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NATO ADVANCED RESEARCH WORKSHOP

OPTICS OF BIOLOGICAL PARTICLES

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Abstracts

NATO ARW: Optics of Biological Particles

Co-Chairs
Valeri Maltsev
Alfons Hoekstra
Gorden Videen

Abstracts of the NATO Advanced Research Workshop
On Optics of Biological ParticlesAk
ademgorodok (Academic Town), Novosibirsk, Russian Federation
October 3 — October 6, 2005

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Objective

Included within this volume are the abstracts to be presented at the NATO Advanced Research Workshop on Optics of Biological Particles. The meeting's focus is on the potential of light scattering in the detection of biological agents, on novel detection systems using polarized light scattering, imaging (microscopy), inelastic scattering, absorption and emission over all EM spectral regions. The aim is to bring together researchers from different fields (physics, biophysics, microbiology, cytology, etc) to foster the flow of information between these diverse groups of researchers. Most of all, we hope that the workshop results in new collaborations that improve research efficiency.

ORGANIZATIONAL STRUCTURE

Chairs

Valeri Maltsev	*Institute of Chemical Kinetics and Combustion, Russia*
Alfons Hoekstra	*University of Amsterdam, Netherlands*
Gorden Videen	*US Army Research Laboratory, USA*

Local Organizing Committee

Valeri Maltsev	*Institute of Chemical Kinetics and Combustion, Russia*
Peter Tarasov	*Institute of Chemical Kinetics and Combustion, Russia*
Dina Goloshchapova	*Institute of Chemical Kinetics and Combustion, Russia*

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NATO Advanced Research Workshop
Programme of the Workshop
«Optics of Biological Particles»
Novosibirsk, Russia

1st day: Monday October 3, 2005

09:45 – 10:00 Alfons Hoekstra "Purpose and Overview of the Workshop"
10:00 – 10:30 Valeri Maltsev "Quantitative biology – the first step in predictive biology. Role of physical methods"

10:30 – 11:00 coffee-break

11:00 – 11:30 G. Videen "Aerosol elastic scattering"
11:30 – 11:50 A. Chernyshev "Determination of dynamic parameters of biological particles with flow cytometry"
11:50 – 12:10 Andrey Fedorenko "Introduction of CytoNova Ltd and SFC Prototype. IPR activity"

12:10 – 14:00 Lunch

14:00 – 14:30 Alexander V. Priezzhev "Optics of red blood cells and cell aggregates"
14:30 – 14:50 Jens Hellmers, Elena Eremina, Thomas Wriedt "T-matrix computation for erythrocyte-like biconcave particles using the Nullfield-Method with Discrete Sources"
14:50 – 15:10 Elena Eremina, Jens Hellmers, Thomas Wriedt "Different shape models for erythrocyte. Light scattering analysis on the base of the Discrete Sources Method"
15:10 – 15:30 Anatoli Borovoi, Vladimir Prokopiev, Nikolai Vagin "Retrieval of distributions of red blood cells over their sizes in the whole blood by small-angular multiple scattering method"
15:30 – 15:50 Bodo Roemer "Use of Scanning Flow Cytometry for advanced classification of erythrocytes in clinical case studies for feasibility"

15:50 – 16:20 coffee-break

16:20 – 16:40 Tarasov P.A., Semianov K.A., Maltsev V.P., Nekrasov V.M. Chernyshev A.V. "Approach to identify and characterize sphered red blood cells"
16:40 – 17:00 Maxim A. Yurkin, Alfons G. Hoekstra "Capabilities of the discrete dipole approximation for simulation light scattering by biological cells"
17:00 – 17:20 Igor K. Lednev, Ming Xu, Vladimir V. Ermolenkov, Wei He "Deep UV Raman Spectroscopic Characterization of Biological Species"

17:20 – 19:00 visit IChK&C

19:00 dinner

2nd day: Tuesday October 4, 2005

09:00 – 09:30 Virginia Foot, Tom Klaentschi, Karen Baxter "Characterisation of Bioaerosols using spatial elastic scattering and fluorescence"

09:30 – 09:50 Vyacheslav M.Nekrasov, Alexander N.Shvalov "Experimental investigations of bacteria cells growth with scanning flow cytometry"

09:50 – 10:10 Z. Filip, S. Herrmann, K. Demnerova "FT-IR spectroscopic characterization of some sporeforming and nonsporeforming health relevant bacteria"

10:10 – 10:40 coffee-break

10:40 – 11:10 V.N. Lopatin, N.V. Shepelevich "Formation of light-scattering patterns of optically soft particles under variation of their parameters"

11:10 – 11:30 Jun Q. Lu, R. Scott Brock, Xin-Hua Hu, Ping Yang, Douglas A. Weidner "Numerical simulations of light scattering from blood cells using FDTD method"

11:30 – 11:50 Dmitriy Petrov, Yuriy Shkuratov, Elena Synelnyk, G. Videen, Jay Eversole, Matthew Hart, and Kathy Scotto "Tmatrix calculations of photopolarimetric properties of biological particles with complicated"

11:50 – 12:10 Nikolai Voshchinnikov, Gorden Videen "Core-mantle spheroidal model of electroporated vesicles"

12:10 – 12:40 Dirk van Bockstaele "Single cell light scattering in the biomedical field: Why would it provide more than just a trigger for fluorescence collection?"

12:40 – Multi-session round-table

3rd day: Wednesday October 5, 2005

09:00 – 09:30 Antonio Palucci "Optical and morphological characterization of natural phytoplanktonic communities"

09:30 – 09:50 F. Barnaba, L. Fiorani, A. Palucci, P. Tarasov "ENEA laser scanning flow cytometer (CLASS) for optical characterization of marine particles"

09:50 – 10:10 Victor Martinez-Vicente and Gavin H. Tilstone "Characterisation of marine bio-particles from in-situ measurements of bulk optical properties"

10:10 – 10:40 coffee-break

10:40 – 11:10 J.D. Eversole, H-B Lin, C.S. Scotto, A. Huston, V. Sivaprakasam, J. Reintjes, and A. Schultz "Optical Characterization of Bioaerosols"

11:10 – 11:30 Matthew B. Hart, Yuriy G. Shkuratov, Gorden Videen, Jay D. Eversole "Measuring the Polarization Opposition Effect from Single Suspended Aggregate Particles"

11:30 – 11:50 Paul H Kaye, Zbigniew Ulanowski, Warren R Stanley, Virginia Foot "Dual-wavelength Fluorescence for Detecting Single Biological Particles"

11:50 – 12:10 Kevin Aptowicz, Gustavo Fernandes, and Richard Chang "Angularly-resolved elastic light scattering: pattern complexity and feature extraction"

12:10 – 14:00 Lunch

14:00 – 14:30 K.Semyanov, A.Zharinov and V.Maltsev "Scattering by Leucocytes, theory, simulations and experiments"

14:30 – 14:50 Valery A. Loiko, Gennady I. Ruban, Olga A. Gritsai, Alexander A. Miskevich "Human white blood cells: investigation by the methods of light microscopy"

14:50 – 15:10 Maxim A. Yurkin, David de Kanter, Alfons G. Hoekstra "Applicability of effective medium approximation to light scattering by granulated biological cells"

15:10 – 15:30 D. Strokotov, I. Skribunov, V. Yurkin, D. van Bockstaele, M. Lenjou, and K.Semyanov "Identification of blood cell subpopulations from angle resolved light scattering"

15:30 – 15:50 A.Zharinov, D. van Bockstaele, M. Lenjou, and K.Semyanov "Characterization of mononuclear blood cell from light scattering"

15:50 – 16:10 I.Skribunov, D. van Bockstaele, M. Lenjou, and K.Semyanov "Identification of leukocytes with Scanning Flow Cytometer"

16:10 – 16:30 I.Kolesnikova and K.Semyanov "Optics of blood platelets"

16:30 – 19:00 coffee-break and poster session

Poster Session

Sooheir S. Korraa, M.H. El Batanouny, A. Khourshid, S.N. Amin, M.I. Moursy "Photodynamic Effect on Leukemic Cells In Vitro"

Javier A. Martínez, M. P. Hernández "Liposomes size measurement using Angular Light Scattering"

H. Volten "Light scattering database including measurements of phytoplankton"

Liviu-Daniel Galatchi "Light Scattering Effects on Both Aquatic Organisms Enzymes and Enzymatic Reactions Made by Pure Enzymes"

Vladimir V. Berdnik, Robert D. Mukhamed'yarov, Julia E. Andrianova "Radiation transfer in stratified biological objects"

A. N. Korolevich, E. K. Naumenko, N. S. Dubina, S. I. Vecherinski, A. Bernjak, M. Belsley, A. Stefanovska "Intensity backreflectance from the blood plexus in the skin under the low-power laser heating"

Yu. I. Glazachev, V.V. Khramtsov "Development of FRAP method with decaying photobleaching intensity"

A.P. Sviridov, V. Chernomordik, M.Hassan, A.Gandjbakhche "Visualization of tissue structures using polarization degree patterns and correlation analysis"

E.E.Gorodnichev, A.I.Kuzovlev, D.B.Rogozkin "Transmission of polarized light in scattering media with large-scale inhomogeneities"

Gorobchenko O.A., Nikolov O.T., Gatash S.V. "Conformation transitions of the blood proteins under influence of physical factors on microwave dielectric method"

19:00 dinner

4th day: Thursday October 6, 2005

10:00 – 10:30 Yongle Pan, Hermes Huang and Richard K. Chang "Enriching Bio-aerosols for Species Identification by Optical or Biochemical Assay Techniques"

10:30 – 11:00 Yuriy Shkuratov "Backscattering of agglomerated biological particles and surfaces thereof"

11:00 – 11:20 Andrey Ovcharenko, Sergey Bondarenko, Yuriy Shkuratov, Gorden Videen, Jay Eversole, and Matthew Hart "Backscattering of surfaces composed with dry biological particles"

11:20 – 11:40 F. Moreno, O. Muñoz, A. Molina, D. Guirado, A. López, L. Costillo, F. Girela, J.L. Ramos, J.M. Jerónimo "The IAA Light Scattering Facility"

11:40 – 12:00 Kristan P. Gurton, Rachid Dahmani, David Ligon, Burt Bronk "Direct measurement of the aerosol absorption and extinction cross section for Bacillus"

12:00 – 14:00 Lunch

14:00 – 14:20 D.Goloshchapova, V.Nekrasov, E.Protopopova, and A.Chernyshev "A study of penetration of a virus into a cell with Dynamic Flow Cytometry"

14:20 – 14:40 R.S. Marks "The 7th sense: bionic fiber-optic biosensors"

14:40 – 15:00 Soheir Saad Korraa, Ekram Abdel Salam, Rabha El Shinawy, Gihan El Sharnouby "Red Light Laser Effects on Blood Cells of Some Pathological Haemodynamic Human Conditions"

15:00 – 15:20 N.V. Shepelevich "Informational content of integral light scattering indicatrix of optically soft particles"

15:20 – 15:40 Ashim K. Roy, Subodh K Sharma "A simple inversion of the Mie particle extinction spectrum"

15:40 – 16:00 Alexander N. Shvalov "Use of Singular Value Decomposition for analysis of light-scattering Indicatrices of small particles"

16:00 – 16:30 coffee-break

16:30 – 16:50 Konstantin V. Gilev, Alexander N. Shvalov "Sphere particle characterization by means of the neural networks"

16:50 – 17:10 Vladimir V. Berdnik, Valery A. Loiko "High-order neural networks for spherical particle characterization by the intensity of scattered light"

17:10 – 19:00 visit IChK&C

19:00 dinner

5th day: Friday October 7, 2005 Transfer to Airport/Railstation and departure

1st day: Monday October 3, 2005

AEROSOL ELASTIC SCATTERING

Gorden Videen and A. Kastov Thuisanz

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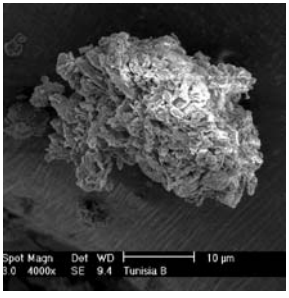
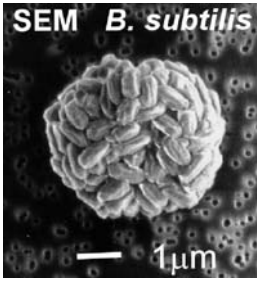
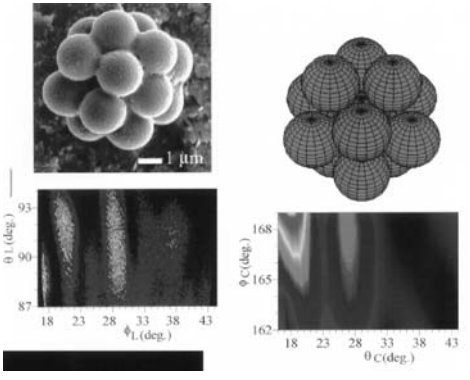
Aerosol particles are not the most pleasant things to try to model. While liquid droplets form the bulk of natural aerosols, many of these are mixed-phase aerosols, containing solid inclusions that spoil the symmetry. Dust particles, like that shown on the right have highly irregular shape, sharp edges and also tend to agglomerate. Accompanying these characteristics are their relatively high refractive index and wavelength-sized dimensions. Taken in sum, they are a modeler's nightmare. Biological particles are another beast, having multiple, irregularly shaped components in addition to intraspecies







variability. Depending on how they are aerosolized, they have a tendency to agglomerate, like that shown below. During this talk, I will discuss some of the modeling efforts undertaken to get a grip on the wide variety of aerosol morphologies that we hope to identify using their light scattering patterns. While these models, in general, are a gross approximation of reality, they are becoming more sophisticated and do provide us with some insight, especially with regard to some scattering features. In some cases, we are surprised to find coincidence between experiment and modeling (right). Other times we may discover that two similar models yield very different results.

DETERMINATION OF DYNAMIC PARAMETERS OF BIOLOGICAL PARTICLES WITH FLOW CYTOMETRY

***A.V. Chernyshev*[1]**

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Flow cytometry is a powerful technique for measuring optical (light scattering and fluorescent) properties of single particles in suspension as they pass by a laser or other light source. It is widely used for the study of systems of biological particles either in static (for characterization of stable systems) or in dynamic (for investigation of biological processes) modes. In biological processes, some optical properties of the involved biological particles are changed. Therefore, it becomes important to find additional parameters of the particles, – «dynamic parameters», – which (1) do not change their values during the process and (2) determine the change of the optical properties of the particles. Working in the dynamic mode, one should take into account that the measurement of single particles with a flow cytometer is a sequence of random events: each particle is taken from the sample to the testing zone at a random time point, the time of measurement of the particle is relatively short, and every particle is measured just once. That means: not the evolution of a

single particle, but the evolution of particles distributions in the system can be monitored with flow cytometry. Consequently, corresponding mathematical models of a biological process are developed in frames of a statistical approach describing the evolution of the distributions of the particles on their static and dynamic parameters in the system.

This approach is demonstrated by application of a scanning flow cytometer for the dynamic study of different processes in the systems of biological particles: (1) immunoagglutination of antigen coved latex particles in the antibody contained media; (2) ligand-receptor binding on cells surfaces; (3) erythrocyte lysis in isotonic solution of ammonium chloride; (4) endocytosis.

OPTICS OF RED BLOOD CELLS AND CELL AGGREGATES

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Blood is a highly structured heterogeneous and dynamic fluid tissue exhibiting complex rheologic and optical properties. Contemporary optical techniques are potentially capable of providing the quantitative assessment of the fundamental role and properties of the molecular and cellular components of blood in maintaining the homeostasis of life organisms or in reflecting their pathological alterations.

Red blood cells (RBC) are the major scattering particles in whole blood due to their predominant volumetric concentration and high scattering cross-section. Theoretically, the angular distribution of light intensity scattered by an individual particle is determined by a scattering phase function (SPF), which can be defined either by a phenomenological formula, or calculated on the basis of certain approximations, accounting for the shape of RBC. Recent results in measurement and calculation of light scattering phase functions of large optically soft particles of different sizes, shapes, and orientations, modeling the single nondeformed, shear-deformed and

aggregated RBC are discussed. Different approaches are compared to calculate light scattering angular distributions resulting from photon migration in multiply scattering layers of concentrated suspensions of such particles. Results are in good agreement with Monte-Carlo simulations of the similar process. Both approaches are used to model the time course of backscattered light intensity, related to the experimentally measured spontaneous aggregation kinetics.

An overview will be given of optical techniques and their applications for the study of single RBC, RBC suspensions and whole blood structure and dynamics at both *in vitro* and *in vivo* conditions, including reversible aggregation, shear-induced deformation and radial redistribution of RBC, concentration and velocity profile alterations in bulk flow conditions, etc. Feasibility of light scattering, laser Doppler and optical coherence Doppler tomography measurements in blood in single and multiple scattering limits will be evaluated.

Potentials of the discussed techniques for biomedical experimental and clinical applications including diagnostics of pathological states, and pain-free experiments will be discussed. As an example, measurements will be discussed performed with blood samples of experimental animals that help to assess the mechanisms of such pathological states as cerebral ischemia.

References

[1] A.V. Priezzhev and M.Yu. Kirillin, «Monte Carlo simulation of laser beam propagation in plain layer of red blood cell suspension. Comparison of contribution of different scattering orders to the angle distribution of light intensity», Quantum Electronics, **32**(10), 883–887 (2002).

[2] O.E. Fedorova and A.V. Priezzhev, «Numerical modeling of light scattering by aggregating erythrocyte suspensions», Moscow University Physics Bulletin, **57**(2), pp. 55–59 (2002).

[3] A.N. Yaroslavsky, A.V. Priezzhev, J. Rodrigues, I.V. Yaroslavsky, and H. Battarbee. «Optics of Blood». Chapter 2 in: *Handbook on Optical Biomedical Diagnostics*, V.V. Tuchin – editor, SPIE Press, Bellingham, 2002, pp. 169–216.

[4] V.N. Lopatin, A.V. Priezzhev, et al. *Methods of Light Scattering in the Analysis of Dispersed Biological Media* (Fizmatlit, Moscow, 2004) (in Russian).

T-MATRIX COMPUTATION FOR ERYTHROCYTE-LIKE BICONCAVE PARTICLES USING THE NULLFIELD-METHOD WITH DISCRETE SOURCES

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To simulate light scattering by the erythrocyte several approaches have been applied, e.g. the Dipole-Dipole Approximation (DDA), the Finite Difference Time Domain (FDTD) and the Discrete Sources Method (DSM). Another popular method so far had been supposed not to be suitable to model light scattering by this kind of shapes: the T-matrix method, also known as Nullfield-Method (NFM) or Extended Boundary Condition Method (EBCM). As this method makes additional investigations like orientation averaging, changing the direction of the incident light or the use of a Gaussian laser beam, easy to accomplish, it would be advantageous for investigating the light scattering by the erythrocyte. Unfortunately its size, aspect ratio and the presence of concavities exceed the limits of the conventional T-matrix method. The shape of the erythrocyte can vary and has no specific form or size, but in general it can be described as an biconcave oblate discsphere with an aspect ratio about 4:1 and an average diameter of 6 μm.

Extending the Nullfield-Method by the use of discrete sources situated in a complex plane [1] now enables to calculate light scattering by oblate particles of even bigger size and higher aspect ratios; also biconcave particles can be simulated [2]. One convenient method to numerically generate such kinds of shapes is the use of Cassini ovals. These curves are characterized in such a way that the product of the distance of two fixed focal points is constant.

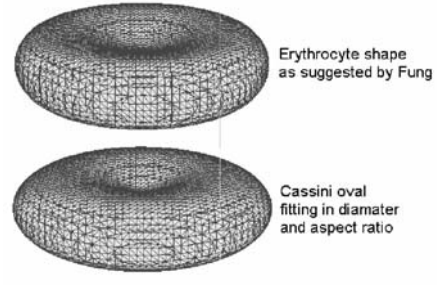

Fig. 1. Comparison between the mostly used erythrocyte description for modeling and a threedimensional shape created by a Cassini oval

In practice it means that one can vary diameter, thickness and concavity by just three parameters. Rotating this two-dimensional curve around its vertical axis generates a three-dimensional shape. By choosing the parameters accurately one can get a particle very close to the shape of the erythrocyte (see Fig. 1.) as it is usually described.

In the presentation the latest results for light scattering by biconcave, erythrocyte-like Cassini ovals simulated by the Nullfield-Method with Discrete Sources will be shown.

References

[1] A. Doicu, Y. Eremin, T. Wriedt, Acoustic and Electromagnetic Scattering Analysis using Discrete Sources (Academic Press, San Diego, 2000).

[2] J. Hellmers, E. Eremina, T. Wriedt, «Simulation of light scattering by biconcave Cassini ovals using the Nullfield Method with Discrete Sources», IOP Journal of Optics A (in print) (2005).

DIFFERENT SHAPE MODELS FOR ERYTHROCYTE. LIGHT SCATTERING ANALYSIS ON THE BASE OF THE DISCRETE SOURCES METHOD

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Light scattering by blood cells is recently of great interest both in mathematical modeling and in practical applications. For example a studying of light scattering by erythrocytes is an appropriate method for detection of some blood diseases. Between other blood cells, erythrocyte has an advantage for modeling, as it demonstrates no internal structure and can be modeled

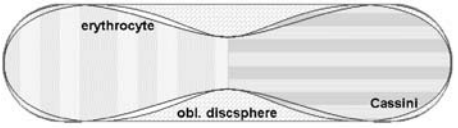

Fig. 1. Different erythrocyte shape models

as homogeneous object with a certain refractive index. Difficulties for erythrocyte modeling are: relatively big size and complicated biconcave shape.

During the last years different methods have been applied to this problem: Finite Difference Time Domain (FDTD), Discrete Dipole Approximation (DDA), Multipole Multiple Technique (MMP), T-matrix and many others. Some of these methods are not applicable to the real biconcave shape of erythrocyte, others are rather time consuming, and some methods are restricted to small dimensions. Between others the Discrete Sources Method (DSM) looks very attractive, as it allows making use of the axial symmetry of the particle and polarization of an incident excitation, which sufficiently reduces time of calculations. Besides, the DSM allows calculation of all the incident angles and polarizations at once [1] unlike volume-based methods, such as DDA and FDTD.

Another problem of modeling is that erythrocyte is an elastic particle which can relatively easily change its form to be able to pass throw capillary. To simplify erythrocyte modeling often classical shapes of oblate discs and spheroids are used. There are also different experimentally based equations of a real biconcave shape of a strainless erythrocyte. In some works erythrocyte is modeled on the base of Cassini ovals.

In this work the DSM is applied to investigate the influence of different erythrocyte shape models (Fig. 1) on light scattering results. For the comparison we chose one of the experimentally based equations, a modified Cassini-based model and a disc-sphere model [2]. Numerical results will be shown at the oral presentation.

References

[1] A. Doicu, Yu. Eremin, T. Wriedt. Acoustic and Electromagnetic Scattering Analysis using Discrete Sources. (Academic Press, London, 2000).

[2] E. Eremina, Y. Eremin, T. Wriedt: Analysis of light scattering by different shape models of erythrocyte based on Discrete Sources Method. Opt. Comm. Vol.244 (2005), 15–23.

RETRIEVAL OF DISTRIBUTIONS OF RED BLOOD CELLS OVER THEIR SIZES IN THE WHOLE BLOOD BY SMALL-ANGULAR MULTIPLE SCATTERING METHOD

Anatoli Borovoi[1], Vladimir Prokopiev[2], Nikolai Vagin[1]

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[2]Institute of High Current Electronics, SB Rus. Acad. Sci., Tomsk, Russia

At present, measurements of sizes of the red blood cells (RBC) are made mainly by various flow cytometric methods. The drawbacks of these methods are the necessity of preliminary sample preparations, qualified personnel, sophisticated facilities, etc. There is an opportunity to investigate an ensemble of a great number of cells by means of light scattering in the case of multiple scattering, too. In particular, the small-angular multiple-scattering method is of practical importance. The advantages of these methods are the possibilities to measure undamaged cells in vitro, and to take into account the natural interaction of the cells with their surroundings.

In this contribution, we are investigating the dynamics of the RBC size distribution in vitro in the process of sedimentation of the whole blood both theoretically and experimentally. This method is reduced to transmission of radiation from a He-Ne laser through the whole blood in vitro. Then distribution of the scattered light within small scattering angles is detected with a CCD camera. The data obtained are processed with our algorithm to retrieve the RBCs distributions. In parallel, a cytometric method using the transparent dry blood samples on a substrate is applied for comparison and justification.

The dynamics of the RBCs distributions during the sedimentation is used for discrimination of the blood from both ill and healthy donors.

USE OF SCANNING FLOW CYTOMETRY FOR ADVANCED CLASSIFICATION OF ERYTHROCYTES IN CLINICAL CASE STUDIES FOR FEASIBILITY

Bodo Roemer[1]

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The analysis of lightscattering patterns (indicatrices) of peripheral human blood cells in dilutions is one of most promising methods for further differentiation of erythrocytes, leukocytes and platelets for future routine hematology analyzers. The technology of Scanning Flow Cytometry (SFC) offers a number of additional parameters where a few of them were analyzed for feasibility research for early clinical use.

Figure 1a:

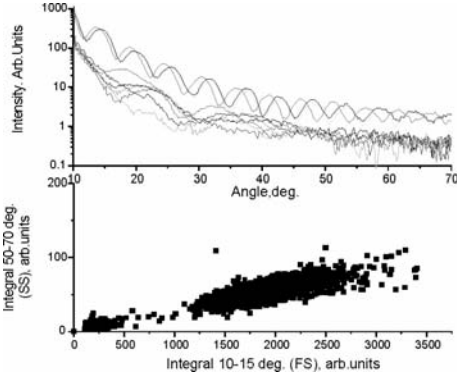
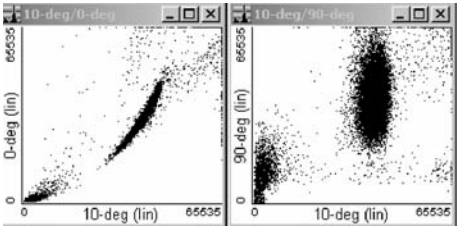


Figure 1b:



Example of RBC/PLT distribution when using standard Cell-Dyn 3200 sphering reagent in SFC [1a] and Abbott Cell-Dyn optics [1b] simultaneously

In collaboration with the Institute of Chemical Kinetics and Combustion, Institutskaya 3, Novosibirsk, 630090 Russia we were able to set up a prototype instrument based on Cell-Dyn 3000 series optics for processing a number of routine samples simultaneously using a SFC flow cell [1a] hosted in an Abbott CDNext platform [1b].

The study topic so far is to use a single dilution without chemical lysis, by just sphering red blood cells using standard Cell-Dyn 3200/4000 diluent reagents for comparison purposes.

We collected a number of blood samples including normals as well as abnormal samples with different clinical disease patterns for observing anemia, thalassemia, iron deficiency and other common disease states. With this we were interested in effects of morphological patterns (microscopy) in anisocytic samples with hypochromic, hyperchromic, microcytic, macrocytic, erythrocytes to indicatrices.

Early in the analyzer setup phase (where this abstract is submitted) we already experienced an excellent separation between sphered erythrocytes and platelets. When using a flow rate of 1 m/s laminar flow and a dilution of 1:1000 we are able to collect good quality indicatrices with 100 to 300 cells per second in normal samples with parameters like size, volume and cell by cell hemoglobin concentration.

References:

[1] Y.R.Kim and L. Ornstein «Isovolumetric sphering of erythrocytes for more accurate and precise cell volume measurement by flow cytometry», Cytometry 3, 419–427, (1983).

[2] P.A. Tarasov «Determination of characteristic parameters of erythrocytes by methods of dynamical flow cytometry», PhD thesis (in Russian), Novosibirsk 2005.

APPROACH TO IDENTIFY AND CHARACTERIZE SPHERED RED BLOOD CELLS

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At present one of the most developed applications of a Scanning Flow Cytometer (SFC) is characterization of individual microspheres [1]. We use

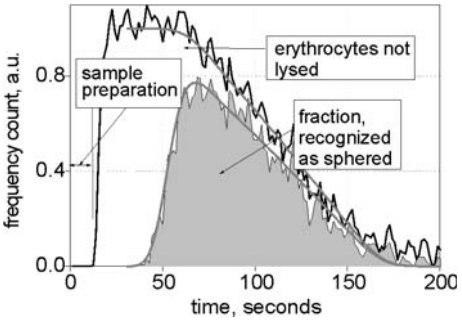

Fig. 1. Kinetics of hemolysis and sphering in isotonic saline of ammonium chloride. At present for cells in sphered fraction solution of inverse light scattering problem is available. Experimental data and model fit (gray lines) are shown

this approach to characterize sphered human erythrocytes. Two types of sphering processes have been studied: isovolumetric sphering in the presence of detergent [2] and characterization of sphered fraction of erythrocytes in the course of colloid osmotic hemolysis in solution of ammonium chloride. Because after isovolumetric sphering cells are not perfect spheres the solution of inverse light scattering problem has been extended to slightly nonspherical particles. For the second process algorithm for identification of sphered fraction of erythrocytes in the course of hemolysis has been proposed. This algorithm uses boundary level of amplitude of the main frequency peak in the normalized Fourier spectrum as a criterion of sphericity. To describe kinetic data model of hemolysis has been developed using distribution function approach: taking into account that values of some parameters are varied from cell to cell. New parameters of population could be obtained from kinetic of hemolysis: surface area, time of opening for hemolytic hole, membrane permeability, and critical tension.

References

[1] K.A. Semyanov, P.A. Tarasov, A.E. Zharinov., A.V. Chernyshev, A.G. Hoekstra, and V.P. Maltsev. «Single-particle sizing from light scattering by spectral decomposition», Appl. Opt. **43**, 5110–5115, (2004).

[2] Y.R. Kim and L. Ornstein «Isovolumetric sphering of erythrocytes for more accurate and precise cell volume measurement by flow cytometry», Cytometry **3**, 419–427, (1983).

CAPABILITIES OF THE DISCRETE DIPOLE APPROXIMATION FOR SIMULATION LIGHT SCATTERING BY BIOLOGICAL CELLS

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Light scattering is a long used non-invasive method to study biological cells. It is used extensively in flow cytometers, where light scattering by single cells is analyzed. However, solving the direct light scattering problem for single cells still presents lots of difficulties that are mainly caused by the size and shape of the cells. The size is in the range from a few wavelengths (for visible light) to several tens of wavelengths. This size range prohibits the use of long wavelength (Rayleigh) or short wavelength (Geometrical Optics (GO)) approximations. The shape in general is very complex with multiple inclusions, which can be smaller, comparable or considerably larger than the wavelength). This limits the use of quick, accurate techniques to very few cases. Internal inclusions with size comparable to wavelength also greatly complicate the application of GO even to very large cells. Another property of biological cells, which simplifies computations, is index-matching (when they are studied in liquid solution, as is usually the case). This property gives rise to several approximations, e.g. Rayleigh-Debye-Gans and anomalous diffraction, however they usually give satisfactory accuracy only to some measured quantities. Reference [1] is a recent review of light scattering by biological particles, where these issues are discussed in more detail.

Although different new approximations are being developed, e.g. replacing certain cells by simpler shapes, validation of such approximations anyway require rigorous simulation of light scattering by complex-shaped particles. There are two types of methods, which are in principle capable of handling any geometry and size of the scatterers. They numerically solve the Maxwell equations in the time – Finite Difference Time Domain (FDTD) – or frequency – Discrete Dipole Approximation (DDA) – domain. Both methods divide the scatterer in subvolumes that should be much

smaller than the wavelength. Therefore their memory and time requirement rises steeply with size of the scatterer. That was always the main restriction for simulating light scattering by complex-shaped biological cells.

FDTD simulation of biological cells has a long history [2, 3], but it is mainly focused on small cells (because of computational requirement described above). The largest simulations found in the literature are for size parameter 80 [3]. We have recently applied DDA to large-scale simulation of light scattering by red blood cells [4] (size parameter up to 40). In this study we are testing the limits of DDA, using simple shapes, e.g. spheres, which can be solved using analytical techniques. At the workshop we will present results of DDA performance and accuracy of both integral characteristics (cross-sections and asymmetry parameter) and angular dependence of all Mueller matrix elements for particles with sizes and refractive in the range of biological particles, i.e. $30 < x < 130$ and $1.02 < m < 1.2$. We will show that DDA is capable of accurately computing light scattering by biological particles. However, especially for the largest sizes, computation times will be very large, even on state-of-the-art massively parallel supercomputers.

References

[1] A. G. Hoekstra and P. M. A. Sloot, «Biophysical and biomedical applications of nonspherical scattering», in *Light Scattering by Nonspherical Particles, Theory, Measurements, and Applications*, M. I. Mishchenko, J. W. Hovenier, and L. D. Travis, eds., (Academic Press, 2000), pp. 585–602.

[2] C. Liu, C. Capjack, and W. Rozmus, «3-D simulation of light scattering from biological cells and cell differentiation», J. Biomed. Opt. **10**, 014007 (2005).

[3] R. Drezek, A. Dunn, and R. Richards-Kortum, «Light scattering from cells: finite-difference time-domain simulations and goniometric measurements,» Appl. Opt. **38**, 3651–3661 (1999).

[4] M. A. Yurkin, K. A. Semyanov, P. A. Tarasov, A. V. Chernyshev, A.G. Hoekstra, and V. P. Maltsev, «Experimental and theoretical study of light scattering by individual mature red blood cells with scanning flow cytometry and discrete dipole approximation», Appl. Opt., 44, 5249–5256 (2005).

DEEP UV RAMAN SPECTROSCOPIC CHARACTERIZATION OF BIOLOGICAL SPECIES

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Amyloid depositions consisting of fibrils with a cross-β structure have been found in many neurodegenerative diseases such as Alzheimer's disease. Protein structural transformations on the molecular level during in vitro fibril formation are accompanied by substantial changes in macroscopic properties, such as formation of a gelatinous phase and the formation of insoluble particles. These changes limit the application of conventional methods for characterizing protein conformational transformations. Raman spectroscopy has been proven to be an efficient technique for characterizing highly-scattering and opaque samples. At deep ultraviolet excitation around and below 200 nm, Raman scattering is resonantly enhanced from the amide chromophore, a building block of a polypeptide backbone, providing quantitative information about the protein secondary structures. [1] A new deep UV Raman spectrometer, which requires only a 100-μl sample with a protein concentration of at least 0.1 mg/ml has been recently built. [2] No special sample preparation is required: the dynamic range has no limitations at the high concentration end.

We report here on the application of deep ultraviolet resonance Raman spectroscopy for structural characterization of a protein at all stages of fibrillation process.

High-quality deep UV Raman spectra of lysozyme were obtained at all stages of fibril formation. The evolution of the protein secondary structure as well as a local environment of phenylalanine, a new natural deep ultraviolet

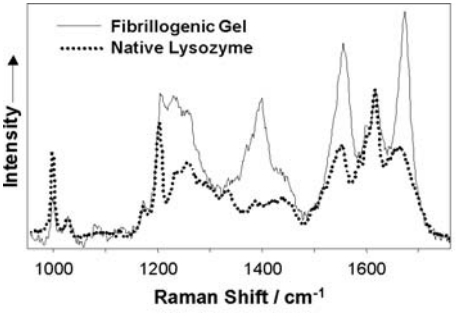

Fig. 1. 197-nm Raman spectra of native lysozyme (pH 2, dotted line) and lysozyme fibrils (solid line) measured at room temperature. The spectra were normalized for comparison using the phenylalanine-tyrosine band at ~1600 cm^{-1}

Raman marker, was documented. Concentration-independent irreversible helix melting was quantitatively characterized as the first step of the fibrillation.

References

[1] I.K. Lednev, A.S. Karnoup, M.C. Sparrow, S.A. Asher, «α-Helix peptide folding and unfolding activation barriers: A nanosecond UV Resonance Raman Study», *J. Am. Chem. Soc.* **121**, 8074 (1999).

[2] I.K. Lednev, V.V. Ermolenkov, W. He, M. Xu, «Deep-UV Raman spectrometer tunable between 193 and 205 nm for structural characterization of proteins», *Anal Bioanal Chem*, **381** (2), 431–437 (2005).

2st day: Tuesday October 4, 2005

CHARACTERISATION OF BIOAEROSOLS USING SPATIAL ELASTIC SCATTERING AND FLUORESCENCE

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Micro-organisms contain bio-chemical fluorophores, which exhibit characteristic fluorescence when irradiated with ultraviolet radiation, and it has been widely demonstrated that the fluorescence from single airborne organisms is measurable. However, other materials which may be present in the atmosphere such as fuel drops and agricultural chemicals, as well as naturally occurring materials like pollens, also fluoresce. In order to characterise particles it is probable that multiple properties of the particle need to be determined, such as size and shape, as well as characterising the fluorescence emission.

A number of instruments have been developed to provide real-time detection and characterisation of biological aerosols. Earlier work at Dstl had investigated elastic scattered light and determined that particles could be put into broad classes using as few as 3 detectors to measure the scatter pattern. These signals could be used to train artificial neural networks and provide detection of unusual events in aerosol distributions.

A Fluorescence and Aerosol Shape Analysis (FLASAS) instrument has extended the number of detectors used to spatially analyse the elastically scattered light and added a single broadband fluorescence measure, so that the instrument can classify particles by their size, shape and fluorescence properties.

The instrument generates a large amount of data with up to 40 measurements of shape, size and fluorescence parameters recorded for each aerosol particle sampled. Automated aerosol particle classification is therefore a difficult problem due to the complexity and volume of data. A number of different techniques have been explored, including feature extraction tech-

niques such as Principal Components Analysis (PCA), various methods of feature selection, and basic numerical analysis such as Discrete Fourier Transforms (DFT). For the classification itself we considered Bayesian classifiers, Neural Networks (multi-layer perceptron) and Support Vector Machine (SVM) algorithms. The presentation will evaluate some of the data analysis and particle classification techniques, including comparisons between Neural Networks and SVM classifiers.

EXPERIMENTAL INVESTIGATIONS OF BACTERIA CELLS GROWTH WITH SCANNING FLOW CYTOMETRY

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Investigations and identification of bacterial cells is a very important problem in biology and medicine. Particularly, it includes detection of influences of medium and antibiotics on growth rate of bacterial cells.

Flow Cytometry is one of the powerful techniques used in the detecting and characterizing of the individual cells and particles of high rate. That is why several attempts have been made to use this technique for identification of bacterial cells from growth rate. Our work presents an application of Scanning Flow Cytometry for determination growth rate of different type of bacterial cells.

Scanning Flow Cytometry allows one to measure entire light scattering pattern (an indicatrix) of individual particles. This so-called indicatrix technology gives the researcher additional possibilities of particle characterization, especially for non-spherical objects. In contrast to ordinary forward and side light scattering continuous angular dependence of light scattered by the individual particle is measured. This technology potentially allows

one to detect the influence of antibiotics on growth rate of bacteria during the time of one division (about some hours).

We measured indicatrix signals for several types of bacteria. Escherichia coli, Candida Utilis, Salmonella typhimurium, Bacillus sphaericus, Bacillus megateriums, Bacillus subtilis, Bacillus thuringiensis, and Escherichia coli infected with protobe have been measured. We carried out kinetics experiments for determination growth rate at 37 °C for two types of bacterial cells culture – Escherichia coli and Candida Utilis. We develop a kinetic model of cell division, taking into account the fact of heterogeneity of cells growth rate.

FT-IR SPECTROSCOPIC CHARACTERIZATION OF SOME SPOREFORMING AND NON-SPOREFORMING HEALTH RELEVANT BACTERIA

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For security reasons, rapid and reliable detection of health relevant biological agents in different environments gains still more importance. The absorption of light in infrared regions of the electromagnetic spectrum appears capable of delivering information on individual structural units of microbial cells, growth-dependent phenomena, storage materials, and endospores [1]. We were able to make respective demonstrations by FT-IR spectroscopy of *Pseudomonas* spp. strains, and differently cultivated *Bacillus subtilis* [2, 3], e.g., recently. These bacteria and also *Escherichia coli*, and *Enterobacter agglomerans* delivered well distinguished IR spectra for structural units of nucleic acids at a waverange of 3300 cm^{-1}, for cell membrane fatty acids at 3000–2800 cm^{-1}, for saturated esters and cell proteins at

1800–1500 cm^{-1}, for proteins, lipids and phosphoesters at 1500 cm^{-1}, and for glycopeptides and phosphate groups of nucleic acids constituents at 1200–800 cm^{-1}. A spectral dominance at 1745 cm^{-1} was observed in *B. subtilis* endospores. Between cell biomass of the both members of the *Enterobacteriaceae* family, and harvested under standard cultural conditions, no spectral differences could be observed, however. On the other hand, the IR spectra of *E. coli* and *E. agglomerans* demonstrated several differences if the bacteria were grown under different nutrient conditions. The advantage and limitations of the FT-IR spectroscopy in detection and characterization of health relevant bacteria should be critically discussed, and experimentally further evidenced.

References

[1] D. Naumann, «Infrared spectroscopy in microbiology», in: *Encyclopedia of Analytical Chemistry*, R.A. Meyers, Ed., (Wiley, Chichester, 2000, pp. 102–131).

[2] Z. Filip, S. Herrmann, «An attempt to differentiate Pseudomonas spp. and other soil bacteria by FT-IR spectroscopy», Eur. J. Soil. Biol. **37**, 137–143 (2001).

[3] Z. Filip, S. Herrmann, J. Kubat, «FT-IR spectroscopic characteristics of differently cultivated Bacillus subtilis», Microbiol. Res. **159**, 257–262 (2004).

FORMATION OF LIGHT-SCATTERING PATTERNS OF OPTICALLY SOFT PARTICLES UNDER VARIATION OF THEIR PARAMETERS

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In this work we review of the basic scientific advances of the Krasnoyarsk biophysics and hydrooptics group, connected with theoretical and experimen-

tal developments of express optical methods for research of natural aqueous ecosystems, and also outcomes of their application for determination of native characteristics of biological particles and their suspensions.

Among these advances are basic theoretical results:

1. development of algorithms of rigorous and approximate solutions of the direct optical problem on particles of arbitrary form and structure (T-matrix method, Mie theory, anomalous diffraction, Rayleigh-Gans-Debye approximation, Wentzel-Kramers-Brillouin approximation etc.);

2. statement of the principle of «optical equivalence» and conditions of its existence;

3. findings of connections of the scattering matrix elements of biological particle suspensions with the factors, forming them (including experimental results);

4. theoretical analysis of shape and structure of differential and integral indicatrixes of spherical and spheroidal particles, including inhomogeneous ones (with reference to methods of «flying» and integral light-scattering indicatrixes).

On the basis of these and other researches a series of optical effects is predicted: an extreme transparency of suspensions of biological particles, anomalous light-scattering of suspensions of oriented nonspherical particles in the region of the first resonance, various polarization effects. Moreover, the theoretical outcomes have allowed us to solve the inverse optical problems connected with experiment and to create appropriate optical devices (in particular, the moving cuvette platform for obtaining and decoding of an information about an integral light-scattering indicatrix).

The obtained results have allowed us to create new express methods of monitoring water quality in natural and artificial reservoirs based on an evaluation of the amount of suspended particles, their dispersity, chemical and biological consumption of oxygen, total amount of bacterial plankton, phytoplankton, etc. The basic results are published in the monographs [1]–[4].

The latest outcomes of researches on self-descriptiveness of the method of «flying» light-scattering indicatrix for transparent absorbing particles and organic and mineral complexes are marked also.

References

[1] Ph.Ya. Sidko, V.N. Lopatin, L.E. Paramonov, *Polarization characteristics of suspension of the biological particles* (Novosibirsk, Nauka, 1990).

[2] V.N. Lopatin, F.Ya. Sidko, *Introduction in the cell suspension optics* (Novosibirsk, Nauka, 1988).

[3] V.N. Lopatin, A.D. Aponasenko and L.A. Shur, *Biophysical Fundamentals of Evaluation of State of Water Ecosystems (Theory, Equipment, Methods, Studies)* (Novosibirsk, Nauka, 2000).
[4] V.N. Lopatin, A.V. Priezzhev, A.D. Aponasenko, N.V. Shepelevich et al. *Light scattering methods in the analysis of aqueous disperse biological media* (Moscow, Fizmatlit, 2004)

NUMERICAL SIMULATIONS OF LIGHT SCATTERING FROM BLOOD CELLS USING FDTD METHOD

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We have recently studied numerically [1], [2] the light scattering properties of blood cells such as un-deformed biconcave shaped red blood cells (RBCs), pressure deformed RBCs in blood microvessels, and B cells at different life stages of the cell cycle using the finite-difference time-domain (FDTD) method. [3]

The RBC assumes a biconcave shape when free of constraints [4] and deforms from its biconcave shape in the blood flow through microvessels in response to the upstream hydrostatic pressure drop across the cell and the opposing drag of the capillary wall. In our RBC studies, the RBC is modeled as a homogenous body with an inelastic but deformable membrane, and the axisymmetric shapes proposed by Zarda et al. [5] have been adopted to describe the shapes of the deformed RBC under different pressure drop. The FDTD method is used to calculate the angular distributions of the Mueller matrix elements of a single RBC, and the dependence of the

Mueller matrix elements on the shape, orientation of the cell, and the wavelength of the incident light has been investigated. Analysis of the data provides valuable insight on determination of the RBC shapes using the method of elastic light scattering measurements, and it also shows that the measurements of the elements S_{22} and S_{34} may provide a sensitive means to detect the shape change of a RBC.

B cells are importance in the understanding and diagnosis of lymphomas and leukemia. B cells take a slightly elongated spherical shape and have a relatively simple cellular structure with their nuclei occupying about 90% of cell volume. In our study, cultured B cells are harvested at different phases of cell cycle, and selected cells are imaged with a laser scanning confocal microscope to produce a set of 2D images of internal structure which are then processed to produce the 3D structures for FDTD calculations. The angular distribution of the Mueller scattering matrix elements are calculated and their dependences on the changes occurred inside the cell at different phases of cell cycle are studied. The potential usages of these results will be discussed.

References

[1] J.Q. Lu, P. Yang, and X.H. Hu, «Simulations of Light Scattering from a Biconcave Red Blood Cell Using the FDTD method», J. Biomed. Opt. **10**, 024022 (2005).

[2] R. Scott, X.H. Hu, P. Yang, and J. Q. Lu «Evaluation of a parallel FDTD code and application to modeling of light scattering by deformed red blood cells», submitted to Optics Express, (2005).

[3] A. Taflove, *Computational Electrodynamics: The Finite-Difference Time-Domain Method* (Artech House, Boston, 1995).

[4] A.J. Grimes, *Human Red Cell Metabolism*, (Blackwell Scientific Pub, Oxford: 1980) pp. 57.

[5] P.R. Zardar, S. Chien, R. Skalak, «Interaction of viscous incompressible fluid with an elastic body», in *Computational Methods for Fluid-Solid Interaction Problems*, T. L. Geers, Ed. (American Society of Mechanical Engineers, New York: 1977) pp. 65–82.

T-MATRIX CALCULATIONS OF PHOTOPOLARIMETRIC PROPERTIES OF BIOLOGICAL PARTICLES WITH COMPLICATED SHAPE

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The T-matrix method is widely used for determination of scattering properties of particles compared with the wavelength [1]. We use a modified T-matrix method to calculate photometric and polarimetric properties of *Aspergillus terreus* spores – biological particles comparable to the light wavelength. An effective code, which allows us to calculate scattering properties of particles with complicated shape, was developed.

A SEM image of *Aspergillus terreus* spores is given in Fig. 1. We described

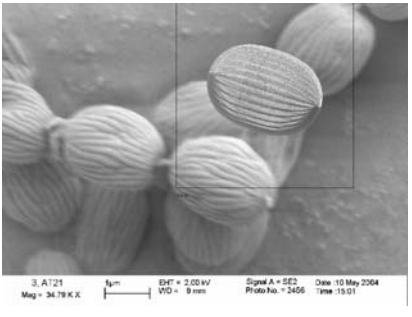

Fig. 1. An example of *Aspergillus terreus* spores. A model particle is shown inside of square the box

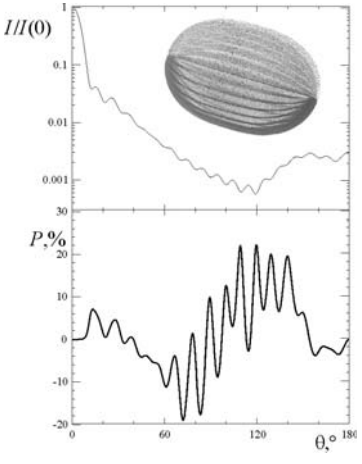

Fig. 2. Normalized intensity and degree of linear polarization for an ensemble of model irregular particles

the shape of the particles by the formula: $R(\theta,\varphi)=X(1+0.16T_2(\cos\theta))(1+0.04T_{30}(\cos\varphi))$, where X is a size parameter of the major axis of particles, $X=2\pi r/\lambda$, λ is the wavelength of incident light, $T_n(z)$ is the Tchebyshev polynomials. A model particle is shown in Figs. 1 and 2. At $\lambda = 683$ nm and the particle size estimated with Fig. 1, the parameter $X = 20$. The particles are non-absorbing with the real part of the refractive index $n = 1.5 + 0i$.

In Fig. 2 scattering angle dependencies of normalized intensity and degree of linear polarization of an ensemble of the model particles are shown, demonstrating many features that can be considered as a "visiting card" of such particles.

References

[1] M. Mishchenko, L. Travis, A. Lacis. "Scattering, absorption, and emission of light by small particles". Cambridge: Cambridge Univ. Press; 2002.

CORE-MANTLE SPHEROIDAL MODEL OF ELECTROPORATED VESICLES

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Membrane electroporation is an electrical technique used in clinical medicine to introduce, very efficiently, therapeutically active drugs and gene DNA into cells and tissues. Optical microscopy data have documented that giant lipid vesicles, exposed to an electric field, are elongated into spheroid-like particles with long axis oriented along the field direction.

We used core-mantle spheroids to calculate extinction cross sections for two polarizations of incident radiation. The consideration is based on the solution to the electromagnetic scattering problem obtained by the method

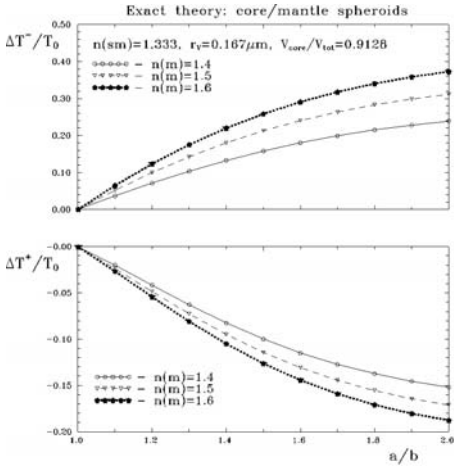

Fig. 1. Reduced and chemical turbidity calculated for vesicles with different refractive indices of membranes. The variations of the vesicle shape occur during electric pulse

of separation of variables in a spheroidal coordinate system [1]. Theory was applied to explain the experiments made at the University of Bielefeld (see, e.g., [2]). The detailed calculations of the reduced and chemical turbidity have been performed for vesicles of different sizes and shapes, various volume ratios and refractive indices of membranes, etc. Some results are shown in Fig. 1. It is concluded that the effects of the change of shape and refractive index of the core can be separated. Thus, the polarized light is a very effective tool for control the penetration of drugs into the models of cells.

The work was partly supported by the TechBase Program on Chemical and Biological Defense, by the Battlefield Environment Directorate under the auspices of the U.S. Army Research Office Scientific Services Program administrated by Batelle (Delivery Order 0395, Contract No. DAAD19-02-D-0001).

References

[1] V.G. Farafonov, N.V. Voshchinnikov, V.V. Somsikov, «Light scattering by a core-mantle spheroidal particle», Appl. Optics **35**, 5412–5426 (1996).

[2] S. Kakorin, E. Neumann, «Electrooptical relaxation spectrometry of membrane electroporation in lipid vesicles», Colloids and Surfaces A: Physicochemical and Engineering Aspects **209**, 147–165 (2002).

3st day: Wedvesday October 5, 2005

OPTICAL AND MORPHOLOGICAL CHARACTERIZATION OF NATURAL PHYTOPLANKTONIC COMMUNITIES

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The ENEA laser remote sensing laboratory in Frascati, in the early stage of running a lidar fluorosensor apparatus, started in a detailed parallel investigation on the organisms accountable for the well known red fluorescence emission. The participation in national and international oceanographic campaigns, pushed us to explore this aspect, also due to the possible capability to distinguishing dominant species dispersed in seawaters by means of our lidar fluorosensor apparatus. In fact, aside from the chlorophyll-a pigment, many other pigments are present in these living organisms and several of them show the ability to fluoresce if stimulated by proper light emission.

Therefore, a systematic laboratory characterization of different phytoplankton cultures has been performed in combination with LIF (Laser Induced Fluorescence) measurements aimed to investigate the possibility of their remote monitoring by means of lidar fluorosensor systems. Cultures of microalgae characterized by different pigment contents have been analyzed in the visible region upon UV laser excitation. High resolution laboratory spectra have been measured in order to obtain the fingerprint of each species. Emission wavelengths related to the main pigments contribution have been identified. Detec-

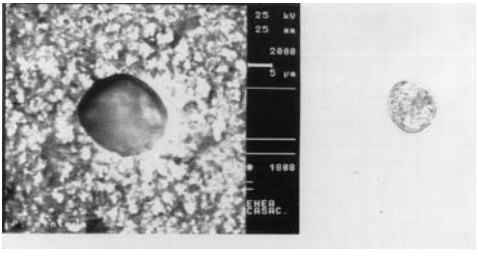

Fig. 1. SEM (left) and fluorescence (right) microscope images of Dinophyceae Prorocentrum minimum

tion limits of the emitted red chlorophyll signal have been evaluated for the different species after dilution in the culture medium and in real sea water [1].

Prior to the LIF excitation aimed at remote characterization, the of algal cultures were morphologically analyzed by fluorescence and scanning electron microscopy. Furthermore the relevant cell number was counted for biomass estimation, and the chlorophylls content was determined by different chemical methods.

References

[1] R. Barbini, F. Colao, R. Fantoni, A. Palucci, S. Ribezzo, C. Micheli. *Laboratory LIF characterization of different phytoplankton species originating harmful blooms*. ENEA RT/INN/97/21.

ENEA LASER SCANNING FLOW CYTOMETER (CLASS) FOR OPTICAL CHARACTERIZATION OF MARINE PARTICLES

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The ENEA laser remote sensing laboratory in Frascati began developing a lidar fluorosensor apparatus for surface water quality monitoring (ELF, ENEA Lidar Fluorosensor) in the early nineties. The following upgraded version of that instrument was employed in five oceanographic campaigns in Antarctica [1] and in transoceanic transects from Italy to New Zealand aboard the research vessel *Italica*. ELF is based on laser-induced fluorescence and continuously provides concentrations of chromophoric dissolved organic matter (CDOM), phytoplanktonic pigments and photosynthetic activity all along the ship track. Recently, comparative studies of

ELF and the ocean color satellite radiometer SeaWiFS (Sea-viewing Wide Field-of-view Sensor) started, with the development of bio-optical algorithms for the retrieval of chlorophyll-a, CDOM and primary productivity imagery from 1997 to 2004.

Actually, ELF operation is restricted to superficial monitoring of the first sea waters layers (15-30 m). Therefore, measurements of physical and optical properties of the natural phytoplanktonic community have to be carried out with submerged local instruments. In this framework, an innovative scanning flow cytometer [2] is under development at ENEA. The preliminary setup of the optical system is shown in Fig. 1. A He-Ne laser (633 nm) and a diode laser (405 nm) are employed for the determination of particle speed and scattering function (indicatrix), respectively. Detections of the first indicatrix signals from particles of known dimension and refractive index (e.g. latex particles) are currently under way to optimize the alignment of the optical elements and to derive a calibration function for the system. First tests of the hydrodynamic system performances allowed us to define the conditions to have a stable internal jet of about 40 μm. Future upgrades include the addition of fluorescence and polarization channels.

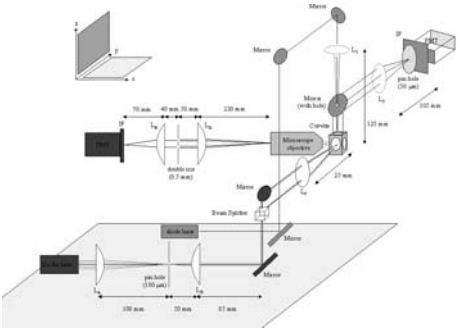

Fig. 1. Preliminary set up of the ENEA laser scanning flow cytometer (CLASS)

References

[1] Barbini R., Colao F., Fantoni R., Fiorani L. and Palucci A., «Lidar fluorosensor calibration of the SeaWiFS chlorophyll algorithm in the Ross Sea», International Journal of Remote Sensing **24**, 3205–3218 (2003).

[2] Maltsev V. P., «Scanning flow cytometry for individual particle analysis», Review of Scientific Instruments **71**, 243–255 (2000).

CHARACTERISATION OF MARINE BIO-PARTICLES FROM IN-SITU MEASUREMENTS OF BULK OPTICAL PROPERTIES

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The conversion of the ocean remote-sensing reflectance into marine optically active constituents involves the understanding of the relationships between the substances present in the seawater and their optical signal. In particular, the problem of identifying the particles that contribute to the bulk backscattering signal (b_b) is still unresolved [1].

Early studies explored the relationship between b_b and phytoplankton [2] and also with bacteria [3] in cultures, using Mie theory. Since then, it has been hypothesised that the most important contributors to b_b are small (~1 μm) particles [4].

To elucidate this question bio-optical data were collected during an optics-dedicated cruise in the Benguela upwelling region. The conventional regression between bulk b_b measurements and macroscopic variables shows strong correlations: $b_{bp}(550)$ vs Total Suspended Matter gives $r^2 = 0.81$. However, this kind of analysis does not allow assessment of the role of smaller particles in producing b_b.

Additionally, a single particle approach has been used [2]. This method uses information on particle size distribution, pigment absorption and attenuation from particles to produce estimates of the efficiency factors of absorption, scattering and b_b(Q_a, Q_b and Q_{bb}). These efficiency factors were then extrapolated to bulk quantities that can be compared with in-situ measurements.

References

[1] D. Stramski, E. Boss, D. J. Bogucki and K. J. Voss, «The role of seawater constituents in light backscattering in the ocean», *Prog. Oceanogr.* **61**, 27–56 (2004).

[2] A. Bricaud and A. Morel, «Light Attenuation and Scattering by Phytoplanktonic Cells – a Theoretical Modeling», *Appl. Opt.* **25**, 571–580 (1986).

[3] A. Morel and Y. H. Ahn, «Optics of Heterotrophic Nanoflagellates and Ciliates – a Tentative Assessment of Their Scattering Role in Oceanic Waters Compared to Those of Bacterial and Algal Cells», *J. Mar. Res.* **49**, 177–202 (1991).

[4] D. Stramski, A. Bricaud and A. Morel, «Modeling the inherent optical properties of the ocean based on the detailed composition of the planktonic community», *Appl. Opt.* **40**, 2929–2945 (2001).

OPTICAL CHARACTERIZATION OF BIOAEROSOLS

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This presentation provides an overview of recent efforts to develop detection capabilities for biological and chemical agent aerosols. The project is a collaboration involving other R&D groups as well as the Naval Research Laboratory, Washington, DC. The project goal is to evaluate and develop advanced aerosol agent detection. The technical goal is to achieve improved rapid biological aerosol discrimination using a combination of primarily optical technologies. Complete point detection is typically conceived as having a low-discrimination, fast response «front-end» or «trigger» followed by a slower «identifier» unit. Optical technologies naturally fit the trigger application for continuous operation, and rapid response without using consumables. Current trigger technology involves fluorescent excitation of individual aerosols in an inlet air flow using a UV wavelength laser. An advanced prototype detection capability with increased discrimination for an expanded range of agent materials will be achieved by fusing one or more complementary optical detection techniques with some form of UV fluorescence. Candidate signatures undergoing evaluation in the RAAD project include: 2-D angular patterns of elastic scattering, high resolution imaging, infrared absorption spectroscopy, multiple wavelength excitation fluorescence, wavelength-dispersed fluorescence, fluorescence lifetime, laser induced breakdown spectroscopy (LIBS), and conventional and enhanced Raman scattering.

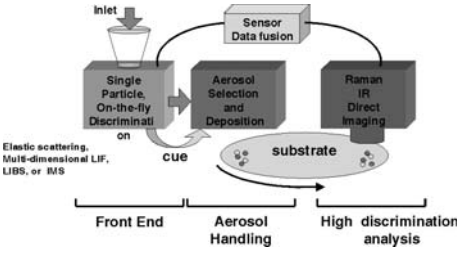

Fig. 1. Conceptual and schematic arrangement for optical aerpsol agent detection system

Once measurement techniques have been identified as potentially supplying significant additional discrimination capability, a fused sensor concept will be developed leading to the construction of a prototype to provide a feasibility demonstration and initial performance evaluation (see Figure 1). Since not all of the candidate optical measurement techniques are amenable to single particle, on-the-fly configuration, the continuous concentration, selection, and detainment of a sample stream is a challenge. Therefore, a key element of the fused sensor concept will be advanced aerosol and aerosol-laden flow manipulation technologies. These types of enabling technologies are being investigated in parallel with the optical signature evaluations. Recent highlights from the candidate optical detection techniques and manipulation technologies will be presented.

MEASURING THE POLARIZATION OPPOSITION EFFECT FROM SINGLE SUSPENDED AGGREGATE PARTICLES

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Our objective is to evaluate a novel method to obtain optical signatures using the Polarization Opposition Effect (POE) for use as a bacterial spore particle (aerosol) discriminator. The angular position and width of the POE

effect depends on particle morphology (shape & surface quality). That is, different angle-dependant polarized scattered light intensities are observed for different particle shape and surface characteristics. The actual signatures for typical atmospheric aerosols, or clusters of biological units such as bacterial or fungal spores have never previously been directly measured. The effort proposed here is an experimental investigation of this effect together with a detailed comparison to computationally predicted signatures in order to evaluate the utility of this optical signature for aerosol detection.

Aggregate particles will be interrogated while being levitated using an electrodynamic trap. The method proposed will allow the simultaneous recording of the separated polarization components for the scattering angles of interest. This is expected to lead to a means to rapidly classify single aerosols.

DUAL-WAVELENGTH FLUORESCENCE FOR DETECTING SINGLE BIOLOGICAL PARTICLES

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There is currently substantial effort through the SUVOS [1] and other initiatives to develop laser diodes and light-emitting diodes capable of continuous sub-300 nm radiation emission. Such devices will ultimately represent optimal excitation sources for compact and fieldable bio-aerosol moni-

tors. However, until such devices are routinely available and whilst solid-state UV lasers remain relatively expensive, other low-cost sources of UV can offer advantages, especially for use in monitoring networks where several tens of discrete point-sampling detectors may be required. This paper describes one such prototype monitor that employs two compact xenon discharge UV sources to excite intrinsic fluorescence from individual particles within an ambient aerosol sample.

Aerosol particles contained within a sample flow drawn from the ambient environment are rendered in single file as they intersect the beam from a continuous-wave 660 nm diode laser. From the magnitude of the scattered light signal produced by each particle, an estimate of particle size is determined. For particles deemed to be in the approximate size range 1 μm to 10 μm, the sequential firing is initiated of two xenon sources (~5 μs apart). These irradiate the particle with UV pulses of ~1 μs duration and $> 300\ \mu J / cm^2$ fluence, centred upon ~280 nm and ~370 nm wavelength, optimal for excitation of bio-fluorophores tryptophan and NADH, respectively. For each excitation wavelength, fluorescence is detected across two bands containing the peak emissions of the same bio-fluorophores. Thus, for each particle, a 2-dimensional fluorescence excitation-emission matrix is recorded together with an estimate of particle size. This data acquisition is achieved in approximately 25 μs for each particle. Current measurement rates are up to ~125 particles/s (limited by the 5 ms xenon recharge time), corresponding to all particles for concentrations up to 1.3×10^4 particles/l. Developments to increase this to ~500 particles/s are in hand. Example results from aerosols of *E. coli*, *B. globigii* spores, and a variety of non-biological materials are given which illustrate the degree of discrimination achievable.

References

[1] SUVOS – Semiconductor Ultraviolet Optical Sources, J.C. Carrano, Director, http://www.darpa.mil/mto/suvos/ (2002).

ANGULARLY-RESOLVED ELASTIC LIGHT SCATTERING: PATTERN COMPLEXITY AND FEATURE EXTRACTION

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The LA TAOS (Large-Angle Two-dimensional Angular Optical Scattering) system captures angularly resolved elastic light scattering from individual aerosol particles in-situ from a single laser shot [1]. The key component of the system is an ellipsoidal reflector that collects light scattered over a solid angle of $75°<\theta<135°$ and $0°<\varphi<360°$ and guides it to an ICCD detector with a format size of 1024×1024, similar to the work of Kaye *et al*. [2]. By incorporating a cross-beam trigger system to define a small trigger volume ($25\mu m \times 25\mu m$), high-resolution LA TAOS patterns are captured with angle inaccuracy of less than a degree. Embedded in each scattering pattern is information on the interrogated aerosol particle's morphology (shape, size, structure, and composition).

Using the LA TAOS setup, ~6000 patterns of ambient aerosols (particle diameter: 0.5 μm – 10 μm) were captured at the Army Research Laboratory in collaboration with Ronald G. Pinnick and Steven C. Hill. The patterns suggest an ever-present diverse background of aerosol particles; however it appears that the patterns can be clustered into certain categories like spherical, deformed-sphere, and fiber-like. In addition, patterns were collected of single and powered-dispersed clusters of *Bacillus subtilis* (BG) spores. These patterns are being studied to determine the extent to which LA TAOS patterns of BG spores are mimicked by the particles in the ambient environment.

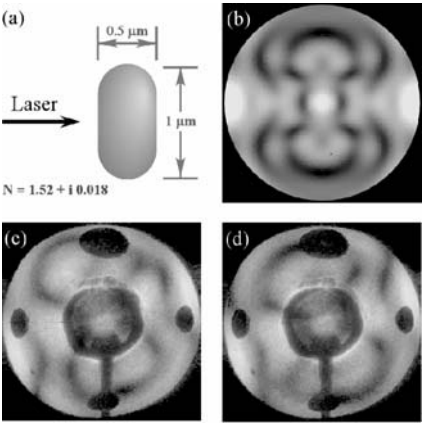

Fig. 1. Scattering pattern from a single BG spore: (a) estimated spore dimensions, (b) numerical simulation, (c) and (d) experimental results

Patterns are currently being analyzed with simple feature extraction routines that analyze the shape and orientation of the island-like features found in a majority of the patterns. In addition, as is shown in Fig. 1, experimental results are being compared with theory. Depicted are two

captured LA TAOS patterns of single BG spores as well as a numerical simulation of the scattering from a spore based on the T-matrix method calculated by Dr. Jean-Claude Auger.

Current experiments in the laboratory are investigating the correlation between the depolarization of scattered light and the absorption bands in the aerosol particle. In addition, we have designed a series of experiments to determine the degree to which LA TAOS patterns are sensitive to surface roughness. We are hoping that these experiments as well as those previously conducted will stimulate a conversation that will help us unravel the LA TAOS patterns to a degree where quantifiable information on particle morphology can be extracted. We acknowledge support by the RAAD program, the Air Force Research Laboratory, and Sandia National Labs Excellence in Engineering Fellowship.

References

[1] Pan, Y.L., et al., *Characterizing and monitoring respiratory aerosols by light scattering*. Optics Letters, 2003. **28**(8): p. 589–591.

[2] Hirst, E. and P.H. Kaye, *Experimental and theoretical light scattering profiles from spherical and nonspherical particles*. Journal of Geophysical Research-Atmospheres, 1996. **101**(D14): p. 19231–19235.

SCATTERING BY LEUCOCYTES, THEORY, SIMULATIONS AND EXPERIMENTS

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Leukocytes which include three basic classes: lymphocytes, monocytes and granulocytes are an important part of the body's immune system, help-

ing to destroy invading microorganisms. Leukocytes classification and analysis are an important diagnostic technique in the detection of many diseases. Ordinary flow cytometry was successfully applied to classify the leukocytes. Granulocytes, monocytes and lymphocytes occupied unique positions in the two-dimensional space created by the forward and side light-scattering intensities. De Grooth *et al*. [1] proposed measurement of depolarization of side light scattering for discrimination inside of a granulocyte subpopulation. Measurement of the depolarized orthogonal light scattering in flow cytometry enables one to discriminate human eosinophilic granulocytes from neutrophilic granulocytes [2]. Finally they measured forward light scattering, orthogonal light scattering, and the fluorescence intensities of unlysed peripheral blood cells, labeled with CD45-phycoerythrin and the nucleic acid dyes LDS-751 and thiazole orange utilizing an ordinary flow cytometer. Erythrocytes, reticulocytes, platelets, neutrophils, eosinophils, basophils, monocytes, lymphocytes, nucleated erythrocytes, and immature nucleated cells occupied unique positions in the five-dimensional space created by the listmode storage of the five independent parameters [3].

Unfortunately, modern diagnostic equipment based on ordinary flow cytometry technique allows making only classifications but does not allow making a quantitative description of leukocytes. Quantitative description of leukocytes can help in the analysis of pathological cases. The next generation of flow cytometers, Scanning Flow Cytometer (SFC), allows measurement of the angular dependence of light-scattering intensity (indicatrix) of single cells. The light scattering indicatrix (LSI) contains information about morphological characteristics of cells: size, shape, internal structure, sizes and refractive indices of cytoplasm, nuclear, organelles, *et cetera*. In order to obtain information about the morphology of a cell it is necessary to solve the inverse light scattering (ILS) problem.

In this study we analyzed the light scattering of leukocytes. Formation of the leukocytes LSI was studied with an aim of development of an appropriate optical model of all three types of leukocytes to solve the ILS problem for their characterization. These cells have a complex structure especially the granulocytes. The monocytes have nonconcentric and nonspherical nuclei. The granulocytes have several nonconcentric and nonspherical nuclei and their cytoplasm contain a lot of granules, which vary in size and concentration. Although use of a realistic model without any simplifications in morphology and without any approximations in the physics is tempting, at present it seems unbearable since it requires significant computational time. The appropriate optical model was two or more layered spheres with the following characteristics: d_i and n_i are the diameter and refractive index of the i-th layer respectively. The choice of the models is based on analysis of experimentally measured LSIs of individual leukocytes.

References

[1] De Grooth, B.G., Terstappen, L.W.M.M., Puppels, G.J., and Greve, J., «Light-scattering polarization measurements as a new parameter in flow cytometry», *Cytometry*, 8, 539–544, (1987).

[2] Terstappen, L.W.M.M., de Grooth, B.G., Visscher, K., van Kouterik, F.A., and Greve, J., «Four-parameter white blood cell differential counting based on light scattering measurements», *Cytometry* 9, 39–43, (1987).

[3] Terstappen, L.W.M.M., Johnson, D., Mickaels, R.A., Chen, J., Olds, G., Hawkins, J.T., Loken, M.R., & Levin, J., «Multidimensional flow cytometric blood cell differentiation without erythrocyte lysis», *Blood Cells* 17, 585–602, (1991).

HUMAN WHITE BLOOD CELLS: INVESTIGATION BY THE METHODS OF LIGHT MICROSCOPY

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Flow cytometers are widely used to identify the disperse particles, in particular, white blood cells (leukocytes) flow cytometers are widely used [1]. Their operation is based on the analysis of laser light, scattered by separate cells in

turn flying with a rate of up to 5000 particles per second. In flow cytometry the analysis of cells is carried out by fluorescent probes and light, scattered in two directions, namely forward and sideward. In scanning flow cytometry the function of the channel of scattering is increased. It is realized by measuring the angular structure of light in a wide interval of angles.

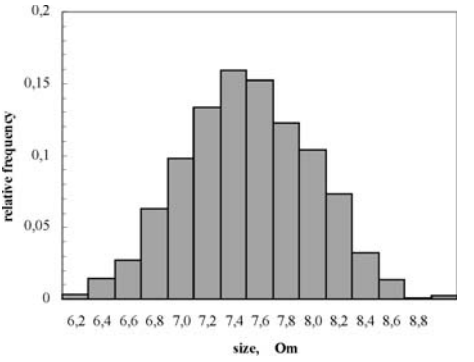

Fig. 1. . Size distribution of lymphocytes for one individual

The problem of retrieval of particle parameters by measuring scattered light is the inverse problem of scattering-media optics. One of the methods to solve the inverse problem of scattering is the neural networks method. The problem of identification of particles is being solved correctly when the shape and structure of particles, and the range of their sizes are known more precisely.

In this work the geometro-optical characteristics of normal human leukocytes have been investigated by methods of the specialized light microscopy at the conditions saving their native sizes. On the basis of the obtained experimental data the histograms of size distributions of lymphocytes, their nuclei, and granulocytes were constructed. The results will be used to develop an optical model of human white blood cells. In Fig. 1 the size distribution of lymphocytes for one donor is presented.

References

[1] V.P. Maltsev, «Scanning flow cytometry for individual particle analysis», Rev. of Sci. Instrument. **71**, 243–255 (2000).

APPLICABILITY OF EFFECTIVE MEDIUM APPROXIMATION TO LIGHT SCATTERING BY GRANULATED BIOLOGICAL CELLS – FIRST RESULTS

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Our primary interest is constructing and validating simplified models to simulate light scattering by granulated white blood cells (granulocytes). These cells have a complex shape (especially the nucleus) and their cytoplasms contain many granules, which vary in size and concentration. Although use of a realistic model (without any simplifications in morphology and without any approximations in the physics) is tempting, at present it seems unbearable since 1) detailed information on the morphology of granulocytes is sparse, especially on the statistical distribution of relevant parameters (e.g. size and refractive index distribution of granules); 2) a realistic model has many free parameters and computationally intensive methods, such as the Discrete Dipole Approximation (DDA), should be used to simulate light scattering by such complex model. While computational time for one set of free parameters is acceptable (few hours), modeling biological diversity, which implies varying each of the free parameters, increases simulation time by many orders of magnitude.

Therefore we have to simplify the model but in doing so it is important to control the deviation of results from those obtained by using a realistic model. The first simplification, which we concentrate on in this study, is replacing the cytoplasm containing granules by a homogenous medium with some average refractive index. Calculation of this refractive index is performed by an Effective Medium Approximation (EMA). All classical EMAs assume that the size of the inclusions is small compared to the wavelength, however in some cases they are applicable for much larger inclusions [1]. The latter, however, can be verified in any particular case only by comparison of EMA

prediction to exact results. Extensive studies of EMA applicability were performed using experimental [1, 2] and theoretical [1, 3] reference results. However they focused on astrophysical applications (refractive index of 1.6 and larger) and used spheres with inclusions as a model.

We are studying the applicability of EMA to granulocytes, therefore we consider a much lower refractive index and a coated sphere model for the cell, where the inner cell corresponds to the nucleus and the outer shell to the cytoplasm with granules. We use DDA to simulate light scattering for different sizes and volume fractions of granules and compare them to Mie theory applied to a coated sphere, where the refractive index of the outer shell is calculated by EMA. Our first results show that Maxwell-Garnett EMA (which corresponds to the studied topology – inclusions inside the matrix) can be successfully applied for small inclusions over a large range of volume fractions. We are currently studying larger inclusions and relaxing the concentric sphere model by allowing an eccentric nucleus. At the workshop we will present a progress report of our study.

References

[1] P. Chylek, G. Videen, D.J. W. Geldart, J.S. Dobbie, and H.C.W. Tso, «Effective medium approximations for heterogenous particles», in *Light Scattering by Nonspherical Particles, Theory, Measurements, and Applications*, M. I. Mishchenko, J.W. Hovenier, and L. D. Travis, eds., (Academic Press, 2000), pp. 273–308.

[2] L. Kolokolova and B.A.S. Gustafson, «Scattering by inhomogeneous particles: microwave analog experiments and comparison to effective medium theories,» J. Quant. Spectrosc. Radiat. Transf. **70**, 611–625 (2001).

[3] N.V. Voshchinnikov, V.B. Il'in, and T. Henning, «Modelling the optical properties of composite and porous interstellar grains», Astronomy & Astrophysics 429, 371–381 (2005).

IDENTIFICATION OF BLOOD CELL SUBPOPULATIONS FROM ANGLE RESOLVED LIGHT SCATTERING

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Flow cytometry has experienced a considerable expansion of available parameters during the last years. Various techniques that allow for the direct analysis of leukocytes by direct labeling with antigen-specific fluorescent labeled antibodies or according to functional parameters will have enormous impact on immunological research and hematopathological diagnosis. Scanning flow cytometry (SFC) [1] with its additional ability to measure morphological and/or biophysical differences between different blood cell subpopulations can make analysis of leukocytes more accurate and more comfortable. The angle resolved light scattering pattern (LSP) measured with SFC contains encoded information about morphological and biophysical properties of cells. Cells having subtle differences in their morphological and/or biophysical properties can potentially be better discriminated by LSP compared to "classical" integrated side scatter measurements. Eventually this could lead to a reduction in the number of antibodies needed for proper subset identification, thus allowing for either less expensive testing or adding additional antigenic evaluations to the test.

The aim of this study is to develop multiparametric methods of classification for peripheral blood (PB) cells based on the LSP only and using them for cell subset identification. We adapted Bayes classification algorithm to classify different types of blood cells. The antigen-specific (antibody) labeling was used for building of a learning sample but not for the classification itself. The integrals of LSP over different angular ranges and spectrum parameters of LSP were used in the classification. The optimization of algorithm was done by correctly definiting parameters. We verified the obtained algorithm on different healthy donors. Though some overlap in cell subsets is present, good evaluations with subset classification errors less then 15% can be made without the need for antigen-specific labeling.

References

[1] Maltsev V.P. and Semyanov K.A., Characterization of Bioparticles from Light Scattering (Vista Science Press, Netherlands, 2004).

CHARACTERIZATION OF MONONUCLEAR BLOOD CELLS FROM LIGHT SCATTERING

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Modern diagnostic equipment based on classical flow cytometric techniques developed rapidly into a cell subset classification tool based on the simultaneous evaluation of several (up to 5 and more) cellular antigens detected by the use of fluorescent labeled monoclonal antibodies. The two light scattering parameters (collected in the forward and side direction) are used for the discrimination of the major leukocytic populations (lymphocytes, monocytes and granulocytes).

Scanning flow cytometry (SFC) allows for measuring the light scattering intensity dependency on detection / observation angle, i.e. the light scattering indicatrix (LSI) in range from 10 to 70 degrees. The LSI contains information about morphological and biophysical properties of cells: size, shape, internal structure, sizes and refractive indices of cytoplasm, nuclear, organelles, etc. In this way the LSI can be regarded as comparable to a microscopic image but with encoded information content. The additional information thus obtained can help in the detection of aberrant (pathological) versus normal cell types.

In order to obtain information about the morphology of a cell it is necessary to solve the inverse light scattering (ILS) problem. Most blood cells are far from homogenous and spherical: unfortunately there are no methods available at the moment for solving the ILS problem for inhomogeneous and nonspherical cells. This study is the first step in attempting to solve the ILS problem for blood mononuclear cells.

Human blood mononuclear cells (MNC) consist of B- and T-lymphocytes, natural killer (NK) cells and monocytes. These cells play a major role in the development and maintenance of humoral, cellular and innate immune response and in destruction of bacteria and virus infected cells, respectively. The monocyte diameter is about 15–20 μm it has a round shape and a concave or bean-shaped nucleus. Its major function is to destroy foreign particles and infected cells by means of phagocytosis. Lymphocytes are round cells, with a diameter range of 7–12 μm and a near-round shaped nucleus. To describe the morphology of mononuclear cells we resorted to a multi-layered concentric sphere model because of their near-round shape. A special algorithm was realized to calculate the light scattering pattern of the model. Then we performed firstly manual and then half-automated Levenberg-Marquadt fitting to minimize the mean square error function of the theoretical and experimental signal by varying diameters and refractive indices of layers of the model. We processed in this way one hundred T-cells, B-cells, natural killer cells and monocytes. The optimal number of layers was estimated as five, because greater numbers gave fitting errors that were comparable in their value with the difference between layer diameters or even exceeded these. We have associated the first two layers with the nucleus, the third layer with the cytoplasm and the last two layers with the membrane and its asperity. After this step the distributions by parameters of each class of cells were built.

IDENTIFICATION OF LEUKOCYTES WITH SCANNING FLOW CYTOMETER

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It is well known, that populations of lymphocytes, monocytes and polymorpho-nuclear leukocytes can be singled out on a biparametric diagram of forward scattering signal (FSC) versus side scattering signal (SSC)

as measured by standard flow cytometric equipment [1]. Scanning flow cytometry allows us to measure the FSC signal and light scattering trace (LST), i.e. intensity dependence on scattering angle, within an angle range from 7 to 70 degrees [2]. The integral of LST over different angle ranges can be used as a superior alternative for SSC in the biparametric FSC/SSC plots. This work demonstrates the possibility of leukocyte discrimination on a biparametric plot of an erythrocytes free human blood sample measured by a scanning flow cytometer. Three populations of leukocytes can be easily gated out (Fig. 1) using the full integral of the angle resolved light scattering trace. «Classical» FSC/SSC biparametric acquisition does not allow for discriminating residual red blood cells (either after density separation or lysing procedures) from the leukocyte population. Using the integral of LST over specific angle ranges can readily accomplish this.

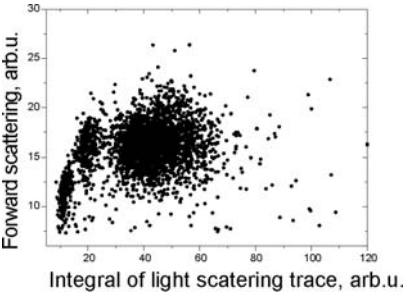

Fig. 1. Dot plot diagram of human blood sample measured by Scanning Flow Cytometer (5000 cells). Erythrocytes were lysed using FACS lysing solution (BD). Full integral of light scattering trace used as second parameter

Reference

[1] H.M. Shapiro, *Practical Flow Cytometry*. 3rd Edition. New York, Wiley-Liss, 1995.

[2] V.P. Maltsev, A.V. Chernyshev, K.A. Sem'yanov, E. Soini, *Absolute real-time measurement of particle size distribution with the flying light-scattering indicatrix method*. Appl. Opt. 35 (1996) 3275.

OPTICS OF BLOOD PLATELETS

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Platelets are small of great importance in maintaining hemostasis, but they also play a significant role in many pathophysiology process, including thrombosis, hemorrhage, inflammation, antimicrobial host defense, and tumor growth and metastasis [1]. Detection of diseases in their early stages is an important and difficult task of medicine. However it becomes possible due to the relationship of normal and abnormal organism operations withing blood.

In this study we analyzed the light scattering pattern (LSP) of individual platelets. Formation of the platelets LSP was studied with the aim of development of appropriate optical model to solve the inverse light scattering (ILS) problem for theirs characterization. An oblate spheroid was taken as an optical model of platelet. Preliminary theoretical calculations using the T-matrix method were made for different parameters of oblate spheroids: volumes, aspect ratios and refractive indices. After measured LSPs of platelets were compared with calculated LSPs. The parameters of spheroids whose LSPs had minimal standard deviation from the measured LSPs were assigned parameters of platelets. The distributions of volume, aspect ratio and refractive index of platelets were built. The refractive index of platelet can be regarded as optical density of one and can be connected with concentrations of any substance in platelet.

References

[1] Michelson A.D. Platelets. New York: Academic Press/Elsevier Science, 2002.

4st day: Thursday October 6, 2005

ENRICHING BIO-AEROSOLS FOR SPECIES IDENTIFICATION BY OPTICAL OR BIOCHEMICAL ASSAY TECHNIQUES

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It has been accepted that the fluorescence spectra of aerosols in the 1–10 μm range can be used to classify whether the aerosol is a bio-threat or a non-bio-threat aerosol. The fluorescence spectra, however, do not provide any species-specific information. To achieve species-specific identification, there are three non-real time methods under consideration. These considerations are as follows: 1) Raman scattering, 2) FTIR absorption, 3) mass spectrometry, and 4) biochemical assay. The first 3 techniques require the sample to be dry while the 4th technique requires the sample to be in a liquid environment. All four techniques are adversely affected if the non-bio-threat aerosols constitute the major portion of the sample. A critical element toward species-specific identification is to first enrich the sample by as much as 10^4 times. Additionally, these enriched particles are selectively concentrated and localized into a small area on a substrate surface or into a small opening in a microfluidic cell.

To achieve enrichment in the dry form, we use an aerodynamic puffer that is cued by a near real-time fluorescence spectra that have satisfied specific preset conditions. The pulsed air packet emitted by the puffer is turbulent and hence spread the deflected particles into a large cone. In order to overcome this spatial spread, a pulsed aerodynamic localizer (PAL) is used. The puffer-PAL combination has been able to confine the enriched bio-threat particle sample to be localized within an area of 0.6 mm diameter. This 0.6 mm diameter is commensurate with the scanning range of the micro-Raman instruments and the IR beam used in FTIR. Ways of improving the efficiency of the puffer-PAL combination to obtain better experimental

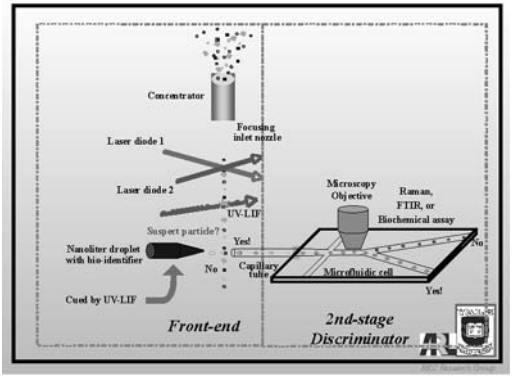

Fig. 1. This is a schematic showing the launcher shooting a particle cued by the fluorescence spectra (Front-end) into a capillary tube of a microfluidic cell (2^{nd}-stage Discriminator). Depending upon the fluorescence intensity of a particle, the bio-threat particle is further deflected electrostatically (2^{nd}-stage Discriminator)

results will be discussed. Certainly for the biochemical assay technique, and to a lesser extent, the mass spectrometry technique, requires the sample to be introduced into a small opening of the instrument used, such as a microfluidic cell.

In order to send a fluorescence-cued particle to the small opening (about 1 mm) of a microfluidic cell, one must deflect that particle very accurately. (See Figure 1) The deflection of a particle by an air puffer does not have the necessary accuracy. Instead, we use a liquid pulsed valve as a launcher of a large droplet (100 μm or larger) to envelope the particle and thereafter having the particle be carried by the trajectory of the large droplet. Because we can launch our droplet with accuracy, we are able to deflect our targeted particle more accurately. The effect of the collision of the particle with the droplet results in diffusion and mixing within the droplet, thereby speeding up the reaction rate. This launcher electrically charges the large droplet (containing specific biochemical binding molecules tagged with fluorescers) and this electrical charge allows electrostatic steering, thereby providing further sorting.

BACKSCATTERING OF SURFACES COMPOSED WITH DRY BIOLOGICAL PARTICLES

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We study the backscattering of particulate surfaces consisting of dry biological particles using two laboratory photometer/polarimeters that allow measurements of brightness and linear polarization degree of particulate surfaces in a phase angle range, 0.2–60° [1]. We measured three samples composed of dry biological particles, *Bacillus subtilis* var. niger (BG) spores (see Fig. 1) and two samples of fungi *Aspergillus Terreus* and *Sporisirum Cruentum* spores in two spectral bands centered near 0.63 μm and 0.45 μm. We find that the surfaces display a prominent brightness opposition effect and significant negative polarization near backscattering angles. The brightness and polarimetric phase curves are different for *Bacillus subtilis*

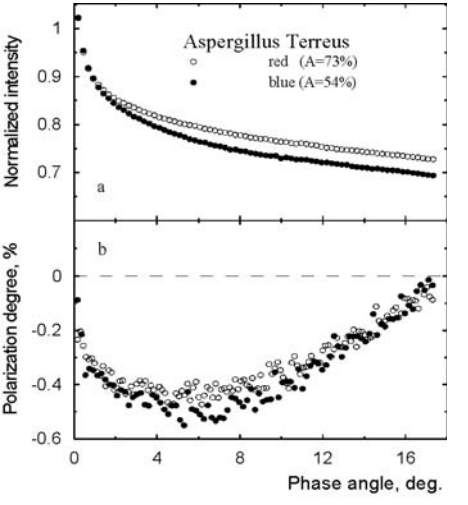

Fig. 1. Photometric and polarimetric data for fungi *Aspergillis Terreus* measured with the small-phase--angle instrument in red and blue light.

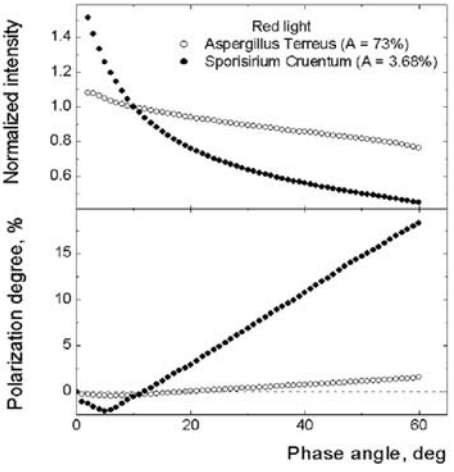

Fig. 2. Photometric and polarimetric data for fungi *Aspergillis Terreus* and *Sporisirum Cruentum* measured with the large-phase-angle instrument in red light

and the fungi. Fig. 1 presents photometric and polarimetric data for fungi *Aspergillis Terreus* measured with the small-phase-angle instrument in red and blue light showing neutral behavior of the negative polarization branch. Fig. 2 presents photometric and polarimetric data for fungi *Aspergillis Terreus* and *Sporisirum Cruentum* measured with the large-phase-angle instrument in red light, demonstrating strong influence of albedo on photopolarimetric properties of biological particles.

References

[1] Yu. Shkuratov, A. Ovcharenko, E. Zubko, O. Miloslavskaya, R. Nelson, W. Smythe, K. Muinonen, J. Piironen, V. Rosenbush, P. Helfenstein, «The opposition effect and negative polarization of structurally simulated planetary regoliths», Icarus 159, 396–416, (2002).

THE IAA LIGHT SCATTERING FACILITY

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A new light scattering laboratory has been built up at the Instituto de Astrofísica de Andalucía (IAA) in Granada, Spain. The facility has benefited from the design of the well-know setup which is currently under operation at the Astronomical Institute «Anton Pannekoek», University of Amsterdam, The Netherlands [1]. Our light scattering facility allows measurement of the full scattering matrix as a function of the scattering angle of a dust sample by using a polarization modulation technique. A tunable Ar-Kr laser is used as a light source, with five wavelengths between 483 and 676 nm. Although the main purpose of this setup is the measurement of dust samples of Astrophysical interest, it is also our intention to open the facility to the study of biological particles, mainly blood cells. In this talk we describe the setup, and our purpose is to discuss what kind of measurements would be interesting to make in the field of biological cells, as well as to learn on the difficulties we would face when dealing with such kind of particles.

References

[1] J.W. Hovenier, «Measuring scattering matrices of small particles at optical wavelengths», in *Light Scattering by Nonspherical Particles*, M.I. Mishchenko, J.W. Hovenier, and L.D. Travis, eds., pp. 355–365, Academic Press, San Diego, California, 2000.

DIRECT MEASUREMENT OF THE AEROSOL ABSORPTION AND EXTINCTION CROSS SECTION FOR BACILLUS *ATROPHAEUS* ENDOSPORES USING FLOW-THROUGH PHOTOACOUSTICS

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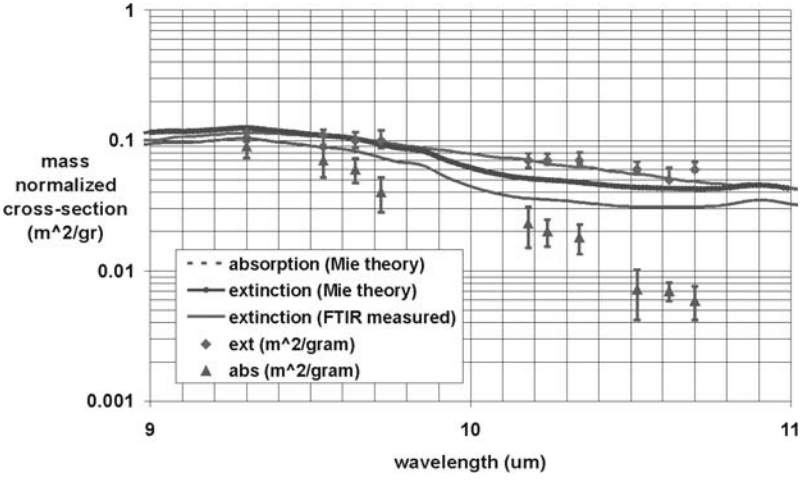
In an effort to establish a more reliable set of relevant optical cross sections for a variety of chemical and biological aerosol simulants, we have developed a flow-through photoacoustic system capable of measuring the absolute mass-normalized extinction and absorption cross sections. Although the results shown here were conducted over the tunable CO_2 laser waveband region, 9.20-10.80 μm, application to other waveband regions are easily achievable. In addition to the Bacillus atrophaeus endospores (BG), various chemical and inorganic background aerosols were also con-



Comparison of measured and Mie theory calculated cross-sections for aerosolized Bacillus atrophaeus endospores

sider i.e., dimethicone silicone oil (SF-96 grade 50), and Kaolin clay powder (alumina and silicate). Results compare well with previously measured spectral extinction measured using FTIR spectroscopy. Comparison with Mie theory calculations based on previously published complex indices of refraction and measured size distributions are also presented.

A STUDY OF PENETRATION OF A VIRUS INTO A CELL WITH DYNAMIC FLOW CYTOMETRY

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Virus-cell interactions must be investigated not only on qualitative level but also at a quantitative level. Complete information about parameters of the interaction is essentially important: (I) for understanding a mechanism of interaction, (II) for building a kinetic model of interaction, (III) for searching new antiviral inhibited virus-cell interactions.

The Venezuelan equine encephalomyelitis (VEE) virus, strain TC-83, and *Vero* cell from the collection of SRC VB "Vector" (Russia) were used in our study. FITC-labeled murine monoclonal antibodies 1D10 against VEE virus, FITC-labeled polyclonal rabbit antibodies (PAbs) against laminin-binding protein (LBP) were used for detection of virus-cell interaction *in vitro* and for evaluation of a number of cell receptors on *Vero* cells. The fluorescence of immune complexes on surface of *Vero* cells and VEE virus-cell complexes were measured by scanning flow cytometry technique.

The scanning flow cytometry technique was used for evaluation of parameters of virus-cell interaction. Concentration of LBP molecules on a single cell was 1.8×10^5 and affinity of the interaction of PAbs against LBP with *Vero* cell is approximately 0.4×10^7 M^{-1}. The affinity of the interaction of the alive VEE virus, strain TC-83, with alive *Vero* cells was 6×10^7 M^{-1}. Competitive analysis with using VEE virions and PAbs against LBP revealed that 50% of cell receptors were occupied by the VEE virus. The maximum number of VEE virion absorbed on the surface of a single cell was estimated at approximately 400 virions for our experiments. Earlier, these

constants were determined using fixed cells, VEE and tick-borne encephalitis viruses by ELISA technique [1, 2]. The affinity constants were similar to our data. It allows us to conclude that scanning flow cytometry technique may be successfully used for investigation of quantitative characteristics of virus-cell interaction.

The scanning flow cytometry technique was used for accumulation of quantitative parameters for individual virus-cell interactions. Quantitative parameters will be used for building kinetic and theoretical models of virus-cell interaction.

References:

[1] Bondarenko E.V. et al. (2003) Molecular genetics, microbiology and virology **4**, 36-39.

[2] Protopopova E.V. et al. (1999) Zentralblatt fur Bakteriologie **289**, 632–638.

THE 7TH SENSE: BIONIC FIBER-OPTIC BIOSENSORS

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We are continually challenged with old and new threats. Our five senses developed through evolution and the 6th sense, non-measurable, but generally believed as existing, cannot help us monitor for most dangers that are either cellular or molecular. So, throughout history, we developed diagnostic tools which served us well. Today, we are supplementing classic methodologies with rapid diagnostics using biosensor (the 7th sense) and biochip technologies. The economic drivers are the following increasing concern with our health, biodefense and old age. We will discuss our developments in this area, which have led us to develop the vision for a BioPen, the first Lab-in-a-Pen.

- The development of chemiluminescent fiber-optic immunosensors to antibodies from Hepatitis C [1], West Nile [2], and Ebola viruses, cholera toxin [3] and Ovarian cancer using either silane or electropolymerisation on ITO-coated fiber-optics

- The development of bioluminescent fiber-optic whole-cell bioreporter biosensors to gentoxicants [4], heavy metals [5] and endocrine disrupting compounds.
- The development of a chemiluminescent phagocyte-based fiber-optic sensor [6].
- The development of nanofluidics [7] for biochip modules.

References

[1] Konry, T., A. Novoa, Y. Shemer-Avni, N. Hanuka, S. Cosnier, A. Lepellec and R.S. Marks (2005) Optical immunosensor based on a poly(pyrrole-benzophenone) film for the detection of antibodies to viral antigen. Analytical Chemistry. 77 (6) 1771–1779.

[2] Herrmann, S. B. Leshem, S. Landes, B. Rager-Zisman and R.S. Marks (2004) Chemiluminescent optical fiber immunosensor for the detection of anti-West Nile virus IgG. Talanta. 66: 6–14.

[3] Leshem, B., G. Sarfati, A. Novoa, I. Breslav and R.S. Marks (2004) Photochemical attachment of biomolecules onto fiber-optics for construction of a chemiluminescence immunosensor. Luminescence. 19: 69–77 & Konry, T., A. Novoa, S. Cosnier and R.S. Marks (2003) Development of an «electroptrode» immunosensor: Indium-tin-oxide-coated optical fiber tips conjugated with an electropolymerized thin film with conjugated cholera toxin B subunit. Analytical Chemistry. 75: 2633–2639 & Marks, R.S., E. Basis, A. Bychenko and M.M. Levine (1997) Chemiluminescent optical fiber immunosensor for detecting cholera antitoxin. Optical Engineering. 36 (12) 3258–3264

[4] Polyak, B., S. Geresh and R.S. Marks (2004) Synthesis and characterization of a biotin-alginate conjugate and its application in a biosensor construction. Biomacromolecules. 5: 389–396 & Pedahzur, R., B. Polyak, R.S. Marks and S. Belkin (2004) Water toxicity detection by a panel of stress responsive luminescent bacteria. Journal of Applied Toxicology. 24: 343–348 & Polyak, B., E. Bassis, A. Novodvorets, S. Belkin and R.S. Marks (2000) Bioluminescent whole-cell optical fiber sensor to genotoxicants: system optimization. Sensors and Actuators. B 3656: 1–9 & Polyak, B., E. Bassis, A. Novodvorets, S. Belkin and R.S. Marks (2000) Optical fiber bioluminescent whole-cell microbial biosensors to genotoxicants. Water Science and Technology. 42 (1–2) 305–311

[5] Hakkila, K., T. Green, P. Leskinen, A. Ivask, R. S. Marks and M. Virta (2004) Detection of bioavailable heavy metals in EILATox-Oregon samples using whole-cell Luminescent bacterial sensors in suspension or immobilized onto fiber-optic tips. Journal of Applied Toxicology. 24: 333–342

[6] Magrisso, M., O. Etzion, G. Pilch, A. Novodvoretz, G. Perez-Avraham, F. Schlaeffer and R.S. Marks (2005) Fiber-optic biosensor to assess circulating phagocyte activity by chemiluminescence. Biosensors & Bioelectronics. In Press.
[7] Ionescu, R.E., R.S. Marks and L. Gheber (2005) Manufacturing of nano-channels with controlled dimensions using protease nanolithography. Nanoletters. 5 (5) 821–827 & Ionescu, R., R.S. Marks and L. Gheber (2003) Nanolithography Using Protease Etching of Protein Surfaces. Nano Letters. 3 (12) 1639–1642 & Taha, Hesham, R.S. Marks, L.A. Gheber, I. Rousso, J. Newman, C. Sukenik and A. Lewis (2003) Protein printing with an atomic force sensing nanofountainpen. Applied Physics Letters. 83 (5) 1041–1043.

RED LIGHT LASER EFFECTS ON BLOOD CELLS OF SOME PATHOLOGICAL HAEMODYNAMIC HUMAN CONDITIONS

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Flow cytometry is playing an increasingly significant role in the practice of the clinical laboratory in several areas like oncology (DNA content measurement), haematology and vascular biology and the pathogenesis of several vascular diseases. Flow cytometry (FCM) allows the simultaneous measurement of multiple fluorescences and light scatter induced by illumination of

single cells or microscopic particles in suspension. Cells in suspension are drawn by a stream of fluid which aligns them before their interception by a beam of light, generally produced by Laser. The generated optical signals (diffused lights and emitted fluorescences), related to biological properties of the cells, are conveyed to photomultipliers which transform light energy into electric currents. The signals are quantified by electronic and processing components which can drive cell sorting. Thus it is important to study the biological effect of laser beam on different types of human pathologic haemodynamic conditions. In this study the effects of fluencies ranging from 1J/cm^2 up 35 J/cm^2 of focal diode laser irradiation (904 nm) and He-Ne laser irradiation (632.8 nm) on blood components of normal, cyanotic and acyanotic blood cells and in cases of acute myeloid and acute lymphocytic leukemias were investigated. Cell viability using flurescine diacetate, proliferation, apoptosis, interlukin 2 release together with reactive oxygen species (ROS) and reactive nitrogen species (RON) generation were estimated before and after 1, 2 and 24 hours of laser irradiation. At low doses of laser irradiation, laser ameliorated oxidative stress in normal blood cells, acyanotic heart disease blood post cardiac catheterization, but increased apoptosis and DNA fragmentation in cyanotic cases, acute lymphocytic and myeloid leukemias 24 hours post laser irradiation. At higher doses of 35 J/cm^2 laser light induced higher percentage of apoptosis in all cases of the study. A hypothesis of free radical mechanisms of stimulatory and inhibitory actions of low energy laser irradiation (LELI), used for therapy of a variety of inflammatory diseases, is formulated. Light absorption induces the production of initiating radicals that are involved in subsequent free radical reactions and subsequent leukocytes stimulation of the greater production of prooxidants and other biologically active products. These products include nitric oxide intermediates (NOI), reactive oxygen intermediates (ROI), which dependent on their induced concentrations, can alter expression (increase or decrease) of proteins involved in multiple signal transduction pathways and induce the expression of inducible proteins, whose genes are highly influenced by external stimuli and could finally lead to apoptosis. Some of these proteins represent protective mechanisms against external stresses, while others amplify adaptation to the induced redox effect.

INFORMATIONAL CONTENT OF INTEGRAL LIGHT SCATTERING INDICATRIX OF OPTICALLY SOFT PARTICLES

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The integral light scattering indicatrix $F(\theta_0)$ presents a flux share that is scattered to the cone with cone angle $2\theta_0$ to the whole flow of the scattered energy:

$$F(\theta_0) = 2\pi \int_0^{\theta_0} I(\theta)\sin\theta d\theta \Big/ 2\pi \int_0^{\pi} I(\theta)\sin\theta d\theta,$$

where $I(\theta)$ – scattering intensity in the direction θ.

In the paper [1] it is shown that in the Rayleigh-Gans-Debye approximation the integral light scattering indicatrix of the homogeneous sphere is invariant in the coordinates $\rho\theta_0$ and a method for determining mean cell dimension from the half level of scattering which is observed $\rho\theta_0 = 1.75$ at has been proposed (ρ is the size parameter of the spherical particle).

In this paper the indirect evidence of relation between $F(\theta_0)$ and relative refractive index m is shown. This relation is based on a presentation of $F(\theta_0)$ in the following form:

$$F(\theta_0) = 1 - 2Q(2\rho\theta_0)/(2\rho\theta_0)^2, \quad (1)$$

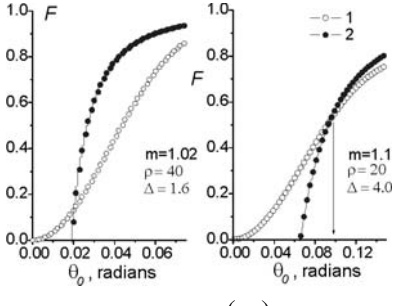

Fig. 1. Relation between $F(\theta_0)$ and relative refractive index m . 1– $F(\theta_0)$ (Mie calculation); 2 – Eq.1 with Q calculated by Mie theory

where Q is a function that coincides formally with Van de Hulst's famous formula for extinction efficiency with $\Delta = 2\rho m - 1$ as the argument [2]. Equating the arguments of these functions leads to the relation $\theta_0 = \theta_m = m - 1$ that is shown in Fig. 1.

The theoretical method of retrieval of m has been demonstrated in this paper. It is based on two integral indicatrices obtained with different wavelengths of in-

cident radiation for the same sample. In this case the value of *m* can be found from equation

$$F_1(\theta_m)/F_2(\theta_m)=Q_1/Q_2.$$

References

[1] Ph.Ya. Sidko, V.N. Lopatin, L.E. Paramonov, *Polarization characteristics of suspension of the biological particles* (Novosibirsk, Nauka, 1990).

[2] H.C. van de Hulst *Light scattering by small particles* (New York, Wiley, 1957).

A SIMPLE INVERSION OF THE MIE PARTICLE EXTINCTION SPECTRUM

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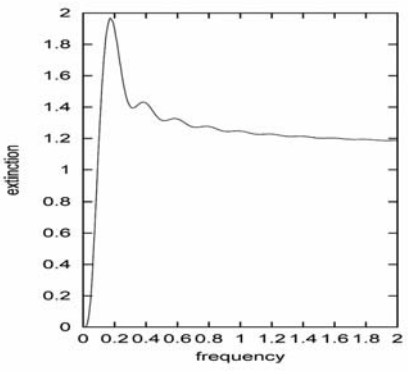
In biomedical optics, a tissue is frequently modeled as a turbid medium where the size distribution of scattering particles is related to pathology of the tissue. The information on size distribution of particles in a turbid tissue is therefore an important diagnostic indicator.

One way to extract information about particle size distribution of the turbid medium is to invert the equation

$$K_{ext}(m, k) = \pi N / Q_{ext}(m, ka)a^2 f(a)da.$$



In the above equation Kext is extinction of light scattered by a dilute suspension of poly-dis-parse spherical particles of relative refractive index *m*, the total number of particles in the suspension is and $Q_{ext}(m, ka)$ is the extinction efficiency of a single particle when light of wave number is scattered by a particle of radius *a*. Both and *m* are defined relative to the medium of suspension and *f(a)* defines the particle size distribution.

A typical $K_{ext}(m, ka)$ versus frequency curve is shown above for a signal modal Gaussian particle size distribution. We have analyzed in details the extinction spectra generated by smooth size distributions (beta, gamma and Gaussian) of Mie particles. We note that extinction spectrum, in general, has some easily identifiable characteristic regions where the extinction - frequency relationship can be approximated by simple empirical formulae involving the first four moments of the particle size distribution function. Our analysis also helps one to understand physically the manner in which the essential features of a particle size distribution get coded into the corresponding extinction spectrum.

USE OF SINGULAR VALUE DECOMPOSITION FOR ANALYSIS OF LIGHT-SCATTERING INDICATRICES OF SMALL PARTICLES

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Spectral analysis of light-scattering patterns (indicatrices) of small particles is one of the most promising methods for size determination of particles. The Hanning window procedure was proposed for preprocessing of an indicatrix before Fourier transform [1]. In this work we propose the Singular Value Decomposition (SVD) for this purpose.

As it is known, the SVD can be used for approximately solving «overestimated» systems of linear equations, when the number of equations m is grater then the number of unknown parameters n. Also, the eigenvectors of $k \le n$ biggest singularvalues form the basis of k-dimensional space, in which this system can be approximately solved for k unknown parameters with best precision.

We used the last property of the SVD for reducing the magnitude difference and for removal of low-frequency interference in the indicatrix.

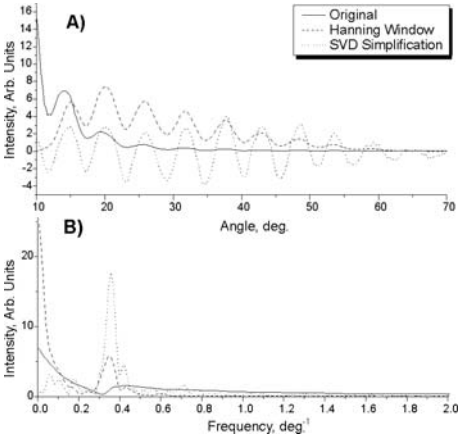

Fig. 1. Example of use of SVD simplification for analysis of indicatrix. a) Experimental signals of sphered erythrocyte, and b) their Fourier image after different preprocessing

For this purpose 5000 (m) indicatrices of spherical particles with parameters within range 10–100 for optical size and 0.5–20 for phase shift parameter have been calculated in the range from 10 to 70 degrees at 256 (n) points. For reducing of errors in the SVD each of 256 indicatrix points was normalized to have mean $\mu = 0$ and variance $\sigma = 1$ through all indicatrices. Using SVD, we calculated 256 singularvalues with corresponding eigenvectors. Two of them with highest singularvalues appeared to have no high frequencies. They were used for removal of low frequency components from normalized indicatrix. The resulting signal with the original and Hanning-windowed signals and their Fourier images are shown in Fig. 1. From the figure it could be found that the frequency corresponding to particle size becomes more visible in comparison with the original and windowed signals.

The same approach has been used for indicatrices of coated spheres and of oblate spheroids. Two main eigenvectors for both of these cases are similar to the ones for the indicatrices of spheres, that allow us to use this approach in all three cases.

References

[1] K.A. Semianov, P.A. Tarasov, A.E. Zharinov., A.V. Chernyshev, A.G. Hoekstra, and V.P. Maltsev «Single-particle sizing from light

scattering by spectral decomposition», Appl. Opt. **43**, 5110–5115, (2004).

SPHERE PARTICLE CHARACTERIZATION BY MEANS OF THE NEURAL NETWORKS

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Artificial Neural Networks (ANN) are commonly used for the approximation of multidimensional functions. They may solve the approximation with considerable high accuracy and fast speed. Solving the inverse lightscattering problem can in a certain sense be considered as a problem of approximating of a multidimensional mapping. In the case of spherical particles it is to approximate the mapping from space of scattering patterns to a two-dimensional space of particle parameters (particle size and refractive index).

We applied the ANN approach to interpret Scanning Flow Cytometry (SFC) data for the case of spherical homogeneous particles. In other words, we searched for the ANN F, such as:

$$F: R^{128} \to R^2 \left(F\left(I(\theta_1), I(\theta_2), \ldots, I(\theta_{128}) \right) = (r, n) \right),$$

where $\theta_1, \theta_2, \ldots \theta_{128}$ are intensities located uniformly on interval $10^0 – 70^0$, $r = 1 – 6$ μm – particle radius, $n = 1.35–1.8$ – refractive index. Wave length λ is equal to 0.488μ m, medium refractive index (water) m_0 is 1.333. A set of

scattering pattern parameters includes spectral characteristics and pattern features.

First, the inverse scattering problem was solved for undisturbed theoretical data. Because of the complexity of the problem, the solution was based on multi-stage networks: the problem has initially been solved with lower accuracy on whole area of parameters, then, with respect to the derived value of refractive index, the problem was solved on a corresponding subinterval with an appropriate neural network.

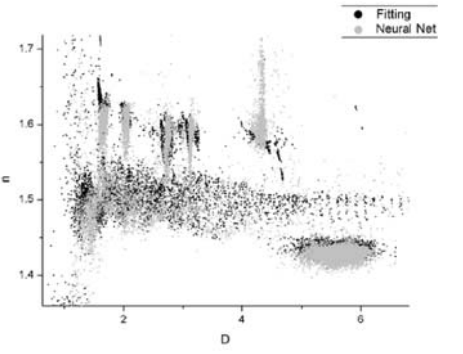

Fig. 1. Results of the characterization of latex particles by fitting and neural network methods

In spite of the relatively high accuracy of derived ANNs, (mean errors for radius and real refractive index are 3% and 1% respectively), tests of the networks on real experimental data showed unsatisfactory high errors in particle parameter estimation, that were much higher than ones for both MSE fitting and FLSI parameterization methods.

To overcome this problem we used several techniques for the distortion of learning multitude for ANN training. They included: high noise level signals, artificial disturbance of theoretical patterns, which could appear in SFC system. Also the most stable input parameters of scattering patterns were selected. These efforts resulted in substantial improvement of the solution (Fig. 1).

HIGH-ORDER NEURAL NETWORKS FOR SPHERICAL PARTICLE CHARACTERIZATION BY THE INTENSITY OF SCATTERED LIGHT

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The problem of retrieval of particle parameters from the angular distribution of light scattered by a single particle is important for different applications.We focus on one particular urgent problen scanning flow cytometry.

There are many approaches to solve this problem for spherical particles. One is the solution of the inverse problem of scattering for a homogeneous spherical particle. To determine parameters of a particle it is necessary to calculate integrals from the measured angular distribution of scattered light multiplied by oscillating functions of the angle in limits from 0° to180°.

The trial-and-error method is widely used. It consists in a multiple solution of a direct problem and selection of particle parameters so that the calculation results as much as possible coincide with the experimentally measured angular distribution of scattered light. The method requires considerable time even for modern computers. To decrease the time of analysis the empirical techniques for solving an inverse problem of scattering in flow cytometry are applied. Recently for a solution of the inverse problem of scattering by spherical and non-spherical particles the neural networks method is used [1], [2], [3].

In the present work the retrieval of diameter D, real n and imaginary κ parts of refractive index of spherical absorbing particle in the ranges $1.26\ \mu m < D < 21.2\ \mu m$, $1.02 < n < 1.38$, $0 < \kappa < 0.03$ by the intensity of light scattered in the range $10° < \theta < 60°$ using high-order neural networks is considered. The advantages of the neural networks are fast training, and an opportunity to use methods for analysis of systems of the linear algebraic equations [1–3]. As the input parameters of a neural network we used the real and imaginary parts of discrete Fourier transform, characteristic frequency of change of angular dependence of scattered light and relative val-

ues of factors of decomposition of angular dependence on the Legendre polynomials. To model intensity of scattered light the Mie theory is used.

We analyze retrieval errors of D, n and κ depending on particle parameters and structural parameters of a neural network. Retrieval errors (standard deviation) of D, n, and κ by a four-dot single-level neural network are 0.07 – 0.19 μm, 0.013 – 0.015 and 0.003 – 0.005, respectively. The greatest errors are characteristic for small particles with a small refractive index. At increase in size of particles, the retrieval errors of the size and refractive index essentially decrease. The two-level neural network allows increasing twice accuracy at small measurement errors, but yields the worse results at significant measurement errors.

References

[1] V.V. Berdnik, R.D. Mukhamedjarov, V.A. Loiko, «Characterization of optically soft spheroidal particles by multiangle light-scattering data by use of the neural-networks method», Opt. Lett. **29**, 1019–1021 (2004).

[2] V.V. Berdnik, R.D. Mukhamedyarov, V.A. Loiko, «Sizing of soft spheroidal particles by multiangle scattered light intensity data: application of neural network», J. Quant. Spectr. Rad. Transfer. **89**, 279–289 (2004).

[3] V.V. Berdnik, V.A. Loiko, «Sizing of spheroidal and cylindrical particles in a binary mixture by measurement scattered light intensity data: application of neural network», J. Quant. Spectr. Rad. Transfer., **91**, 1–10 (2005).

Poster Session

PHOTODYNAMIC EFFECT ON LEUKEMIC CELLS IN VITRO

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This study was designed to investigate the effect of PDT by using photophorin (10 – 4 mMol/ml) and Methylene Blue (10 – 4 mMol/ml) with He:Ne laser (632.8) and diode laser (650) respectively on blood mononuclear and leukemic cells (acute lymphatic and myeloid leukemia). Cells were incubated with each photosensitizer for 1 hour and then received exposure (35 J/cm^2) from the proper laser source. Viability assessment was carried out before, immediately, 24 hour and 48 hours post irradiation. Determination of the resultant viability was carried out using light, fluorescent microscopes together with flow cytometry. After 48 hours viability assessment of PDT effect of Methylene Blue on leukemic cells was 8.8 %, 29.38% and 25...29 % for light microscope, fluorescent microscope and flow cytometry. Meanwhile, viability assessment of PDT effect of Photophorin on leukemic cells was 9.27%, 8.8% and 9.4 % for light microscope, fluorescent microscope and flow cytometry. This showed that PDT efficacy was maximum after 48 hours. Investigations with flow cytometry revealed that

both photosensitisers induced apoptosis rather than necrosis in both types of PDT protocols 48 hours post irradiation and provided higher yield of results than did light and fluorescent microscopy.

LIPOSOMES SIZE MEASUREMENT USING ANGULAR LIGHT SCATTERING

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Determinations of liposome size in colloidal suspension are obtained using Angular Light Scattering. The determination of particle-sizes in colloidal suspension by this technique presents a lot of advantages, i.e.: the relative simplicity of the experimental installations, few amounts of particles (<2mg/cm^3) and its non-destructive character. It makes the method very attractive for pharmaceutical and biotechnological industries applications.

Liposomes are close membranes capsules consisting of single or multiple bilayers of phospholipids, used extensively by biotechnologists to encapsulate proteins. The method used for liposome size determinations in colloidal suspension is based on the measurement of the light scattered intensity for several angles when the sample is illuminated by a beam of polarized monochromatic light. [1, 2] The intensity of the scattered light is related with

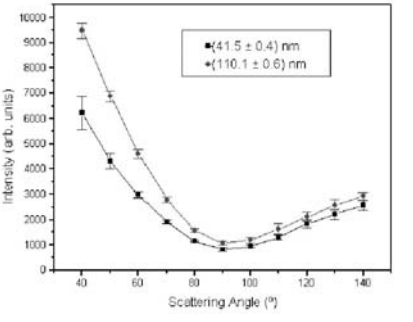

Fig. 1. Liposomes scattering measurements

the liposome radius by Mie Theory [3, 4] using a hollow sphere model. For liposome suspended in water, the intensity of the scattered light measured in each angle θ is the superposition of the scattered intensity for each particle. (Fig. 1). In consequence, the intensity of the light scattered is related with a particle-size distribution function, by a first kind Fredholm equation. Solving a non-linear system of equations, assuming a Dirac delta distribution of particles in the sample, we calculate the particles radius from the measurements of the scattered light by the particles in the sample.

References

[1] J. H. van Zanten, and H. G. Monbouquette, "Characterization of Vesicles using Classical Light Scattering", Journal of Colloid and Interface Science, **146**, 330–336 (1991).

[2] L.B. Scaffardi, J.O. Tocho, L.L. Yebrin and C. Santos Cantera: Optics Engineering Vol. 35 (1996), p. 52.

[3] C. F. Bohren and D. R. Huffman, *Absorption and Scattering of Light by Small Particles* (Wiley, New York, 1983).

[4] H.C. van de Hulst, *Light Scattering by Small Particles* (Dover, New York, 1981).

LIGHT SCATTERING DATABASE INCLUDING MEASUREMENTS OF PHYTOPLANKTON

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Light scattering measurements of biological particles such as phytoplankton are scarce. Yet measured data is important to guide the development of computational methods and for use in applications such as models describing radiative transfer in water.

We will make our light scattering measurements of phytoplankton and silt in water [1] available on the Amsterdam Light Scattering Database (http://www.astro.uva.nl/scatter/) [2] to make these data more accessible to the community. The laboratory measurements pertain to scattering properties of 15 different types of coastal and inland water phytoplankton species and two types of estuarine sediments. We include in the database the scattering function as well as the angular distribution of a ratio of scattering matrix elements, which in practice equals the degree of linear polarization of the scattered light if the incident light is unpolarized. Laser light with a wavelength of 632.8 nm was used, and a scattering angle range from 20 to 160 degrees was covered.

The morphology and the structural features of the particles studied play an important and complex role in their light-scattering behavior, posing an interesting challenge to modelers of light scattering by biological particles. We hope that the easy availability on the World Wide Web of these data will trigger further use e.g. in the context of water-quality studies and to test results of theoretical models.

References

[1] H. Volten, J.F. De Haan, J.W. Hovenier, W. Vassen, R. Schreurs, A. Dekker, H.J. Hoogenboom, F. Charlton, R. Wouts, "Laboratory measurements of angular distributions of light scattered by phytoplankton and silt", Limnology and Oceanography, **43**, 1180–1197 (1998)

[2] H. Volten, O. Muñoz, J.W. Hovenier, J.F. de Haan, W. Vassen, W.J. van der Zande, L.B.F.M. Waters, "WWW scattering matrix database for small mineral particles at 441.6 and 632.8 nm", Journal of Spectroscopy and Radiative Transfer, **90**, 191–206 (2005)

LIGHT SCATTERING EFFECTS ON BOTH AQUATIC ORGANISMS ENZYMES AND ENZYMATIC REACTIONS MADE BY PURE ENZYMES

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UVB and UVA activate the enzymatic reactions following a mechanism related to the catalytic action of UV radiations on some chemical reactions. The energetic content of certain chemical links is in close connection with the potential energy delivered by the solar radiation (visible and UV). The presence of such chemical links in a chemical grouping of an enzymatic substrate determines the instability of the group as a result of sun exposure. The enzymatic reactions are inactivated by radiations with different waveengths, causing disastrous effects on living organisms [1].

We found out that ultraviolet B radiations increase the activity of the pure hydrolase (α amylase, E.C.3.2.1.1.) and Merck peroxidase (E.C.1.11.1.7.) by means of free radicals generated from synthetic polymers walls of the experimental tubes. The activation is higher in UVB than in UVA. UVB and UVA increase the intensity determined on nude alga *Tetraselmis suecica* [2], on exponential phase of cultivated bacteria *Escherichia coli* O_{157}, *Acinetobacter calcoaceticus*, and after the short time of exposure in the thermo-stated conditions, the cell structure destruction by means of free radicals of activated hydrolases.

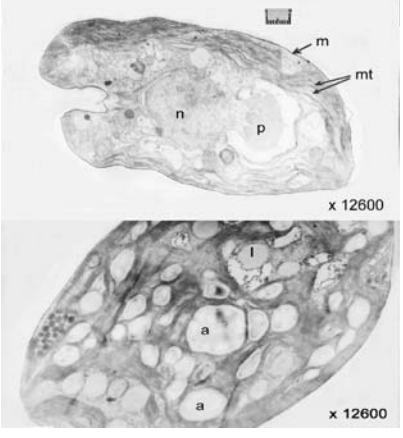

Fig. 1. Nude alga *Tetraselmis suecica* (upper – the normal aspect; below – the sun amplified light post-irradiation aspect) (original)

The nude alga *Tetraselmis suecica* is more resistant and store starch and lipids (Fig. 1.). It has the skill to convert the energy of radiations in the chemical energy of synthesis products. Some individuals of *Tetraselmis suecica* become encysted by creating a thick inhomo-

geneous sub-silique, under which a new silique appears. Other cells increase their glucide (intra-plastids starch granules) and lipid reserves of provisions (oleosoma appear in the central part of the cell and affect the tillacoide lamellar structure; plasto-globules appear as well). Tetraselmis suecica, which lives in the surface region of the sea's waters, has a grown resistance on 10 times amplified natural solar radiation action. After 10 minutes of exposure, it succeeds in removing the energy of the absorbed radiation into the energy of the provisions products, which are stored as starch and lipid granules.

Cultivated bacteria on poor specific media have a small development in 300–800 nm. If the bacteria are cultivated on reach media, which absorb UV (Martin medium) they are developed by n^3 rule, instead n2 in the first stage, after irradiations of bacteria culture bottle (transmittance 235–800 nm).

References

[1] S.C.O.P.E., 2002a, "Effects of Increased Ultraviolet Radiation on Biological Systems – report", UNEP, Budapest, Hungary, p. 1–40.

[2] NECHIFOR, M. and MESTER, R., 2004, "Efectul radicalilor liberi asupra activitatii enzimelor sistemului antioxidant", Buletinul SNBC, Bucharest, Romania, p. 170.

RADIATION TRANSFER IN STRATIFIED BIOLOGICAL OBJECTS

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We develop a package of equipment and software for studying radiation propagation in the stratified biological media. The hardware includes a de-

vice, allowing to measure angular and spectral dependences of scattered radiation in an interval of waveslengths 0.4–0.7 microns.

The program part is based on using a stratified media model. The model of the stratified media is widely applied to the studying of biological objects. For example, to study of a sunlight interaction with leaves of plants one applies a two-layer model of the disperse media [1]. For the analysis of interaction of laser radiation with a coverlet one use multilayered models. For modeling propagation of radiation in the stratified media we use the radiative transfer theory, accounting for Fresnel reflections on boundaries and the dependence of characteristics of elementary volume on the direction of radiation propagation. The structural model of the multicomponent media includes scattering by several types of particles and absorption by several types of pigments, such as chlorophylls and carotinoids. For the description of characteristics of an elementary volume strict methods for calculation of single scattering characteristics by polydisperse homogeneous and multilayered particles and the approached methods for calculation of scattering characteristics by non spherical particles are used.

The method of the numerical decision of the radiative transfer equation in the stratified media, based on techniques of layers addition and a spline – approximation of phase functions is developed [2], [3]. The method reducing the time of calculation of scattered radiation intensity in media with strongly anisotropic scattering characteristic for biological objects. It is especially important for training the neural networks intended for restoration of characteristics of biological objects by scattered radiation. The spectral dependences for reflection and transmission coefficient are calculated in visible ranges as functions of the leaf structure.

References

[1] V.V. Berdnik, R.D. Mukhamed'yarov. Radiation transfer in plant leaves. Optics and Spectroscopy. **90**, 580 (2001).

[2] V.V. Berdnik, V.A. Loiko. Radiative transfer in a layer with oriented spheroidal paricles. J. Quant. Spectrosc. Radiat. Transfer. **63**, 369 (1999).

[3] V.V. Berdnik, V.A. Loiko. Modelling of radiative transfer in disperse layers of medium with a highly stretched phase function. J. Quant. Spectrosc. Radiat. Transfer. **61**, 49 (1999).

INTENSITY BACKREFLECTANCE FROM THE BLOOD PLEXUS IN THE SKIN UNDER THE LOW-POWER LASER HEATING

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The absorption coefficients μ_a of the overwhelming majority of biological tissues are significant in the visible region. For example, for human blood (Hb) (λ = 633 nm) μ_a = 4.87 cm^{-1}, 2.7 cm^{-1} for the dermis and 3.6 cm^{-1} for intima. Therefore we can suppose that the absorbed laser radiation can produce local small heating in the zone of irradiation by the low-power laser radiation and consequently change its characteristics.

Recently in [1] was shown that low-power laser irradiation of the blood in absorption range of haemoglobin leads to fragmentation of erythrocyte aggregates in the blood *in vitro*. It would be interesting to analyse this effect carefully and *in vivo* conditions.

Generally backscattered radiation is a complex phenomenon which depends on the hematocrit, volume of identical scatterers, structure factor, the characteristic spatial organisation of the scatterers and backscattering cross-section of a single scatterer [2]. The intensity of light backscattered when low-power laser radiation is incident on the skin was investigated.

The effect of exposure time on skin temperature inside and outside of, the irradiated region of a He-Ne laser (wavelength 632.8 nm, power 1.1 mW) was studied experimentally. The small changes in temperature expected from the exposure to low-power laser radiation it was measured during the laser irradiation by a thermal detector (Thermilinear@ Temperature probes, YSI-700 Series, YSI Incorporated, Ohio, USA) with a resolution of 0.0016 °C.

The statistical analysis of the results obtained shows that the character/behaviour of the intensity of backscattered radiation Rt is different for the patients, but the value of R_t increased during the irradiation time.

According to the earlier-proposed optical model of erythrocyte aggregation [3] the scattering properties of erythrocytes and its aggregates were approximated by the properties of spheres of equivalent volume. The shape of the isolated erythrocytes was approximated by a circular cylinder with a concave base. It was considered a model of formation of aggregates in the form of rouleaux.

The dependences of the back-scattered intensity from the degree of aggregation for the different erythrocyte refractive index, the refractive index of plasma and attenuation coefficient of the erythrocytes was studied. The theoretical calculation using the existing optical model of erythrocyte aggregation has suggest that the fragmentation of erythrocyte aggregates is the most probable mechanism leading to the enhanced backscattering.

References

[1] A.N. Korolevich, L.G. Astafueva., N.S. Dubina, S.I. Vecherinsky, A. Bernjak, M. Belsley and A. Stefanovska. Fragmentation of erythrocyte aggregates by low intensity laser heating. J. Opt. A: Pure Appl. Opt. **7** S308–S317 (2005).

[2] V. Twersky. Low-frequency scattering by correlated distributions of randomly oriented particles. J. Acoust. Soc. Am. **81**, 1609–1618 (1987).

[3] E.K. Naumenko. Influence of erythrocyte aggregation on the scattering properties of blood. Journal of Applied spectroscopy, **70**, 419–426 (2003).

DEVELOPMENT OF FRAP METHOD WITH DECAYING PHOTOBLEACHING INTENSITY

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Fluorescence recovery after photobleaching (FRAP) is widely used for studying the lateral organization and diffusion of fluorescent probes or species with native fluorescence in the numerous membrane structures. The classical method, first introduced in 1974, also called the bleach-and-probe, is based on observations of kinetics of recovery of integral fluorescence collected from the small spot where previously the concentration of fluorescent probes has been partially bleached with a short but intensive laser pulse.

We present further development of this method. The idea is to use laser irradiation of greater intensity then merely exciting but decaying with time. The normalized kinetics of photobleaching (defined as a ratio of observed fluorescence to intensity of laser irradiation) decays in the beginning. Then it turns into kinetics of recovery with decreasing of laser irradiation. It leads to the existence of the minimum of the kinetics while the classical approach does not. Fig.1 demonstrates the experimental curves where the solution of fluorescein in the glycerol has been used as a sample.

The method has been evaluated for the case of classical Smoluchowsky equation of diffusion, Gaussian lineshape of laser beam and hyperbolic decay for laser irradiation. The analytical solution in the approximation of weak photobleaching has been found. The accuracy of this approximation has been analyzed by a comparison with solutions computed with finite differentiates method.

The setup was constructed on the base of a fluorescence microscope and argon laser. The laser irradiation intensity was controlled by an electro-optical modulator. The experiments have been performed on the aqueous glycerol solutions and lipid multibilayers.

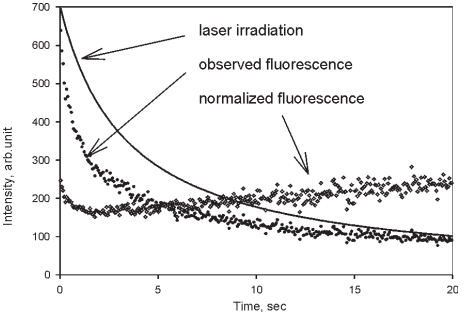

Fig. 1. Measurement of diffusion of fluorescein in the glycerol with modified FRAP method

In summary, this modification has advantages in comparison with the classical one. It leads both to a better signal-to-noise ration and experimental curve. All this should improve studying the lateral diffusion of fluorescence probes in membranes, especially looking forward to the analysis of the multicomponent and anomalous diffusion in the native membrane structures.

VISUALIZATION OF TISSUE STRUCTURES USING POLARIZATION DEGREE PATTERNS AND CORRELATION ANALYSIS

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Mapping the degree of polarization can provide valuable diagnostic information about the superficial and subsurface structures of the skin and other tissues [1]. It can be used to extract tissue characteristics that one can not obtain by conventional visual or photographic methods [2]. These studies show the potentials of polarized light diagnostic to reveal the hidden structure of earlier fibrosis, which was developed in the skin of athymic nude mice after a single dose of X-ray irradiation as well as to enhance an internal structure of the teeth. For polarization degree mapping the sample was illuminated with linearly polarized probe light and two digital photographs were taken through an analyzer oriented parallel or perpendicular to the polarization vector of illuminating beam. Data processing of the raw spatial distributions of the degree of polarization based with Fourier filtering of the high frequency noise improved subjective perception of the revealed structure in the images. The

new approach of Pearson correlation analysis is developed to visualize the regions with statistical similarities of the polarization degree image. It allowed estimation of their size and directionality as well as filtering the image from the high spatial frequency noise. Method and apparatus of polarization degree imaging has potential use for histology and morphology to nondestructively visualize the internal microstructure of the thick tissue probes or objects covered with scattering envelope.

References

[1] S.L. Jacques, J.C. Ramella-Roman, K. Lee "Imaging skin pathology with polarized light", *J. Biomed. Opt.*, 7(3), 329–340, (2002).

[2] A.P. Sviridov, V. Chernomordik, M. Hassan, A. Russo, P. Smith, A. Gandjbakhche, "Enhancement of early skin fibrosis structure from polarized patterns" in *OSA Biomedical Topical Meetings*, Miami, USA, ThD6, (2004).

TRANSMISSION OF POLARIZED LIGHT IN SCATTERING MEDIA WITH LARGE-SCALE INHOMOGENEITIES

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The report is devoted to a novel approximate method for solving the vector radiative transfer equation in media with large-scale (more than a wavelength) scattering inhomogeneities.

Although considerable attention has been focused on the study of multiple light scattering, explaining polarization effects in turbid media with various inhomogeneities is still a topical problem. It has been found experimentally, that the depolarization lengths for linearly and circularly polarized beams depend strongly on the inhomogeneity sizes [1]. The theoretical

explanation of this effect [2] offers a possibility to develop an approximate analytical method for solving the transfer equation.

Two different mechanisms of depolarization – the «geometric» mechanism and the «dynamic» one-can be recognized in the case of light scattering by large-scale inhomogeneities. The «geometric» depolarization is due to Rytov's rotation of the polarization plane along each random ray. The «dynamic» depolarization is due to the difference in the phases of two cross-polarized scattered waves (only the «dynamic» process occurs in scattering of a circularly polarized beam). The difference between the rates of the «geometric» and the «dynamic» processes is the key point for decoupling the vector radiative transfer equation.

The representation for the Stokes vector that was first proposed by Kuscher and Ribaric [3] is used. Our approach is based on the assumption that single scattering of light by large-scale inhomogeneities occurs through small angles and the off-diagonal elements of the scattering matrix are small as compared with the phase function. This allows us to decouple approximately the vector radiative transfer equation. In the leading approximation the system of coupled equations for the Stokes vector falls into three independent equations for the basic modes, namely, for the intensity, for the basic mode of linear polarization (a certain combination of the second and the third Stokes parameters) and for the circularly polarized mode (the fourth Stokes parameter). All these equations are similar to the scalar transfer equation and differ only in the effective extinction coefficients. In the succeeding approximation the interaction between the basic modes must be taken into account, and we arrive at the equations for additional modes («overtones»). In these equations the basic modes appear as sources.

We discuss analytical calculations of the Stokes parameters in the asymptotic state. Final expressions are given in an explicit form. The results of numerical calculations of the material coefficients (depolarization lengths, etc.) with the use of the Mie theory and experimental data for the scattering matrix are presented. We show that the angular dependence of the basic linearly polarized mode is always sharply anisotropic. Only the waves that undergo deflections through relatively small angles remain polarized. The calculations of the polarization degree of multiply scattered circularly and linearly polarized light are compared with experimental data [1, 4].

References

[1] D. Bicout, C. Brosseau, A.S. Martinez, J.M. Schmitt, Phys.Rev. **E49** (1994) 1767.

[2] E.E. Gorodnichev, A.I. Kuzovlev, D.B. Rogozkin, JETP Lett. **68** (1998) 22; Laser Phys. **9** (1999) 1210; IRS 2000: Current Problems in Atmospheric Radiation, W.L. Smith and Yu.M. Timofeev (Eds.). A.

Deepak Publishing. Hampton, Virginia, pp.287-290; SPIE Proceedings, **5829**, (2005) 87.
[3] I. Kuscer, M. Ribaric, Optica Acta, **6**, (1959) 42.
[4] V. Sankaran, M.J. Everett, D.J. Maitland, J.T. Walsh, Opt. Lett., **24** (1999) 1044.

CONFORMATION TRANSITIONS OF THE BLOOD PROTEINS UNDER INFLUENCE OF PHYSICAL FACTORS ON MICROWAVE DIELECTRIC METHOD

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In modern biophysics the performance of research on the influence of such physical factors as ionizing radiation and temperature on blood proteins conformation is an extremely important and urgent problem because of the use of gamma-irradiation at high and low temperatures in various areas of medicine and biology. Such studies will enable us to expand our understanding of the processes that occur and the influence of these physical factors on bio-objects. It will allow us to find out the optimal technologies of sterilization and preservation of bio-preparations derived from human donor and cord blood with the maximum activity storage. Such technologies will enable us to create strategic reserves of preparations and to use them for clinical purposes.

The ability to undergo conformation transitions and the opportunity to change a three dimensional structure is an important property of macromolecules that define their functionality. A structural state of macromolecules and their hydration extensively determine the function activity of protein and can change under the effect of the various physical factors. There-

fore the purpose of this work was the examination of the influence of gamma-irradiation and temperature on conformation, hydration and dielectric properties of blood proteins. The aqueous solutions of bovine serum albumin and human fibrinogen have been studied. The samples were gamma-irradiated by a ^{60}Co source. The native samples were taken as a control. In examination the high sensitive experimental method of a resonator microwave dielectrometry [1] has been used. The calculation of protein hydration has been carried out by the method of the disperse system dielectric permittivity theory.

The real ε' and imaginary ε'' parts of the complex dielectric permittivity $\varepsilon^* = \varepsilon' - i\varepsilon''$ of the serum albumin and fibrinogen in aqueous solutions at various concentrations irradiated with doses 5–200 Gy were measured by the microwave dielectric method at a frequency of 9.2 GHz within the temperature range of 4–70 °C. The corrections based on measurements of conductivity contribution were made for inorganic ions presence. It has been determined that the character of the thermal conformation changes of albumin and fibrinogen and also the water molecule dielectric relaxation time in solutions depend on protein concentration and radiation dose. It was revealed that at the temperatures 30–34 °C and 44–47 °C for serum albumin and at the temperatures 24 °C and 32 °C for fibrinogen there is the increasing of the protein hydration which can be caused by conformation transitions of the macromolecules.

The investigation of dielectric properties of gamma-irradiated fibrinogen solutions show that at the range of doses 5–200 Gy the value of ε' varies in limits 1 % from quantity of ε' of the control sample. The dielectric permittivity of ε' with a radiation dose rising was reduced. However in the range of doses 30–60 Gy the nonmonotone magnification of ε' was observed that can be caused by conformation transition of macromolecules of fibrinogen.

References

[1] E.V. Hackl, S.V. Gatash, O.T. Nikolov, «Using UHF-dielectrometry to study protein structural transitions», J Biochem Biophys Methods. May 30, **63(2)**, 137–148 (2005).

MORPHOMETRIC LYMPHOCYTE MODEL IN APPLICATION TO SCANNING FLOW CYTOMETRY

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Typically, flow cytometers analyse cells by using fluorescent light and light scattered in forward-and side directions. The role of the scattering-channel is enhanced in scanning flow cytometry. It is achieved by measuring the angular structure of the scattered light in a wide interval of scattering angles. The angular pattern of light scattered by a single particle depends on its refractive index distribution, morphology, and size.

The problem of retrieval of particle parameters (in particular, the problem of cell recognition) by measuring scattered light is the inverse problem of scattering theory. The solution of such a problem for heterogeneous particles, e.g. for white blood cells, is rather complex especially when one needs to obtain results in the on-line mode with reference to scanning flow cytometry. The more precise data on the refractive index distribution, size, and morphology parameters we have the more reliable will be the result of the cell recognition by means of the angular structure of the scattered light. Therefore we need a cell model including detailed information on the shape, size, and refractive index of the cell. The model for the ensemble of cells has to include the size distribution function as well.

Different models are used to calculate light-scattering of nucleated biological cells. The models gradually change from the simple ones to more complex ones taking into consideration some fine features of cell morphology [1, 2]. Cell geometry significantly impacts scattering properties, emphasizing the need for careful consideration of the appropriate cell model [1]. Meanwhile, morphometric parameters of lymphocytes are not adequately explored. Indeed, there are data on leukocyte sizes in smears, i.e. under conditions determining distortion of the sizes of cells. This circumstance is important in the framework of solving of the inverse scattering problem rather than in other applications of smears. There are also data of the Coulter counters, in which the cells under investigation are in suspen-

sion. The signal of such counters does not depend directly on the cell size, however. Moreover, some fine features of lymphocyte morphology potentially important in respect of light scattering, are absent in the available literature data.

In this work the morphometric characteristics of leukocytes of healthy individuals have been investigated. The cells were separated by gradient density technique. The viability of selected cells was 95–100%. The morphometric parameters of lymphocytes in suspension are measured by methods of the light microscopy using microscope Leica DMLB2 and microscope-mounted digital camera DC 150 with 5 MPixels resolution. From the obtained data, the cell model is constructed. The model takes into consideration the cell nucleus eccentricity.

References

[1] R. Drezec, A. Dunn, and R. Richards-Kortum, «A pulsed finit-difference time-domane (FDTD) method for calculating light scattering from biological cells over broad wavelength ranges», Optics Express **6**, 147–157 (2000).

[2] G. Videen, D.R. Prabhu, M. Davies, F. Gonzales, and F. Moreno, «Light scattering fluctuations of a soft spherical particle containing an inclusion», Applied Optics **40**, 4054–4057 (2001).

TOTAL INTERNAL REFLECTION ELLIPSOMETRY AND SURFACE PLASMON RESONANCE TECHNIQUE IN DETECTION OF LOW MOLECULAR WEIGHT TOXINS

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Earlier [1–2] we developed an optical instrumental analytical approach based on porous silicon photoluminescence and intended for the express determination of such low weight substances as simasine, 2,4-dichlorophenoxyacetic acid (2,4-D) and nonylphenol. It provided a very simple analysis but its sensitivity was not sufficient for practice demands. Now we try to use other optical approaches (surface plasmon resonance – SPR and total internal reflection ellipsometry – TIRE) for the direct determination of simasine and mycotoxin T-2. SPR is well know approach and TIRE (also called plasmon enhanced ellipsometry) combines the advantages of the spectroscopic ellipsometry and the Kretchmann type SPR geometry of total internal reflection recently proposed. The TIRE experimental set-up was built on the base of a commercial M2000, J.A.Woollam Co, spectroscopic rotating analyzer instrument, operating in the 370–1000 nm wavelength range. The measurements were performed *in-situ* in a specially designed 1.5 cm^3 cell attached to the bottom of a 68° trapezoidal glass prism (BK7, n =1.515) with the gold coated glass slide attached to it via index matching fluid. The cell has inlet and outlet tubes allowing the injection of different aqueous solutions (n = 1.33) into the cell. The cell was sealed against the sample through a rubber O-ring. The immune assay technique was exploited for *in-situ* registration of the above low molecular weight toxins with specific antibodies immobilised onto the gold surface via (poly) allylamine hydrochloride layer using electrostatic self-assembly technique. It was shown that the method of TIRE was employed and proved to be more accurate and sensitive when compared to SPR. TIRE method is therefore found to be more suitable for the registration of low molecular weight toxins, such as simazine, atrazine and T2 mycotoxin. TIRE measurements were repeated many times on a number of samples with polyclonal and monoclonal antibodies, and different concentrations of T2 in the range of

concentrations from 1.5 to 1500 ng/ml. The thickness of layers following the final step of adsorption sequence, i.e., the change in thickness due to binding of toxins, the thickness of T2 layer of 8.686 nm is found to be larger than those for atrazine (4.711 nm) and simazine (5.774 nm), which is consistent with the molecular weight (size) of the above molecules.

References

[1] N.F. Starodub, V.M. Starodub Biosensors based on photoluminescence of porous silicon. Overall characteristics and application for environmental monitoring. J. Sensor Electronics and Microsystem Technol., 2, 63–83 (2004).

[2] N.F. Starodub, V.M. Starodub Biosensors based on photoluminescence of porous silicon. Application for environmental monitoring. J. Sensor Electronics and Microsystem Technol., 1, 63–71 (2005).

APPLICATION OF OLYGOMERES AT THE ANALYSIS OF BIOLOGICAL MOLECULES BY BIOSENSORS

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The problem of achievement of high sensitivity, selectivity and standardization of responses in the development of biosensors is very important. The main aspects of this problem may be solved by use of olygomeres. These may provide high-density immobilization of biological molecules on a surface, their structuring and orientation of recognizing sites toward solution as well as standardization of transducer surface and prevention of degradation of sensitive material. In this report, data about the use of some olygomers and specific receptors for development of biosensors are discussed.

Surfaces from Au and Si_3N_4 were used at the development of optical biosensors, in particular, based on the effects of surface plasmon resonance (SPR) and planar polarization interferometry (PPI). Polyalylamine hydrochloride, polystyren sulphonate as self-assembled polyelectrolites (PESA), Nafion, dodecanthiol (DDCTh), lectins (from *Phaseolus vulgaris*, *Solanum tuberosum*, *Helix pomatia*, *Tuberosum vulgaris*) and staphylococcal protein A were used for the formation of intermediate layers between transducer surfaces and selective biological materials. Antibodies (Ab) to human IgG, human myoglobin as well as retroviral proteins (p24 and jp51), and some enzymes were chosen as selective biological materials in the development of biosensors intended for express diagnostics of immune deficiency, ishemia of human and bovine leucosis.

It was stated that the presence of PESA layer on both types of transducers provides higher (approximately 10 times) levels of specific signal of immune biosensors in comparison with those cases when their surfaces were not treated (bare Au) or treated by GA (Si_3N_4), or even treated by DDCTh (Au). This was achieved due to the increase of the density of recognizing molecules on the transducer surface. Protein A and lectins allow antibodies to structure and orient their recognizing sites toward solution. It is possible in result of the presence of specific site for binding of protein A and some carbohydrates in second constant domain of IgG. It was revealed that pig IgG has the greatest affinity to the lectin of *Tuberosum vulgaris* and mouse Ab – to the lectin of *Helix pomatia*. The effective thickness of a layer of immobilized immune components increases in series: bare surface, treated one by PESA, surface contained PESA with some lectins and last surface contained PESA with protein A. In particular, the higher layer is observed in case of the formation of immune complex of IgG-anti-IgG. It is the result of the presence of a great number of antigenic determinants on the IgG surface. The thickness of anti-IgG-IgG layer is near to that observed at the binding of IgG-anti-IgG if protein A or lectins were introduced into PESA. It testifies that $F(ab)_2$ fragments in this case are oriented towards solution. It was demonstrated the selective immobilisation of the glycolysated protein of jp51 from the mixture of retroviral proteins. It allows us to discriminate animals, which are ill or were preliminary immunized by vaccines contained protein of p24. Both PESA and DDCTh give possibility to standardize state of transducer surface. At the same time covering of selective layer by Nafion prevents spontaneous and enzymatic degradation of biological molecules as well as leads to increase sensitivity of some enzymatic biosensor.

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