample, are non-destructive, fast and require the least sample preparation, making spectroscopic techniques tools of great interest in biological studies. Herein important chemometric algorithms that have been used in virological studies are also evidenced as a good alternative for analyzing the spectra, discrimination and classification of samples. Techniques that have not yet been used in the field of virology are also suggested. This methodology emerges as a new and promising field of research, and may be used in the near future as diagnosis tools for detecting diseases caused by viruses.

2. Theoretical and Experimental Studies

With the advancement of technology and consequently advanced spectroscopy, the interest of researchers in spectroscopic techniques in biological studies has grown. This field of science is known as biospectroscopy, and means the use of spectroscopy to analyze biological objects. Several studies have been conducted involving identification of bacteria [3,4], viruses [5,6],cancer diagnosis [7], and even in the field of forensic entomotoxicology [8], demonstrating that spectroscopic techniques are capable of detecting biochemical changes in biological matrices.

Viruses are submicroscopic infectious agents and obligate intracellular parasites. They are totally dependent on a host cell because they are not able to generate energy to conduct all biological processes (fig.1). Virus particles (virions) come in a variety of sizes and shapes.

However, approximately spherical shapes with diameters in the range between 50nm and 100nm are especially common. Many nearly spherical viruses are revealed by X-ray crystallography to have icosahedral symmetry. A typical virus particle contains genetic material, RNA or DNA, surrounded by a protein coat (capsid). Such an object should have reasonably distinct vibrational frequencies, the study of which may be of interest. Excitation of these vibrations could have applications in either the diagnosis or treatment of viral diseases. The sole discussion of these vibrational modes conjectured that ultrasound in the GHz range could be resonantly absorbed by HIV virus particles, leading to their destruction [9].

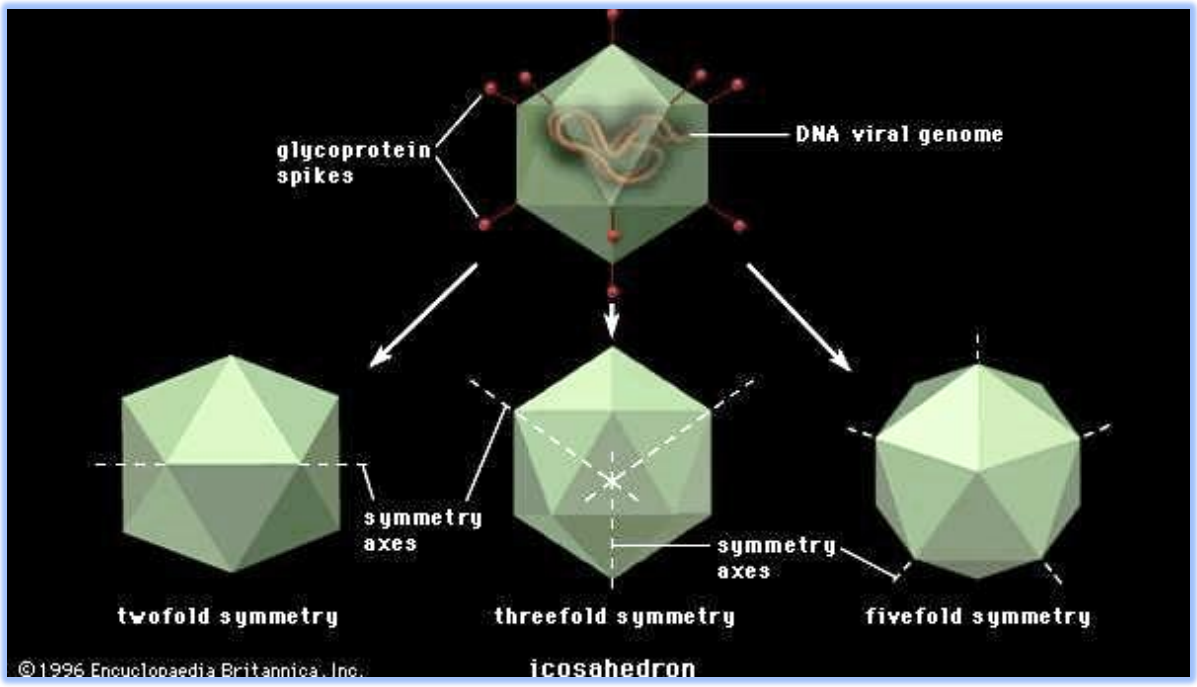

Fig.1. Example of Virion: Virus icosahedron A virus icosahedron (20-sided structure) shown in the (left) twofold, (centre) threefold, and (right) fivefold axes of symmetry. Edges of the upper and lower surfaces are drawn in solid and broken lines, respectively. Encyclopaedia Britannica'

The two methods most commonly used in clinical diagnoses of viruses are enzyme-linked immunosorbent assay, with the best known being the ELISA method and real-time polymerase chain reaction (PCR). These methods have brought benefits such as high levels of repeatability and reproducibility, ease in handling and robustness [10]. However, they have also some negative points. As example, both methodsrequires high quality reagents; in some situations they are not suitable for identifying specific viral species/strains and they are also destructive to the samples. Thus, there is a need for techniques that are as advantageous as ELISA and PCR techniques, and which have fewer disadvantages. The potential of spectroscopic techniques in the detection and identification of virus-infected cells has been studied using statistical methods as a sensitive, rapid and reliable methodology. The ability to discriminate between contaminated and non-contaminated objects in a short time with high sensitivity which characterized biospectroscopy determine its high prospect for studying viruses and similar pathogens [11,12].

An expected difficulty in the use of biospectroscopy in virology is related to the fact that humans have a great diversity of virus circulating in their organism, and each human has a unique microbiome. Because of it, obtaining a fingerprint would be more difficult in view of the specificity of each organism. The solution to this problem seems to be the use of a broad and well-trained database, and changes obtained by multivariate statistical analysis, differentiating these alterations [13,14].

The main spectroscopic techniques that have been used in virological studies are nuclear magnetic resonance spectroscopy (NMR) [15], Raman spectroscopy [16], infrared spectroscopy (IR) [17] and molecular fluorescence spectroscopy [18]. These techniques are known to provide rapid responses and reliable data, as well as having powerful structural elucidation capability.

Such advantages highlight the possibility of identifying and classifying different types of virus using spectroscopic techniques. In this paper, studies using biospectroscopy coupled to statistical methods of classification in virological investigations are emphasized. First, we will discuss the most commonly used spectroscopic techniques, and then we will discuss the computational processes used to extract useful information from the obtained spectra (spectral preprocessing, multivariate classification algorithms, performance evaluation).

3. Discussion and Analyze

In order to understand the possible pathway of biospectroscopy development it is necessary to use the new science and technology tool calls bionanotechnology. The main objective of this modern discipline is cellular uptake of nanosize molecules functioning within the cell. If the size of molecules is bigger than 10nm are taken by the cell trough a clathrin-assisted mode of endocytosis called pinocytosis, while particles of size greater than 200 nm in diameter are usually phagocytosed by the macrophages. Phagocytosis occurs in specialized cells called phagocytes, which includes macrophages, neutrophils, and other white blood cells, which destroys the molecular association. Invagination produces so called phagosome which usually fuses with one or more lysosomes containing hydrolytic enzymes.

In comparison with cellular molecules (nano-ensembles) the size of viruses varies from 20 to 300 nanometers. Practically all viruses by the sizes are smaller, than bacteria. However, the largest viruses, for example a virus of cow smallpox, have the same sizes, as well as the smallest bacteria (hlamidiya and rikketsiya) who too are obligate parasites and breed only in living cells. Therefore, as distinctive features of viruses in comparison with other microscopic causative agents of infections the sizes or obligatory parasitism, and features of a structure and unique mechanisms of replication (reproduction themselves) serve not. Viruses are masterpieces of nanoengineering with a basic common architecture that consists of the capsid – a protein shell made up of repeating protein subunits- which packs within it the viral genome. Nano-sized biological agents and pathogens such as viruses are known to be responsible for a wide variety of diseases such as flu, AIDS and herpes, and have been used as bioreagents [19,20]. For today there are experimentally certified data that Viral nanoparticles are emptied virus cells that can carry drugs directly to cancer cells to kill them [21]. Scientists have engineered viral nanoparticles from plant viruses, insect viruses, and animal viruses [22]. Viral nanoparticles could revolutionize cancer treatment, acting not only as a safer, more specific form of cancer treatment, but also as a new imaging tool. The nanoparticles could create a type of drug delivery that is extremely tumor specific with greatly reduced side effects. The viral nanoparticles would be more soluble and have higher drug efficacy than current treatments [23,24].

Viruses and other biological species can be characterized according to size, shape, and optical/spectroscopic properties. These properties allow them to be distinguished from other biological species and from other particulates such as dust particles. In response to new tasks which face medicine development of a rapid and efficient diagnostic test is considered a high priority. In this direction the decisive word belongs to development of nanotechnologies which have a great potential for use in methods of detection, diagnosis and treatment. The gold nanorods (AuNR) are of particular interest, especially considering their optical properties and chemistry of the surface, which allows easy connection to organic molecules adapted to specific needs. For research of mechanisms of action of viruses and pathogenic microorganisms the study of their properties is very important including oscillations pervade biological systems at all scales. In bacteria, oscillations control fundamental processes, including gene expression, cell cycle progression, cell division, DNA segregation and cell polarity.

Oscillations are generated by biochemical oscillators that incorporate the periodic variation in a parameter over time to generate an oscillatory output. Spatial oscillators incorporate the periodic variation in the localization of a protein to define subcellular positions such as the site of cell division and the localization of DNA. There are some data which are focuses on the mechanisms of oscillators and the design principles of temporal and spatial oscillatory systems [25].

Current optical detection methods which are well developed for single micrometer size particles, cannot be applied to nanoparticles due to a strong signal dependence on particle size. Typically, such sensors consist of a light source which illuminates a sample volume of an aerosol or a liquid flow containing the particles of interest. An off-axis detector measures power of scattered light. The latter is a function of particle properties such as size, concentration, and optical density. In the tens of nanometers size regime particles act as dipoles, therefore the power of scattered light is proportional to the sixth power of particles size. Lowering the detection size limits for the existing detectors places an impossible requirement on noise optimization. Therefore, a signal which has weaker particle size dependence can allow access to smaller particles [26].In the field of virology, for example, it is critical to accurately quantify virus particles to study the effects of drug therapy in patients; and also, to study viral fitness, replication, and inhibition. There are several virus quantification techniques available to virologists, such as the quantitative PCR (polymerase chain reaction) method [27], the plaque titer method [28] and the image enhanced microscopy (IEM) technique [29]. However, a problem common to most of these techniques is that the analysis of a sample involves several tedious steps, which can take several hours to multi plays to complete. The fast detection and characterization of nanoparticles, such as viruses or environmental pollutants, are important in fields ranging from biosensing to quality control. However, most existing techniques have practical throughput limitations, which significantly limit their applicability to low concentration analysis. There are some experimental dates that an integrated nanofluidic scheme for preconcentration and subsequent detection of nanoparticle samples within a continuous flow-through system. In These experiments using a Brownian ratchet mechanism increase the nanoparticle concentration 27-fold. Single nanoparticles are subsequently detected and characterized by optical heterodyne interferometry.

A wide range of potential applications can be foreseen, including real-time analysis of clinically relevant virus samples and contamination control of processing fluids used in the semiconductor industry [30].For nanoparticle structures identification a rather interesting

method is Vibrational Spectroscopy (VS), which provides the most definitive means of identifying the surface species generated upon molecular adsorption and the species generated by surface. In principle, any technique that can be used to obtain vibrational data from solid state or gas phase samples (IR, Raman etc.) can be applied to the study of surfaces - in addition there are a number of techniques which have been specifically developed to study the vibrations of molecules at interfaces (EELS, SFG etc.) [31, 32]. There are, however, only two techniques that are routinely used for vibrational studies of molecules on surfaces - these are: IR Spectroscopy (of various forms, e.g. RAIRS, MIR) and Electron Energy Loss Spectroscopy (EELS). There are both advantages and disadvantages in utilizing EELS, as opposed to IR techniques, for the study of surface species It offers the advantages of high sensitivity, variable selection rules, spectral acquisition to below 400 cm-1 but suffers from the limitations of use of low energy electrons. Raman spectroscopy is used to study low-wave-number (≤20cm−1) acoustic vibrations of the M13 phage. A well-defined Raman line is observed at around 8.5cm−1.

The experimental results are compared with theoretical calculations based on an elastic continuum model and appropriate Raman selection rules derived from a bond Polaris ability model. The observed Raman mode is shown to belong to one of the Raman-active axial modes of the M13 phage protein coat. It is expected that the detection and characterization of this low-frequency vibrational mode can be used for applications in biomedical nanotechnology such as for monitoring the process of virus functionalization and self-assembly.Recently, a technique which departs radically from conventional approaches has been proposed. This novel technique utilizes biological objects such as viruses as nano-templates for the fabrication of nanostructure elements. For example, rod-shaped viruses such as the M13 phage and tobacco mosaic virus have been successfully used as biological templates for the synthesis of semiconductor and metallic nanowires. Low wave number (<or= 20 cm-1) acoustic vibrations of the M13 phage have been studied using Raman spectroscopy [33].

The experimental results are compared with theoretical calculations based on an elastic continuum model and appropriate Raman selection rules derived from a bond polarizability model.Aside from serving as a protective layer, capsids are involved with various other aspects of their respective virus life cycles including timely viral genome encapsulation (self-4assembly and genome packaging), cell-to-cell virus transport, entry into host-cell (e.g., via cell receptor binding), genome release into host cell, etc. Despite their central importance to the life cycle, the various evolutionary pressures acting on spherical capsids are not well known. Half a century of empirical data has uncovered a large array of capsids sizes that range from tens to many thousands in subunit composition. Spherical capsids of all observed sizes may be obtained from a grouping of twelve pentamers (symmetric clusters of five subunits) separated by a variable number of hexamers (clusters of six subunits). This is the case for the T~7d papilloma viruses where all capsomers are made up of five subunits but they are in both hexavalent and pentavalent configuration, and larger viruses whose ‘‘hexamers’’ are actually trimers of ‘‘fused’’ or covalently bonded dimers.Capsid size may be characterized by two integers, h and k, which describe the number of hexamers (hzk{1) one would have to ‘‘walk over’’ to get from one pentamer to an adjacent pentamer within a completed capsid.

The utility of the class system is not entirely lost, however; specific angle patterns within the capsid ensures the existence of distinct hexamer shapes (each shape is colored distinctly in. Evidence indicates that capsid formation is nucleated, often starting with a single

capsomer species (e.g., pentamers; for the purposes of this paper, a capsomer is a generally symmetric cluster of either five or six subunits), which then proceeds to completion by the addition of small subunit clusters (or single subunits). In T~1 capsid, where subunits are in identical/equivalent environments, nucleated assembly will be possible with no additional machinery. However, the formation of two or more capsomers from a single interaction site will require the employment of additional machinery to ensure high yields of the native state. For example, quasi-equivalent switches are required for the proper assembly of capsids containing two distinct capsomers: a pentamer and one type of hexamer. The addition of a second hexamer shape necessitates the requirement of a second mechanism such as auxiliary proteins for proper assembly. For spherical virus capsids requiring more distinct hexamer shapes. Additional mechanisms to stabilize those new shapes at exactly the right positions within the forming capsid are likely to be also needed dominantly form.

Because capsids from different classes display markedly different geometries, they are bound to display different physical properties. The periodic nature of capsid hexamer contents also useful in understanding ‘‘T-switching’’: a process that permits canonical capsid subunits to more easily sample capsids containing similar hexamer shapes. This allows for a segue to understanding currently intractable and deadly pleomorphic viruses like Ebola and arenaviruses. For example, from the above T-switching rule, the available diversity of an arena virus may only be explained if we assume that the biologically relevant form of the arenavirus is the T~12 capsid. Non-icosahedral capsids. Although the framework presented doesn’t appear to readily explain non-icosahedral capsids (some are just ‘‘slightly’’ non-icosahedral, such as the natively prolate phi29 capsids, while others are wildly different in form, such as Ebola with its natively filamentous shape), those capsids, like their icosahedral counterparts, also display capsomer sub-structures.

In light of this, the geometric constraints analogous to endo angles that affect capsomer shape may be useful in obtaining insights into non-icosahedral capsid morphology, behavior, and classification. It will be exciting to see whether incorporating the non-icosahedral capsids into an expanded capsid periodic table will be possible. All canonical capsids (made up of trapezoidal subunits) may be built from a single type of pentamer and a repertoire of distinct hexamer shapes (colored distinctly only once in each capsid. The hexamer shape is described by the number of endo angles it displays. It is necessary to underline that effect of destroy of human immunodeficiency virus (HIV) and other enveloped viruses is based on the highly symmetric structure (e.g. icosahedral and others) of many viruses, which leads to a well-defined resonant frequency of ultrasound in the GHz range and which may be specifically absorbed by these structures and may subsequently lead to their irreversible damage.In order to clarify the possible role of nanoparticles in diseases recently associated with them (such as Crohn’s disease, neurodegenerative diseases, autoimmune diseases, and cancer), nanoscale characterization techniques should be used to a larger extent to identify nanoparticles at disease sites in affected organs or tissues, and to establish pertinent interaction mechanisms. Rapid, selective, and sensitive detection of viruses is central to implement an effective response to viral infection, such as through medication or quarantine. Established methods for viral analysis include plaque assays, immunological assays and transmission electron microscopy. These methods have not achieved rapid detection at a single virus level and often require a relatively high level of sample manipulation that is inconvenient for infectious materials.

Yet, the ability to detect rapidly, directly, and selectively individual virus particles has the potential to significantly impact healthcare, since it could enable diagnosis at the earliest

stages of replication within a host's system. One promising approach for the direct electrical detection of biological macromolecules uses semi-conducting nano-wires or carbon nano-tubes configured as field-effect transistors, which change conductance upon binding of charged macro-molecules to receptors linked to the device surfaces. One of the simplest medical nanomaterials is a surface perforated with holes, or nanopores. These pores are large enough to allow small molecules to pass but are small enough to impede the passage of much larger virus particles. The next step was cylindrical gold nano-tubules with inside diameters as small as 1.6 nm. When tubules were positively charged, positive ions were excluded and only negative ions were transported through the membrane. With a negative voltage, only positive ions could pass. The combining voltage gating with pore size, shape, and charge constraints allows achieving precise control of ion transport with significant molecular specificity. Lieber's group has reported direct, real-time electrical detection of single virus particles with high selectivity using nano-wire field- effect transistors to measure discrete conductance changes characteristic of binding and unbinding on nano-wire arrays modified with viral anti-bodies [34].

The integrity of such devices allows increasing the number of the detection viruses. The analysis of the manifold literature shows, that task of the detection pathogenic micro-organisms is timely. Therefore, our available method would be one brick in the solution of the problems like that. Simultaneous acquisition of the vibrational and electronic fingerprints of molecular systems of biological interest, at the interface between liquid media, or at the air/solid, air/liquid interfaces in conditions similar to those encountered in nature or in model environments requires the use of sensitive and specific spectroscopic probes. Such a characterization is difficult to achieve with conventional linear optical spectroscopies due to their rather poor sensitivity to the low number of molecules (Raman) or their maladjustment to water environment (infrared absorption), at the exception of PM-IRRAS in specific work conditions.

In addition, these techniques are for most of them only partially surface specific. One of the promising solutions of this problem is the use of the nonlinear Two-Color Sum-Frequency Generation Spectroscopy (2C-SFG) that meets the desired spectroscopic requirements.

The goal of this approach is to probe membrane models of various forms and in various environments: (i) lipid monolayers and bilayers; (ii) deposited on substrates, floating on water as Langmuir layers and at a liquid-liquid interface; (iii) alone and in interaction with molecules, including peptides and proteins; (iv) submitted to controlled stress(chemical, pH, electrochemical potential).The increasing amount of available data of protein three-dimensional atomic structures, determined mostly by X-ray crystallography (related to the fast expansion of that field around third generation synchrotron storage rings) and NMR, has given much information about role of many proteins in biological processes. However, it has been pointed out that knowing the structure does not directly lead to the knowledge of the function, and that the protein alone, without its environment or its partners of interaction, is not totally informative. Additionally, some proteins cannot be satisfactorily crystallized and thus cannot be accessed by X-ray crystallographic methods. Among them, membrane proteins need their membrane partners to fully play their role and are often not able to crystallize. In situ studies, and their according investigation techniques, are therefore favored for such objects. In the following, in situ should not be understood as in vivo, but imply rather that the objects are designed and studied in an environment mimicking what they experience in vivo.

On the other hand, due to their essential role as the barrier between the cell cytoplasm and the extracellular medium, membranes themselves also get a lot of attention regarding their shape, stability, structure, composition, modifications under stresses (pH, temperature, electric potential) and interaction with proteins, water and chemicals in solution. The electrical behavior of bilayers makes them good candidates as membrane biosensors when attached to a conducting surface (semiconductor or metal). There are lots of possibilities to get average information on a given parameter of a membrane and its evolution under a given stress (e.g. diffusion of light, electrochemical methods, microbalance measurements). Specific in situ techniques allow direct investigation of key functional behaviors of synthetic membrane models (lipid mono and bilayers in an aqueous environment interacting either with selected proteins, ions or organic molecules) [35-36]. The strong absorption of the water vapor and the poor detection properties of conventional FTIR spectroscopy led to the discarding of this technique for the study of such interfacial systems. This evidence for the limited range of infrared spectroscopic tools dedicated to the study of such fragile objects in their specific environment was written only about ten years ago. From that time, there has been a lot of progress from the spectroscopic point of view.

In addition to IR absorption spectroscopy (conventional or attenuated total reflection (ATR) configuration), three other IR-based spectroscopies have been able to address the issue of a molecular layer on water with a signal-to-noise ratio sufficient to extract scientific information from experimental data. PM-IRRAS, an IR absorption technique initially developed to study the nanosurface of metals, has been applied to that of liquids. Being less sensitive to IR radiation absorption and easier to detect, Raman spectroscopy is often used on biological environment, although the low count rate on monolayers requires long acquisition times.It has been recently reported the detection of viruses by acoustic oscillations [37,38].

However, the process of “rupture event scanning”, which was report, involves the separation of a virus particle from antibodies by ultrasound. This is distinct from the excitation of the vibrational modes of the virus particle itself, and occurs at much lower frequencies. There have also been some experimental studies of ultrasonic absorption by empty viral capsids [39, 40]. These experiments reveal an enhanced absorption in the MHz range as proteins reassemble into a capsid, but do not find a resonant peak in this frequency range. At the same time, it was emphasized that these and other results show that viral capsids are flexible and change size or shape in response to vibrations or to changes in temperature or pH [41].

One the most promising methods of biospectroscopyis SFG. Theseultrashort pulsed lasers based optical measurement method is unique for investigation of vibrational modes of different viruses and other pathogenic microorganisms as well as study of nature of their oscillation processes and parameters of oscillation. Non-linear optics and its resonance technologies is possible direction of organization of treatment of pathogenic microorganisms in their different living media. Contrary to the previous ones, this second order nonlinear process is intrinsically specific to an interface, and involves no contribution from molecules in a centrosymmetric bulk, i.e., in solution or in gas phase. It has been extensively applied to solid interfaces in vacuum, controlled atmosphere and electrochemical conditions.

For a few years, technological development of picosecond and femtosecond tunable laser sources have led both to an increase of the number of SFG experimental setups around the world and to a progressive application to fragile or buried interfaces. In addition to unique SFG setup is research based on usage of the CLIO Free Electron laser [42,43]. This latter allows probing specific vibrations located in the near and far infrared, which is again unique

to date.Although molecular fluorescence spectroscopy has been little used in studies in the field of virology, it is also an interesting approach with great potential in this perspective. This technique analyzes the fluorescence capacity of a sample [44], where a beam of high energy light (usually in the ultraviolet region) is irradiated on the sample to be excited into a higher electronic energy level; then the fluorophore molecule will rapidly lose energy to this environment through non-radiative modes (called internal conversion) and will return to the lowest vibrational level of the lowest electronic excited state. The molecule persists at this vibronic level for a period of time known as the fluorescence lifetime, and then returns to the fundamental electronic state by emitting a photon with energy lower than the irradiated one [45].

The excitation and emission spectrum are recorded by the instrument and is generally used to build excitation-emission (EEM) fluorescence matrices.Another commonly-employed form of fluorescence technique is fluorescence correlation spectroscopy (FCS), which is used for temporal and spatial analysis of molecular interactions of biomolecules present in solution at extremely low concentrations. This technique is based on the principle that a fluorophore molecule has a specific free diffusion rate that is directly related to its size. This basic principle, for example, can be used to study protein interactions. As with other spectroscopic techniques, molecular fluorescence spectroscopy provides rapid results with high sensitivity and specificity, and is non-destructive, making this technique a tool of interest in the field of virology.

4. Computational Methods of Investigation

Spectroscopically interrogating biological samples analyze definitely needs to use computational tools which facilitate the information collection and extraction. For this, it is necessary to address the different computational methods and tools and among them there are employed the methods:

- Preprocessing and multivariate analysis techniques, which consists the correction and improvement of the signal-to-noise ratio of the spectrum, commonly employed before data analysis. [fig.2].

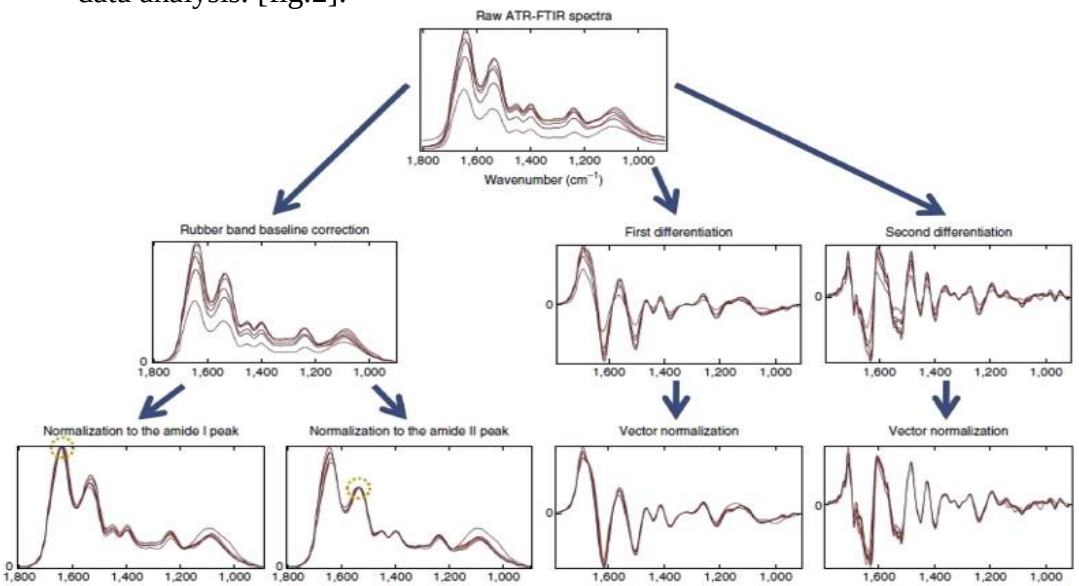

Fig.2. Visual effect of different pre-processing on a set spectrum. Source: Baker M.J., Trevisan J., Bassan P., et all. Using Fourier transform IR spectroscopy to analyze biological materials. Nat. Protoc. 2014;9:1771–1791.

- Spectral cut and Baseline correction, which include determination of exact region of interest and diminish of the wavenumbers which are not absorbed and light scattering occurs due to the non - homogenous particle size [46].
- Spectral normalization techniques are used when it is necessary to remove spectral changes responsible for the thickness or concentration of the sample, making the normalized spectra become comparable to each other. Among the possible normalizations, there is the min-max normalization, which can be applied when there is a known peak that is stable and consistent between the specimens; or scaling methods to equalize the importance of each variable in multivariate data. [47].
- Multivariate analysis techniques are employed to analyze multivariate data, meaning data having two or more variables per object. Examples are first-order data (such as IR, Raman spectrum) and second-order data (such as fluorescence).
- Principal component analysis (PCA)is an unsupervised multivariate analysis technique widely used in biological studies. This technique is used to reduce the dimensionality of the sample's data and generate a new visualization. Dimensionality reduction occurs through a linear transformation of the original variables, generating orthogonal variables called principal components (PC).
- Cluster analysis (CA) techniques are unsupervised methods of pattern recognition that aim to group the spectra into groups when there is no information about the classes. These techniques are exploratory therefore they group the samples based on their similarity between spectra. CA techniques include k-means clustering (KMC), fuzzy c-means cluster analysis (FCA) and hierarchical cluster analysis (HCA) [48].
- Partial least squares (PLS) is a multivariate calibration technique that finds factors (latent variables, LVs) in the spectra set that explain the maximum variance in the reference variables set, using the simultaneous decomposition of the two. For this, PLS finds a set of new maximally correlated variables orthogonal to each other, similar to PCA.
- Linear discriminant analysis (LDA) is a supervised technique widely used for class discrimination. It maximizes the between-class variance over the within-class variance in order to create a linear decision boundary between them.
- Successive projections algorithm (SPA)is a progressive variable selection technique. This means that it starts with a variable (wavelength or wavenumber, for example) and adds new variables in each interaction until an optimal number is selected. This technique uses multicollinearity minimization as a criterion for variable selection.
- The genetic algorithm is a technique that mimics Darwin's theory of evolution, where evolution occurs by natural selection in which the more adapted organisms have a greater chance of survival. In the case of GA, the variable selection process begins with a randomly formed population of variables [49]. Each chromosome is assigned an aptitude through a mathematical function called fitness function, where chromosomes with the highest fitness value are copied and the chromosomes with the lowest fitness value are eliminated in a step called the selection step. After the selection step, genetic operators are probabilistically applied. The mutation operator makes a variable that is selected to be unselected or vice-versa, and the crossover operator crosses the chromosomes. (fig. 3).
- Sensitivity (SENS) can be defined as the confidence that a positive result for a sample of the labeled class is obtained; specificity (SPEC) is the confidence that a negative result for a sample of non-labeled classes is obtained; positive predictive value (PPV) measures the proportion of positives that are correctly assigned; negative predictive value (NPV) measures the proportion of negatives that are correctly assigned; Youden's index (YOU) evaluates the classifier's ability to avoid failure; positive likelihood ratio (LR+) represents

the ratio between the probability of predicting a sample as positive when it truly is positive and the probability of predicting a sample as positive when it is actually not positive; and the negative likelihood ratio (LR−) represents the ratio between the probability of predicting a sample as negative when it is actually positive and the probability of predicting a sample as negative when it is truly negative [50].

- Diagnostic virology continues to evolve rapidly. Viral testing is now essential for the care of a number of patient groups, including hospitalized patients with acute respiratory infections; transplant recipients and other immunocompromised patients; patients infected with human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV); and infants with possible congenital infection. Multiple test methods continue to be used, but molecular tests are emerging as the dominant technology. A variety of commercial molecular assays have been or are in the process of being approved or cleared as in vitro diagnostic tests by the Food and Drug Administration (FDA) [51].

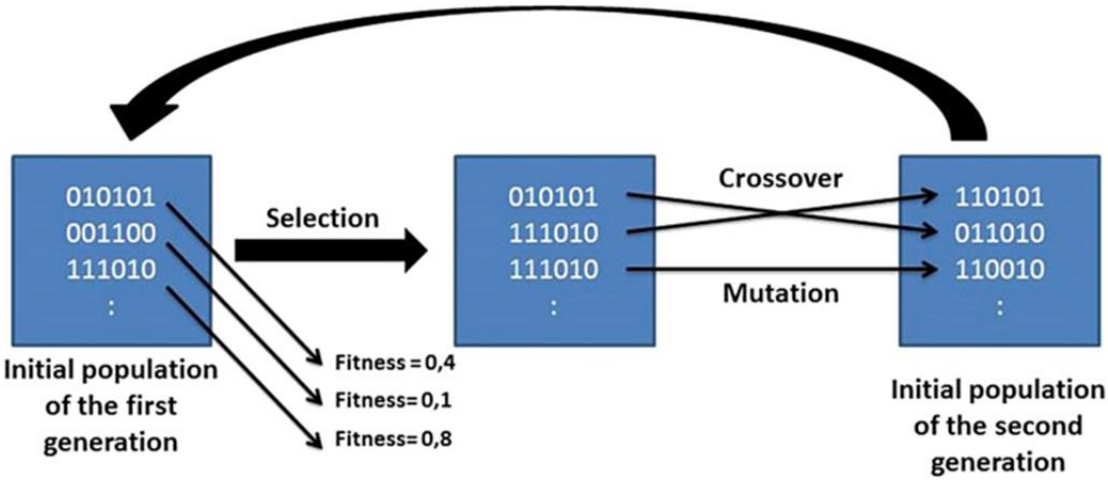

Fig.3. Operational scheme of the genetic algorithm (GA). In this scheme an initial population with 3 chromosomes is shown. A fitness value is assigned for each chromosome through the fitness function (F). Note that the chromosome with less fitness is discarded in the selection stage, while the larger one is doubly copied and the second largest fitness receives a copy. It is observed that the chromosome is mutated through the mutation operator in the second moment, and the other two chromosomes are crossed through the crossover operator.

During the last years in response of global spraying the new structural types of corona viruses the method of estimation unique vibration/oscillation properties, determine the unique "fingerprints" of pathogenic micro-organisms, especially viruses. The main task of the works done by international group including Georgian, French, German British and Greek researcherswas elaboration of optical resonance spectrometry method of detection and treatment of pathogenic nanobiostructures including corona type viruses [52-53].

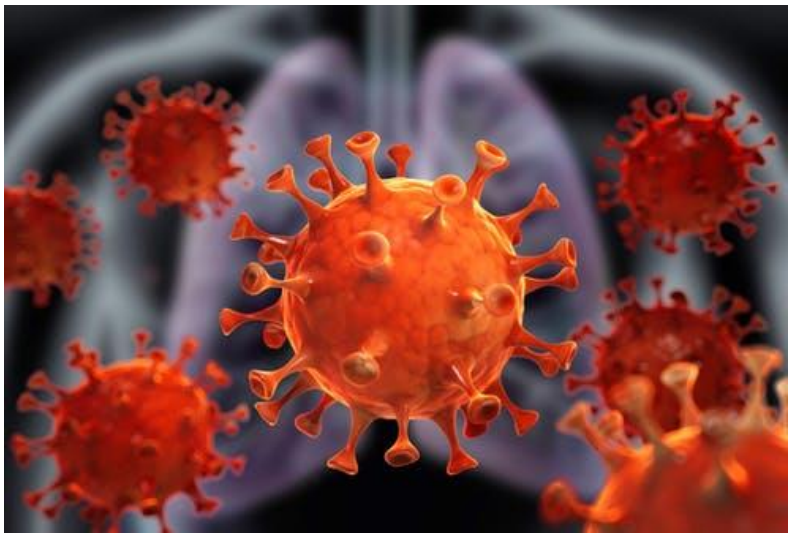

Fig. 4. Corona virus.
.https://www.google.com/url?sa=i&url=https%3A%2F%2Ftheconversation.com%2F4-unusual-things-weve-learned-about-the-coronavirus-since-the-start-of-the-pandemic-.

Viruses and their genomes are mostly enclosed and protected by spherical capsids - symmetric coats or shells composed primarily of multiple copies of protein subunits (Fig.5).Aside from serving as a protective layer, capsids are involved with various other aspects of their respective virus life cycles including timely viral genome encapsulation (self - assembly and genome packaging), cell-to-cell virus transport, entry into host-cell (e.g., via cell receptor binding), genome release into host cell, etc. Despite their central importance to the life cycle, the various evolutionary pressures acting on spherical capsids are not well known. Half a century of empirical data has uncovered a large array of capsids sizes that range from tens to many thousands in subunit composition. Spherical capsids of all observed sizes may be obtained from a grouping of twelve pentamers (symmetric clusters of five subunits) separated by a variable number of hexamers (clusters of six subunits). This is the case for the T~7d papilloma viruses where all capsomers are made up of five subunits but they are in both hexavalent and pentavalent configuration, and larger viruses whose ‘‘hexamers’’ are actually trimers of ‘‘fused’’ or covalently bonded dimers. Capsid size may be characterized by two integers, h and k, which describe the number of hexamers (hzk{1) one would have to ‘‘walk over’’ to get from one pentamer to an adjacent pentamer within a completed capsid. The utility of the class system is not entirely lost, however; specific angle patterns within the capsid ensures the existence of distinct hexamer shapes (each shape is colored distinctly in. Evidence indicates that capsid formation is nucleated, often starting with a single capsomer species (e.g., pentamers; for the purposes of this paper, a capsomer is a generally symmetric cluster of either five or six subunits), which then proceeds to completion by the addition of small subunit clusters (or single subunits). In T~1 capsids, where subunits are in identical/equivalent environments, nucleated assembly will be possible with no additional machinery. However, the formation of two or more capsomers from a single interaction site will require the employment of additional machinery to ensure high yields of the native state. For example, quasi-equivalent switches are required for the proper assembly of capsids containing two distinct capsomers: a pentamer and one type of hexamer. The addition of a second hexamer shape necessitates the requirement of a second mechanism such as auxiliary proteins for proper assembly. For spherical virus capsids requiring more distinct

hexamer shapes. Additional mechanisms to stabilize those new shapes at exactly the right positions within the forming capsid are likely to be also needed dominantly form.

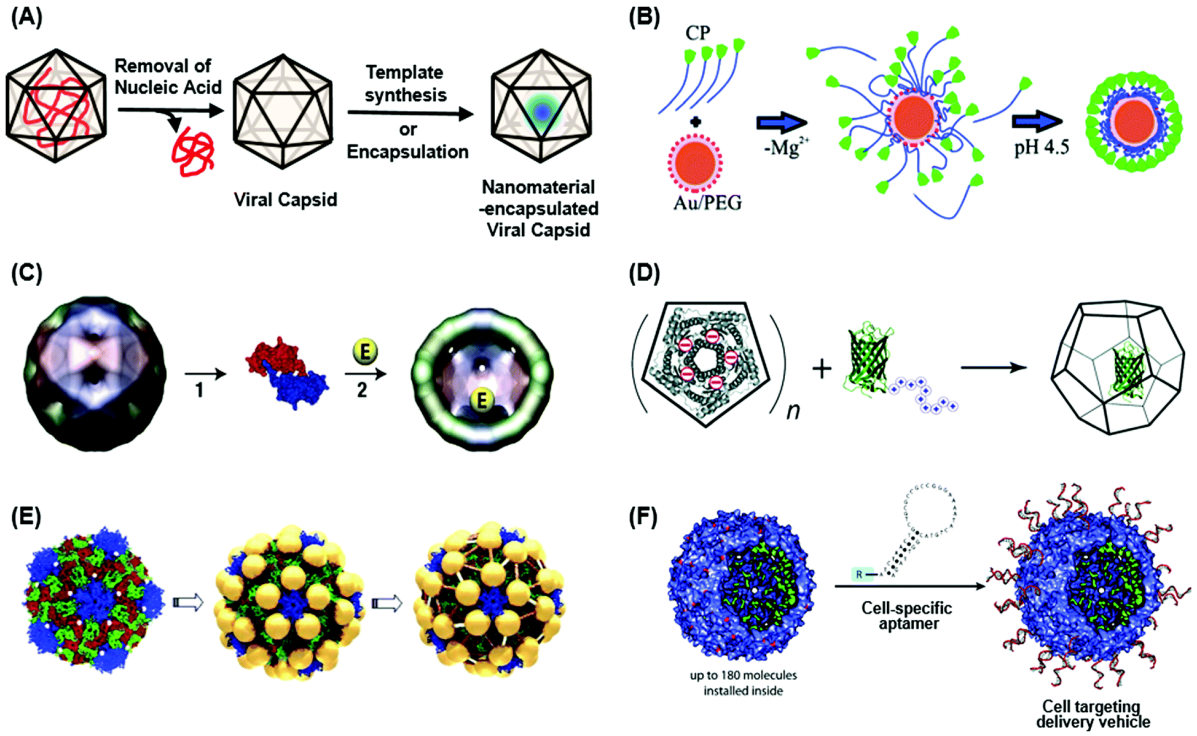

Figure 5. Viral Capsids and their forms

Because capsids from different classes display markedly different geometries, they are bound to display different physical properties. The periodic nature of capsid hexamer contents also useful in understanding ‘‘T-switching’’: a process that permits canonical capsid subunits to more easily sample capsids containing similar hexamer shapes. This allows for a segue to understanding currently intractable and deadly pleomorphic viruses like Ebola and arenaviruses. For example, from the above T-switching rule, the available diversity of an arena virus may only be explained if we assume that the biologically relevant form of the arenavirus is the T~12 capsid. Non-icosahedral capsids. Although the framework presented doesn’t appear to readily explain non-icosahedral capsids (some are just ‘‘slightly’’ non-icosahedral, such as the natively prolate phi29 capsids, while others are wildly different in form, such as Ebola with its natively filamentous shape), those capsids, like their icosahedral counterparts, also display capsomer sub-structures. In light of this, the geometric constraints analogous to endo angles that affect capsomer shape may be useful in obtaining insights into non-icosahedral capsid morphology, behavior, and classification. It will be exciting to see whether incorporating the non-icosahedral capsids into an expanded capsid periodic table will be possible. All canonical capsids (made up of trapezoidal subunits) may be built from a single type of pentamer and a repertoire of distinct hexamer shapes (colored distinctly only once in each capsid. The hexamer shape is described by the number of endo angles it displays. It is necessary to underline that effect of destroy of enveloped viruses is based on the highly symmetric structure (e.g. icosahedral and others) of many viruses, which leads to a well-defined resonant frequency which may be specifically absorbed by these structures and may subsequently lead to their irreversible damage.Modeling and Simulation of oscillation effects in different viruses including corona like onesis based on studies of biostructure’s physical

characteristics, scattering and absorption properties.Estimation of electromagnetic (EM) spectrum and resonance wave length ranges is important for characterization of nano-micro-scaled particles and determination of biostructures unique spectral signatures, essential in bio-agents detecting and identification systems, and becoming as a great challenge in real systems investigations.Method of estimation of spectral response on EM field & particle interaction is based on solutions of electrodynamics two (2D) or three (3D) dimensional boundary tasks. Analytical expressions of EM fields are derived from rigorous solutions of Maxwell's and Helmholtz's equations and defined through the dimensionless parameters, diameters over an excitation wave-length. It makes possible to apply the classical well-known approach to sub-micro particles characterization.One of the ways for estimation of resonance frequencies range is the method determining theeigenvalues and corresponding eigenvectors obtained by algebraic system of functional equationswhich could be written based on the solution of electrodynamic task considering EM wave-VL particlescattering [54]. Algebraic system has more complicated form when VL particle is of core-shellstructure with different electric parameters. Resonant wavelength range is predicted theoretically inthe vicinity of values corresponding the maximums of intensity of particular scattered partial waves.Estimation of resonant wavelength range based on determination of far-field characteristics such asscattering or absorption cross sections, the intensity of energy, wholly representing the response onwave-particle interaction is considered as the reasonable and decisive solution preferable for studyingthe spectroscopic properties and determination of possible spectral signatures of bioparticles, viruses.Method of studying spectroscopic characteristics based on elaborated physical models of virusesrepresents the new possibility for estimation of spectroscopic properties and resonance wavelengthrange of viral nanoparticles, virions.Thus, two main mediums of different structures and properties constitute the virion: capsid of proteincapsomeres and nucleic acids into the capsid. Type, number and arrangement of capsomers and lengthof nucleic acid are essential in defining the size of capsid designed mostly in near-symmetricalgeometry, having the unique self-assemble mechanism.A simple calculation shows, that the ratio of inner (core) and outer spherical volumes of icosahedralcapsid of bacteriophage T7 (of diameters inner 1 d = 42.6 nm and outer 2 d =56.6 nm) is approximately0.426 [55], so quite a large portion (0.57) of volume in virion is occupied by the capsid proteins.Capsid's size is large enough than the ''discrete'' nature of protein subunits, therefore the influence ofthat on capsid whole geometry could probably be less significant. This fact allows virion to bemodelled as a spherical core-shell particle of smooth inner or outer surfaces. Core-shell VLP model ofspherical geometry could be used as the first approximation of shape-structure of icosahedral unenvelopedvirion. The structure and geometry of capsids as well as processes happening insidea layer probably dominate in determination of physical, spectroscopic properties of nano-sizedparticles, virions.

Theoretical approach of studying spectroscopic properties of VLPs is based on classical Maxwell'sEM theory, separation of variables method for solving Helmholtz's (wave) equation. Solution ofHelmholtz's equation leads to the Bessel's and/or Legendre's equations [56]. EM fields in differentareas of VLP are written as the sums of multipole-waves with unknown multipole coefficients.Application of boundary conditions to EM field components on core-shell surfaces and labourconsumingmathematical transformations leads to rigorous theoretical solution of EM scatteringproblem on single VL particle. Analysis of EM field expressions show that arguments of functionsdetermining the multipole coefficients and scattered fields depend only on relative values of particlesdiameters over wavelength. Therefore, it makes possible to expand the research area, and the findingsof well-established

Mie theory [57] considering the light scattering on a homogeneous sphere be applied to nanoparticles of core-shell morphology and particles of biological origin as well.

Spectral response on EM wave-VLP interaction is determined by estimation of far-field ($r \ll 2d/\lambda$) characteristics representing the angular distribution of scattered (absorbed) energy. Scatteringproperties are characterized via expressions such as: the total scattering cross section

$$\sigma_T = 2\pi \frac{1}{k_3^2} \sum_{s=1}^{\infty} \left(\left| A_s^e \right|^2 + \left| A_s^m \right|^2 \right) \frac{s^2(s+1)^2}{(2s+1)}$$

Proposed theoretical solution, analytical expressions of EM fields make possible to estimate the fields in the areas of core, shell and surrounding areas of VLPs. based on machine learning and modeling techniques have been used for generating a simulated spectrum of nanoparticle of given size and available literature optical constants.

Computer simulation (based on MatLabR2013b software) was carried out for TMV particles characterization. Parameters of TMV particle are obtained from scientific publications based on different measuring technics. Length of TMV virion is 280-300 nm, outer (d_2) and inner (d_1) diameters of capsid are 18 nm and 4 nm, correspondingly. Two models - homogeneous cylindrical (of diameter) and homogeneous of core-shell structure (of diameters - outer and inner), are used for simulation study of TMV virion (Fig.6). Computer simulation shows, that expected resonant spectral response is observable on far-field characteristics (Fig.7,8), resonant vibrational frequencies of whole TMV particle may be associated to scattering cross-section maximums. Values and locations of maximums strongly depend on dielectric (Fig. 7) and magnetic (Fig.8) parameters, distance between the neighbor maximums increases the longer wave lengths range is.

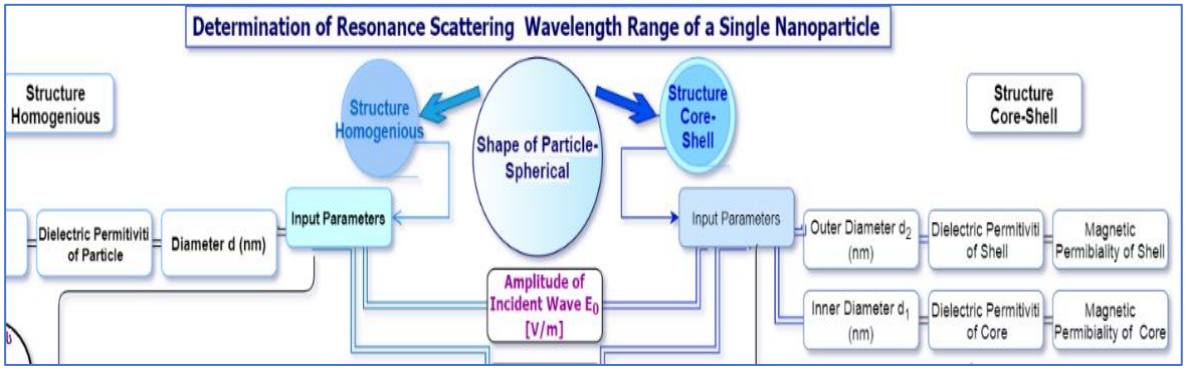

Figure 6. Fragment of the Algorithm for Releasing the Vibration Frequency Method for Viruses

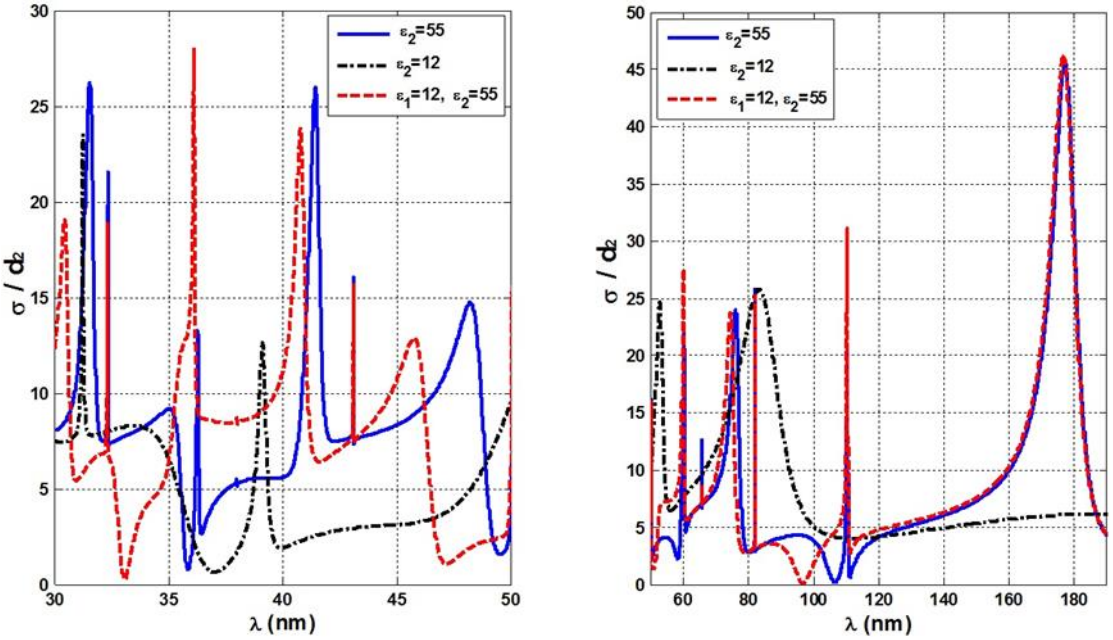

Figure 7. Fig Forward scattering cross section (σ / d_2) vs excitation wave length (λ).Cylindrical model: diameter d_2 =18 nm, dielectric permittivity solid blue), 12 (dash-dot black); Core-shell model (dash red): diameters $d_2 = 18$ nm (outer) and $d_1 = 4$ nm (inner), dielectric permittivity: $\varepsilon_1 = 12$ (core), $\varepsilon_2 = 55$ (shell); $\varepsilon_3 = 1$ (surrounding medium).

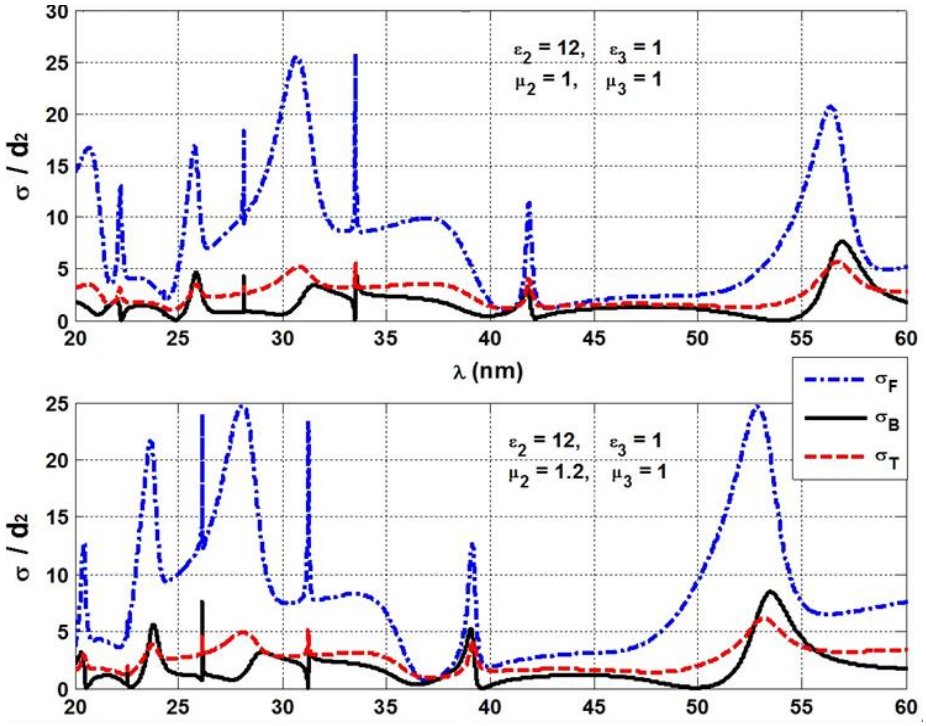

Figure 8. Scattering cross sections (σ / d_2) vs excitation wave length (λ): Forward σ_F (dash-dot blue); Backward σ_B (solid black); Total σ_T (dash red).

Cylindrical model of TMV virion: diameter d_2=18 nm, dielectric permittivities of particle $\varepsilon_2 = 12$ and surrounding medium $\varepsilon_3 = 1$, magnetic permeabilities of particle $\mu_2 = 1$.2 (a), 1 (b) and surrounding medium $\mu_3 = 1$.

Near-field distribution presented in a form of isolines of EM field amplitudes (Fig. 9), indicates the locations of energy maximums inside and outside of particle. Investigation of EM field distribution makes possible to have insight vision of nanobioparticles [59-61].

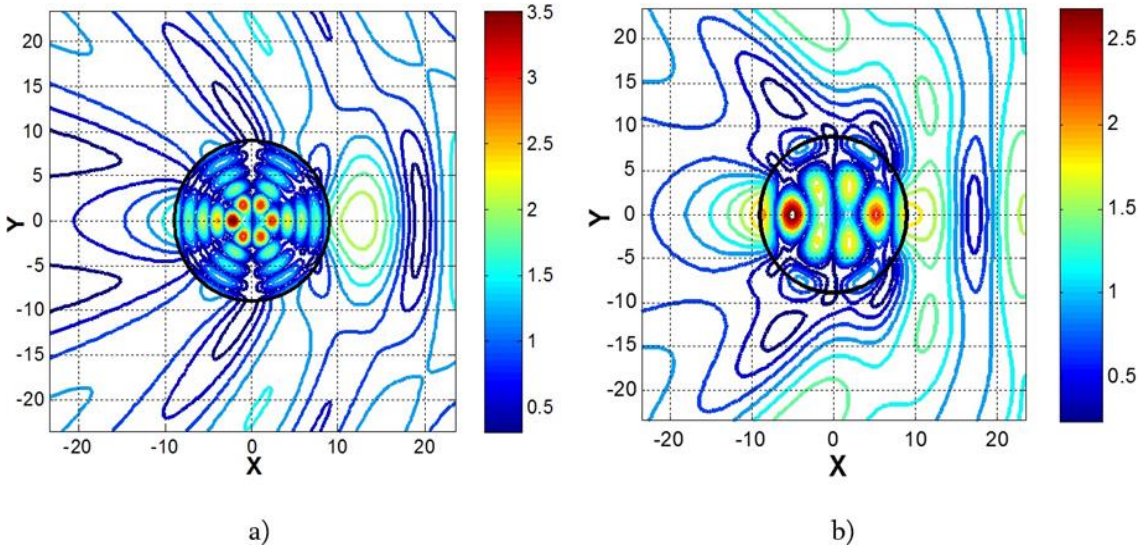

Figure 9. 3 Isolines of EM field amplitudes for cylindrical model of TMV virion, in the range of (-λ,+λ).
Diameter of cylinder: d_2=18 nm; dielectric permittivity: of cylindrical particle - $\varepsilon_2 = 55$ (a), 12 (b), surrounding medium - $\varepsilon_3 = 1$, excitation wave length λ = 23.5 nm, (X,Y) plane is perpendicular to the axis of cylinder.

Proposed computing model is useful for investigation of spectroscopic properties of nanobioparticles and appreciation of possible resonant wave length ranges correlating with scattering/absorbing efficiency of VLP.

Experimental data obtained by applying exquisite techniques and outcomes of theoretical or simulation models should complement each other and verify factors such as possible anisotropy ofcore-shell areas of virion, surface roughness and inhomogeneity unforeseen in simplified approaches.

5. Conclusions

Spectroscopic methods have the characteristic of providing fast results and reliable information related to the composition of the samples. The studies presented here have shown promising results in a field of science that needs to be better explored. It has been shown that multivariate analysis techniques are of great importance to analyze spectroscopic data, providing the potential to identify and classify biological samples. We do hope that with advancement in this field of study, spectroscopic methods and toolswill be used in bio medicine in the near future. Methods of light therapy of different diseases based on estimation of EM field characteristics and resonant wave ranges based on computer simulation of

nanobioparticles characterization will be widely implemented, andpossibility of determination of resonant (own) frequencies of entire system of molecules including virionswill be a key point for that.

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