UNIVERSITY OF MUMBAI

DEPARTMENT OF BIOPHYSICS

ORGANISES

BIOPHYSICS WEEK

On the occasion of National Science Day

(1<sup>st</sup>-3<sup>rd</sup> March 2016)

(LECTURES CUM WORKSHOP ON ADVANCE TECHNIQUES IN BIOPHYSICS)
BIOPHYSICS WEEK

What is Biophysics?

Biophysics is that branch of knowledge that applies the principles of physics and chemistry and the methods of mathematical analysis and computer modeling to biological systems, with the ultimate goal of understanding at a fundamental level the structure, dynamics, interactions, and ultimately the function of biological systems.

Biophysics seeks to explain biological function in terms of the physical properties of specific molecules such as protein, nucleic acids, sugars, fatty acids etc. These building blocks of living organisms, assemble into cells, tissues, and whole organisms by forming complex individual structures with dimensions of 10, 100, 1000, 10,000 nm and larger.

Biophysics explains biological functions in terms of molecular mechanisms: precise physical descriptions of how individual molecules work together like tiny “nanomachines” to produce specific biological functions.

Challenges for biophysicists:

Biological system is a complex dynamic system, it occurs on a wide range of spatial and temporal scales. It is very important and necessary to understand the physical and the chemical processes occurring in the biological systems. The dimension of biological processes ranges from small organic molecules to large multi protein complexes, from cellular processes to the interaction of organisms with their environment. One needs to understand that a small conformational changes results into a various sequential phenomena. Physics make a contribution by providing tools, fundamental laws, mathematical framework and computational approaches to explain the possible answers.

Who can study Biophysics?

Biophysics is interdisciplinary course. It can be availed by student from various branches of science such as Physics, Chemistry, Biotechnology, Microbiology, Biochemistry applied Biology etc. The idea behind the Biophysics course is to understand the various biological processes from all aspects-Biological, Physical and Chemical. One can consider it as a training ground for a new kind of research.

Benefits to the students in the field of Biophysics?

Biophysics is an emerging field in science. One can join research and development field in the areas of structure base drug development, bio-imaging, pharmaceuticals, forensic, cell & physiology, radiobiology, bioinformatics and various other fields.

About Workshop:

On the occasion of National Science day, the Department of Biophysics is celebrating a Biophysics Week. A 3 day seminar cum workshop/demonstrations on various platforms of biophysics such as structural biophysics, Spectroscopy, Radiation Biophysics, Physiological Biophysics, molecular Biophysics and Bio-imaging ( advanced Microscopy) etc.
Who can participate?

This intense course is primarily designed for students who are pursuing Third year Bachelor of Science degree in the stream of Physics/Chemistry/Biology of recognised universities/institutions. Students should provide a bonafide certificate from the principal/concerned authority.

Workshop fee: Rs. 150/- (Includes tea, coffee, working lunch)

The student must get confirmation before registration for the availability of seats. Cash or Demand Draft in favour of The Finance and Account officer, University of Mumbai, Vidyanagari.

Last Date for registration: 25th February 2016.

Only 40 participants would be selected based on your registration information. Registration is on first come first basis. Accommodation will not be provided by the department/University, outside students has to make their own accommodation arrangements.

For any further Queries & registration contact us on email: biophysicsweek2016@gmail.com

URL: http://archive.mu.ac.in/science/bio_physics/home.htm

Contact Person:

Vinod Jaiswal: 09699569168
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Priyanka Pal: 08976135103
| **Full Name** |  |
| **Class & year** |  |
| **Communication Address with email & Tel number** |  |
| **Registration fees Details cash/DD** |  |
| **College Name with detailed address** |  |
| **Research Interest (mention only areas)** |  |
| **Why do you want to join this program?** |  |
| *(write in 25 words only)* |  |
| **Signature** |  |
# PROGRAM SCHEDULE

**Venue: Department of Biophysics, University of Mumbai**

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<th>Day I: 1(^{st}) March 2016</th>
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<tr>
<td><strong>1(^{st}) Half</strong>&lt;br&gt;Time: 9.30 AM to 1.00 PM</td>
<td>Lecture 1: Introduction to Biophysics&lt;br&gt;Speaker: Prof Prabhakar Dongre&lt;br&gt;Prof &amp; Head, Department of Biophysics&lt;br&gt;University of Mumbai</td>
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<td>Lecture 2: Physiological Biophysics&lt;br&gt;Speaker: Dr Sanjeev Rao, (MBBS, MS CVTS)&lt;br&gt;IIT Bombay</td>
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<td><strong>Tea break</strong></td>
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<td>Lecture 3: Fluorescence spectroscopy&lt;br&gt;Dr Basir Ahmad&lt;br&gt;UM DAE Centre of Excellence in Basic Sciences.</td>
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<td>Lunch break</td>
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<td><strong>2(^{nd}) Half</strong>&lt;br&gt;Time: 2.00 PM to 5.00 PM</td>
<td>Demonstration 1: Fluorescence Spectroscopy</td>
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<th>Day II: 2(^{nd}) March 2016</th>
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<td><strong>1(^{st}) Half</strong>&lt;br&gt;9.30 AM to 1.00 PM</td>
<td>Lecture 4: Radiation Biophysics&lt;br&gt;Speaker: Dr. J R Bandekar&lt;br&gt;Former Head, Radiation Biology &amp; Health Division, BARC, Mumbai</td>
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<td>Lecture 5: Raman Spectroscopy&lt;br&gt;Speaker: Dr C Murli Krishna&lt;br&gt;PI &amp; Scientific Officer ‘F’&lt;br&gt;Advanced Centre for Treatment, Research &amp; Education in cancer (ACTREC), TMC Navi Mumbai</td>
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<td><strong>Tea break</strong></td>
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<td>Lecture 6: Bio-imaging&lt;br&gt;Speaker: Smt Sharda Sawant&lt;br&gt;Scientific Officer ‘E’&lt;br&gt;Advanced Centre for Treatment, Research &amp; Education in cancer (ACTREC), TMC Navi Mumbai</td>
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<td>Lunch break</td>
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<td>2nd Half</td>
<td>Demonstration 3: Raman Spectroscopy</td>
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<tr>
<td>Time: 2.00PM to 5.00PM</td>
<td>Demonstration 4: Fluorescence Microscopy/ Electrophoresis / UV dose/ energy measurement</td>
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<th>Day 3: 3rd March 2016</th>
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<tr>
<td>1st Half</td>
<td>Feedback, Q &amp; A, Prize distribution &amp; conclusion</td>
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<tr>
<td>Time: 9.30AM to 12.00PM</td>
<td>Feedback, Q &amp; A, Prize distribution &amp; conclusion</td>
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**SCIENCE EXHIBITION**

<table>
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<th>Day 1,2 &amp; 3</th>
<th>Working model presented by students in the area of biophysics.</th>
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<tr>
<td>9.30 am to 6.00 pm</td>
<td>Working model presented by students in the area of biophysics.</td>
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Lecture 1

Introduction to Biophysics

Biophysics is the science of physical principles underlying all processes of life, including the dynamics and kinetics of biological systems. Biophysics covers all scales of biological organization, from molecular to organismic and populations. Biophysical research shares significant with biochemistry, nanotechnology, bioengineering, computational biology and systems biology.

Biophysicists used variety of techniques to address the biological problems. Fluorescent imaging techniques, electron microscopy, x-ray crystallography, NMR spectroscopy, atomic force microscopy (AFM) and small-angle scattering (SAS) both with X-rays and neutrons (SAXS/SANS) are often used to visualize structures of biological significance.

Protein dynamics can be observed by neutron spin echo spectroscopy. Conformational change in structure can be measured using techniques such as dual polarisation interferometry, circular dichroism, SAXS and SANS. Direct manipulation of molecules using optical tweezers or AFM, can also be used to monitor biological events where forces and distances are at the nanoscale.

Lecture 2

Physiological Biophysics,

Physiological biophysics is very diverse that include systems and cellular physiology, cell and molecular biology, biophysics and structural biology. A common emphasis is on quantitative understanding of biological process on a physical level. Some of the areas covered by physiological biophysics are as follows.

• Membrane physiology and biophysics (ion channels, receptors, transporters, protein-lipid interactions, surface phenomena, model membranes),

• Intercellular and intracellular signalling (neurotransmission, endocrine regulations, messengers, secretory mechanisms, regulatory proteins, calcium signalling pathways, nitric oxide),

• Excitability and contractility (neurophysiology, smooth, cardiac and skeletal muscle physiology, excitation-contraction coupling, cellular motility),

• Biophysical analysis of cellular function (bioenergetics, volume regulation, thermodynamics, mathematical models),

• Metabolic regulations (enzymology, ATPases, plant and microbial metabolism),

• Cellular aspects of pharmacology, toxicology and pathology (receptor-drug interaction, the action of drugs and toxins on signalling and regulatory functions, free radicals, lipid peroxidation, ischemic preconditioning, calcium paradox),
Fluorescence Spectroscopy

PHENOMENON OF FLUORESCENCE

Fluorescence is the emission of light from any substance, and occur from electronically excited state, it is divided two categories – fluorescence and phosphorescence depending on the nature of the excited state. In excited singlet states, the electron in the excited orbital is paired by to the second electron in the ground state orbital. Consequently, return to ground state is spin allowed and occur rapidly by emission of photon. The emission rate of fluorescence are typically $10^8$ s$^{-1}$. Phosphorances is the emission of light from triplet excited state, in which the electron in the excited orbital has the same spin orientation as the ground state electron. Transitions to the ground state are forbidden and the emission rates are slow, so phosphorescence life time is in millisecond.

In fluorescence spectroscopy, a molecule absorbs a light of shorter wavelength and emits a light of longer wavelength. Fluorescence spectroscopy is a technique of considerable practical importance. Measurements of fluorescence can provide important information regarding the molecule, its quantity and local environment, etc. Fluorescence spectroscopy finds widespread use in basic and applied researches of chemical and biological sciences. Analytical techniques based on fluorescence can yield low detection limits and are very sensitive, highly specific, often economical and relatively simple to perform. The high specificity arises from the fact that fluorophores exhibit specific excitation (absorption) and emission (fluorescence) wavelength.

INSTRUMENTATION

All fluorescence instruments contain three basic items: a source of light, a sample holder and a detector. In addition, to be of analytical use, the wavelength of incident radiation needs to be selectable and the detector signal capable of precise manipulation and presentation. In simple filter fluorimeters, the wavelengths of excited and emitted light are selected by filters which allow measurements to be made at any pair of fixed wavelengths. Simple fluorescence spectrometers have a means of analysing the spectral distribution of the light emitted from the sample, the fluorescence emission spectrum, which may be by means of either a continuously variable interference filter or a monochromator. In more sophisticated instruments, monochromators are provided for both the selection of exciting light and the analysis of sample emission. Such instruments are also capable of measuring the variation of emission intensity with exciting wavelength, the fluorescence excitation spectrum.

In principle, the greatest sensitivity can be achieved by the use of filters, which allow the total range of wavelengths emitted by the sample to be collected, together with the highest intensity source possible. In practice, to realize the full potential of the technique, only a small band of emitted wavelengths is examined and the incident light intensity is not made excessive, to minimize the possible photodecomposition of the sample.

The main components of a spectrofluorimeter, indicated in below figure are:
1) A continuous source of radiant energy (mercury lamp or xenon arc)
2) A monochromator usually a prism (P1), to choose the wavelength with which the sample is to be irradiated.
3) A second monochromator (P2) which is placed after the sample enables the determination of the fluorescent spectrum of the sample.
4) A detector, usually a photomultiplier suited for wavelengths greater than 500nm
5) An amplifier to amplify the signal.

**PRINCIPLE:**

In fluorescence spectroscopy, a molecule absorbs a light of shorter wavelength and emits a light of longer wavelength.

According to the Frank-Codon principle, the molecule will most probably be excited to one of the higher vibrational states of the excited electronic state. The molecule in an excited vibrational energy level loses energy rapidly (in $10^{-12}$ or less) and moves to a lower (and finally to the lowest) vibrational energy level in the same excited electronic state. Once the molecule is in the lowest vibrational level of the excited electronic state, $S_1$, the molecule can return to one of the vibrational states of the ground singlet state, $S_0$ through an emission of photon radiation, called fluorescence, or via other relaxation pathways.

In fluorescence, the acquired (absorbed) electronic energy is lost via the emission of a photon while transition occurs from lowest vibrational level of first excited singlet (i.e. $v = 0$ of $S_1$) to one of the vibrational states of the ground singlet state, $S_0$. These radiative processes take about $10^{-9}$ second.

**APPLICATION OF FLUORESCENCE SPECTROSCOPY**

1) Fluorescence spectroscopy is used in chemical, biochemical, medical, research fields for analyzing organic compounds.
2) Used for fluorescence anisotropy and polarization measurement
3) Used to for quenching studies i.e. for protein tryptophan fluorescent measurement
4) It can be adapted to microscopic level using microfluorimetry.
Radiation Biophysics.

As a science, radiation biophysics is concerned with the action of ionizing radiation on biological systems. In contrast to radiobiology, which deals with the biological and medical consequences of radiation, radiation biophysics concerns itself mainly with the physical and chemical primary processes, and biological effects are attributed to them. Further neighboring fields are radiation chemistry and photobiology: the former because radiation-induced chemical alterations generally initiate the biological-metabolic radiation effects, the latter because visible and especially ultraviolet light is often able to generate effects similar to ionizing radiation.

What about the biology of radiation? What was being done to examine the biological effects of these rays? Next to nothing except by sad accident. Pierre Curie carried out a few small experiments on the biological effects of the emanations from radium. In particular, he reported before the Académie Française on his studies on the effects of radium emanations on developing tadpoles. He found that these emanations produced severe developmental abnormalities in the growing tadpoles. Little attention was paid to these findings, and even when early radiologists developed skin lesions and lost fingers there was little attempt to systematically examine the effects of radiation on living systems.

In 1902 it was first formally reported that radiation of the skin with X-rays could lead to skin cancer, but even then, few scientists were motivated to proceed with studies of the systematic biology of radiation. The first truly systematic study of the pathological effects of ionizing radiations on animal systems was done by Heineke, whose studies reported on mice, guinea pigs, rabbits, and dogs. Special attention was paid to the reproductive systems of irradiated rodents by Albers-Schönberg (1903) and by Halberstéider (1905). Bergonie and Tribondeau (1906), after extensive studies of the testes of rodents, formulated what has come to be known as the "Law of Bergonie and Tribondeau." Remarkably, their statement of some principles of radiation effects on cells remains sound today, with some important exceptions. They stated that if cells have a high mitotic rate, that under normal circumstances they will undergo many mitoses, and that they are generally of a "primitive" type, then they will be radiosensitive. We can identify many exceptions at this time, but the core meaning of the rule remains useful. Radiation biophysics as a quantitative science saw the light of day in the 1920s with Dessauer's (1922) efforts to quantitatively investigate the effects of radiation. These early studies were centered around a statistical analysis of dose-response curves in an effort to understand the mechanisms of radiation action. Except for the truly insightful studies on the mutagenic action of ionizing radiation, by Mffler in 1927 (reviewed in Mffler, 1950), little else happened before World War II. One of the great constraints on the biological investigation of radiation effects in these early times lay in the limited ability to quantitate dose. In the immediate postwar years, due in part to the atom bomb and its impact, radiation biology and biophysics came into its own with the work of pioneers such as Lea and his colleague Catchside, as well as the work of the German biophysicist Zimmer. It is interesting to observe that most of the early workers in this field were physicists who turned to biology, perhaps because their biological colleagues were slow to enter this difficult field. In particular, the proposal of a model for repair of sublethal damage by Elkind and Sutton (1960) revolutionized our thinking about the initial injury to DNA and the subsequent complicated repair processes.

We are now on the verge of a new cycle of significant discoveries related to the effects of ionizing radiation. If we re-examine the field of radiation bioeffects a decade from now, we may find that this decade is in many ways similar to the 1895-1905 period, which was the "golden age" of discovery for radiation and radioactivity research.
Radiation Biophysics includes following topics

1) Interaction of Radiation with Matter
2) Interaction of Radiation with Living Systems
3) Elements of Nuclear Physics
4) Biological effects of Radiation
5) Biological aspects of Radiotherapy
6) Diagnostic Radiology
7) Medical biophysics
8) Principle of Radiation Protection

Lecture 5

Raman Spectroscopy.

C. V. Raman, in 1928, observed that scattered radiation of different wavelengths from incident radiation are obtained for some molecules. The difference was later related to the structure of the molecule where it was found that the frequency differences are just the vibrational frequencies of specific molecular vibrations. Most of the scattered radiation has, of course, the same wavelength as that of the incident radiation, due to elastic collisions. This scattered radiation is known as Rayleigh scattering. Inelastic collisions yield scattered radiation that can either be of longer wavelengths than incident radiation (stokes shift) or shorter wavelengths (antistokes shift). A simplified energy diagram that illustrates these concepts is shown below.

![Energy Diagram](image)

Figure 1: Origin of Raman Spectroscopy

Stokes radiation occurs at lower energy (longer wavelength) than the Rayleigh radiation, and anti-Stokes radiation has greater energy. The energy increase or decrease is related to the vibrational energy levels in the ground electronic state of the molecule, and as such, the observed Raman shift of the Stokes and anti-Stokes features are a direct measure of the vibrational energies of the molecule.

Application of Raman Spectroscopy

Raman spectroscopy is used in many varied fields – in fact, any application where non-destructive, microscopic, chemical analysis and imaging is required. Whether the goal is qualitative or quantitative data, Raman analysis can provide key information easily and quickly. It can be used to
rapidly characterise the chemical composition and structure of a sample, whether solid, liquid, gas, gel, slurry or powder.

Lecture 6

Bio-imagining/Molecular Biophysics.

Biological imaging has entered a new era and is presently having a profound impact on the way research is being conducted in the life sciences with the recent development of fluorescent probes and new high-resolution microscopes. Bio-imaging covers a wide range of techniques such as CT, MRI, PET, SPECT, and even X-rays. Image acquiring and processing is the fundamental base of bio-imaging. Biologists have come to depend more and more on imaging. They can now visualize subcellular components and processes in vivo, both structurally and functionally. Observations can be made in two or three dimensions, at different wavelengths (spectroscopy), possibly with time-lapse imaging to investigate cellular dynamics. Bio-Imaging techniques include PET, MRI, or many fluorescence methods, to image an entire body to the cellular or molecular level, which then enables the use of Microscopy for even greater detail. Bio-Imaging techniques are typically non-invasive, and are designed to minimally interfere with life processes. Bio-Imaging utilizes light, fluorescence, electrons, ultrasound, X-ray, and magnetic resonance as illumination sources to increase image detail.

Fluorescence microscopy is one of the powerful tools of the bio-imaging. It requires the use of so-called fluorochromes or fluorophores, which absorb light in a specific wavelength range, and re-emit it with lower energy, that is, shifted to a longer wavelength. Today, a very large number of different dyes with absorption from the UV to the near-infrared region are available, and more fluorophores with new properties are still being developed. The principal advantages of this approach are a very high contrast, sensitivity, specificity, and selectivity. The use of fluorescently stained antibodies and the introduction of a variety of fluorescent heterocyclic probes synthesized for specific biological applications brought about an unprecedented growth in biological applications of fluorescence microscopy. Introduction of fluorescent proteins sparked a new revolution in microscopy, contributed to the development of a plethora of new microscopy techniques, and enabled the recent enormous growth of optical microscopy and new developments in cell biology.

Principle:

In fluorescence, a molecule absorbs a light of shorter wavelength and emits a light of longer wavelength. Thus fluorescence in a microscopic object has to be excited by incident light of a shorter wavelength. Thus, a fluorescence microscope is constructed in a way that allows excitation of fluorescence, subsequent separation of the relatively weak emission from the strong exciting light, and finally, the detection of the fluorescence. An efficient separation of the exciting from the fluorescence light, which eventually reaches the electronic detector, is mandatory for obtaining high image contrast.
Features of Fluorescence Microscopy

1) Image Contrast
2) Specificity of Fluorescence Labeling
3) Sensitivity of Detection

Application of Bio-imaging

1) Positron emission tomography (PET) is a nuclear medical imaging technique for quantitative measurement of physiologic parameters in vivo based on the detection of small amounts of positron-emitter-labelled biologic molecules.
2) A SPECT (Single Photon Emission Computed Tomography) is one of the bioimaging techniques where a gamma camera or a set of gamma cameras mounted on a gantry so that the detector can record projections from many equally spaced angular intervals around the body.
3) The principles of CT are conceptually simple. Physically, X-rays can traverse a cross-section of an object along straight lines, are attenuated by the object, and detected outside it. During CT scanning, the cross-section is probed with X-rays from various directions; attenuated signals are recorded and converted to projections of the linear attenuation coefficient distribution of the cross-section. These X-ray shadows are directly related to the Fourier transform of the cross-section, and can be processed to reconstruct the cross-section.

ELECTROPHORESIS:

Electrophoresis is a technique for separating or resolving charged molecules such as amino acid, peptide, proteins, and nucleic acid in a mixture under the influence of electric field. Charged molecules in an electric field move or migrate at a speed determined by their charge to mass ratio.

According to the laws of electrostatic, an ion with charge Q in an electric field of strength E will experience on electrical force $F_{ele}$ is given by

$$F_{ele} = Q \cdot E$$

The resulting migration of the charged molecules through the solution is opposed by frictional force

$$F_{frictional} = V \cdot f$$

Where $V$ is rate of migration of charged molecules and $f$ is frictional coefficient

Frictional coefficient depends on the size and shape of the migrating molecules and viscosity of the medium.

In constant electric field the above force on charged molecule balances each other. Therefore

$$Q \cdot E = V \cdot f$$

$$\mu = \frac{V}{E} = \frac{Q}{f}$$
The migration of charged molecule under the influence of electric field is generally expressed in terms of electrophoretic mobility (μ) which is the ratio of migration rate of molecule to the applied magnetic field. So, according to the above equation if two molecules have the same mass and shape, the one with the greater net charge will move faster towards an electrode.

Types of electrophoresis

1) Moving boundary electrophoresis
2) Zone electrophoresis
3) Immuno-electrophoresis

Applications

The vast applications of electrophoresis are most evident in the health or medical industry, including antibiotic and vaccine analysis. Protein and DNA analysis are also important electrophoresis applications. Aside from allowing researchers to map and see the differences in the genetic code of species on earth, electrophoretic DNA analysis also provides a reliable tool in forensic investigations.

DNA Analysis

- Electrophoresis is one way of analyzing DNA, deoxyribonucleic acid, which is the code that contains all the traits you inherited from your parents. DNA is arranged in sequences, for instance, one sequence represents the color of your eyes and another sequence represents the color of your skin. Through electrophoresis, specific DNA sequences can be analyzed, isolated and cloned. The analyzed DNA may be used in forensic investigations and paternity tests.

Protein Analysis

- Electrophoresis has advanced our understanding on the structure and function of proteins. These molecules are needed by our body cells and may be analyzed, for instance, by getting blood and urine samples. Then through electrophoresis, the amount of proteins in your blood or in your urine is measured and compared to established normal values—lower or higher than the normal levels usually indicates a disease.

Antibiotics Analysis
The application of electrophoresis in antibiotic studies dates back to the 1950s. Further studies led to improved electrophoretic techniques and new antibiotics. These drugs, such as penicillin, are among the widely prescribed drugs against bacterial infections. With electrophoresis, experts are not only able to synthesize new antibiotics but are also able to analyze which types of bacteria are antibiotic-resistant.

**Vaccine Analysis**

Vaccine analysis is one of the many important applications of electrophoresis. There are several vaccines that have been purified, processed and analyzed through electrophoresis, such as the influenza vaccine, hepatitis vaccine and polio vaccine. The exact steps done in the vaccine analysis, however, cannot be determined due to confidentiality reasons of the pharmaceutical companies.

**References:**

- Handbook of fluorescence spectra of aromatic molecule by Berlman
- Principle of Fluorescence Spectroscopy by JR Lakowicz
- Radiation Biophysics by Edward Alpen
- Biophysics an Introduction by Rodney C
- Introductory Raman Spectroscopy by John R Ferraraao and others
- Text book of Physiology By A Guyton
- Text Book of Biophysics by Rao
- Biophysics by Vasantha Pattabhi