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CONTENTS

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National Centre for Information and Documentation

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NATIONAL SCIENTIFIC PROGRAMMES WITH EUROPEAN DIMENSIONS

PROGNOSTIC MODELS FOR ASSESSMENT OF RISK OF CENTRAL SLEEP APNOEA IN PATIENTS WITH CHRONIC HEART FAILURE. EFFECTS FROM THE NON-INVASIVE VENTILATION

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Abstract

Central sleep apnoea - Cheyne-Stokes respiration (CSA-CSR) is among the most important factors responsible for the high morbidity and preterm mortality of patients with chronic heart failure (CHF). It is present in 30-50% of CHF patients. Despite its significant contribution to the prognosis of CHF and its high frequency, few large scale investigations on its prediction and early diagnosis are present. The main reason for its underdiagnosing is the fact that its diagnosis needs expensive and labour-consuming polysomnographic investigation. The aim of the project is to further investigate some recently reported parameters, informative of CSA risk in CHF, as well as to look for new parameters with predictive capabilities, based on the up-to-date knowledge on CSA pathogenesis.

The following parameters of the cardiorespiratory system will be investigated following scrutinized analysis of its functional profile:

- *Characteristics of tidal volume dynamics (cyclic pattern), exhaled CO_2, ventilatory equivalents at rest, during exercise and sleep.*
- *Gas exchange response - oxygen uptake, exhaled CO_2, ventilation and ventilatory equivalents on different stages of exercise testing (onset, anaerobic threshold, recovery).*
- *Changes in heart rate and the equilibrium sympathetic-parasympathetic regulation of heart rate; chronotropic incompetence and heart rate variability analysis.*

Basic parameters from the mentioned investigations and their derivatives will be juxtaposed to chemodynamics condition (ejection fraction, functional capacity); sleep architecture (non-REM and REM), apnoea-hypopnoea index and desaturation characteristics (amplitude, duration, specific characteristics), quality of life, etc. Grounded on the acquired results, an integrative system (panel of parameters) for CSA risk prediction in CHF will be proposed. This panel will be followed-up after therapy with positive airway pressure and oxygen therapy, if needed. The expected results would contribute to the early diagnostics and purposeful treatment of CSA-CSR in CHF.

INTRODUCTION

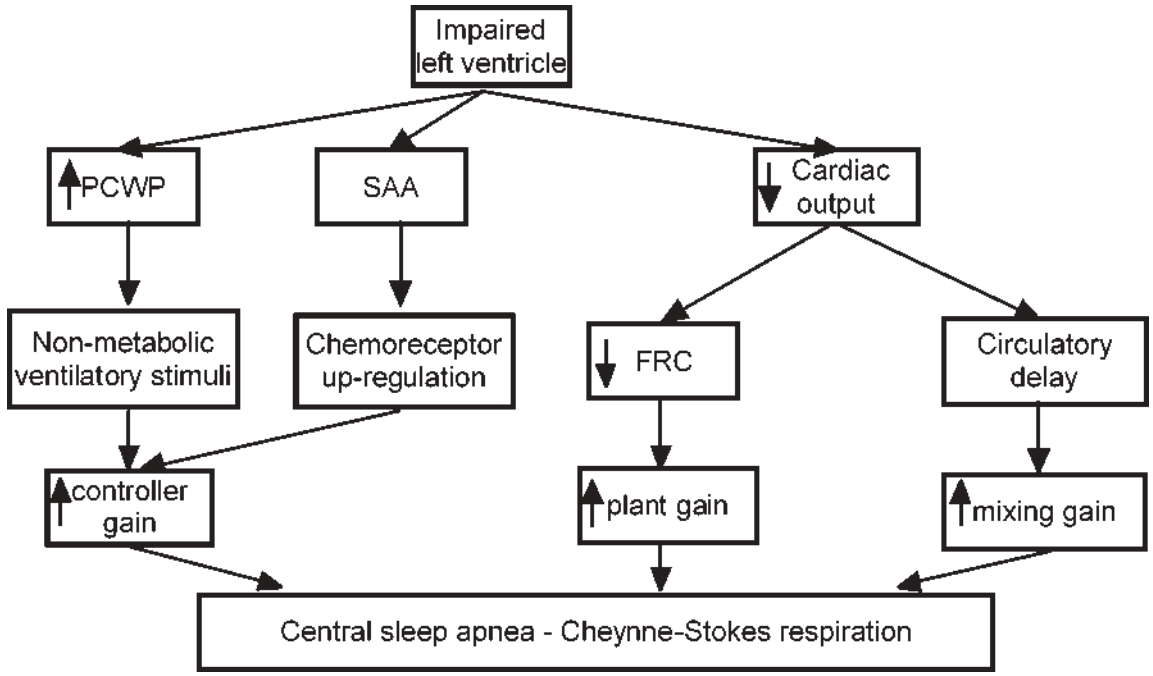
The problem with the early functional diagnostics of central sleep apnea (CSA) in patients with chronic heart failure (CHF) has been a matter of extreme interest worldwide since the turn of the century. The bulk of scientific publications on this problem has been published in the last 5 years. A considerable part of the authors pointed out the significance of the problem and the necessity for additional research on the topic and particularly on the construction of predictive models for CSA. Currently, leading teams have

been involved with achieving this goal. No such research has been done in Bulgaria so far, which puts the scientific community in the country in an unfavorable position as compared to its European and global counterparts.

Cheyne-Stokes respiration is a type of periodic breathing defined, according to the American Academy of Sleep Medicine criteria, as: at least 3 consecutive cycles of crescendo-decrescendo breathing with cycle length approximately 60 seconds and at least one of the following: 5 or more central apneas or hypopneas per hour of sleep or duration of the cycles at least 10 minutes.[1]

CSA-CSR is a sign of respiratory instability, particularly characteristic during sleep, when respiratory control is predominantly metabolic, based on a negative feedback mechanism, involving PaO_2 and $PaCO_2$.[2]

The pathophysiology of CSA-CSR in CHF is presented on Fig. 1.



PCWP - pulmocapillary wedge pressure; SAA - sympathetic-adrenal activation; FRC - functional residual capacity

Fig. 1. Pathogenesis of CSA-CSR in CHF (based on Naughton[2])

Significance of the central sleep apnea – Cheyne–Stokes respiration in chronic heart failure

CSA-CSR is the most common type of CSA.[2] Its clinical significance is determined by its frequency, the lack of routine screening and/or easily available diagnostic method and the negative impact on morbidity and mortality in CHF patients.

Frequency of CSA–CSR

Sleep disordered breathing in CHF is quite common: CSA-CSR is present in 30-50% of the patients, depending on the definition used, and obstructive sleep apnea (OSA) - in 20-40%.[2] CSA frequency in CHF varies with CHF severity.[3]

Significance of CSA–CSR for the development and prognosis of CHF

Negative impact of CSA-CSR on CHF is supported by multiple studies showing increased morbidity and mortality in CHF with CSA, compared to CHF without CSA, given that CHF is of comparable severity. [4,5,6] In a study on 353 patients the CHF+CSA showed significantly lower survival in comparison to CHF without sleep apnea and CSA/OSA without CHF (2.5 vs 4.5 vs 6.5 years).[4] These results are in concordance with the opinion of Somers et al stating that cardiovascular diseases and CSA are potentiating each other.[7] Sleep apnea is a known risk factor for

arterial hypertension and increased morbidity, and probably for increased cardiovascular disease mortality. [8,9] This fact implicates its treatment even in mild cases, aiming to avoid the development of the stated concomitant diseases.[8,9] Ancoli-Israel et al give the same recommendation, pointing out elderly CHF patients as a risk group for SA. [4]

Pathophysiological mechanisms linking CSA with worse prognosis in CHF include: hypoxemia, sympathetic-adrenal activation and sleep fragmentation with frequent arousals.[4] Sympathetic-adrenal activation during sleep decreases cardiomyocyte life, has arhythmogenic effect and worsens ventricular energetics [6], while frequent desaturations and multiple arousals impose structural changes in the heart and left- and right- ventricular hypertrophy.[4] CSA-CSR causes impairment in CHF patients' physical capacity, which by confining their physical activity leads to further progression of the main condition. [3,10,11]

Irrespective of the mentioned facts, some authors propose the hypothesis that CSA-CSR in CHF is not solely pathophysiological, but also a compensatory-adaptive mechanism, bringing the following arguments: a) adaptation to the present hypoxemia; b) increase in the pulmonary oxygen storage by the gradual increase in the endexpiratory volume during the crescendo period of ventilation; c) under hypoxic conditions, muscle contractility is improved in the presence of alkalosis; d) the increased non-obstructive ventilatory effort during CSA increases cardiac stroke volume; d) 20-30 sec ventilatory pauses during CSA are sufficient to prevent fatigue in myopathic respiratory muscles [12].

Diagnostic problems in CSA-CSR

Methodological problems are mainly in the distinguishing between central and obstructive phenomena, based solely on the data from breathing and thoracic and abdominal channels, especially concerning clasification of hypopneas. The modern approach to a more precise diagnosis involves non-invasive measurement of the respiratory effort by pulse transit time measurement, instead of the gold standard - esophageal catheter.

Unlike the case in obstructive sleep apnea, "clinical profile" of patients with CSA is scarce. On the one hand, the characteristic loud snoring is absent, and on the other - subjective daytime sleepiness may be absent [13].

Against the backdrop of increased mortality and morbidity rate in patients with CHF+CA+CSR, it goes without saying that a timely diagnostics and therapy of patients with SDB is paramount. As of today, the golden standard for diagnosis of the above-mentioned conditions is laboratory polysomnography, which can determine the various SDB[14]. It is, though a very expensive, labor-consuming, highly specialized analysis, and besides that the laboratories at hand cannot handle the existing flow of patients with CHF at the registered frequency of 1-2% [11,15].

Therefore, a number of scientists declare the need of a screening method for prediction of CSA-CSR in CHF [4,11,14]. This is further justified by the fact that these patients show excellent therapeutic results to different modes of non-invasive ventilation with positive airway pressure [16].

Standard parameters used in the assessment of CHF severity (NYHA functional class and ejection fraction) and the quality of life in these patients (assessed by Minnesota Living With Heart Failure Questionnaire - MLHFQ), do not differ between CHF patients with or without SDB [10,11,13,14,17] and are not usable for a screening method.

In the ideal case, a predictive system for CSA-CSB would be based on parameters from tests, which are a part of the diagnostic approach to CHF, minimally involving additional financial or human resource. Such tests are cardiopulmonary exercise testing (CPET), heart rate variability (HRV) analysis, modern laboratory parameters - brain natriuretic peptide (BNP) and high sensitivity C-reactive protein (hsCRP).

At the moment, parameters that had proved their distinguishing capability (concerning the presence of CSA) are the cyclic pattern of breathing at rest [3,18] and in the first 4 minutes of veloergometry[3], the slope of the relationship between ventilation and carbon dioxide (VE/VCO_2 slope) during CPET[10,13], HRV[11,15], BNP[5,17]. Shortcomings of their single use to predict CSA-CSR are their insufficient specificity and sensitiv-

ity, especially in mild cases [11,17]. Contradictory results reported by Roche et al show extremely high sensitivity (92.9%), specificity (94.1%) and predictive power (93.3%) of periodic breathing pattern during the early part of CPET [3]. However, we think that presence or absence of variations in ventilation, oxygen uptake and CO_2 output, assessed visually is too subjective to serve as a widely used screening method.

We think that present data for the effect of CSA-CSB on the above-mentioned diagnostic parameters justify the search for a multicomponent screening system, based on these and other parameters (e.g. chronotropic parameters from CPET, hsCRP). The complex approach probably would increase specificity and sensitivity of the screening.

Therapeutic approaches for CSA–CSR

Therapy with applying positive air pressure in the airways (Continuous Positive Airway Pressure - CPAP) is the most profoundly explored therapeutic strategy for CSA-CSR in patients with CHF.[2] Mechanisms responsible for the effect of that treatment are yet to be fully clarified, but the following are assumed: 1) preventing the narrowing in the pharyngeal area during CSA, hence preventing hyperventilation, 2) improved oxygenation due to increased lung volume, 3) improved cardiac function as a result of decreased pre-load and after-load (as a consequence of intrathoracic pressure elevation and transmural pressure reduction), 4) decreased work of the respiratory muscles [2,19]. In a large study involving 258 subjects (CANPAP), followed up for a period of 2 years, the beneficial effect of the CPAP treatment has been proven, regarding ejection fraction (EF), physical capacity, quality of life and sympatic activity. Mortality, on the other hand, is comparable between the patients, receiving CPAP therapy and the control group [20]. A consecutive post-hoc analysis of the results of that study show a significant decrease in the mortality rate in the sub-group where treatment has resulted in lowering the AHI<15 and concludes that the good response to the CPAP therapy (AHI<15) is an indication for treatment of such patients [21].

There is, however, a part of the CSA-CSR patients, who do not respond so well to therapy and that imposes the search for other alternatives, for example, therapy with oxygen, with bi-level positive airway pressure (BiPAP), adaptive servo-ventilation (ASV). Comparative characteristics of the different therapeutic approaches are presented in Table 1.

Adequate management of CHF is also an important factor in treating CSA-CSR. It is related to improving circulatory time, as well as to controller gain and plant gain lowering, by means of decreased stimulation of the irritative and juxtacapillary receptors, improved perfusion of the respiratory muscles and the lungs, resulting in better ventilation/perfusion proportion.

SCIENTIFIC TASKS

In the current project we plan to explore an unsolved medical problem, connected with the early diagnostics of central sleep apnea in chronic heart failure. In conjunction with the implementation of this fundamental idea the project pursues the following specific scientific objectives:

Task 1. Defining the possibilities of various cardio-pulmonary and clinical and laboratory indicators for prediction of central sleep apnea - Cheyne-Stokes respiration in patients with chronic heart failure. For this purpose the following investigations will be carried out:

1. A panel of preliminary tests to determine study participation eligibility of each patient, according to the defined inclusion/exclusion criteria, shown on Fig. 2.

2. Full-night polysomnogrpahy for diagnosing the presence of CSA-CSR and dividing the patients into different groups.

3. Cardio-pulmonary exercise testing (CPET) with gas exchange analysis. Cycling patterns in tidal volume (V_T), ventilation (V_E), oxygen uptake (VO_2), carbon dioxide output (VCO_2) at rest and in the first 4 minutes of exercise will be assessed. Analysis of the ventilatory efficiency in the course of CPET - ventilatory equivalents for O_2 (VEVO2) and CO_2 (VEVCO2) at peak exercise and AT, slope of the relationship VE-VCO2 (VEVCO2 slope), oxygen uptake efficiency slope (OUES) will be performed. The chronotropic incompetence during exercise will be presented by

Table 1. Advantages, disadvantages and evidence for the influence of different methods for treatment of Cheyne-Stokes respiration in chronic heart failure on mortality, quality of life and other important parameters

Therapeutical approach	Advantages	Disadvantages	Effect on mortality and quality of life	Positive effects
CPAP	best known[2]	50-69% therapeutical response[20,22]; 2-4 weeks for assessment of the therapeutical response[22]	↑quality of life[20]; ↓mortality[21]	↓AHI[2,20,21,22]; ↑EF[20]; ↑functional capacity[20]; ↓SAA[20]
O_2	Effectiveness, compared with CPAP[20]	high oxygen concentration (>50%)[2]; negative effect on the cardiac output[2]; no scientific data on long term effects[2]	↑ quality of life [23]; ↓ hospitalizations[23]; ↓ need of IR admissions[23]; ↓ hospital stay[23]	↓AHI [23,24,25]; ↓desaturation index [23,24,25]; ↑oxygen saturation (sleep)[23,24,25]; ↓BNP[25]; ↑EF[25]; ↑functional capacity[25]; SAA - contradictory data[24,25]
BiPAP	↑ compliance	insufficient data; may provoke central apneas/hypopneas[6]	no data	no data
ASV	combines CPAP and BiPAP advantages; in >80% sufficient therapeutical response[26]	expensive; no long-term data	↑ quality of life	↓ BNP and SAA; ↑ EF ; ↓nicturia[27,28]
CO_2		↑ arousals[29]; water retention	no data	↓ AHI[29]
Acetazolamid	↑ compliance	insufficient long term data	no data	↓AHI[30]

CPAP - (therapy with) continuous positive airway pressure, O_2 - oxygen therapy, BiPAP - (therapy with) bi-level positive airway pressure, ASV - adaptive servoventilation, CO_2 - carbon dioxide therapy, AHI - apneo-hypopneic index, BNP - brain natriuretic peptide, EF - ejection fraction, SAA - sympathetic-adrenal activation.

calculation and analysis of heart rate response (HRResp), heart rate reserve (HRReserve) and chronotropic index (CRI). Exercise capacity will also be assessed.

4. Heart rate variability (HRV) analysis for evaluation of the frequency characteristics of the pulse at rest and during sleep, with emphasis on very low frequency band (VLF-bands).

5. Brain natriuretic peptide (BNP) measurement.

Task 2. Integrating single predictors into a unified system for screening of patients with chronic heart failure for concomitant central sleep apnea - Cheyne-Stokes respiration. We plan to create panels of parameters following hierarchical principle with respect to their applicability to the level of healthcare:

1. Ambulatory level - specialist in cardiology.
2. Ambulatory level - general practitioner.
3. Hospital level.

After that, we will perform prospective study of the positive and negative predictive value, specificity and sensitivity of the model, applying it to patient groups with different functional class of cardiac dysfunction.

Task 3. Gauging the effect from central sleep apnea - Cheyne-Stokes respiration therapy on the quality of life, physical ability and the main functional parameters. We will determine the effect of the therapy on quality of life, psychological status, exercise capacity and polysomnographic parameters (apneahypopnea index (AHI), sleep quality parameters, etc.).

STUDY DESIGN

A schematic representation of the study design is presented on Fig. 2.

EXPECTED EFFECT AND RESULTS

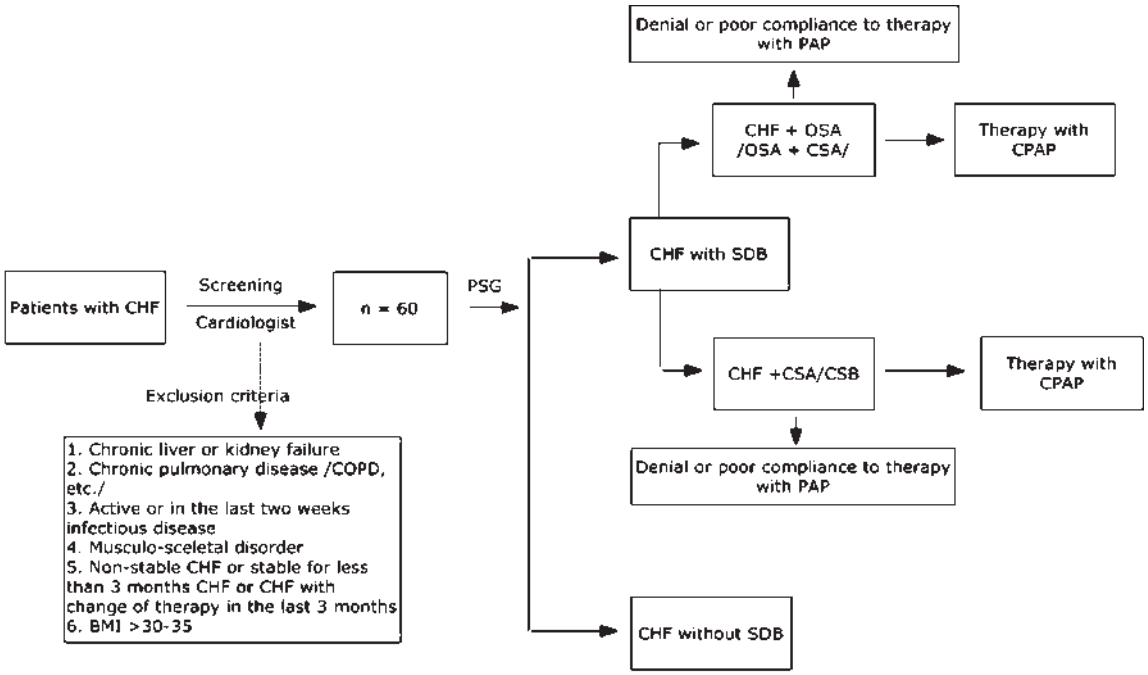
At the end of the study it is expected to be created:

• System for risk stratification of CSA in patients with CHF, allowing for early detection without the need for performing polysomnography as a screening method.

• Algorithm for application of this system in ambulatory practice and in specialized laboratories.

The system will include parameters reflecting breathing, circulation and reactive homeostasis of the organism with respect to rest exercise and sleep.

The created system will be introduced in cardiologists' practice in the hospital, the region and



Legend:
SDB - sleep-disordered breathing; CHF - chronic heart failure; OSA - obstructive sleep apnea; CSA - central sleep apnea; CSB - Cheyne-Stokes breathing; PSG- polysomnography; BMI- body mass index; BiPAP-bi-level positive airway pressure; CPAP - continuous positive airway pressure; ASV-adaptive servoventilation.

Fig. 2. Design of the study

eventually the whole country. It is anticipated that this will lead to reduction of morbidity and mortality in patient population with CHF. The results of the study will be disseminated in other centers in the country and abroad.

We expect the results will serve for establishing an information database for studying the pathogenesis and therapeutic options of CSA-CHF.

CONCLUSION

CSA-CSR is associated with worsened prognosis and quality of life in patients with CHF and therefore should be diagnosed and treated properly. Practically applicable screening is not available at the moment and ideally would be based on routine cardiological investigations. The project aims at creating such a multidimensional screening system which is expected to have significant health and economic impact.

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ПРОГНОСТИЧНИ МОДЕЛИ ЗА ОЦЕНКА НА РИСКА ОТ ЦЕНТРАЛНА СЪННА АПНЕЯ ПРИ БОЛНИ С ХРОНИЧНА СЪРДЕЧНА НЕДОСТАТЪЧНОСТ. ЕФЕКТИ ОТ НЕИНВАЗИВНАТА ВЕНТИЛАЦИЯ

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Резюме

Един от важните фактори за високата морбидност и преждевременната смъртност при болните с хронична сърдечна недостатъчност (ХСН) е централната сънна апнея-Чейн-Стоуксовото дишане (ЦСА-ЧСД). Тя се среща при 30-50% от пациентите с ХСН. Въпреки значението й за прогнозата и високата й честота, малко са мащабните проучвания, посветени на нейното предвиждане и ранно диагностициране. Основната причина за недостатъчното й диагностициране е, че диагностиката й налага скъпо и трудоемко полисомнографско изследване. Целта на проекта е въз основа на съвременните данни за патогенезата на ЦСА в хода на СН да се проучат някои набелязани вече в литературата показатели, както и да се потърсят нови параметри, които носят информация за риска от ЦСА при ХСН.

От страна на кардиореспираторната система след щателен анализ на нейния функционален профил, ще се изследват:

- *Особености в динамиката (цикличен патерн) на дихателния обем, издишания CO_2, вентилаторните еквиваленти при покой, натоварване и сън.*
- *Отговорът на газовата обмяна - кислородна консумация, издишан CO_2, вентилация и вентилаторни еквиваленти на различни етапи от физическото натоварване (начало, анаеробен праг, възстановяване).*
- *Промени в кардиофреквенцията и състоянието на симпатикусово-парасимпатикусово регулиране на сърдечната честота; хронотропната инкомпетентност и честотен анализ на пулсовата вариабилност.*

Основните показатели от тези изследвания и техните производни ще се съпоставят със състоянието на хемодинамиката (фракция на изтласкване, функционален капацитет); архитектониката на съня (нон-РЕМ и РЕМ фази), апнеично-хипопнеичния индекс и характеристика на десатурациите (тежест, продължителност, специфични особености), качеството на живот на пациентите и др. Въз основа на получените резултати ще се предложи интегриран панел (система) от показатели за отчитане на риска от ЦСА при ХСН. Той ще бъде проследен и след лечение на болните с неинвазивна вентилация и евентуално с кислородотерапия. Получените резултати от проучването ще позволят да се направи крачка в ранната диагностика и целенасочено лечение на ЦСА-ЧСД при ХСН.

PSYCHOPHYSICAL AND ELECTROPHYSIOLOGICAL APPROACHES IN SEARCH OF NEW EVIDENCE FOR SEPARATE S–OFF PATHWAY IN HUMAN VISUAL SYSTEM. INVESTIGATIONS WITH HEALTHY VOLUNTEERS AND PATIENTS WITH GLAUCOMA

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Abstract

A basic principle of the visual system organization is information transmission about light changes along two different pathways - ON и OFF, transmitting signals for luminance increase (increment) and luminance decrease (decrement) respectively from the separate areas of the retina. Daylight vision in primates (including humans) is subserved by three types of cones, short wavelength (S-cones), middle wavelength (M-cones) and long wavelength (L-cones). The segregation of the information from the M and L cones along ON and OFF pathways takes place as early as the cone-bipolar cell synapse. The existence of ON channel of short wavelength sensitivity photoreceptors has also been proved, while the existence of S-OFF channel is a matter of debate. The present project proposes to extend a fundamental research, undertaken in the "Processing of visual information" laboratory, in search of new evidence about the existence of S-OFF pathway. An interdisciplinary approach will be applied combining psychophysical and electrophysiological methods in conditions of selective stimulation of shortwave sensitive cones. Visual evoked potentials (VEP) will be recorded, using large size stimuli, covering the entire monitor screen, and a long stimulus duration, which will allow separating onset from offset responses. The results obtained might be interpreted as electrophysiological evidence about the existence of separate S-OFF pathway. VEPs to blue brightness increment and decrement stimuli with size consistent with the area of complete spatial summation (Ricco's area) will also be registered. The results obtained will be an electrophysiological correlate of psychophysical data about differences in spatial summation in S-ON and S-OFF channels received by Vassilev et al. (2003). The data from this experiment, conducted with both healthy volunteers and patients suffering from glaucoma, will provide new opportunities to develop faster and more convenient clinical test for early diagnosis of glaucoma, based on VEP changes in perception of S cones selective stimuli. Creation of such a test is of exceptional importance, because standard diagnostic approaches are not sensitive in the initial stages of the disease, while early detection of glaucoma is essential in fighting its progression.

INTRODUCTION

Vision is the main source of information about the surrounding world for humans and higher primates. A basic principle of the visual system organization is information transmission along two different pathway types - ON pathway, transmitting signals for luminance increase (increment) and OFF pathway, transmitting signal for luminance decrease (decrement) respectively from separate retinal areas. Daylight vision in primates (including humans) is subserved by three types of photoreceptors, short wavelength (S), middle wavelength (M) and long wavelength (L) sensitive cones. The existence of S-ON bipolar cells (Mariani, 1984; Kouyama & Marshak, 1992; 1997) and ganglion cells (Dacey & Lee, 1994; Dacey, 1993) is well proved. However, the existence of S-OFF bipolar and ganglion cells is a matter of debates. According to most electrophysiological findings from the lateral geniculate nucleus (LGN), S-OFF cells are encountered much more rarely than S-ON cells (recently Smajda, Buzas, FitzGibbon, & Martin, 2006). This asymmetry disappears in primary visual cortex (Mullen, Dumoulin & Hess, 2008), a finding

that has no explanation yet.

Contrary to the subcortical asymmetry of S-ON and S-OFF pathways, a number of psychophysical studies has shown that the visual system is equally sensitive to luminance increment and decrement signals that stimulate selectively short wavelength sensitive cones.

In the Institute of Neurobiology, BAS, series of experiments were conducted in order to prove the existence of separate S-OFF pathway (Vassilev, Zlatkova, Manahilov, Krumov, & Schaumberger, 2000; Vassilev, Mihaylova, Racheva, Zlatkova, Anderson, 2003; Racheva, Vassilev, 2008, 2009; Racheva, 2011). Spatial summation for S-cone ON and OFF signals as a function of retinal eccentricity was studied and a psychophysical correlate of unequal density of S-ON and S-OFF cells was found. For the first time data about spatial summation of S-OFF stimuli at different retinal eccentricity were shown. It was found that spatial summation varied for increments and decrements along the retinal eccentricity in a different way, suggesting that the underlying mechanisms processing the S-ON and the S-OFF stimuli are different. In subsequent experiments reaction time (RT) to near-threshold stimuli was measured. In order to avoid spatial contrast between the test stimulus and the background they were of the same spatial size and overlapped each other. The results obtained showed that human visual system is more sensitive to stimulus onset than to stimulus offset when duration of S-cone selective stimuli was shorter, probably as a result of the slow adaptation of the short-wavelength-sensitive system. Indeed, the response to the stimulus offset appeared when stimulus duration became longer. All these data might be interpreted as a result of the longer time constant of the blue cones system (Hughes & DeMarco, 2003), rather than as a sustained response of a system sensitive to the stimulus presence. The response of the S-ON and S-OFF cells in LGN is more probably sustained, while the response of neurons in magnocellular (M) geniculate layers is more probably transient (Reid & Shapley, 2002). If the type of the response correlates with the time course of adaptation, than the adaptation of cells transmitting S-cone signals should be the slowest.

Jankov (1988) recorded visually evoked cortical potentials (VECPs) to stimulus increments applying Stiles two-color method of selective stimulation of the S and L-cones. VEPs to a red light were of large amplitude and were clearly visible at both onset and offset of the stimulus. Offset components to violet light were recorded rarely (with 2 of 14 subjects) and were always of small amplitude.

Study of S-cones and their pathways is of great interest because short wavelength system is morphologically, genetically, physiologically and psychophysiologically distinct from the other two systems and it is the most sensitive to retinal diseases. Several studies have demonstrated that glaucoma is associated with disturbances in blue color perception (Johnson, Adams, Casson, Brandt, 1993; Horn, Jonas, Budde, Junemann, Mardin, Korth, 2002; Aldebasi, Drasdo, Morgan, North, 2003; Drance, Lakowski, Schulzer, Douglas, 1981).

Primary open-angle glaucoma is a common disease and is one of the most frequent causes of blindness. Early diagnosis is crucial for combating the disease progression. Routine screening tests are able to detect only a part of the glaucoma cases. The standard diagnosis tests like subjective perimetry depend to a great extent on the patient's cooperation. Moreover, these tests are not sensitive in the early glaucoma stage (Vistamehr, Shelsta, Palmisano, et al., 2006; Cockburn, 2000). In last years objective techniques for eye diagnosis have been developed (Horn et al., 2002; Crognale, 2002; Rodarte, Hood, Yang et al., 2006; Tobimatsu, Celesia, 2006). One of these approaches is the objective assessment of visual functions through analysis of electrophysiological responses from the visual cortex, using the method of VEPs (Tobimatsu, Celesia, 2006, Aldebasi et al., 2006, Crognale, 2002, Sartucci, Murri, Orsini, Porciatti, 2001).

Resent research (Bessler, Klee, Kellner & Haueisen, 2010) analyzes VEPs recorded under selective stimulation of the separate color channels using the silent substitution method. The results obtained show impressive differences in the VEPs to a blue channel stimulation in patients with glaucoma and age-matched healthy subjects. However, the isoluminant stimulation

could not guarantee perfect S-channel selective stimulation over large retinal areas, because of the differences in the distribution of the three types of cones at different eccentricities. This puts forward the necessity to optimize the electrophysiological approach for early glaucoma detection by choosing a more exact method for S-cone selective stimulation.

AIM AND TASKS OF THE PROJECT

The aim of the present project is to continue fundamental research on human color vision, initiated in the "Processing of visual information" laboratory. Moreover, on the base of the results obtained an attempt will be made to develop a fast, easy applicable and non-invasive test for early diagnosis of glaucoma. The **specific tasks** of the project include:

1. Finding new evidence about the existences of separate S-ON and S-OFF channels in the human visual system through psychophysical and electrophysiological methods.

2. Finding new opportunities to develop faster and convenient clinical test for early diagnosis of glaucoma based on changes in the perception of S-cones selective stimuli and VEP changes to such stimuli in patients with glaucoma. With this purpose it is planned to re-establish collaboration between the "Processing of visual information" laboratory and Department of Ophthalmology, Alexandrovska Hospital, Sofia, and Ophthalmological Clinic of University hospital "Saint Anna", Sofia.

To achieve the aims of this project the following **scientific tasks** were set:

1. To search for new direct electrophysiological correlates of psychophysical data about the existence of S-OFF pathway (e.g. Racheva & Vassilev, 2008). The results of the proposed experiment could resolve the controversy about the nature of the S-OFF channel - transient or sustained. Our assumption is based on promising data obtained in Jankov (1988), who however used relatively short increment stimuli only. With this purpose in our experiment decrement stimuli with longer duration will be also used thus allowing to distinguish between onset and offset responses. Significant problem when using stimuli of long duration and small size is the displacement of their retinal projections due to eye movements and fluctuations in lens accommodation. These movements cause a transient stimulation of different retinal areas and make it difficult to distinguish responses to the onset from the responses to the offset of the stimulus. In order to avoid these problems in the experiments proposed the stimulus and the background will occupy the whole screen of the monitor and will overlap each other completely.

2. Further investigation of spatial summation along the retinal eccentricity for S-cone selective light increments and decrements. The difference in spatial summation for these two stimulus types along the retinal eccentricity, first observed in our laboratory (Vassilev et al., 2003), was recently discussed in the book of Stockman & Brainard (2010) as an important finding in support of the existence of two different S-ON and S-OFF pathways in human vision. However, there are no other experimental findings concerning the spatial-summation differences for increment and decrement S-cone selective stimuli. That is why our aim is to study spatial summation differences along the retina by the method of VEP employing stimuli of different size, presented at different eccentricities. Stimulus size will be consistent with the area of complete spatial summation (Ricco's area).)

3. Development of tests for early glaucoma diagnostics. With this purpose we will conduct experiments analogous to these in the second experiment (paragraph 2) with patients suffering from glaucoma. The two-color method of Stiles will be used, instead of the isoluminant method (Bessler et al. 2010) because of some important disadvantages of the isoluminant method - impossibility to vary accurately stimulus chromaticity; difficulty or even impossibility to present stimuli at different distance from the fovea, since isoluminant couples will vary individually between subjects and along the visual field for each subject. Moreover, Bessler et al. (2010) use quite large test stimulus (22° in diameter), which covers areas with different points of isolumination. Consequently, the question arises whether S channel was selectively stimulated, and if difficulties in the data processing described by the authors, could be due to incorrect mixing of regions with different points of

isolumination. The present project proposes to optimize the electrophysiological method for early diagnosis of glaucoma. VEP will be registered using a small stimulus size which will accurately reflect the specific retinal changes with increasing eccentricity. Furthermore, instead of using the silent substitution method the short wave system will be isolated by the method of Styles (Wyszecki & Stiles, 1982), which has proven its efficiency in various studies.

METHODS

Method of Styles. In the experiments proposed a two-color threshold method of Stiles will be employed for selective stimulation of the S-cones (Wyszecki & Stiles, 1982), i.e. the blue test stimuli will be presented after adaptation to a bright yellow background. This method was modified previously by Vassilev et al. (2000) by adding a dim blue light to the background, thus allowing presentation of both increments and decrements.

2008). Similar apparatus will be constructed for the purposes of the present experiments. The source of the blue light will be a monitor and the stimuli will be generated on its screen by a Visual Stimulus Generator VSG 2/3 of Cambridge Research Systems and Psycho program of the same company. The bright yellow component in the experiments will be provided by slide projector. Both sources will be superimposed at the eye by a neutral semitransparent mirror. The flow of the slide projector will pass through a yellow glass filter, practically not transparent in the short-wave area of the visual spectrum.

The yellow background intensity in the experiments proposed will be 360 cd/m^2. Our previous experiments have shown that the threshold/background intensity curve reached a plateau above 300 cd/m^2, thus becoming independent of the background level (Vassilev et al., 2003) (Fig. 2). This indicates that above 300 cd/m^2 saturation level of the M and L cones was

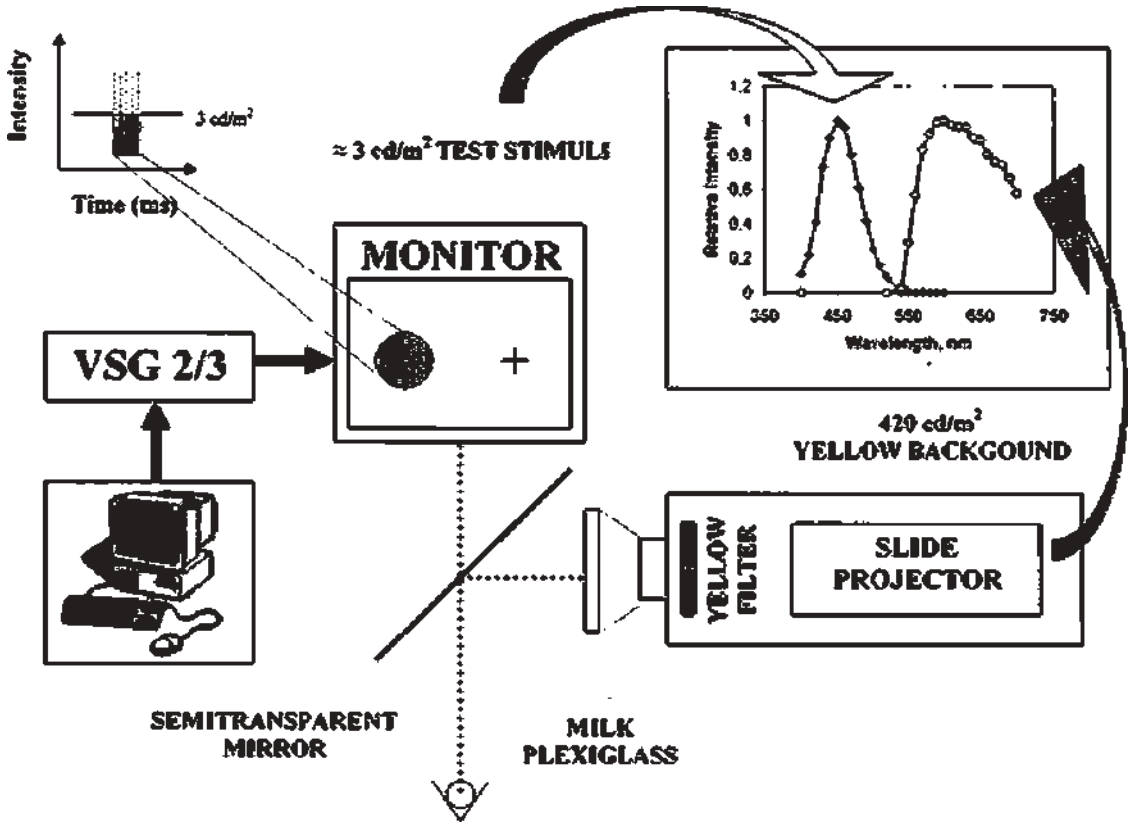

Fig. 1. Apparatus for S cones selective stimulation (from Vassilev et al., 2000)

Fig. 1 schematically describes the apparatus used in previous laboratory experiments (Vassilev et al. 2000, 2003, Racheva & Vassilev, reached and test stimuli were detected by S-cones only. M and L cones are sensitive to the yellow background and therefore the threshold

no longer depends on background intensity. Therefore, 360 cd/m^2 yellow background intensity is bright enough to suppress M and L cone sensitivities.

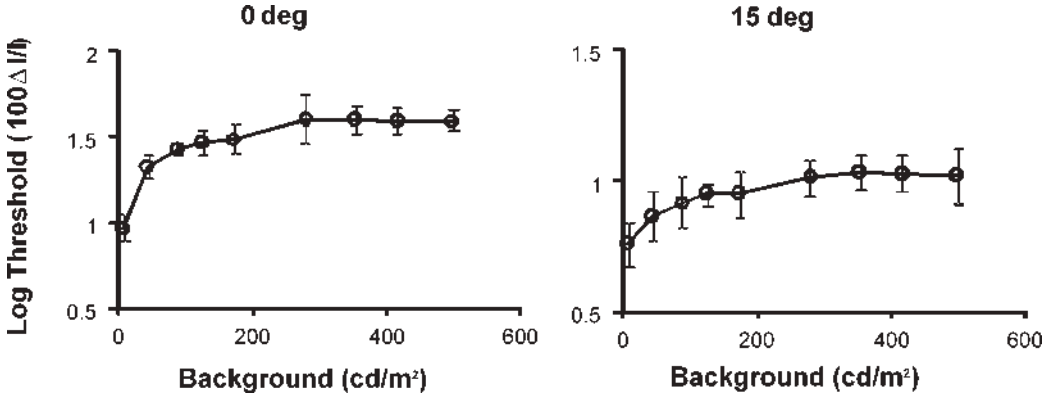

Fig. 2. Increment threshold for a blue test stimulus as a function of the luminance of the yellow background component. The luminance of the blue background was fixed at 3 cd/m^2. Data obtained at 0 and 15 deg from fovea. The test stimulus diameter was 0.5 deg in the fovea and 2 deg at the periphery. Vertical bars - the 95% confidence intervals. Arrows - the background intensity at which spatial summation was studied. Subject: KR. (Adapted from Vassilev et al., 2003)

Experimental procedures and conditions will be similar to those used in Vassilev et al. (2000, 2003) and Racheva & Vassilev (2008). All experiments will be performed in a dark room. Viewing will be monocular, with the right eye, through the natural pupil. Observers will be adapted for 10 min in darkness and then to the background for 2 min. Blue luminance increments and decrements will be used as test stimuli and their spatial and temporal parameters will be different and will vary depending on the experiment purpose.

Electroencephalograms (EEGs) will be recorded using a dense array of scalp electrodes around the striate cortex. The EEG recording epoch will depend on the stimulus duration and will start 500 ms prior the stimulus onset. An age-matched healthy subject group of approximately the same number of participants will be employed to compare their experimental data with the group of patients with glaucoma.

Several schemes will be used to test the correlation between behavioral performance and cortical activity. First, event-related potentials (ERPs) will be extracted from the non-event-related noise by averaging single-trial EEGs which are time-locked to the event. Second,non-phase-locked (induced) oscillatory activity will be analyzed by averaging the spectral power of single-trial EEGs. Next scheme will show event-related synchronization/ desynchronization. For extraction of time-locked activity associated with stimulus presentation, each synchronized with stimulus trial of EEG record will be filtered in appropriate frequency range: delta, theta, alpha, and beta. Then the absolute value of the filtered activity before and after visual stimulus onset will be averaged for all stimuli. The obtained curve can be interpreted in terms of event-related synchronization and desynchronization in selected frequency range. The last approach concerns the change of fractal dimension of EEG due to visual stimuli. Each synchronized with visual stimulus trial of EEG record will be divided into overlapping windows and for each window the fractal dimension (FD) will be calculated by Higuchi method. FD is parameter related to coupling and decoupling of neuronal oscillators (Basar, 1983) due to sensory-motor and cognitive information processing. Thus, for each stimulus new FD time-series will be calculated. The time-series will be averaged similar to the ERPs.

EXPECTED RESULTS AND IMPACT

We expect that the results obtained from the proposed experiments would be of both theoretical and practical importance. It is expected to receive direct electrophysiological evidence about the existence of separate S-OFF pathway and thus to confirm our recent psychophysical data. The new data would contribute to resolving the

discussion whether the blue-yellow channel processing signals from S-cones is composed of two separate neural mechanisms (S-ON and S-OFF pathways). The possible existence of separate S-ON and S-OFF pathways in the human visual system would also be important for understanding the principles of color vision,as well as for optimization of current approaches in medical practice and video technologies.

Moreover, early diagnosis of such socially important disease as glaucoma is essential in preventing its progression. Because short wave cones are less numerous, the blue system is most vulnerable for retinal diseases. In glaucoma early initial deficits in S-cones system stimulation could probably be manifested. Due to subcortical asymmetry in S-ON and S-OFF cells, it could be expected that glaucoma will affect first S-OFF pathway. As a result of the present project a new faster non-invasive method for early glaucoma diagnosis, which can be used in clinical practice, is expected to be developed. The software developed during the project implementation will also enable to perform future research on the topic.

Individual participants in the present project will increase their scientific skills through mutual exchange of methodological approaches. During the project implementation workshops will take place where project participants will report their activities. The results will be published in scientific journals and will be reported on national and international forums. The planned cooperation with health care system units will enable future developments to be tested and used in medical practice in a short time.

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ПСИХОФИЗИЧНИ И ЕЛЕКТРОФИЗИОЛОГИЧНИ ПОДХОДИ В ТЪРСЕНЕТО НА НОВИ ДОКАЗАТЕЛСТВА ЗА СЪЩЕСТВУВАНЕТО НА ОТДЕЛЕН S-OFF ПЪТ В ЗРИТЕЛНАТА СИСТЕМА НА ЧОВЕК. ИЗСЛЕДВАНИЯ ВЪРХУ ЗДРАВИ ДОБРОВОЛЦИ И ПАЦИЕНТИ С ГЛАУКОМА

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Резюме

Основен принцип на организацията в зрителната система е разделянето на информацията за промени в светлинната стимулация по два отделни канала – ON и OFF, провеждащи сигнали съответно за увеличаване (инкремент) и намаляване (декремент) на осветеността от отделните участъци на ретината. Дневното зрение при примати (включително и човек) се осъществява от три вида фоторецептори – късовълнови (S), средновълнови (M) и дълговълнови (L) колбички. Докато разделянето на информацията от M и L колбичките по ON и OFF пътища се извършва още на ранно ниво синапс M и L колбички – биполярни клетки, при късновълновите фоторецептори е доказано наличието само на ON канал, а съществуването на OFF канал е спорно. В настоящия проект се предлага продължаване на фундаменталните изследвания, започнати в лаборатория "Преработка на зрителна информация", в търсене на нови доказателства за наличието на S-OFF път при късовълновата система. За тази цел ще се използва интердисциплинарен подход, комбиниращ психофизични и електрофизиологични методи, в условията на селективна стимулация на късовълновите колбички. Ще се регистрират зрително предизвикани потенциали (ЗПП) при използването на големи по размер стимули, заемащи целия екран на монитора. Стимулите ще бъдат с голяма продължителност, което ще позволи отделянето на отговорите към включване от тези към изключването на стимула. Получените резултати ще бъдат преки електрофизиологични доказателства за съществуването на отделен S-OFF път. Във втория експеримент ще бъдат регистрирани ЗПП при сини яркостни инкрементни и декрементни стимули с размер, съобразен със зоната на пълна пространствена сумация (зоната на Рико). Получените резултати ще бъдат електрофизиологичен корелат на психофизичните данни за разликите в пространствената сумация в S-ON и S-OFF каналите, получени от Vassilev и съавт. (2003). Данните от тези експерименти, проведени както със здрави доброволци, така и с пациенти, страдащи от глаукома, ще дадат нови възможности за разработването на по-бърз и удобен за клиничната практика тест за ранна диагностика на глаукома, основан на промените в ЗПП при възприятието на селективни за S-колбичките стимули. Създаването на подобен тест е от изключителна важност поради факта, че ранната диагностика на глаукомата е от решаващо значение за борбата с това заболяване, а стандартните диагностични подходи не са чувствителни в началните етапи на заболяването.

CONCERNING GENETIC VARIABILITY AND USABLE ISOZYME MARKERS FOR CHARACTERIZATION OF *A. MELLIFERA* L. POPULATIONS AND *B. MORI* L. BREEDS IN BULGARIA

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Abstract

Genetic variability in different populations of Bulgarian honey bee Apis mellifera from the all six main regions in the country have been studied using analysis of six enzymic systems corresponding to six loci (MDH 1, ME, EST 3, ALP, PGM and HK). All of the studied loci were found to be polymorphic. Three to six alleles were detected for the different allozymic systems: three alleles - at MDH-1, ALP and HK loci; four alleles - at ME and PGM loci, and six - at EST-3 locus. Genetic markers, usable for discrimination of Bulgarian honey bees were described in this study.

A genetic analysis of three enzymic systems (PGM, MDH and HK) was conducted on 10 breeds of B. mori. Suitable isoenzyme markers were specified for evaluation of interbreed and intrabreed polymorphism and breed differentiation for this species. The phylogenetical relationships were studied. It was established that breeds E 27, Japanese 106 and Jena may be used in future breeding programs as donor breeds as they are genetically distant from all others.

INTRODUCTION

The most economically significant species of insects Apis mellifera L. and Bombyx mori L. are subject to purposeful selection activity and fundamental scientific research in our country in the recent years.

According to Ruttner's morphometric analysis (1988), *A. m. macedonica* subspecies occur in Bulgaria but according to Petrov (1990), a native type, named as *"A. m. rodopica"*, exists in the country. Since 1930 honey bees in Bulgaria have been studied for the selection purpose (Lazarov 1935; 1936) and during the period 1971-1990 the local bee was threatened by queen breeding and importation of foreign bees. These activities have had an impact on the genetic variability of the honey bees throughout the country. Although there are different studies concerning the degree of genetic diversity of local Bulgarian honey bee, they are mainly based on classical morphometry (Petrov 1990; 1995; 1996; 2000) and partially on isoenzyme analysis (Ivanova et al., 2007; 2010a,b; Ivanova and Bouga 2009). Even though some comparison between selectively reared in Bulgaria lines have been done (Ivanova et al. 2010a; 2011), genetic structures of honey bee populations from all over the country have not been compared yet and differencies between Bulgarian honey bees and other *A. m. macedonica* populations are not enough clear. Hence, the present research focuses on: 1) detection of the genetic variability among honey bee populations in Bulgaria and 2) search for genetic markers usable for discrimination of Bulgarian honey bees.

Utilization of isozyme markers provides the opportunity to study genetic diversity of the mulberry silkworm (Chatterjee & Datta, 1992; Chatterjee et al., 1993; Eguchi, 1995), as well as to differentiate the various breeds (Abraham et al. 1992; Goldsmith 1995). Genetic resources of more than 230 local and introduced breeds and lines of *Bombyx mori* L., with various geographic origins have been maintained in Bulgaria. Their biodiversity has been studied mainly on the basis of some main selection properties - qualitative and quantitative. The isozyme polymorphism with the breeds grown in Bulgaria is weakly studied. Their differentiation on the basis of isozyme markers has not been studied either. This research helped us to identify suitable isozyme markers to analyze the gene pool, genotypic

structure and the degree of genetic heterogeneity of mulberry silkworm breeds grown in Bulgaria and the phylogenetic relationships existing among them.

MATERIALS AND METHODS

1. Electrophoretic analysis

Apis mellifera L.

Totally about 2800 worker bees were tested for this analysis. More than 100 different populations from 24 provinces in all the six main regions of the country were included in this investigation. The thorax or total body homogenization, electrophoresis in 7.5% polyacrylamide gel, buffers and electrophoretic conditions for each enzymic system and histochemical staining were done according to Ivanova (1996) and Ivanova et al., 2010b. Six enzymic systems were studied: MDH (malate dehydrogenase, EC 1.1.1.37); ME (malic enzyme, EC 1.1.1.40); EST (esterase, EC 3.1.1), ALP (alkaline phosphatase, EC 3.1.3.1); PGM (Phosphoglucomutase, EC 5.4.2.2) and HK (Hexokinase, EC 2.7.1.1).

Bombyx mori L.

Ten breeds of silkworm with different origin and named as Belopol 1/18, Belopol 2/21, Gergana 1 and Gergana 2 - from Bulgaria, M-6 - from Azerbaijan, Japanese 106, Asahi and Kinshu - from Japan, Jena - from Austria and E 27 - from Egypt, were tested. All individuals were nourished at a standard regime of silkworm breeding in Sericultural Experiment Station (SES) in Vratza and Agriculture University in Plovdiv. Breeds Jena and E 27 are with colored cocoons; all others are with white cocoons. 30 to 40 larvae were selected randomly from each breed on the fifth day of the fifth instar and were used in the study. The spectrum of phosphoglucomutase (PGM) (EC 5.4.2.2) and hexokinase (HK) (EC 2.7.1.1) from silk glands and malate dehydrogenase (MDH) (EC 1.1.1.37) from haemolymph were studied by means of 7.5% PAGE (Daevis, 1964). Isolation of the haemolymph and the silk glands was done according to Stoykova et al. (2003) and Staykova et al. (2004). Method of Shaw and Prasad (1970) was used to visualize the malate dehydrogenase. Methods of Spencer et al. (1964) and Eaton et al. (1966) were used to visualize the phosphoglucomutase and hexokinase respectively.

2. Statistical and clustering methodology

Allele frequencies, mean number of alleles per locus, proportion of polymorphic loci, observed (H_o) and expected (H_e) heterozygosity, deviation from the Hardy-Weinberg equilibrium and Nei's genetic distance (D) (Nei, 1972) were calculated using BIOSYS-1 (Swofford and Selander, 1981) package. Phylogenetic trees were constructed using Nei's (1972) genetic distance, by UPGMA (Sneath and Sokal, 1973) method using the PHYLIP (Felsenstein, 1993) software package. F_{ST} statistics (Wright, 1965) were also used to estimate the degree of population subdivision, using BIOSYS-1 (Swofford and Selander, 1981) software package.

RESULTS AND DISCUSSION

***Apis mellifera* L.**

Totally, for the studied Bulgarian honey bee population, three alleles were detected at MDH-1 (MDH^{65}, MDH^{80} and MDH^{100}), four - at ME locus (ME^{90}, ME^{100}, ME^{106} and ME^{115}), six - at EST-3 locus (EST^{80}, EST^{88}, EST^{94}, EST^{100}, EST^{105} and EST^{118}), three - at ALP (ALP^{80}, $ALP^{\mathbf{90}}$ and $ALP^{\mathbf{100}}$), four - at PGM (PGM^{80}, PGM^{100}, PGM^{114} and PGM^{125}) and three - at HK (HK^{87}, HK^{100} and HK^{110}) locus. Data about the allozyme polymorphism detected and the allele frequencies calculated are presented in Table 1. Mean sample size per locus, mean number of alleles per locus, proportions of polymorphism, observed and expected heterozygosity in the populations tested are presented in Table 2.

Concerning the different studied loci, our results showed that the most frequent alleles in the Bulgarian populations were MDH^{100}, ME^{100}, EST^{100}, PGM^{100}, HK^{100} and ALP^{80} (except for the populations from South East part of Bulgaria, where $ALP^{\mathbf{100}}$ was more frequent than ALP^{80} or with similar frequency). All these alleles could be used as genetic markers.

On the basis of similar studies it was found that MDH-1 locus is polymorphic with two or three alleles in *A. m. macedonica* populations from Greece (Dedej et al., 1996; Bouga et al., 2005; Ivanova 2010) and that MDH^{80} allele has quite high frequency in these populations. At the same time, according to our results, the frequency of this allele varied between 0.0 and 0.097 in

Bulgarian populations.

Concerning ME locus, Dedej et al. (1996) reported no polymorphism in Greek populations, but according to Bouga et al. (2005) this locus is polymorphic with two alleles in *A. m. macedonica* from Greece. In the present study, four alleles of this locus were detected in Bulgarian populations.

Three alleles of EST-3 locus were detected in *A. m. macedonica* from Greece (Bouga et al., 2005; Ivanova, 2010), but in our research six alleles were found. The alleles EST^{80}, EST^{105} and EST^{118} were detected only in Bulgarian honey bee populations.

Concerning the ALP polymorphism, two alleles (ALP^{100} and ALP^{80}) were detected in Greece (Bouga et al., 2005) and in Bulgaria (Ivanova et al., 2010a,b). In the present research, a third allele - ALP^{90} was observed and its frequency varied between 0.0 and 0.136.

PGM locus was found to be polymorphic with two alleles (PGM^{100} and PGM^{114}) in previously studied populations from Bulgaria (Ivanova et al., 2007, 2010a,b) and in Greece (Ivanova, 2010). Two more alleles of the same locus (PGM^{80} and PGM^{125}) were detected in the present study.

HK locus was found to be polymorphic with two alleles (HK^{100} and HK^{110}) in *A. m. macedonica* from Greece (Ivanova, 2010). In the present study a third allele - HK^{87} was found in Bulgarian population and its frequency varied between 0.007 and 0.027.

All mentioned above private alleles in Bulgarian populations (EST^{80}, EST^{105}, EST^{118}, ALP^{90}, PGM^{80}, PGM^{125} and HK^{87}) could be used also for comparisons, discrimination and characterization of Bulgarian honey bees. In this aspect, together with the most frequent alleles (MDH^{100}, ME^{100}, EST^{100}, PGM^{100}, HK^{100} and ALP^{80}) they could be useful as suitable genetic markers for selection and conservation purposes.

The mean number of alleles per locus varied from 2.5 (North West) to 3.7 (South East). The estimated percentage of polymorphic loci ranged from 50% (South West) to 83.3%

Table 1. Number of samples studied (N) and data about the allele frequencies in the populations

Locus	South West	South Central	South East	North East	North Central	North West
MDH-1						
(N)	438	788	504	406	599	88
65	0.451	0.451	0.419	0.458	0.496	0.318
100	0.549	0.543	0.577	0.542	0.503	0.585
80	0	0.007	0.004	0	0.001	0.097
ME						
(N)	441	585	398	355	366	76
100	0.93	0.921	0.908	0.921	0.937	0.875
106	0.045	0.069	0.044	0.065	0.049	0.125
90	0.02	0.01	0.048	0.014	0.014	0
115	0.005	0	0	0	0	0
EST-3						
(N)	442	758	480	372	527	69
80	0.008	0.036	0.011	0.02	0.013	0
100	0.974	0.923	0.953	0.952	0.956	0.949
88	0	0	0.005	0.007	0	0.029
118	0.016	0.022	0.004	0.013	0.011	0.022
94	0.002	0.015	0.014	0.008	0	0
105	0	0.005	0.013	0	0.019	0

ALP						
(N)	386	618	385	318	550	68
80	0.517	0.513	0.403	0.539	0.585	0.537
100	0.47	0.47	0.461	0.418	0.408	0.463
90	0.013	0.017	0.136	0.042	0.006	0
PGM						
(N)	424	640	406	363	392	69
100	0.953	0.922	0.915	0.946	0.916	0.92
114	0.038	0.077	0.081	0.054	0.08	0.08
80	0	0	0.001	0	0	0
125	0.009	0.002	0.002	0	0.004	0
HK						
(N)	494	894	573	445	822	86
87	0.007	0.027	0.011	0.022	0.021	0.017
100	0.97	0.966	0.966	0.962	0.964	0.965
110	0.023	0.007	0.023	0.016	0.015	0.017

(South Central and North West). (Table 2). The observed and expected heterozygosity (H_o and H_e) ranged from 0.166 (North West) to 0.218 (North East) and from 0.224 (South West) to 0.264 (South East and North West), respectively (Table 2). There are significant deviations of genotype frequencies from Hardy-Weinberg expectations at most of the loci in most populations ($P \geq 0.01$). Chi-Square (df: 1-6) tests showed that the deviations were generally in favour of homozygotes.

The estimated mean F_{ST} value was 0.0068, which shows high level of genetic diversity within populations. The values of genetic distance (Nei, 1972) were calculated using the allele frequencies (Table 1) and ranged from 0.001 to 0.008. In UPGMA dendrograms, North West and South East populations were grouped separately. All other populations were clustered together in two branches: first - North Central and North East and second - South Central and South West (Fig. 1).

Table 2. Observed and expected heterozygosity in the populations tested (Standard errors are included)

Population	**Mean sample size per locus**	**Mean no. of alleles per locus**	**Percent Polymorphic loci(P=0.95)**	**H_o**	**H_e**
South West	437.5±14.2	3.2±0.3	50	0.21±0.094	0.224±0.089
South Central	713.8±48.7	3.3±0.3	83.3	0.203±0.079	0.254±0.082
South East	457.7±30.3	3.7±0.5	66.7	0.215±0.086	0.264±0.093
North East	376.5±17.9	3±0.4	66.7	0.218±0.089	0.241±0.087
North Central	542.7±67.2	3.2±0.2	66.7	0.18±0.07	0.237±0.083
North West	76±3.7	2.5±0.2	83.3	0.166±0.053	0.264±0.085

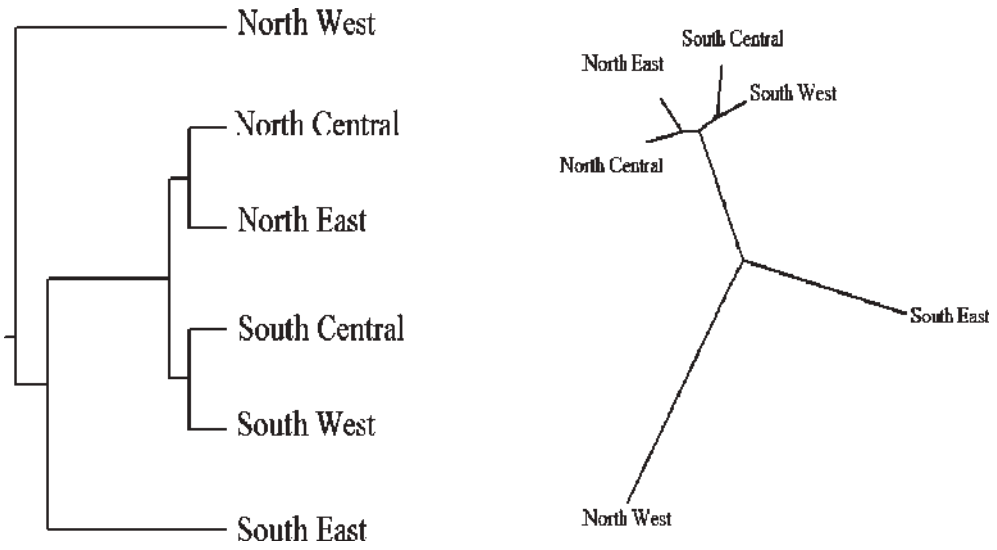

Fig. 1. Relationships of honey bee populations studied as shown in UPGMA (Sneath and Sokal, 1973) dendrograms

Bombyx mori L.

In two of the studied enzyme systems - phosphoglucomutase and malate dehydrogenase, we found out intrabreed and interbreed polymorphism (Table 3). We established interbreed polymorphism by means of hexokinase.

Phosphoglucomutase from various organs of *B. mori* was determined by one locus for which a three-allelic polymorphism was described (Staykova, 2006; Staykova, 2008). With the breeds included in this study we established presence of all three alleles (Pgm A_1, A_2 and A_3) in the gene pool of the breeds Belopol 1/18, Belopol 2/21 and Gergana 1 (Table 3). With Kinshu, Jena and E 27, allele Pgm A_1 was missing. Pgm A_2 was seen in the gene pool of all the studied breeds, and with Gergana 2, M-6, Japanese 106 and Asahi this allele was fixed. Among these breeds with established polymorphism, the highest frequency of allele Pgm A_1 was established for Gergana 1, allele Pgm A_2 - for Jena, and allele Pgm A_3 - for Kinshu.

Table 3. Allele frequencies in breeds tested

Breeds	Locus							
	Pgm			Mdh			Hk	
	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2
Belopol 1/18	0.038	0.438	0.525	0	1	0	0	1
Belopol 2/21	0.038	0.603	0.359	0	1	0	0	1
Gergana 1	0.265	0.500	0.235	0	1	0	0	1
Gergana 2	0	1	0	0	1	0	0	1
M-6	0	1	0	0	1	0	0	1
Japanese 106	0	1	0	0	1	0	1	0
Asahi	0	1	0	0.031	0.859	0.109	0	1
Kinshu	0	0.306	0.694	0.113	0.855	0.032	0	1
Jena	0	0.676	0.324	0	1	0	1	0
E 27	0	0.425	0.575	0	1	0	1	0

By means of electrophoresis in polyacrylamide and cellulose acetate gel Marcato et al.

(1990) found polymorphism by the loci coding the phosphoglucose isomerase and mannose phosphate isomerase, and lack of polymorphism by the loci coding malate dehydrogenase, isocitrate dehydrogenase and other enzymes. Egorova and Nasirillaev (1993) reported the presence of MDH polymorphism at the hemolymph of *B. mori*. For two of the breeds studied by us we also found polymorphism on the malate dehydrogenase from the hemolymph (Table 3). In the gene pool of the Japanese breeds Asahi and Kinshu we established the presence of three alleles (Mdh A_1, A_2 and A_3). In the gene pool of all other breeds, the Mdh A_2 allele was fixed, which agrees with the established by Marcato et al. (1990).

Yanagawa (1978) studied the properties of hexokinase from various tissues of the mulberry silkworm (fat body, muscles, spermaries, midgut and Malpighian tubules) and established the tissue specificity in distribution of the multiple molecular forms. In the group of breeds of various origins studied in Bulgaria we established interbreed polymorphism by the hexokinase of the silk glands and the lack of intrabreed polymorphism (Table 3). In the gene pool of Japanese 106, Jena and E 27 we ascertained the presence of the Hк A_1 allele, whereas in the gene pool of all other breeds we ascertained the Hк A_2 allele.

The average number of alleles per locus calculated by BIOSYS-1 (Table 4) varies from 1.0 (with Gergana 2, M-6 and Japanese 106) to 2.0 (with Kinshu). The degree of polymorphism (according to criteria 0.99) was highest for breed Kinshu (66.70%), and lowest - with Gergana 2, M-6 and Japanese 106 (0%), where we observed monomorphism in all studied loci. The established heterozygosity (H_o) varied from 0.000 (with Gergana 2, M-6 and Japanese) to 0.151 (with Kinshu). With all analyzed breeds with established polymorphism the expected heterozygosity (H_e) was higher than the one we obtained. The test for conformance to Hardy-Weinberg equilibrium manifested significant differences between the obtained and expected genotype frequencies, which were due to the established higher frequency of the homozygotes. The lower extent of established heterozygosity and the higher frequency of homozygote individuals were related to inbreeding effects.

The average value of F_{ST} (0.6131) calculated on the basis of the established isozyme polymorphism showed that 61.31% of the genetic variability was observed between different breeds, which also corresponded to the degree of interbreed differentiation, whereas 38.69% of the genetic variability was intrabreed. The highest extent of interbreed variability was accounted by hexokinase (1.000).

On the grounds of the allele frequencies, the genetic distance by Nei (Nei, 1972) was also calculated, which was the base for constructing a

Table 4. Mean number of alleles per locus, proportion of polymorphic loci, observed (H_o) and expected heterozygosity (H_e)

Breeds	Mean sample size per locus	Mean no. of alleles per locus	Percent Polymorphic loci (P=0.99)	H_o	H_e
Belopol 1/18	40.0	1.7±0.7	33.3	0.050	0.179
Belopol 2/21	39.0	1.7±0.7	33.3	0.060	0.171
Gergana 1	34.0	1.7±0.7	33.3	0.088	0.211
Gergana 2	30.0	1.0±0.0	0.0	0	0
M-6	30.0	1.0±0.0	0.0	0	0
Japanese 106	36.0	1.0±0.0	0.0	0	0
Asahi	32.0	1.7±0.7	33.3	0.052	0.084
Kinshu	31.0	2.0±0.6	66.7	0.151	0.231
Jena	34.0	1.3±0.3	33.3	0.078	0.148
E 27	40.0	1.3±0.3	33.3	0.050	0.165

UPGMA dendrogram (Fig.2). It shows that within the group of studied breeds two main clusters were formed. In the first cluster the breeds E 27, Japanese 106 and Jena were distributed, and in the other - all others.

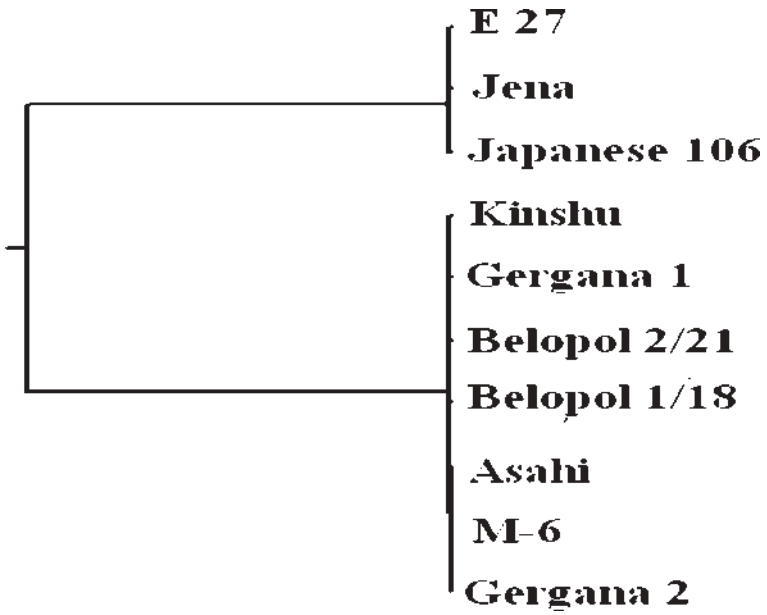

Fig. 2. Relationships of studied breeds as shown in UPGMA (Sneath and Sokal,1973) dendrograms

CONCLUSIONS

Concerning *Apis mellifera*, the obtained results in the present study give us the grounds to draw the flowing deductions:

- The allozyme systems studied in this investigation are useful for characterization of genetic variability in honey bee populations and show a high level of genetic diversity within Bulgarian populations.
- The indicated in this study allozyme genetic markers are appropriate for comparisons, discrimination and characterization of Bulgarian honey bees and would be useful for selection and conservation purposes.

The accomplished study for establishing suitable isozyme markers at valuation of the gene pool of the mulberry silkworm and the obtained results give us the grounds to draw the flowing deductions and conclusions:

- Hexokinase (HK) is a suitable marker for analyzing interbreed polymorphism and breed differentiation.

* Phosphoglucomutase (PGM) and malate dehydrogenase (MDH) are more suitable for studying the intrabreed polymorphism and establishing the degree of intrabreed genetic variability.

- The established isozyme markers could be used in the future breeding and selection work on creating initial populations and performing interbreed hybridization.
- Breeds E 27, Japanese 106 and Jena could be included in selection programs as donor breeds, as they are genetically distant from all others.

Acknowledgments

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ОТНОСНО ГЕНЕТИЧНАТА ИЗМЕНЧИВОСТ И ИЗПОЛЗВАНИТЕ ГЕНЕТИЧНИ МАРКЕРИ ЗА ХАРАКТЕРИЗИРАНЕ НА ПОПУЛАЦИИ *A. MELLIFERA* L. И ПОРОДИ *B. MORI* L. В БЪЛГАРИЯ

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Резюме

Проучена е генетичната изменчивост в популации медоносни пчели от цялата страна чрез изоензимен анализ по шест изоензимни системи (MDH 1, ME, EST 3, ALP, PGM и HK). Констатиран е полиморфизъм по всичките шест изследвани локуси, както следва: три алела - за MDH-1, ALP и HK локусите; четири - за ME и PGM локусите и шест - за EST-3 локуса. Отчетени са изоензимни генетични маркери, подходящи за използване при характеризиране на българските медоносни пчели.

Чрез изследване на три изоензимни системи (PGM, MDH и HK) е проведен генетичен анализ на 10 породи черничева копринена пеперуда B. mori L. Уточнени са подходящи изоензимни маркери за оценка на вътрепородния и междупороден полиморфизъм и породната диференциация при този вид. Изследвани са филогенетичните връзки. Установено е, че породите E 27, Japanese 106 и Jena могат да се използват в бъдещи селекционни програми като донорни породи, тъй като са генетично отдалечени от всички останали.

IMMUNOMODULATORY EFFECTS OF SELECTED LACTOBACILLUS STRAINS AGAINST INFLAMMATION IN HUMAN INTESTINES

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Abstract

The aim of the present study was to evaluate the effect of LAB strains on IL-8 secretion in intestinal epithelia with/ without stimulation by TNF-alpha.

Intestinal epithelium is capable of releasing some proinflammatory cytokines such as IL-8, especially when stimulated by cytokines like TNF-alpha and IL-1. Some Lactic acid bacteria (LAB) strains could modulate intestinal mucosal immune response playing anti-inflammatory role in the intestinal immune signaling.

A large number of strains with yoghurt and intestinal origin were evaluated in their ability to reduce the production of IL-8 from human epithelium cell line Caco-2. The strains demonstrating the highest reduction of IL-8 after stimulation of epithelium cells with TNF-alpha were: B. longum Bif8, L. gasseri G8, E. faecalis E2, L. bulgaricus B67, and S. thermophilus T43. It should be noted the yoghurt origin of the latest two strains. The stimulation index of these strains was between 0.6 and 0.8 in presence of TNF-alpha, and between 0.94 and 1.07 without co-stimulation with TNF-alpha. In order to complete the information about anti-inflammatory potential of the strains, they were evaluated in induction of IL-10 using macrophage cell line model U-937. The strains B. longum Bif8, L. gasseri G8, E. faecalis E2 induced the production of IL-10 - 3.2, 2.1, and 0.2 ng/ml, respectively.

It was proven that some LAB strains inhibit the secretion of IL-8 from epithelial cells. These strains will be included in products for further clinical trials of their anti-inflammatory effect.

INTRODUCTION

Intestinal epithelium is an important factor of gut mucosal barrier,and participates in innate immunity. Intestinal epithelia are capable of releasing some proinflammatory cytokines such as IL-8 when stimulated by cytokines like TNF-a, and can response to enteric pathogens and release some proinflammatory cytokines which in turn direct the movement of inflammatory cells of the lamina propria [8]. Probiotics, including bifidobacterium, lactobacillus play an essential role in the completeness of intestinal mucosa barrier. For example, some probiotic strains could modulate intestinal mucosal immune response, some could play protective roles by inhibiting the

adhesion of pathogenic bacteria to intestinal epithelia [6]. The present study was to investigate the effect of probiotics on IL-8 secretion of intestinal epithelium induced by TNF-a, and its possible mechanism. Additionally, selected LAB strains were evaluated in induction of IL-10 using macrophage cell line model U-937.

MATERIALS AND METHODS

Bacteria Different strains LAB, previously identified at strain level by help of pulsed field gel electrophoresis [5], were provided by the laboratory "LBB collection" in LB Bulgaricum PLC. The strains were grown at 37 in static, non-aerated BHI-agar to reach the mid-log phase. Bacteria were harvested by centrifugation at 2 500 g for 15 min at 20 . After two washes in sterile PBS pH 7.4, at 25 , the bacteria were resuspended in PBS. Cell counts in the bacteria suspension were estimated by optical density at 600 nm absorbance (BioMerieux, Germany). Then the bacteria were added to the cell culture wells at appropriate dilution to reach a final concentration of 108 cfu/ml of medium.

Cells and bacteria coculture Caco-2 cells were grown in DMEM with 10% fetal calf serum, and divided into four groups: control, TNF-a (group T in short), LAB group (group L). When grown to confluence in single layer, cells were washed three times with PBS pH 7.4, to remove culture medium and non-adherent cells. The bacteria in culture medium were transferred into individual wells respectively. TNF-a (10 ng/ml) was added into each well of groups T and L 1 hour later. The supernatants were collected and centrifuged for measurement of IL-8 after 3 hours.

U-937 cells were grown in RPMI with 10% fetal calf serum. The cells were differentiated to macrophages by help of phorbol-myristate-acetate (PMA) at final concentration of 1 mkg/ ml. The adhered macrophages were washed with PBS pH 7.4 to remove PMA and non-adherent cells. The bacteria in culture medium were transferred into individual wells respectively. The supernatants were collected and centrifuged for measurement of IL-10 after 18 hours.

IL–8 enzyme–linked immunosorbent assays IL-8 enzymelinked immunosorbent assays (ELISA) were performed according to the manufacturer's instructions. In short, polyclonal goat anti-human IL-8 antibodies were used as capturing antibodies, biotinylated polyclonal rabbit anti-human IL-8 antibodies as detecting antibodies. Streptavidin-HRP and TMBS were added as color indicator. Plates were read at 450 nm of wavelength right after color reaction was stopped

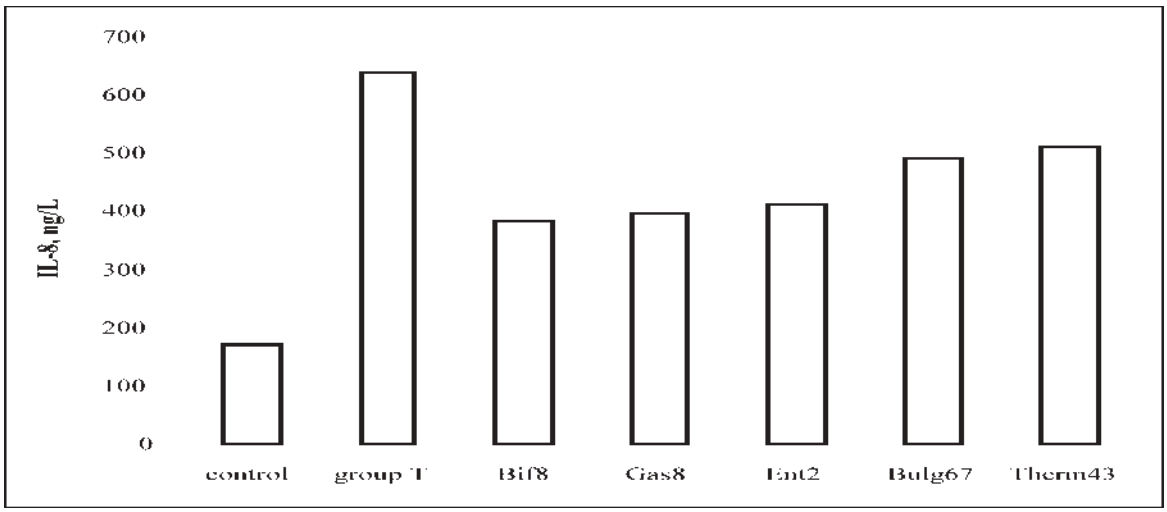

Fig. 1. Concentrations of IL-8 in each group (mean±SD). Natural interleukin-8 expression was seldom found in Caco-2 cells of the control group. When stimulated by TNF-a (10 ng/ml), Caco-2 cells secreted a large number of IL-8, and the concentration of IL-8 in group T was significantly increased than that in control (P<0.001). However, there was some difference in the concentration of IL-8 among group T and L. There was less interleukin-8 secretion in Caco-2 cells when preincubated with selected LAB strains (group L) compared with group T (P=0.002, 0.01, respectively).

with acid. All procedures were performed at room temperature.

IL-10 enzyme-linked immunosorbent assays IL-10 enzymelinked immunosorbent assays (ELISA) were performed according to the manufacturer's instructions (Diaclone, USA).

RESULTS AND DISCUSSION

IL-8 secretion. Concentrations of IL-8 in supernatants of each group are shown in Figure 1. The concentration of IL-8 in control was only 172.2±42.1 ng/L. When stimulated by TNF-a, Caco-2 cells secreted a large number of IL-8, and the concentration of IL-8 in group T was 639.5±62.3 ng/L. However, when preincubated with different LAB strains, Caco-2 cells produced either the same quantity or less IL-8, compared with group T. The concentrations of IL-8 were 383.8±76.7 ng/L, 397.2±71.5 ng/L, 412.1±68.6 ng/L, 492.1±56.6 ng/L, 511.4±55.4 ng/L in group L for the strains *B. longum* Bif8, *L. gasseri* G8, *E. faecalis* E2, *L. bulgaricus* B67, and *S. thermophilus* T43, respectively.

Intestinal epithelia constitute mucosal barrier of the bowel,and participate in inflammatory or immune responses in gut [4, 10]. In some gastrointestinal infectious and inflammatory conditions, such as inflammatory bowel disease (IBD), acute gastroenteritis, inflammatory cells including monocytes, lymphocytes, were activated and accumulated in lamina propria. The cells secrete excessive inflammatory products, such as TH1 type cytokines, chemokines and a lot of active oxides. Overproduction of cytokines could affect the biological action of epithelial cells. For instance, TNF-a could induce epithelial cells to secrete IL-8, and express membrane Toll-like receptor 4 (TLR4) excessively [12, 20]. TLR4 could enable intestinal epithelia hyper reactive in response to lipopolysaccharides (LPS),the component of bacteria walls, and IL-8 had leukocytes chemotactic and stimulatory properties [1]. As more inflammatory cells infiltrate, the inflammatory reaction is therefore amplified. The normal flora of human gastrointestinal tract contains diverse populations of bacteria which play an essential role in the development of gut mucosal barrier and innate immunity. Some intestinal microflora could exert a protective role against pathogens [13]. Aberrance of gut microflora has been reported in IBD and acute gastroenteritis [7, 16]. The aberrant microflora disregulates mucosal immune reaction. Invasion of some virulent strains into epithelia could break down the integrity of intestinal mucosa, and induce inflammatory cell infiltration [18]. Some researchers found that manipulating the normal intestinal flora using probiotics had a beneficial effect on health by altering the microbial environment, and some components of the flora could down-regulate inflammation when supplemented to patients with gastrointestinal diseases [11, 17]. Some studies have been undergoing to explore the possible mechanisms of probiotic action on gut epithelium and mucosal immune system. In order to imitate the inflammatory condition of gut in vitro, we used TNF-a to stimulate human colonic adenocarcinoma Caco-2 cells, which has basically the same biological properties as normal colonic epithelia. As some probiotic strains could adhere to human intestinal cell surface [2, 3], five probiotic strains *B. longum* Bif8, *L. gasseri* G8, *E. faecalis* E2, *L. bulgaricus* B67, and *S. thermophilus* T43, inhibited the secretion of IL-8 in Caco-2 cells when stimulated with TNF-a one hour after coculture with the five probiotic strains. It indicated that the strains could trigger anti-inflammatory pathways within the gut epithelium. The epithelia attached to the strains showed immune hypo reaction to TNF-a, and produced less IL-8.

IL-10 secretion. As the induction of the cytokine IL-10 is recognized to be anti-inflammatory, we evaluated the IL-10 induction effect by the five LAB strains, which previously demonstrated the best inhibition of the synthesis of IL-8, on differentiated to macrophage U-937 cell line. Concentrations of IL-10 in supernatants of each group are shown in Figure 2.

When stimulated by the five LAB strains, U-937 cells secreted a different quantity of IL-10. The concentration of IL-10 when U-937 cells were preincubated with the five previously selected LAB strains, demonstrating the best inhibition of IL-8, was 3.2, 2.1, 0.2, 0.05, and 0.0 ng/ml for the strains *B. longum* Bif8, *L. gasseri* G8, *E. faecalis* E2, *L. bulgaricus* B67, and *S. thermophilus* T43, respectively.

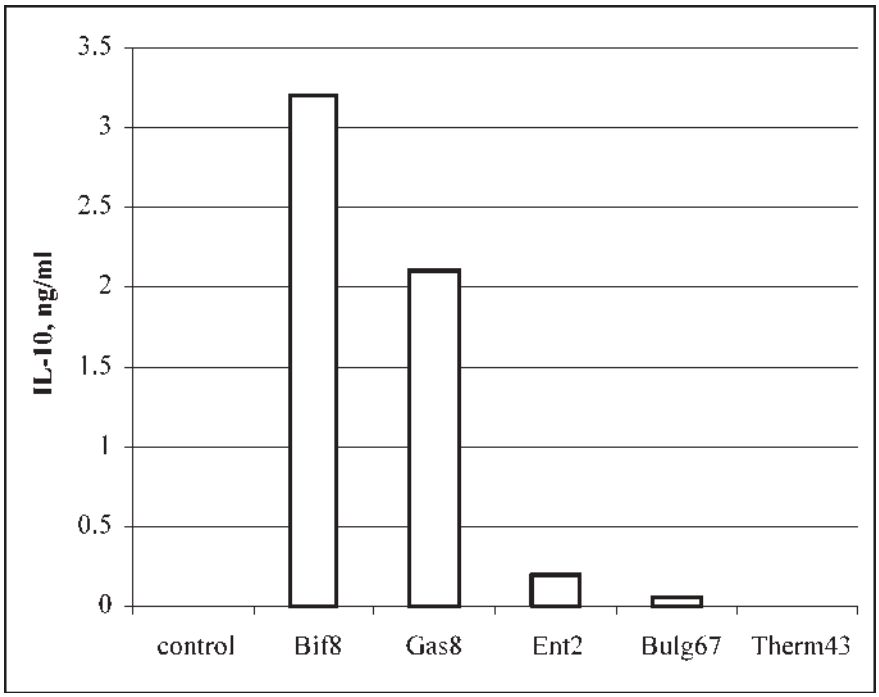

Fig. 2. Concentrations of IL-10 induction by the five best IL-8 inhibitors

CONCLUSIONS

The selected five LAB strains successfully inhibited IL-8 secretion in intestinal epithelia in the experiment. At present, some probiotic compounds have been used in management of some diseases, such as maintenance therapy in IBD [9, 15]. Although the mechanism of probiotic action has not been fully understood, the beneficial effects were consistent with an anti-inflammatory state conferred by probiotics [14, 19]. These five LAB strains will be included in products for further clinical trials of their anti-inflammatory effect.

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ИМУНОМОДУЛИРАЩИ ЕФЕКТИ НА СЕЛЕКТИРАНИ ЩАМОВЕ ЛАКТОБАЦИЛИ СРЕЩУ ИНФЛАМАЦИИ В ИНТЕСТИНАЛНИЯ ТРАКТ

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Резюме

Целта на изследването е да се оцени ефекта на щамове лактобацили върху секрецията на интерлевкин 8 (IL-8) от интестиналния епител с и без стимулиране с тумор-некрозис алфа (TNF-alpha).

Интестиналният епител е способен да синтезира някои проинфламаторни цитокини като IL-8, особено в присъствие на TNF-alpha и IL-1. Някои млечнокисели бактерии (МКБ) могат да модулират интестиналния имунен отговор, играейки антиинфламаторна роля.

В настоящото изследване голяма група щамове с интестинален и киселомлечен произход бяха изпитани по отношение способността им да понижават продукцията на IL-8 от човешката епителна клетъчна линия Caco-2. Щамовете, които демонстрираха най-висока редукция на продукцията на IL-8 след стимулиране на епителните клетки с TNF-alpha са: B. longum Bif8, L. gasseri G8, E. faecalis E2, L. bulgaricus B67, и S. thermophilus T43. Трябва да се отбележи киселомлечният произход на последните два щама. Стимулиращият индекс на гореспоменатите щамове бе в диапазона 0.6-0.8 в присъствие на TNF-alpha, и между 0.94 и 1.07 в отсъствие на TNF-alpha. За да се допълни оценката за антиинфламаторния потенциал на щамовете, те бяха изпитани за способността им да индуцират производството на IL-10 от макрофаговата клетъчна линия U-937. Щамовете B. longum Bif8, L. gasseri G8, E. faecalis E2 индуцираха синтеза на IL-10 - 3.2, 2.1, and 0.2 ng/ml, съответно.

Доказано бе, че някои щамове МКБ инхибират секретирането на IL-8 от епителни клетки. Тези щамове ще се включат в продукти за бъдещи клинични изпитания на техния антиинфламаторен ефект.

IMMOBILIZATION OF *TRICHOSPORON CUTANEUM* R57 CELLS ONTO SiO_2/HPC HYBRID MATERIALS AND REMOVAL OF Mn^{2+} IONS

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Abstract

New silica hybrid materials containing tetraethoxy siloxane (TEOS) as inorganic precursor and hydroxypropyl cellulose (HPC) as an organic compound were prepared. The quantity of organic substance was 5 and 50 wt% to silica. The structure of obtained hybrids was investigated by FT-IR and SEM analysis. The synthesized hybrid materials were applied as matrices for cells immobilization by attachment of filamentous yeast Trichosporon cutaneum R57. This strain showed considerable ability to remove manganese ions from aqueous solutions. The experimental data on the equilibrium specific uptake with free strain cells were used to plot the adsorption isotherm. Kinetic experiments on biosorption of manganese ions with immobilized cells were performed at Mn2+ initial concentrations 275 and 550 mg/L. These values were selected based on the free strain cells performance shown by the adsorption isotherm. A pseudo second order kinetic model was employed to fit the experimental data. Higher specific uptake was established with immobilized cells and higher content of HPC in the immobilization material.

INTRODUCTION

Heavy metal pollution due to anthropological practices represents an important environmental problem - more toxic metal ions are released in the nature and disrupt the ecosystem. Manganese is an essential transition metal that is required by organisms ranging from simple bacteria to humans. Manganese function is a component of several enzymes involved in carbohydrate, lipid and protein metabolism. High amounts of Cd^{2+}, Cu^{2+} and Mn^{2+} ions have a toxic effect. Exposure to high concentrations of manganese leads to manganism, Parkinson's disease and Alzheimer's [1]. The importance of microbial activity in the remediation of Mn-contaminated waters was frequently observed. Several strains of Mn^{2+}-oxidizing bacteria were used for treatment of manganiferous mine waters [2]. Mariner et al. [3] identified Mn^{2+}-oxidizing fungi in addition to bacteria, useful for treatment of mine waters.

Metal remediation through common physicochemical techniques is not efficient in case of effluents containing complexing organic matter and low metal contamination [4]. Microbial remediation of metal ions can be either catalytic or non-catalytic. It precedes through four different mechanisms, namely biosorption, bioaccumulation, redox reaction and complex formation [5]. Bioremediation techniques include cellular sequestration and accumulation, or extra-cellular precipitation. Microorganisms provide a large contact area that can interact with metals in the surrounding environment [6, 7]. The microbial biomass contains chemically active sites that are responsible for sequestering metals from the surrounding solution [4]. The heavy metal tolerance of the microorganisms depends on their ability to sustain toxic ions by absorbtion to metabolically less active compartments such as cell walls or vacuoles, or by evolving an effective mechanism for ion extrusion outside of the cells [8]. Living microorganisms have been effectively immobilized in silica sol-gel matrices, and successfully used for preparation of heavy metal biosorbents [9-15]. High potential of *Trihosporon cutaneum* strain R57 for heavy metals removal - copper, cadmium and chromium from contaminated waters has been revealed in several previ-

ous investigations [16-19]. The immobilization trough attachment on the surface of hybrid matrices (although decreasing the specific rate of the process) has been shown to retain the high strain biosorption capacity [19].

The goal of the present work is to study the properties of new SiO_2/hydroxypropyl cellulose hybrid matrices and their suitability for *Trichosporon cutaneum* strain R57 cell immobilization, aiming removal of manganese ions from waste waters.

MATERIALS AND METHODS

Microorganisms and growth conditions

Filamentous yeast strain *Trichosporon cutaneum* R 57 maintained in the culture collection of Bulgarian National Bank of Industrial Microorganisms and Cell Cultures under N2414 was used in this study. The cultivation was carried out in a medium under conditions described elsewhere [7].

Hybrid materials preparation

A pre-hydrolyzed solution of tetraethoxy siloxane (TEOS) with H_2O and HCl was prepared, then dissolved pectin was added and pH was adjusted to 7 by phosphate buffer. The molar ratio SiO_2:H_2O:C_2H_5OH:HCl was $1:2:8:1x10^{-3}$. The hybrids contained 5 and 50 wt% HPC to silica. The hybrid materials were aged at room temperature and used as matrices for cells immobilization.

Cells immobilization and biosorption experiments

Biosorption of manganese ions was performed in a batch system as described previously [19]. Immobilization of cells by attachment was carried out at the 6^{th} hour of the strain cultivation when the hybrid materials were added to the culture medium. In order to study the equilibrium state and kinetics of biosorption by free and immobilized cells, manganese ions were supplied to the cultivated strain at the 24^{th} hour of cultivation in the form of sulfates in concentrations of 5 mM and 10 mM $MnSO_4$. The cultivation experiments were carried out at 30^0C.

The heavy metals biosorption was performed under vigorous stirring in order to restrict external diffusivity limitations. The experimental data for the concentration of residual heavy metals in the liquid phase at given time were used to calculate the specific heavy metal uptake, $q = V(C_0 - C)/m$ where q is the quantity of metal ions adsorbed by a given quantity of biosorbent (mg g^{-1}) in time t (min); V is the volume of the liquid phase (L); C is the concentration of metal ions, mg L^{-1}; C_0 is the initial concentration of metal ions, mg L^{-1} and m is the biosorbent dry weight (g). To determine the kinetic parameters for the adsorption of manganese ions by immobilized cells of the studied strain, the experimental data were used to fit a pseudo second order kinetic model (Eq. (1)).

$$\frac{t}{q} = \frac{1}{k_{ads} q_{eq}^2} + \frac{1}{q_{eq}} t \qquad (1)$$

The values for the equilibrium heavy metal uptake q_{eq} (metal uptake concentration at equilibrium, mg.g^{-1}) and the specific adsorption rate constant k_{ads} (g mg^{-1} min^{-1}) were determined from the slope and the intercept of the plot t/q versus t. The equilibrium parameter q_{eq}, which is a characteristic for the specific uptake capacity (i.e. the efficiency) of the biosorbent and the rate constant provide tools for process evaluation and engineering.

Analytical measurements

Structural groups of the obtained hybrid materials were determinated by a Fourier Transform infrared (FT-IR) spectroscopy with MATSON 7000 Fourier Transforming Infra-Red spectrometer using KBr tablets. Scanning electron microscopy (SEM) was employed to study the morphology of the obtained hybrids. SEM images were taken with a Microscope LEO 1530, Gemini at an acceleration voltage of 10 kV. The obtained materials were covered with gold using BAL-TEC/SCD 050 Sputter coater and subsequent degassing using Vacuum Pump Leybovac PT 360/16 before the investigation.

Manganese ions concentration in the medium was measured by Prodigy High Dispersion ICP Leeman Labs. After supplying manganese ions to the cultivated strain, samples for ICP analysis were taken at every 5, 10, 15, 30, 60 and 120 min. Before analysis, all samples were centrifuged to remove the solids.

For microscope observations, the cells were washed twice with distilled water, stained with 2% solution of methylene blue for 20 min at

room temperature, washed again with distilled water and dried for 24 hours at 37°C. Further, the samples were analyzed by using bright field microscope Olympus BX53, Camera SC30 (Japan).

RESULTS AND DISCUSSION

The FT-IR spectra of the precursors and the obtained hybrids are illustrated in Fig.1. As it can be seen, the broad band at ~3400 cm^{-1} is due to Si-OH stretching on the surface of silanols hydrogen and also due to vibration of Si-O-Si units [20-22]. The bands at 3210 cm^{-1} can be attributed to silanol groups [20]. The band at ~1635 cm^{-1} can be attributed to vibration of H-O-H which interacts through hydrogen bonds with silanol groups. SiO_2 network can be identified with bands at around 1057 cm^{-1}, 790 cm^{-1} and 460 cm^{-1} due to vibrations of Si-O-Si bonds [20]. The bands at ~940 cm^{-1} are attributed to Si-OH groups [21-24]. The main difference can be seen for the asymmetric bond at ~3260 cm^{-1} which becomes broader probably due to the formation of hydrogen bonding between organic and inorganic part [20-22].

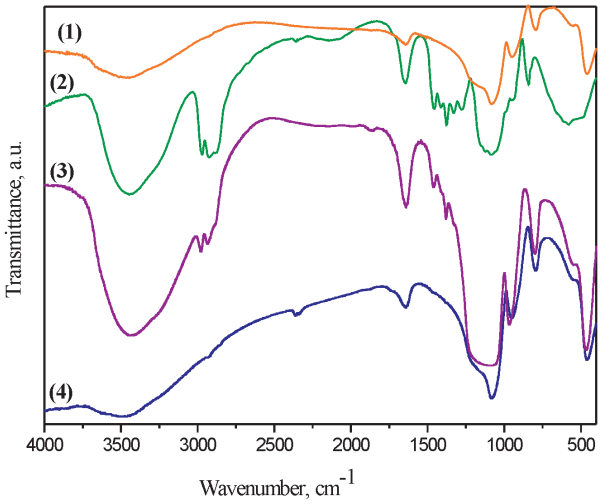

Fig. 1. FT-IR spectra of SiO_2 derived from TEOS (1), HPC (2) and obtained hybrids with 5 wt% HPC (3) and 50 wt% (4)

The surface morphology and microstructure of the hybrid materials was observed by SEM (Fig. 2). From the SEM images it can be seen that the hybrids containing 5 wt % cellulose ether have a smooth surface with wavy character and heterogeneous inclusions, unevenly distributed in the main matrix. The formed microheterogeneities are with average size ~0.5μm. The addition of 50 wt % HPC (Fig. 2, b) led to formation of a homogeneous structure. The hybrids have the so-called "fracture structure", consisting of micro cracks, evenly distributed in the matrix.

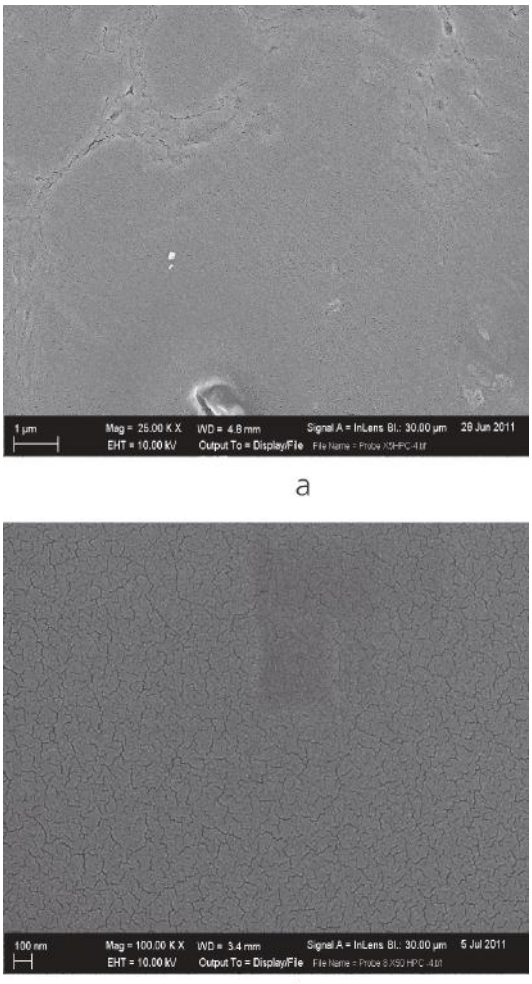

Fig. 2. SEM images of SiO_2/ HPC hybrid materials containing 5 wt% (1) and 50 wt% (2) cellulose ether

The equilibrium state during adsorption of manganese ions by free yeast cells was studied in the range for the equilibrium concentration in the liquid phase, C_e, from 0 to 446 g/L. The experimental isotherm (Fig. 3) illustrates the dependence of the equilibrium specific metal uptake q_{eq} on C_e. Up to concentration of 215 mg/L, the specific uptake increased monotonically. For concentrations in the range from 215 to 380 mg/L (from 5 to 8 mM) the function reaches plateau and further increase in the ions concentration in the liquid phase did not result in higher

specific uptake. Additional adsorption was observed at concentrations higher than 8 mM in the liquid phase.

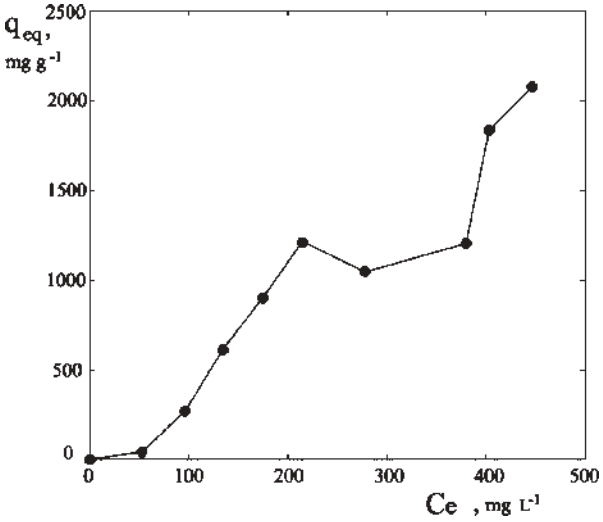

Fig. 3. Experimental isotherm for biosorption of manganese ions by free cells of *Trichosporon cutaneum* strain *R57*

For industrial scale applications, it is very important to immobilize the yeast cells on an inert carrier while maintaining their activity. The feasibility of the proposed in this work materials and methodology for immobilization was proved by series of kinetic experiments, which included immobilization materials with 5% and 50 % content of HPC at initial concentration of the Mn^{2+} ions in the batch reactor 5 mM and 10 mM.

morphology. The experimental data were fitted to the second order Eq. (1). The results are summarized in Table 1 and illustrated in Fig. 5.

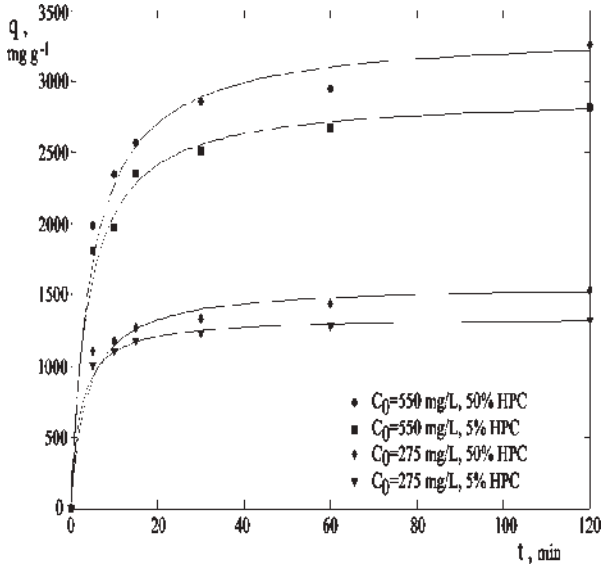

Fig. 5. Kinetics of manganese ions adsorption by immobilized *Tr. cutaneum R57* cells

As it can be seen from Table 1, the correlation coefficient, R values for the t/q vs. t plots are sufficiently high in all cases (R>0.998), which demonstrated the applicability of the second order kinetic model. The good model description is also supported by the observed negligible deviation between the measured equilibrium specific uptake, q_{eq}^{exp} , and the calculated one q_{eq}^{calc}

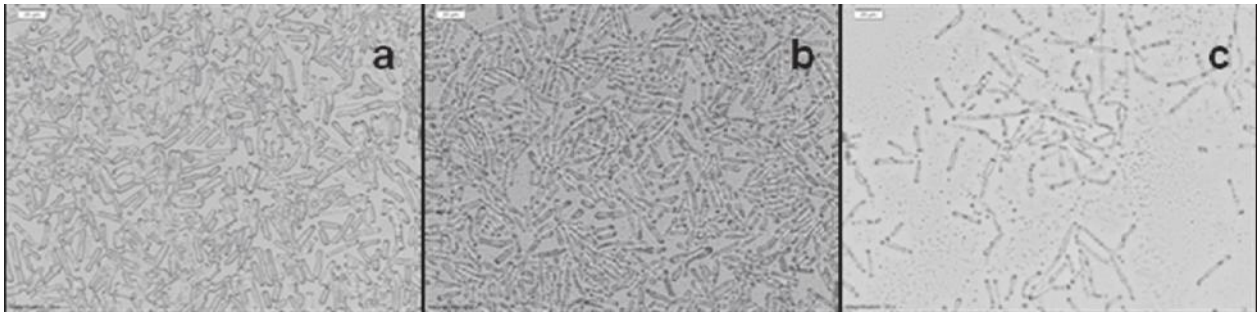

Fig. 4. Microscopic photographs of immobilized *Tr. cutaneum R57* cells onto 50 wt% SiO_2/ HPC hybrid materials; a- Control, b- in the presence of 5 mM $MnSO_4$, c- in the presence of 10 mM $MnSO_4$. Bar is 20 μm

As it can be concluded based on Fig. 4 and indirectly from Fig. 3, the concentration of 5 mM can be considered as inhibitory threshold value. The concentration of 10 mM is moderate inhibitory value, since it was observed an abnormal (less than 3%).

A careful observation of the data presented in Table 1, Figs. 3 and 5 reveals that the immobilization of the cells not only retained but slightly increased their adsorption capacity. This effect is

Table 1. Second order kinetic model parameters

Immobilization material HPC content, wt %	C_0, mg l^{-1}	R	$k_{ads} \times 10^4$ min^{-1}	$q_{eq}^{calc} \times 10^3$ mg g^{-1}	$q_{eq}^{exp} \times 10^3$ mg g^{-1}	$\frac{\left\|q_{eq}^{exp} - q_{eq}^{calc}\right\|}{q_{eq}^{exp}} 100$ %
5	275	0.9999	3.3366	1.308	1.3203	1.6
50	275	0.999	1.7049	1.5689	1.5357	2.2
5	550	0.9997	0.84946	2.8977	2.8158	2.9
50	550	0.9984	0.63087	3.3422	3.4376	2.8

more prominent with higher HPC content in the immobilization matrix (50% compared to 5%) and higher initial concentration of the metal ions in the medium.

The biosorption process includes physico-chemical interactions between metal ions and several anionic ligands present on the biomass such as carboxyl, phosphoryl, carbonyl and sulfhydryl groups. The results in this study clearly supported the benefit of incorporating *Trichosporon cutaneum* strain R57 cells into the studied matrices for removal of manganese ions from waste waters. Thus this study may be regarded as an initial step in the development of technologies for bioremediation of waste water polluted with heavy metals.

CONCLUSION

The obtained encouraging results on enhancing the biosorption capacity of the *Tr. cutaneum* R57 strain cells, the relatively high specific surface, appropriate physicochemical properties and mechanical stability of the synthesized hybrid materials revealed the potential benefits from their application in industrial scale applications for bioremediation of Mn^{2+} contaminated waste waters.

Acknowledgements

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ИМОБИЛИЗИРАНЕ НА *TRICHOSPORON CUTANEUM* R57 ВЪРХУ SiO_2/ ХИДРОКСИПРОПИЛЦЕЛУЛОЗА ХИБРИДНИ МАТЕРИАЛИ И ОТСТРАНЯВАНЕ НА Mn^{2+} ЙОНИ

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Резюме

Синтезирани са нови хибридни материали, съдържащи тетраетокси силан (TEOS), като неорганичен прекурсор и хидроксипропил целулоза (HPC), като органичен компонент. Съотношението на органичния компонент беше 5 и 50 мас. %. Структурата на получените хибриди бе изследвана с помощта на инфрачервена спектроскопия (FT-IR) и сканираща електронна микроскопия (SEM). Синтезираните хибридни материали бяха използвани като носители за клетъчна имобилизация чрез прикрепване на филаментозни дрожди Trichosporon cutaneum R57. Този щам е показал способност за отстраняване на манганови йони. Опитните данни за равновесната концентрация на адсорбата при използване на свободни клетки, като адсорбент, са използвани за построяване на адсорбционната изотерма. Проведени са кинетични експерименти по биосорбция на манганови йони с имобилизирани клетки при начални концентрации на адсорбтива 275 и 550 mg/L. Тези стойности са подбрани въз основа на поведението на свободните клетки, илюстрирано чрез адсорбционната изотерма. Кинетично уравнение от псевдо-втори порядък е използвано за описание на опитните данни. Установени са по-високи стойности за концентрацията на адсорбата при имобилизирани клетки и при по-високо съдържание на хидроксипропил целулоза в матриците, използвани за имобилизация.

INVERSE FIELD SOURCE PROBLEMS IN BIOMAGNETICS

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Abstract

Electromagnetic field distribution in biological structures is challenging multidisciplinary problem which may offer many new opportunities for medical diagnosis and therapy due to modern scientific approaches. Investigation of electromagnetic fields, processes and phenomena is significantly improved by new highly precise computational models and field reconstruction strategies. These problems can be broadly divided into two main topics. First is the field of biomagnetic or bioelectric fields produced naturally into the biological structures. Second wide topic contains all problems of interaction of outer electromagnetic

fields with biological structures. In this paper some new approaches and results in determination of bioelectromagnetic fields and bioelectromagnetic interaction are presented. These researches are focused exclusively in the area of inverse source and material properties reconstruction in inaccessible for direct measurements regions. Results from computational techniques are presented and discussed.

INTRODUCTION

Investigation of processes and phenomena during electromagnetic medical diagnosis and therapy is significantly improved by new highly precise computational models and field reconstruction strategies. These problems can be broadly divided into two main topics. First is the field of biomagnetic or bioelectric fields produced naturally into the biological structures. Second wide topic contains all problems of interaction of outer electromagnetic fields with biological structures.

Naturally accruing bioelectromagnetic fields are widely used for functional diagnosis. Applications such as Electrocardiography (ECG), Electroencephalography (EEG), Electrical impedance tomography (EIT) or even Magnetoencephalography (MEG) and Magnetocardiography (MCG) are generally connected with accurate field measurements out of body surface. These data must be later processed for field sources or properties distribution reconstruction. The methods for accurate field and source reconstructions are of great importance for precise diagnosis process.

Interaction of external electromagnetic fields with the human body has gained increasing attention throughout the past years. Electromagnetic phenomena are used day by day as examination and therapeutic tools in various medical applications. On the other hand, owing to the increasing number of electromagnetic and wireless devices in daily surroundings possible adverse health effects of electromagnetic fields could appear. Diagram of the proposed classification with some common bioelectromagnetic applications is presented in Fig. 1. This classification based on field source location against biological object is very suitable for gathering electromagnetic field problem formulations in clear structure.

Up-to-date development of electromagnetic devices, bioinformatics, biomaterials, measurement systems, information technologies, etc. give possibilities for optimal investigations of fields, processes and phenomena in biological structures during their interactions with electromagnetic devices in order to realize precise medical diagnosis and therapy.

Modeling and optimization of fields and processes during energy interaction between electromagnetic device with nonlinear, inhomogeneous and complex biological structures lead to produce of high quality and energy safe medical electromagnetic devices and to realizing of precise medical diagnosis and therapy [1-3].

In this paper the interaction of low-frequency electromagnetic fields with the different organs and parts of human body is analyzed using field modeling, simulations and visualization. Computer modeling of electromagnetic fields distributed in biological tissues is of paramount importance for investigations of processes and phenomena during medical diagnosis and therapy.

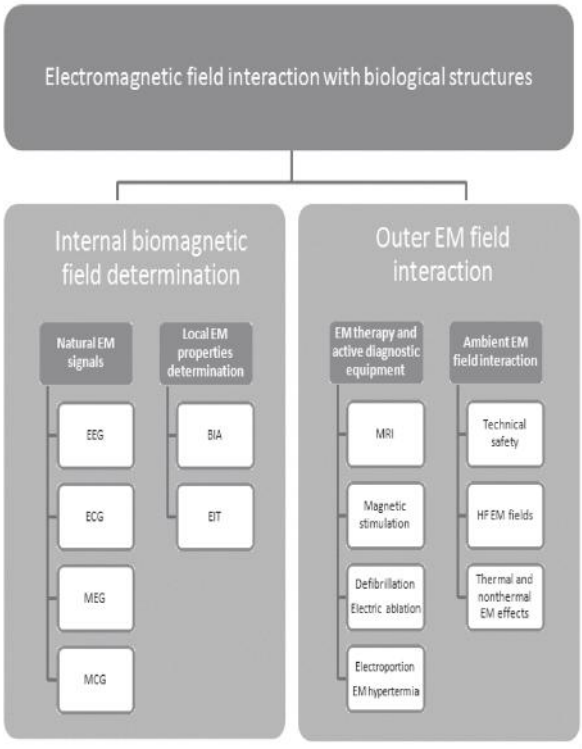


Fig. 1. Electromagnetic (EM) field and source problems classification and applications

Modeling of electromagnetic field distributions in biological objects must be handled by modern numerical method covering all specific anatomical properties of modeled application. Finite element method (FEM) is a powerful numerical method capable to solve such complex problems taking into account all characteristics and special features. Recent achievements of the FEM give possibilities to model fully three-dimensional, nonlinear, inhomogeneous, or anisotropy multi-tissue, and multi-joint systems of biological structures.

In this paper some new approaches and results in determination of biomagnetic fields and biomagnetic interaction are presented. These researches are focused exclusively in the area of inverse source and material properties reconstruction in inaccessible for direct measurements regions. Results from computational techniques are presented and discussed.

to the complicated structure of the human body, consisting of organs with varying dimensions and geometrically complicated shapes, all exhibiting different electrical properties, the major task from the simulations point of view is to achieve a very fine spatial resolution in the computations. Developed for computations 3D human model, containing all major anatomical systems and organs is presented in Fig. 2.

For computer modeling of electromagnetic fields distributions in human body the electromagnetic properties of live tissue have to be incorporated into 3D geometrical models of human organs. The specific electromagnetic material properties are introduced for each tissue of the developed model. Properties of tumor and other specific tissues which may have great dispersion due to many individual characteristics are of special interest. These electromagnetic properties are accurately measured and determinate for investigated cases [6-8].

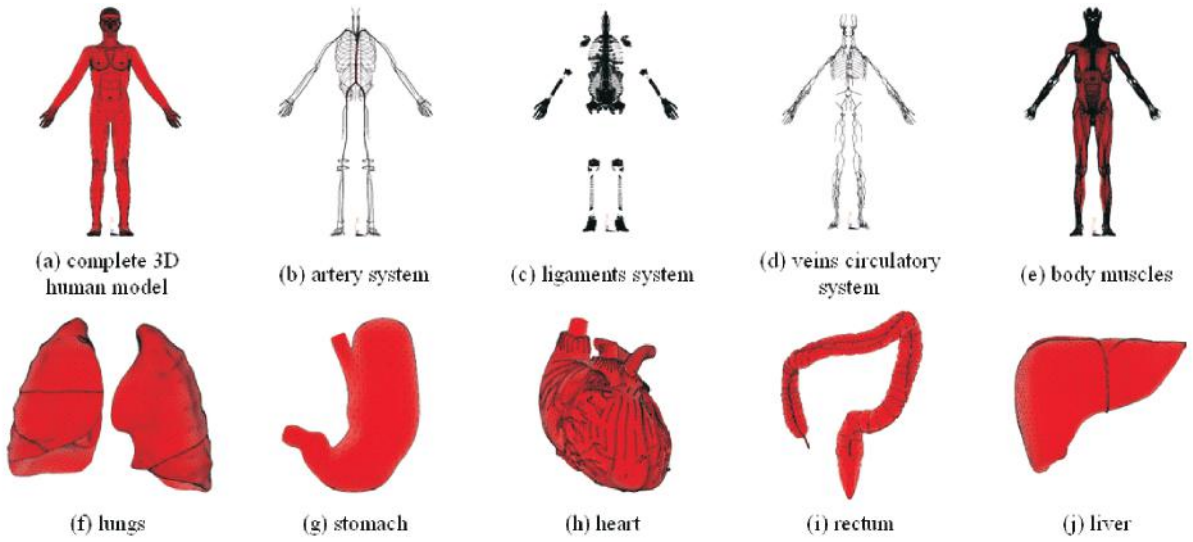


Fig. 2. 3D human anatomy model with some of included organs and subsystems

3D HUMAN ANATOMY MODEL

In order to achieve accurate field distribution results the realistic 3D geometry vector models are developed. The data from modern medical imaging techniques, such as CT and MRI have been used for building of a precise three-dimensional high-resolution anatomical model of the human body [4]. Recently, different techniques have been utilized to convert automatically 3D image data into 3D FEM models or into numerical meshes suitable for FEM analysis [4-5]. Due

INVERSE SOURCE RECONSTRUCTION PROBLEM FOR MCG

Measurement of biologically produced magnetic fields was unknown before the invention of SQUIDs. This is because the fields in question are of such a small value, typically 1nT to less than 1pT. There are many magnetic fields which have been measured, ranging from the susceptibility of tissue to applied magnetic fields to ionic healing currents and those associated with neural or muscle activity. [9]

MCG utilizes SQUIDs to measure the magnetic fields produced in the heart by ionic current flow arising from cardiac muscle contractions. Current MCG techniques have spatial resolutions of 1-5mm but the time resolution is between 1-5ms allowing real time imaging. The main problem comes from the actual computing of the current density. For every magnetic field recorded there is infinite number of current densities that would give rise to such a field. Heart is considered to be a much simpler shape which contains current sources. Actually heart volume is investigated as magnetically homogenous, fine structure mesh where current sources or electric dipoles are searched. A solution is then computed fitting certain boundary conditions (such as distribution having the least energy). In spite of these simplifications, an accurate current distribution can be obtained.

Here fast inverse approach for reconstruction of current sources by locally measured magnetic field data is presented. Results for reconstructed current dipoles for heart volume according to the measured surface data are presented in Fig. 3-a-c. The magnetic field distributions were measured using SQUID biomagnetometer system containing 31 magnetic sensors. Complete SQUID data set is presented at [9].

(1) $$\frac{1}{\mu}\nabla^2\mathbf{A} = -\mathbf{J}$$

(2) $$\mathbf{A} = \mu\int_{\Omega} G\mathbf{J}d\Omega$$

where G is 3D Green's function given by equation (3)

(3) $$G = \frac{1}{4\pi r}$$

where r is the distance radius-vector between coordinates of each measurement mesh nodes and current source nodes,

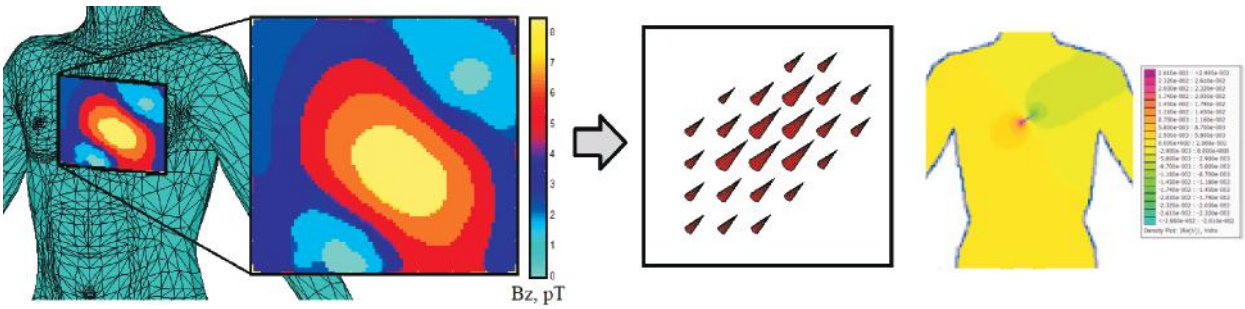
(4) $$r_{ij} = \sqrt{(x_j - x_i)^2 + (y_j - y_i)^2 + (z_j - z_i)^2}$$

where (x_i, y_i, z_i) are coordinates of each measurement mesh node;

(x_j, y_j, z_j) are coordinates of current source nodes.

In order to realize the reconstruction approach the whole body domain Ω is divided in elementary volumes $dV_j (j = 1,...,m)$. The magnetic field distribution is measured in n points over the chest surface, Fig. 3.

For determination of field sources distribution (2) is transformed in system of linear equations (5).



(a) Measured magnetic flux density data

(b) reconstructed current densities

(c) voltage distribution

Fig. 3. Measured magnetic flux density, z-direction in chest region, reconstructed current density vectors in heart layer and corresponding voltage distribution in heart layer

The developed approach is suitable for current source distribution reconstruction in magnetically homogenous, linear non-magnetic media ($\mu = \mu_0$). Solution of (1) for magnetic vector potential A is expressed by (2)

(5) $$\mathbf{Y} = \int_{\Omega} C\mathbf{X}d\Omega,$$

where $\mathbf{Y}$ is the vector with measured values $\mathbf{Y_i}$ presented by (6) and (7).

(6) $$\mathbf{Y} = [Y_1, Y_2, ..., Y_n]^T,$$

(7) $$\mathbf{Y} = \sum_{j=1}^{m} c_j x_{ij} = c_1 x_{s1} + c_2 x_{s2} + ... + c_m x_{sm},$$

where x_j is given by equation (8)

(8) $$\mathbf{x}_{sj} = \mathbf{X}_{sj} dV_j.$$

In elementary domain dV a constant current density value is used $X_{sj}=const.$

c_i is determined with equation (9) by Green's function G_{ij} for $i=1,...,n$ and $j=1,...,m$.

(9) $$\mathbf{c}_j = [G_{1j}, G_{2j}, ..., G_{nj}]^T.$$

Finally c_i is given by equation (10).

(10) $$\mathbf{c}_j = \frac{\mu_0}{4\pi}\left[\frac{n_j e_{1j}}{r_{1j}}, \frac{n_j e_{2j}}{r_{2j}}, ..., \frac{n_j e_{nj}}{r_{nj}}\right],$$

where

n_j is the normal direction vector with direction of x_{sj};

e_{ij} - direction vector between current source j - point to field measurements i - point;

r_{ij} - radius vector between current source j - point to field measurements i - point.

Accurate 3D images of the reconstructed current density within used initial data are presented in Fig. 3. The magnetic field distributions were measured using SQUID biomagnetometer system containing 31 magnetic sensors [9]. Magnetic field flux density is measured in a moment of the T-wave. Current density vectors are reconstructed in a structural mesh of 10x10 nodes, with spacing 10mm, which presents a 2D quadratic plane in heart region, Fig. 3-b.

MODELING OF MRI GRADIENT FIELD INTERACTION WITH THE HUMAN BODY

Effects of low-frequency electromagnetic fields encountered specifically during MRI are examined. The primary biological effect at typical switching gradient frequency of 1kHz is non-thermal peripheral nerve stimulation. These effects are successfully simulated with 3D human model. [10-11]

Electromagnetic field distribution inside the conductive tissue region depends on the time varying magnetic flux density. The activation source is the electric field **E** induced in the tissues and expressed by using Faraday's law

(11) $$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t},$$

where $\mathbf{B}$ is magnetic flux density, $\mathbf{B} = \mu\mathbf{H}$, μ is magnetic permeability, $\mathbf{H}$ is magnetic field intensity.

According to $\nabla \mathbf{B} = 0$ and introducing magnetic vector potential $\mathbf{A}$ by $\mathbf{B} = \nabla \times \mathbf{A}$ from (11) is obtained

(12) $$\nabla \times (\mathbf{E} - \frac{\partial \mathbf{A}}{\partial t}) = 0.$$

Using electric scalar potential V_ε, (12) can also be expressed as

(13) $$\mathbf{E} = -\frac{\partial \mathbf{A}}{\partial t} - \nabla V_\varepsilon.$$

The induced current density $\mathbf{J_e}$ satisfies the Ohm's law is

(14) $$\mathbf{J}_e = \sigma \mathbf{E} = -\sigma(\frac{\partial \mathbf{A}}{\partial t} + \nabla V_\varepsilon).$$

According to Ampere law can be written

(15) $$\nabla \times \mathbf{H} = \mathbf{J} - \sigma \mathbf{E}.$$

The governing equation for magnetic vector potential-electric scalar potential (A-V, A) formulation is

(16) $$\nabla \times \frac{1}{\mu} \nabla \times \mathbf{A} = \mathbf{J} - \sigma(\frac{\partial \mathbf{A}}{\partial t} + \nabla V_\varepsilon).$$

Three-dimensional harmonic and transient electromagnetic problems with Zero value Dirichlet boundary condition for the free space boundary according FEM formulation are utilized for the field analysis in human tissues.

The induced currents in tissue are expressed by (14).

Heating effect on the tissue can be calculated with Joule heating Q.

(17) $$Q = \rho J^2,$$

where $\rho=1/\sigma$, is specific electrical resistance.

Open magnetic circuit MRI system shown on Fig.4 is investigated. A three-dimensional harmonic and transient magnetic problem according FEM for magnetic vector potential-electric scalar

potential (A-V, A) formulation was used for the analysis. Switching gradient frequency of 1kHz is applied.

Constructed realistic 3D anatomy models are imported in ANSYS software and the electromagnetic models are built according to the described FEM formulation. The electromagnetic model represents the organs field distribution under outer source. Electromagnetic properties for different model regions are acquired by impedance measurement system [8].

Some modeling results are presented in Figs. 5-8.

Real and imaginary parts of magnetic vector potential distribution and magnetic flux density distributions are shown in Fig. 5 and Fig. 6, respectively. Gradient coils influence over the chest region can be seen by maximum values above the coil system.

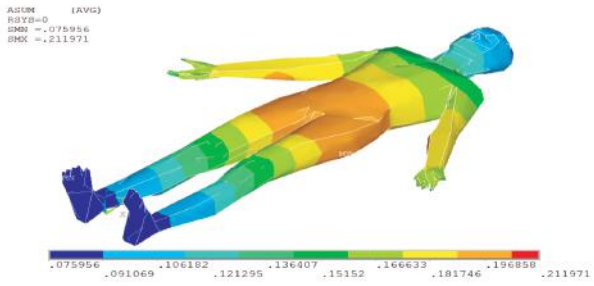
Real and imaginary parts of scalar electric potential distribution are shown in Fig 7-a, b.

Induced current density distribution in 3D human model is shown in Fig 8.

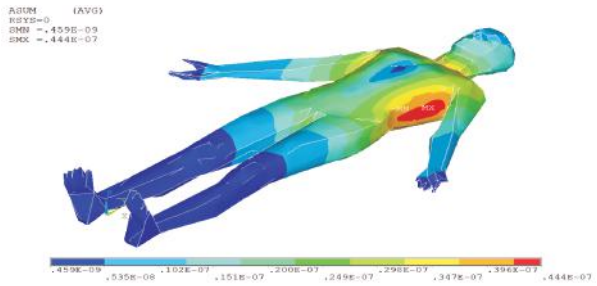
(a) Open MRI system

(b) Coil system

Fig. 4. Open magnetic circuit of investigated MRI system main with correcting and gradient coils

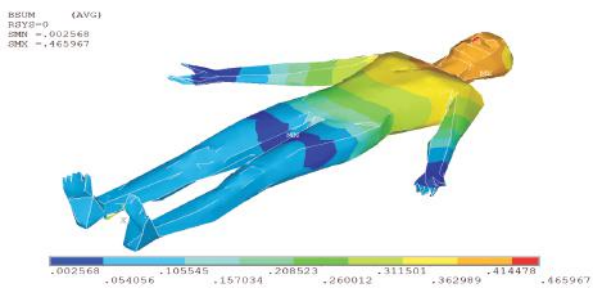


(a) MVP - real part

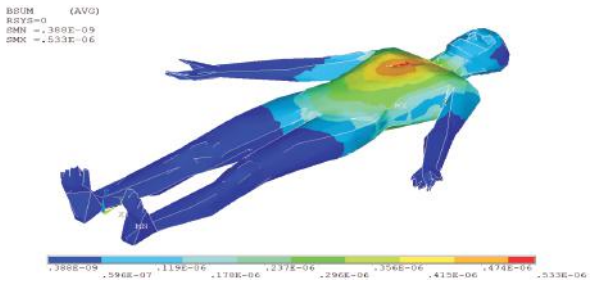


(b) MVP - imaginary part

Fig. 5. Magnetic vector potential (MVP) distribution in 3D human model

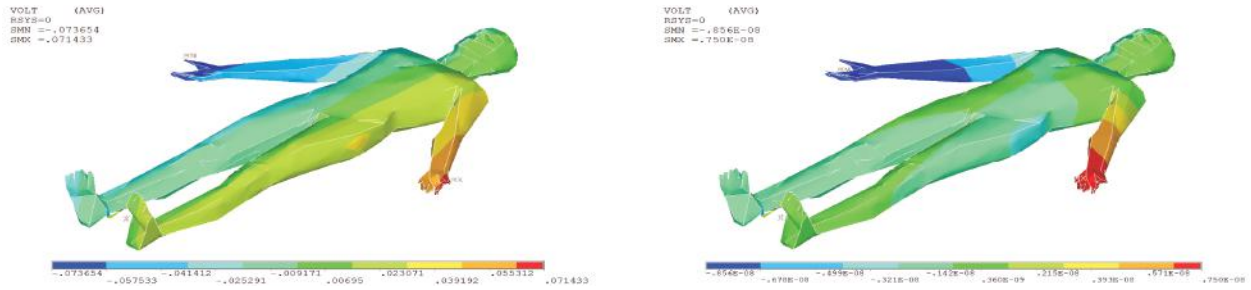


(a) Magnetic flux density - real part



(b) Magnetic flux density - imaginary part

Fig. 6. Magnetic flux density distribution in 3D human model



(a) Scalar electric potential - real part (b) Scalar electric potential - imaginary part

Fig. 7. Scalar electric potential distribution in 3D human model

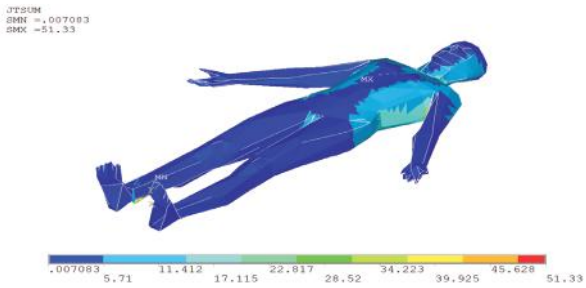


Fig. 8. Induced current density distribution in 3D human model

ELECTRIC ABLATION

Electric ablation is used to destroy pathologic tissue by heating of the targeted tissue with directly applied electric current. For tumor treatments the electrical ablation is currently the most commonly used thermal ablation therapy applied in a clinical setting. Cryoablation, which is another fairly common tumor ablation modality, uses freezing instead of heat to kill tissue [12]. Current studies suggest that electrical ablation is much better than chemotherapy alone [13]. Radiofrequency ablation and microwave ablation are clinically used for minimally invasive treatment of inoperable tumors of liver, as well as other organs such as lung, kidney and bone [14].

We develop a model for investigation of tumor liver ablation. The tissue regions and electromagnetic characteristics are determined and incorporated in the model. The tissues are directly heated by electrical ablation device and the thermal conduction of tissues is dominating in the thermal process. The model is designed to use common properties of ablation devices currently applied in clinical practice.

Electric field modeling

The governing equation for electric field distributions is the Laplace equation Eqn. 1 [5] with respect to electric scalar potential V,

(17) $(\sigma - j\omega\varepsilon)\nabla^2 V = 0$,

where σ is specific electric conductivity, ε - permittivity of investigated tissues.

The electric field E is determined by (18)

(18) $E = -\nabla V$.

FEM is applied for analysis of electric field.

Field sources are pair of electrodes, operating at constant frequency of 500kHz with rms voltage of 10kV.

Thermal field modeling

The thermal field at steady-state is governed by the Poisson's equation with respect to the temperature T

(19) $\lambda\nabla^2 T = -Q$,

where Q is the heat source defined by electric field, i.e., by the solution of the electric field problem;

λ is the thermal conductivity.

Heat source density Q defined by (20),

(20) $Q = \sigma V^2$.

Coupled field problem

For tumor temperature determination a first-order coupling between two field models (electric and thermal) is made. The coupling of the two fields is in one direction. The heat source depends on the results of the electric field

analysis. Indirect coupling between the two problems (electric field and thermal one) has been utilized. The two problems are solved successively and for thermal field solution the heat sources are defined by the solution of the electric field problem. The architecture of the couple electric-thermal field problem solution is shown in Fig. 9.

CONCLUSION

Some new approaches and results in determination of bioelectromagnetic fields and bioelectromagnetic interaction are presented here. These researches are focused exclusively in the area of inverse source and material properties reconstruction in inaccessible for direct measurements regions.

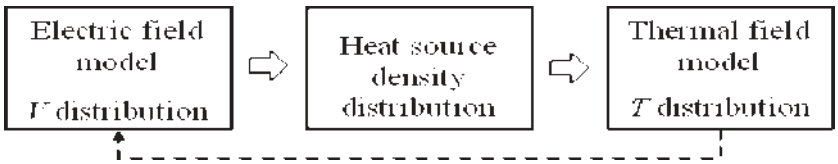
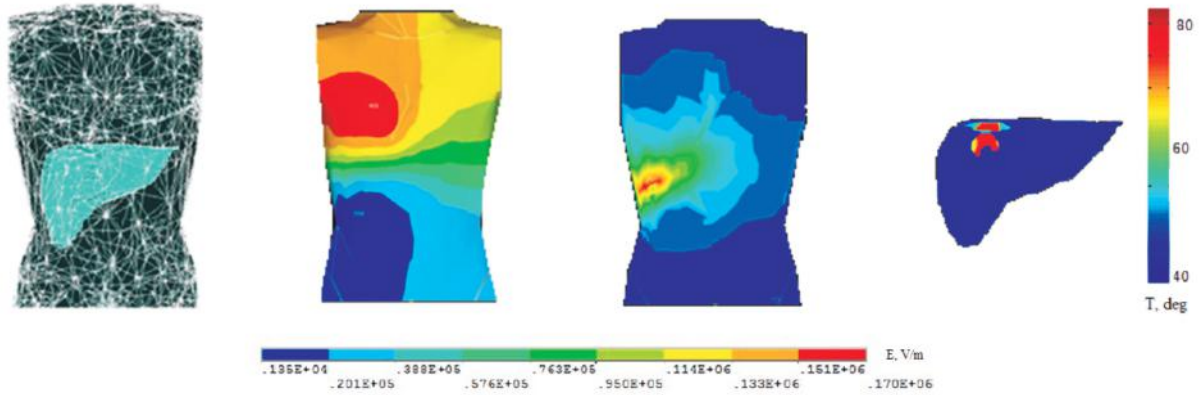


Fig. 9. Architecture of the couple field problem solution



(a) 3D model (b) electric scalar potential (c) inside electric field (d) temperature distribution

Fig. 10. Electric ablation model results: 3D model, electric scalar potential distribution in surface and cross-section area, temperature overheating distribution in liver and tumor area.

Results from coupled field modeling process are presented in Fig.10. Electric scalar potential distribution in 3D model surface is shown in Fig.10-(b). Electric scalar potential distribution in 3D model cross-section is shown in Fig.10-(c). Treated tumor is a complexly shaped object with maximal size of about 10-15mm. Final temperature distribution in liver region is shown in Fig.10-(d), where the treated tumor is heated to nearly 40° C above body temperature. Thermal impact in tissue between electrodes outside tumor volume may also be observed in the top side of liver. This negative effect must be minimized for good electrical ablation treatment planning. Optimal individual therapy strategy could be organized including this kind of field modeling procedure.

Proposed applications, forward and inverse problems for them are very suitable for determination of energy interaction optimization between biological structures and electromagnetic devices which will be determined at different conditions and modes.

Three-dimensional adequate mathematical models taking into account all essential features of the processes at corresponding modes of electromagnetic device operation as well as media parameters of biological structure give possibility for factor and condition influence study over these fields, processes and phenomena. Quantitative results for electromagnetic and thermal field distribution in inner organs can be easily made. Complete procedure of electromagnetic field model building is applicable for device

and process optimization due to a desired electromagnetic or corresponding thermal impact over a known object.

Proposed approaches are envisaged to be developed on the base of the three-dimensional models with application of up-to-date methods and technologies in the area of numerical methods, signal and image processing, optimization, image reconstructions and visualization. The models developed could be applied for solving other problems in different scientific areas. The models and approaches must be generalized in theoretical aspect for solution of forward and inverse problems in electromagnetism and bioelectromagnetism.

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ОБРАТНИ ЕЛЕКТРОМАГНИТНИ ЗАДАЧИ В БИОЕЛЕКТРОМАГНЕТИЗМА

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Резюме

Определянето на електромагнитното поле в биологичните обекти е важна интердисциплинарна задача, решаването на която може да предложи много нови възможности за медицинска диагностика и терапия благодарение на разработените нови методи и средства. Изграждането на нови прецизни изчислителни модели и подходи за обратна реконструкция на полета дават възможност за изследване на полета и процеси с висока точност. Тези задачи могат да бъдат разделени на два основни класа. Първият е областта биомагнитните или биоелектрическите полета естествено създадени в биологичните обекти. Вторият обширен клас обхваща всички задачи на взаимодействие на биологичните обекти с външни електромагнитни полета. В статията са представени някои нови подходи и резултати в изследването на електромагнитното поле в биологични обекти. Тези изследвания са съсредоточени предимно в областта на обратните задачи за определяне на източници, свойства и разпределения на електромагнитни полета в недостъпни за директно измерване области. Представени и дискутирани са резултати от разгледаните изчислителни методи.

AN INTEGRAL SOLUTION FOR TELEMEDICINE AND HOSPITAL INFORMATION SYSTEM

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Abstract

The aim of this paper is to present results obtained in the context of joint research with Medical University Sofia about investigations, design, organisation and future expansion of a hospital information system. This research is in the area of e-Health and Telehealth and is oriented to complex investigation of new concepts and methods for continuous acquisition of patient's vital data, transmission, collection and binding of that data for diagnostic and disease tracking purposes as well as investigations on relevance of life quality and healthcare based on the e-Health technologies.

INTRODUCTION

The aim of this paper is to present results obtained in the context of the "Medical data acquisition, processing and collection for e-health solutions" joint research project for investigation, design, organisation and future expansion of a hospital information system [1].

The research started with deep investigation of current status of hospital information systems on the territory of the Medical University Sofia and implementation of a pilot version of integrated solution. Elimination of the usual paper-based information exchange to e-health and IT-based one is one of the major project topics.

Medical University Sofia is a huge hospital complex situated on a large territory and has tens of different clinics, laboratories and buildings.

Results of this investigation and some of implemented solutions are discussed hereafter. We focused on the following main problems:

• Interface incompatibility between different medical systems and apparatus - Medical systems from different vendors are incompatible. Most of them are designed to transfer data directly to computer application with HMI.

• "Data are distributed, heterogeneous and changeable"- Hospital medical systems collect a diverse variety of patient information represented in many digitized or hard-written types. Creation and support of patient's analyses library is a problem solved under presented project.

• Data validity, security and protection - Data validity is very important to make decision-making process stable and safe. This includes time validity and safety and security of delivery. Data access and privacy are very important and have to provide end-to-end security and validation in the system.

• Tracking patients when they are out of the hospital - technical, medical and economical aspect.

• IT problems of a) archiving and b) digitalisation of paper-based video-images.

• Improvement of analyses of medical video-images.

Of course, this list cannot pretend to cover all possible aspect of hospital information systems, solutions, problems and similar. The structure of implemented intelligent hospital information system is presented briefly at the end of this material.

INTEGRATION OF AVAILABLE MEDICAL APPARATUS AND SOFTWARE SYSTEMS IN NEW SYSTEM

Starting with the investigation of how to integrate available equipment in one system we contacted directly vendors' representatives for detailed technical data. The equipment includes from very modern to 10-15 years old machines. Interfaces to humans and to other computers vary much. Computer interfaces include RS232 / RS485 and Ethernet wired connections, floppy-disk data exchange and on-screen or printed

output. Protocols are very different, too. All this stands as big challenge to the integration team. We contacted technical groups supporting other hospitals and found that even products from one and the same vendor are not fully compatible on interface and protocol levels. As an example DICOM-based image systems can be shown. DICOM has variety of dialects and needs additional processing to make all sources fully compatible with all visualisation systems.

Data integration

A hospital medical system collects a variety of patient information represented in many digital or hand-written types. All over the world, a huge number of standards of organisation and representation of the Electronic Health Record exist (HER) [2, 3]. Unfortunately, many of them depend on local law regulations and even in European Community they are not synchronised. Hospital data (not originally included in HER) are harder to track - they include information from many sources - diagnoses, many types of laboratory results, imaging results - X-ray / ultrasound / scanners / Doppler, medications, consultations. These data are very distributed. In a large hospital like the Medical University Sofia original data are held on the laboratories and clinics servers. They have to be exchanged and bound in patients' records. Such a database did not exist before the beginning of this project. One of the biggest problems was the fact that data are very changeable, the amount increased all the time and the structure depends on uncontrollable variety of external (mostly human) factors. Today, an information system based on unified generalised structure which covers all types of available data is implemented in the MU Sofia medical information system [4, 6].

Tracking patients when they are out of the hospital

One big objective of the presented project was to implement tracking patients when they finish the hospital phase of medication. Experimentation field was clinic of pulmonary diseases. Technological idea was to equip patients with mobile sensors controllable via smart phones and to collect and transmit data using SMS and/or data channels. Currently in the non-military areas of application the most common mobile/wearable biosensors available on the market are 1 or 3 lead ECG sensors, spirometers, pulse and blood pressure meters, thermometers, SPO2 meters and glucometers. All of these sensors were investigated [7].

Three to two years ago these devices had mainly a wired connection to a computer. Their weight was not negligible, either. Today these types of devices are greatly improved. They have WiFi or Bluetooth wireless communication capabilities. The weight is tens of grams. Power consumption is also much reduced. All this is a basis for increasing the remote acquisition of human vital data and doing some in situ pre-diagnostics or pre-analysis.

Basic structure of a remote data acquisition node

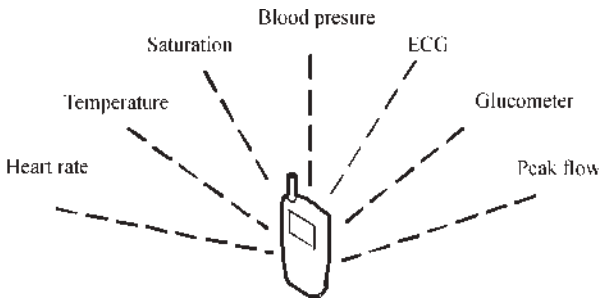


Fig. 1. Typical structure of a vital data acquisition node based on a smart phone

Today smart phones are equipped with Bluetooth and/or WiFi communications. Depending on the software platform and operating system they can execute Java applets and/or C/C# applications. Devices provided by different vendors support different communication protocols. This requests the installation of additional communication drivers on the phone (over Bluetooth or WiFi). The most common versions are based on Bluetooth communication. Depending on the drivers not every sensor can be connected to every smart phone.

Many providers offer closed multi-layer systems which does not allow their sensors to be used with other systems and also excludes the possibility to extend the system with other sensors. When such providers do not offer a full list of sensors, remote patient support becomes limited to the available peripherals.

Another problem is that some data acquisition

drivers are very aggressive and start to connect and transmit acquired data to the upper system levels without user permission. This increases the exploitation price and lowers battery life.

Fortunately today providers are starting to sell their sensors with open source libraries or libraries of modules configurable by the customer (or IT personnel). We studied families of sensors provided by Intelesens Ltd., Medic4All, THOR Medical systems, Card Guard, Aerotel and AIT GmbH.

A typical structure of a remote data acquisition node based on smart phone is shown on Fig.1. It includes the most common available today sensors for vital data.

Depending on the functionality of the sensors data can be received on demand or continuously (sessions with unlimited time length).

Modern mobile phones have large memories and can save very big amounts of acquired data autonomously. This reduces the need for permanent connection to the hospital servers.

Typical layered system is shown on Fig. 2.

Figure 2 presents a new element - a data

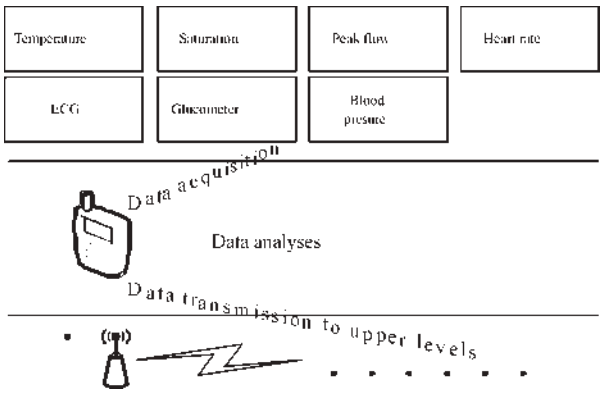


Fig. 2. Layered structure of a vital data acquisition system

analysis module. Additionally a bi-directional connection to the upper system layers provides the possibilities to send commands to the phone-based system and to enhance the quality of data acquisition.

The following are our conclusions about technical aspects:

• Many providers offer closed multi-layer systems which does not allow their sensors to be used with other systems and also excludes the possibility to extend the system with other sensors. When such providers do not offer a full list of sensors, remote patient support becomes limited to the available peripherals.

• Another problem is that some data acquisition software for smart phones is very aggressive and starts to connect and transmit acquired data to the upper system levels without user permission. This increases the exploitation price and lowers battery life.

• Uninterrupted usage of any type of sensor is impossible. Phone's battery cannot last more than 6 hours on intensive Bluetooth or WiFi communication. Investigation oriented to sports control envisaged that this technology can be used only for approximately short training processes.

• Performance analysis of the computational power targeted smart phones envisaged that in case of ordinary load we can conclude that the smart phones studied can run in real-time many on-site analyses.

Conclusions about medical and economical aspects:

• From the very beginning of experiments, supervising medical doctors concluded that this remote control over their patients' data helped them to prevent possible dangerous situations but this needs a lot of human effort. A system to control vital parameters is needed. It cannot be simple "in/out of boundary" control but complicated multi-variable decision-making system. Presently, some work is in progress but it still needs a lot of investigations.

• Instructions to patients about interpreting very simple raw data from sensors decreased number of hospital visits based on apprehensions. Psychologically, patients felt much comfortable when they knew somebody keeps an eye on them. Thus hospital expenses were decreased.

One important remark is that because of financial limitations, the number of obtained sensors is relatively low and the investigation was useful for experimental conclusions and future directions but is not enough representative for statistical purposes.

DATA VALIDITY, SECURITY AND PROTECTION

Developing and implementing hospital infor-

mation systems including remote data acquisition requires strong data protection. But before this, some other problems important for the patients have to be resolved. First is data validity. Investigating paper-based process we found that in many cases information is wrong or interpreted inadequately or simply lost somewhere in document paths. This is a well-known administrative problem but here it is important because human health depends on it.

Where the biggest problems are:

• Remote data acquisition via smart phones needs data validation and time stampings. Because sensors are worn by patients and acquisition and communication software is run by them too, improper measurements and operations are common.

• Inter-hospital data exchange is not always marked properly. Results for one patient can be assigned to other. This is a usual human error - to mark a box near the exact one or to change some letter or digit.

On the one hand, medical doctors are overloaded and normally hate to go through deep and strong security system. From patients' side, data protection and security are very important. These opposite restrictions make solutions hard to design. Currently, we think that only hardware identification systems fast enough and easy to use can solve identification problems and access rights, but even this opens a new domain of problems.

Digitalisation of film and paper archives

Designing the solution for integrated hospital information system required us to think how to transfer current archives in a digital form. Of course, the problem for archive digitalisation has existed for more than 20 years. The main problem we had to solve was digitalisation of video imaging library. Investigation about possibilities to digitise images of different types envisaged that the on-hand solution to use flat-bed scanners is not applicable. Available scanners for large X-ray pictures start from 15000 USD. Services for film scanning are from 2 to 10 USD depending on picture and requirements. Financial limitations directed us to design low-cost scanners for all kinds of films and pictures. Their price is much lower and resolution is better than film grain. This system is equipped with software for images post-processing. Some results are presented in [8].

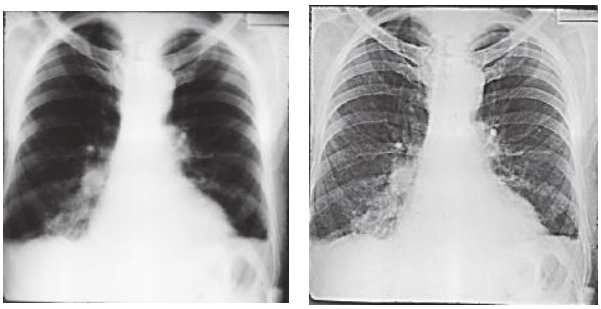
A very important additional problem is the size of a digital archive. For medium level Bulgarian hospital (covering 30000-50000 people) only DICOM library is more than 5-6 Tbs/year. Today, disk devices have huge capacities but nevertheless increasing hospital data server with 10TB/year is problematic.

A major problem of diagnosis methods based on X-ray images is images' quality. This applies to all diseases where one of the following changes in image can be observed: changes with a small X-ray density, the appearance of shadows in the image, soft shadows, structural formations with a certain shape (linear, circular or otherwise), etc. Pulmonary diseases are targeted here because in many cases X-ray image is the only way to diagnose in time diseases like peripheral lung cancer, secondary tuberculosis, pulmonary embolism, bronchiectasis, echinococcus of the lung, etc. The low quality of X-ray images is one of the reasons for hypo- or hyper-diagnosis as a result of incorrect deciphering. This problem is even more pronounced using the old type X-ray images (analog images). New digital X-ray machines do not increase the main quality of images. They are only faster and the image is better measurable.

Digitalisation of medical images is a very hard task. Our target was perception improvement of processed X-ray images under elimination medical artifact: only after eliminating the possibility of the occurrence of medical artifacts developed techniques and methods can be used for the purposes of computer-assisted diagnostics. The difference between human eyes and digital cameras as a vision device is due mostly to different color spaces and the specific properties of human vision known as 'visual weight' and 'approximate color consistence' [8]. As a result in many cases when human percepts X-rays as grayscale images, digital cameras produce color image. In all these cases a conversion color-to-grayscale is needed. This is risky because methods for this conversion can produce artifacts and some of them can be characterized as medical artifacts [9].

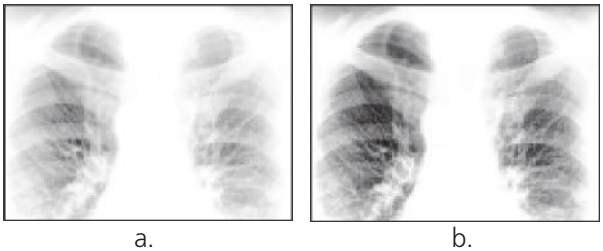
To improve the quality of the digitalized X-

rays numerous studies on the filtering methods, methods for contrast enhancement, and combined methods were carried out. Tests were conducted with second-order structures, too: the X-ray image of the lung has many transitions between areas of soft tissues and bones, but they have large differences in contrast and brightness in the final image; this generates high frequency components the result of which is artifacts in the image. Some results of these investigations are shown in Figures 3, 4 and 5.



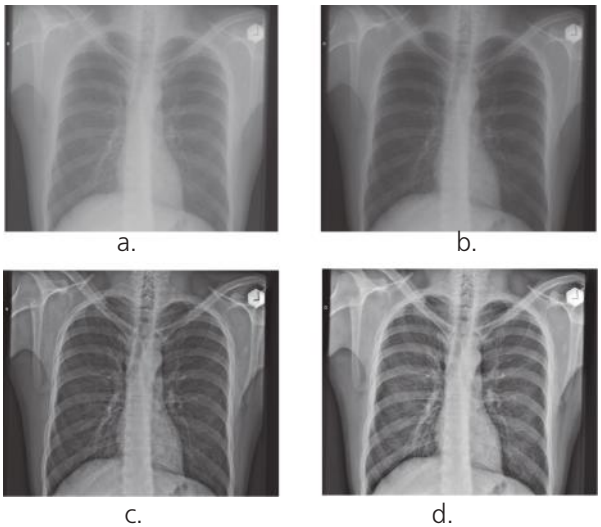
a. b.

Fig. 3. X-ray image with underexposure: (a) original (b) after correction



a. b.

Fig. 4. Fading of X-rays as a result of improper storage: (a) original (b) after correction



a. b.

c. d.

Fig. 5. Improved image quality of scanned X-rays: (a) original (b) filtered (c) contrast correction (d) hybrid technique (adaptive algorithms)

The result of our investigation is development of semi-automated techniques for improving quality of digitalized X-rays as an alternative of today used fully manual control by physician approaches.

GENERAL STRUCTURE OF HOSPITAL INFORMATION SYSTEM

The **Intelligent Hospital Information System** structure and subsystems are shown on Figure 6. It offers the following features:

- Unified environment for data exchange between installed apparatus and systems in the hospital
- Tracking the full process of hospitalisation of every single patient
- Data collection and storage for every medication and procedure
- Offers Remote Medical WWW Services for out-of-hospital health tracking and care
- Management of all procedures and medications
- Administrative tracking of all patients
- Remote messaging of medical personnel about health status of selected patients based on remote vital data acquisition and control
- Extension background for expert medical systems for analysis and control of health status for every single patient

The future target of this work is to establish an environment for transformation of the treatment data to knowledge system which will improve the following elements:

- To increase the quality of the healthcare and services
- To offer to medical personnel access to the information resources via a heterogeneous communication environment (mesh)
- To offer structured information for increasing diagnosis quality
- In- and out-of hospital life-long patients' tracking

Doctors' network

This network has to provide doctors access to huge data sources (e.g. pictures, video films, etc.) in the hospital. It is useful for accessing data bigger than 50MB via fixed network resources.

Now bandwidth of 1GB is possible and this guarantees on-line diagnostics and data exchange between sources, doctors' terminals and storage servers.

Clinic's server

It controls access over the local network, database consistency and tracks patients at home care.

Fixed hospital network

Fixed clinical network provides connectivity for all machines and apparatus in the hospital from one side and servers and personnel's terminals from the other side. Additionally it guarantees better redundancy and offers possibility to control internal clinical networks loading.

Wireless medical sensor network

This network enables access for the medical personnel to data servers. Additionally, sensors and apparatus generating small amount of data can be mobile on hospital territory. This guarantees unbroken control when patients can carry their vital data acquisition sensors or simply to move patients over clinics without loss of connectivity.

Management server

It controls all administrative processes, hosts all records about manipulations (total and associated to every patient), personnel and patients archive, etc. Based on this, doctors can do different analyses and increase quality of medication.

Main database server

The SAN network offers: on-line information for all patients; fast access to disease history and hospital archive.

Data analyses server

This is the core for future advances. Data mining services will be positioned there. A hospital grid with specialised servers will be built on this basis.

Acknowledgement

This work is started and partially funded by Bulgarian NSF under D002/113-2008 and DRNF 02/3-2009 projects.

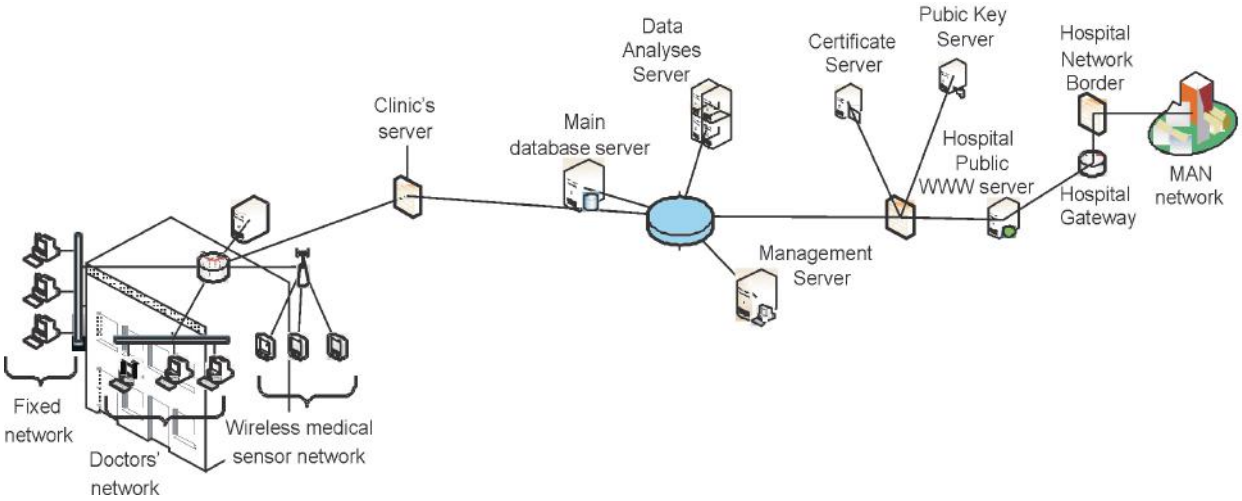


Fig. 6. General structure of IHIS

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ИНТЕГРИРАНО РЕШЕНИЕ ЗА ОБЕДИНЕНИЕ НА БОЛНИЧНА ИНФОРМАЦИОННА СИСТЕМА И ТЕЛЕМЕДИЦИНСКИ ТЕХНОЛОГИИ

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Резюме

Статията разглежда резултатите, получени в рамките на съвместен проект между Технически университет - София и Медицински университет - София. Разглеждат се началните проучвания, дизайна, организацията и бъдещото развитие на болнична информационна система. Това проучване е в областта на телемедицината и на електронното здравеопазване. То е ориентирано към широкомащабно проучване на нови концепции и методи за непрекъснато събиране на данни за жизнените показатели на пациентите, тяхното валидиране, предаване, обработка и съхранение с диагностични цели и за проследяване на заболяванията, както и за проучване на отражението на новите електронни и информационни технологии върху здравеопазването и подобряването на качеството на живот.

BULGARIAN ADDED VALUE TO ERA

THE GENOMIC RESEARCH CENTER – A LONG STORY TOLD IN SHORT

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It is the idea what matters. If you want to accomplish something, you need a good idea. Everything else is secondary. The University of Plovdiv (UoP) has always been among the best Bulgarian universities. Nevertheless, Bulgarian science has long been centered in specialized research centers, such as the Academy of Sciences and the Agrarian Academy. This is one of the largest gaps which should be filled in. This lack of collaboration between different groups and faculties, as well as the lack of interdisciplinary studies constitute considerable problems. My "childhood dream" from years ago was to develop a high quality research infrastructure at the University, with which to compete with the big players in the game. For the last 20 years, we have perused the idea of developing research in the fields of Molecular Biology and especially Plant Molecular Biology and Biochemistry - laboratories, equipment, but most importantly - human potential. I was lucky enough to understand that humans come first - you may have funds and equipment but without trained personnel you would still not succeed.

This idea has to be developed in the conditions of severe funds restrictions. We have started almost from scratch - as a Department of Plant Physiology with 5-6 people, obsolete and old-fashioned equipment and shabby laboratories mainly used for teaching with methods from 50 years ago. We had no other option but to send our people all over Europe for training and research. This primarily led to the specialization of people in diverse research areas of Molecular Biology. Also, we tried to select good quality researchers and only the most devoted "survived". After returning, people created their research groups and this is how we founded the Genomics Research Center (GRC) at the Faculty of Biology, University of Plovdiv and recently a spin-off under the same name.

Times came when national financing started to rise, we participated in several EU programs for University teaching enhancement (TEMPUS), research programs including NATO and Swiss National Science Fund, but our main success was our participation in a number of Framework Program projects for which the GRC got a Pythagoras award (2009) as a regional centre with the highest Framework Programs 6 participation. Inevitably we came to understand that we needed a really large project to strengthen the capacity of the Center with which concentrate our studies in a more focused area. Systems Biology, and especially Genomics and Bioinformatics, seemed like the logical choice for a topic for the project. The main breakthrough came when the GRC received serious financing from the Bulgarian National Science Fund, one of the projects being aimed at establishing a regional center for genomics and bioinformatics. Unfortunately, the Fund literally "broke" and the center was not established as planned.

The EU program REGPOT was an ideal choice to make the dream come true. After several difficult and highly competitive submissions (three

years consequently, which in itself is another thrilling story), we have finally succeeded in securing a project with the acronym BioSUPPORT from this program with real financing to back it up. Just as an university's findings are useless if kept within a department, so specialists' knowledge is ineffective if not brought into a wider picture. In keeping with this thought, BioSUPPORT emphasizes that collaborative knowledge is a key in the future of the GRC and stresses that their biggest goal is to foster the integration of the University into the wider stage of European research. The variety of successful and worthy projects currently being conducted at the University is a strong indication of the need to work together. BioSUPPORT emphatically endorses this focus, aiming to extend it beyond the confines of the Faculty and highlight the importance of networking to provide a reinforced web of success. Such a collaborative spirit will not only develop the reputation of University of Plovdiv internationally, but will also facilitate knowledge transfer, as well as advance the field greatly.

Now, the situation at the GRC at the UoP is rather complicated. At a time when many countries and institutions are making cuts, the University of Plovdiv and the GRC are focused on expansion. This inevitably leads to difficulties in securing the necessary funding and resources. The current situation in Bulgaria is not much different from that of many other European countries. Over the last three years, the national research financing has been cut considerably. As mentioned above, the Genomics Research Centre has won seven large projects from the Bulgarian National Science Fund (BNSF) and, together with financing from the Framework Programs, has started extensive development of research infrastructure and human potential in the area of molecular biology and bioinformatics. This success is a direct result of the long-lasting process of human potential consolidation, from the Department of Plant Physiology of 1989 with just 5-6 researchers to the GRC of today with over 40 people.

Thanks to the largest ever financing from FP7 as the BioSUPPORT project and despite the significant restructuring of the BNSF during this time, the UoP now is in the rather exciting position of having more research funds than ever before. Even better, this is one of the most exciting research areas in biology and for the most developed research entity - the GRC. With this financing, we are "doomed" to further expand hoping that this project and our efforts will have a sustainable future. To secure this future, we hope to be able to establishing a Technology Centre with the help of the Structural Funds, as well as a Technology Park with the help of industry investment, with both structures comprising almost the same researchers involved in BioSUPPORT. We hope that the Cooperative Program from the FP7 and future Horizon 2020 will provide additional funding.

Some of the main goals of establishing the GRC were to consolidate our researchers in UoP, in addition to researchers from groups at Faculties of Physics and Chemistry, as well as to find a common basis for collaboration. In this way, I hope to help transform the University in a center of excellence in one specific scientific field. For the last 20 years, this has been the area of systems biology. Along with food biotechnology, these were the best developed areas at the University, distinguishing it from other large Bulgarian universities. The main aim of the project is to reinforce the research infrastructure and human potential in those areas and to consolidate science in these fields of study. Ideally, the project would turn the University in one of the best research institutions in national research and development. Another long-term goal is to overcome the gap between education and research at the University, especially in the field of natural sciences.

Young researchers and PhD students benefit most from the project and the center. Significant part of the financing from the REGPOT project is for mobility, giving young people opportunity to work in some of the best research institutions in Europe. This allowed them to participate in projects not possible in UoP, the results of which they could then publish in good peer review research papers and now the GRC has a good scientific record. Infrastructure development also benefits young researchers by mitigating the brain drain of Bulgarian scientists in recent years. This brain drain from the GRC in UoP has been

much lower over the last 10 years and this is for several reasons - good research infrastructure, better project-based salaries, and good human atmosphere.

Systems Biology comprises a broad range of inter-disciplinary research areas in biology and constitutes the application of dynamic systems theory to the field of molecular biology. It covers Genomics, Metabolomics, Proteomics, Bioinformatics and a wide range of genetics investigations which, when implemented in the area of crop studies and biomedical problems, represent clear societal benefits. To a great extent, Biotechnology is an implementation of the Systems Biology. The region of Plovdiv has always been famous for its agriculture, crops breeding and food industry development. Consequently, the development of food biotechnologies is directly relevant to regional problems and development. This is the reason we received support from the municipality and the regional governors' office during the project submission and execution. This support, in the form of community land provided for our idea to organize a Technology Park in the field of agriculture and biotechnologies show clearly that we address some important societal needs of the region.

The most important task left before BioSUPPORT is finished is the organization of several new labs, the structuring for which started immediately after first project finished. Those are the Laboratories of Metabolomics and Proteomics, Confocal Microscopy, Next Generation Sequencing and Atomic Force Microscopy. Part of it is finalizing equipment purchase organized during the first period of the project. Another important task is the continuation of the mobility missions until the completion of the project. Experienced researchers from UoP will also to attend several international congresses and conferences. One task which was rather underestimated during the first period was dissemination of the results of the project, but more work will be done on the subject. An important task towards the project's end is ensuring of sustainability of the obtained results.

Most of our 21 European partners and supporters of the project for establishing the GRC were also previous contacts of different research groups at the University. Nevertheless some old connections were strengthened and collaboration was extended to other research groups and research areas. One example is the strengthening of our collaboration with Mugla University, Turkey from insect taxonomy to insect molecular biology and plant physiology, as well as the participation of other research groups. Collaboration with the University of Angers, France was expanded with additional connections to participate in other FP7 projects, in addition to a new research group from UoP - the Faculty of Physics, now included in this collaboration. Additionally, we have improved our collaboration with Groningen University considerably and one of the post docs from this partner was employed with a contract to UoP. The University of Potsdam also supports the GRC in the field of metabolomics and is training a person to take over the Metabolomics and Proteomics Lab at UoP, as well. A much more intensive exchange was conducted with the University of Alicante, Spain and as a result, Prof. Antonio Canals was appointed "Doctor Honoris Causa" of University of Plovdiv. The John Innes Centre, U.K. are also very active in their participation in establishing the Center, taking part in introducing a new research area at UoP - Plant Derived Vaccines, as well as taking part in other collaborative projects. The idea to create an association between the UoP and some small and medium enterprises (SMEs) like Komihris Ltd., MultiCoats Ltd., Biovet JSC with an idea of Technology Centre establishment has also been proposed. Generally speaking, all previous liaisons are substantially intensified.

The established research GRC infrastructure already stands as a solid, internationally recognized entity, as evident from the "Molecular Basis of Plant Stress" research conference in Bulgaria at the end of 2011. This conference (http://cmbps.bio.uni-plovdiv.bg/), with the scientific organizer Prof. Tsanko Gechev from the UoP-GRC, attracted more than 120 participants representing 23 countries and five continents. There, distinguished speakers, some of them ISI highly cited (such as Pof. Klaus Apel from the Boyce Thomson Institute, Ithaca, USA, and the European

The young researchers and PhD students from the GRC during one project workshop

Plant Science Organization president Prof. Heribert Hirt), together with high profile young researchers with papers in Nature and Science (Prof. Christophe Laloi, Dr. Francesco Licausi, Dr. Hiroaki Fujii) delivered exciting lectures on the latest developments in stress physiology and plant development. A number of PhD students also contributed with lectures and posters, ensuring a good balance between experience and youth.

From a scientific point of view, the conference was a great success. Participants not only presented their results, but were also able to interact with each other, exchange ideas, and even discuss future collaborations and mutual projects. The quality of the keynote lecturers, as well as the impressive number of universities and research institutes, attracted sponsors such as Thermo Scientific, Monsanto, LemnaTech, Sigma-Aldrich, Illumina, Carl Zeiss, LKB, BASF, Conviron, Evogene, Merck Millipore, as well as several Bulgarian companies. The international peer reviewed journal Cellular and Molecular Life Sciences (Impact Factor 7.09) will publish a multi-author review 'Molecular Basis of Plant Stress', where most of the keynote speakers will contribute particular topics. In addition, the Oxford open access journal Annals of Botany PLANTS will publish a special issue titled 'Molecular Basis of Plant Stress' dedicated to the conference. This issue will contain peer reviewed papers based on some of the best contributions delivered at the conference. Thus, the meeting was also a great success from a publication point of view, as well.

One of the main focuses of the project and the developed GRC is the area of Bioinformatics. The focus of the Bioinformatics Lab (http://bioinfo.uni-plovdiv.bg/) is on the development of new methods, algorithms and computational software for exploring and interpreting genomic data in a framework of automated sequence analysis. Current emphasis is on the small RNAs (microRNAs and small interfering RNAs) in plants and animals. Activities include analysis of small RNA libraries from deep-sequencing data, expression regulation profiling, novel miRNA gene discovery and annotation and their targets, as well as identification of stress-related small RNAs. The lab provides comprehensive models and publicly available computational tools integrating various levels of genomic data analysis of small RNAs. It also hosts several publicly available original bioinformatics tools and services:

- MicroInspector software - a scanning software for detection of miRNAs target sites;
- starPRO DB - promoter siRNA target sites database;
- miRPlan/CLUSTER/DUPLEX software - de

novo identification of plant miRNAs from deep-sequencing data.

• MirTour - discovery of plant miRNA homologs in EST/GSS datasets;

• BrachyBrowse - Brachypodium siRNAs visual browser database.

The lab is also used in the computational biology classes of BSc, MSc and PhD-student as well as them with a specialized Linux computer room, including HP ProLeant remote servers with pre-installed bioinformatics software packages.

As an infrastructure hub, a network center (NC) is established. This facilitates collaboration between different workgroups by providing webpage hosting, project management and issue tracking systems, mail groups, etc. The NC consists of high-performance servers placed in a renovated server room.

An important emphasis of the GRC's development is its connection with the industry. One of the benefits from our strong cooperation with SMEs and the food industry is the ability to apply for financing together. In our current situation, direct benefit from the SMEs and the industry is difficult. On the one hand, the current economic crisis makes companies hesitant to invest in science in general, and on the other, there aren't very many big biotech companies in Bulgaria to begin with. One strategy available to us is to strive for an association between the UoP and several SMEs - Komihris Ltd, MultiCoats LTD (participant in the project), GRC Ltd (spin-off SME). We can then apply for Structural Funds Call subsidies with which to establish a Technology Centre. Another approach is to initiate joint ventures with SMEs, such as our recent idea to establish a testing laboratory via an action between the Faculty of Chemistry (UoP) and Komihris Ltd., (supporting SMEs for the Biosupport project submission) where the investment will come from the company and the expertise - from the University side.

The strengths of the Genomics Research Center (GRC) at UoP should now be evident. To call that the research conducted at the GRC "focused" would be doing the department something of an injustice. It covers vast areas of bioscience, concentrating on the food we eat, the medications we take and the environment we develop. Together with the recent development of an entire new laboratory from scratch, it certainly becomes clear that the department has collaborative development at its heart. Indeed, after two decades of research excellence, the GRC plays a key part in its industry and even today the department is still making great strides, constantly developing its facilities and its specialists.

Which are the main areas and laboratories that comprise the GRC? Below I will explain the main areas in recent science which GRC is addressing now and will develop also in the near future, as well as some of the challenges faced along the way. Consult the table at the end of the article for a summary explanation.

Plant Breeding Technologies for Drought Resistance

Global climate change is predicted to increase variability of water supply dramatically, both in spatially and in time, and will thus affect the growth of crops. Several European regions are already at severe risk of drought, extreme temperatures, and of other types of abiotic stress linked to water (e.g. as a result of periodic flooding or salt stress). This technology will address water stress affecting crop plants and will develop agricultural plant varieties better equipped to withstand it,enhancing the production of biomass and bioproducts. It will target commercially important crops using state-of the-art knowledge on molecular and genetic processes involved in plant tolerance and adaptation to water stress, which are derived from some model plants (e. g. Resurrection plants) for use in developing more robust crops with improved biomass yield productivity and quality traits under adverse and/or erratic environmental conditions. This technology is aimed at improving the agricultural production to better adapt it to the erratic, unpredictable conditions under the effects of climate change and should ideally involve active participation of European SMEs and industry, increasing its competitiveness.

Increasing the Food Quality and Safety via Production of Doubled Haploids and Hybrid Seeds

High-precision breeding by combining doubled haploids and marker-assisted selection,

which converts genetic diversity into functional homogeneity, may become the dominating technology in breeding. The production of high value crops would offer a good rate of return on investment. Traditional crops can be produced at a lower cost and might, therefore, enable European farmers to reduce prices and take advantage of the foreseen expansion of world markets. Thus, in the long run, this technology would contribute to job security, as well as reduce in the need to subsidise European agriculture, a long-term goal of European policy.

Doubled haploids are an accepted component of modern plant breeding, both for variety and F1 hybrid breeding. This is a newly developing research field at the GRC which will bring this important breeding tool to European and non-European plant breeding institutions and allow industry to improve the conversion of biological diversity into a large scale F1 hybrid seed production. It will provide a sound technological basis for the production of doubled haploid plants for use in a wide range of crop plants. European seed companies will benefit from the action through reduction in the number of years needed to generate inbred parental lines. Thus, time and money in breeding are saved, and plant breeding can adapt faster and more specifically to consumer demands.

A particular feature of this approach is that it allows both a transgenic and a non-transgenic high-precision breeding approach, thus reacting to changes in the ethical and political arenas that surround food production in the EU and worldwide appropriately. It also meets the preferences of some European consumers regarding the absence of transgenes in food.

Plant Breeding for Biomass and Biofuel (Paulownia and Brachypodium breeding)

The *Paulownia* plant, recently introduced in Europe, can be used as a replanting tree for areas devastated by forest fires, for soils reclamation, and so on, but its main advantage is that it grows incredibly fast and has high quality of wood for the timber industry. It can be also used for biomass production with very high yield. The aim of this technology is the production of a high number of saplings and seedlings of the species *Paulownia elongata* and *Paulownia tomentosa*, as well as their frost-resistant hybrid, by using different in vitro techniques. Also, different biotechnology approaches can be applied to the creation of new or hybrid varieties of faster growing, drought, salinity and high temperature resistant Paulownia.

Brachypodium distachyon represents an excellent model species for grasses. It is a small, easily propagated, temperate grass with a rapid life cycle and a small genome. It is a self-fertile plant that can be transformed with high efficiency using *Agrobacteria* and callus derived from immature embryos. In addition, considerable genetic and genomic resources are becoming available for this species in the form of mapping populations, large expressed sequence tag collections, T-DNA insertion lines and, in the near future, a complete genome sequence. The development of *Brachypodium* as a model species is of particular value in the areas of cell wall and biomass research, where the differences between dicots and grasses are greatest. Here, we explore the effect of mild conditions of pretreatment and hydrolysis in *Brachypodium* stem segments as a contribution to the establishment of sensitive screening of the saccharification properties in different genetic materials.

Development of Transient Expression Systems for Rapid Production of Biopharmaceuticals (Vaccines)

Transient protein expression systems are particularly suitable for rapid response products and production of personalized medicines and vaccines because of their flexibility, rapid realisation and production. They scale up easily, enabling the provision of large product quantities in a short time.

Transient expression platforms will reduce the cost and development time for effective vaccines and personalised medicines, and will provide the means for rapid response to bioterrorism attacks, emerging pandemics and to individual needs of patients.

The world needs recombinant vaccines that provide enhanced protection against emerging and re-emerging diseases such as influenza, malaria and tuberculosis. Moreover, personalized medicines are required in order to make our healthcare system more effective and afford-

able. Existing protein production platforms are expensive and have limited capacity and scalability. Transient expression platforms overcome these limitations by combining the advantages of traditional protein production systems (containment, precise growth conditions) with speed, scalability and economy. Therefore, this technology offers an innovative, scalable and sustainable platform for inexpensive production of safe and effective biopharmaceuticals, exceeding the performance of current production technologies. Importantly, harmonized international regulations would greatly improve the prospect of commercialization of transient expression technologies.

The research group involved in this biotech company has an experience in different antigens expression in plants using stable and transit expression which is a part of the EU FP7 project PLAPROVA.

De Novo Sequencing of Eukaryotic Genomes

The power of high-throughput DNA sequencing technologies is being harnessed by researchers in order to address an increasingly diverse range of biological problems. The scale and efficiency of modern sequencing provides unprecedented progress in many areas ranging from the analysis of genomes themselves to how proteins interact with nucleic acids. This series highlights the breadth of next-generation sequencing applications and the importance of the insights gained through these methods.

As part of the BioSUPPORT project, the GRC will obtain next-generation sequencing equipment. This unique equipment will enable sequencing and analysis of the genome of an individual person. Scientists will eventually be able to use genomic information to predict what diseases a person may suffer from throughout life, as well as attempt to either minimise the impact of that disease or avoid it altogether through the implementation of personalised, preventive medicine. Full genome sequencing will rapidly lead to Predictive and Personalised Medicine and will mark a significant leap forward in the clinical genetic revolution. Moreover, we will be able to sequence de-novo genomes of important organisms. We intend to sequence one 'resurrection' plant, *Haberlea rhodopensis*. This is an incredibly drought-resistant plant from which genome sequencing could provide a 'recipe' for creation of highly drought-tolerant crops.

Cancer Research (Oncogenes Regulation)

Cancer is currently one of the leading causes of death and has caused many public health concerns in the present. Latest research has made important progress in illuminating one of the molecular mechanisms and damaged genes involved in cancer. One such example is the discovery of microRNAs (miRNAs), leading to a sudden escalation in research implicating these tiny RNA molecules as key factors in cancer biology. Recent studies indicate that miRNAs will most likely have a diagnostic, prognostic, and therapeutic value. Research has provided evidence for potential use of miRNA expression profiling as a novel tool in cancer diagnosis. Currently, there are three commercially available molecular diagnostic tests for cancer based on microRNAs, all from Rosetta Genomics.

Molecular Studies of Schizophrenia and Autism fop Medical Practice Implementation

Despite the apparent assumption that the only suitable material for testing and validation of biomarkers associated with mental illness is the brain, a new direction in the search for biomarkers for autism and schizophrenia is profiling the expression of the non-protein encoding genes of micro RNA in cells from peripheral blood. Micro RNA molecules are small regulatory RNA molecules that show a change in the pattern of expression in many diseases. The specific micro RNA profile of bodily fluids such as peripheral blood showed a high diagnostic value, which offers benefits such as a relatively non-invasive diagnostic test. Micro RNA molecules as potential biomarkers have objectively measurable biological characteristics that can be used as indicators of normal or pathological processes. Peripheral blood cells as a dynamic reflecting system allow profiling of micro RNA expression, which makes possible their use as potential biomarkers. From this perspective, micro-RNA molecules are considered as a potential new class of biomarkers for many diseases including disorders of the central nervous system (CNS).

The use of these discoveries leads us to more

personal medicine. The diagnostic value of single biomarkers is limited by restricted sensitivity and specificity, while the total micro-RNA profile obtained by multiplex analysis of the use of micro-RNA microarray and RT-PCR platforms is characterized by high specificity and informativeness and makes possible both identification of individual micro-RNA and micro-RNA complex profiles with a high degree of accuracy. The methods presented here provide a basis for the development of technologies based on micro-RNA for early detection, identification and assessment of a disease, which are essential for successful treatment of diseases, particularly in patients where timely therapeutic interventions are extremely critical.

Biological Pest Control

Biological control of pests and diseases is in agriculture relies on natural predation rather than introduced chemicals. This can be done by introducing various predatory insects, mites, parasitic wasps or nematodes that infect the pest with a fatal bacterial disease. This technique is mainly used in greenhouses, but some biological controls, especially pathogenic nematodes, can also be used outdoors. The technology proposed here comprise the use of two approaches:

a) Using different species of parasitic wasps by studying their molecular biology and taxonomy to find the most useful species to the important plant crops.

b) Using the *B. thuringiensis* delta-endotoxins to eradicate agriculturally significant pest insects, such as the European corn borer and Colorado beetle. This can be done by spraying or by means of plant transformation.

Bioinformatics

The focus of the Bioinformatics Lab (SMART - small RNA Team, http://bioinfo.uni-plovdiv.bg/) at Dept. of Plant Physiology and Molecular biology is the development of new technologies, algorithms and computational software for exploring and interpreting genomic data in a framework of automated sequence analysis. Our current emphasis is on small RNAs (microRNAs and small interfering RNAs) in plants, animals and humans.

One of the research activities of SMART comprises analysis of small RNA libraries deep-sequencing data (NGS) from several important cancer types. By analyzing these libraries we are able to find a collection of miRNAs aberrantly expressed in these cancers as compared to normal tissues, suggesting that they might play a role as oncogenes or tumor suppressors in the development and/or progression of cancer and thus have potential as prognostic markers.

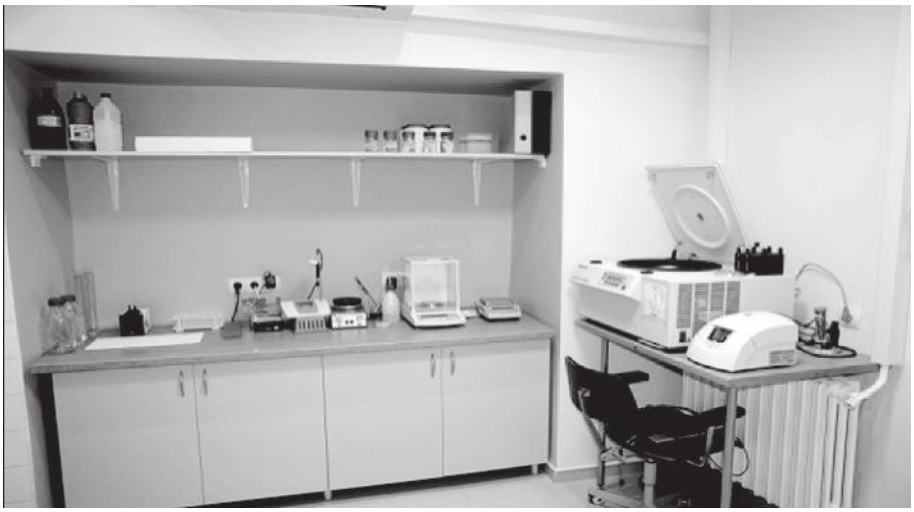
In addition to this prognostic role, miRNAs may also constitute a viable strategy for cancer therapy, as many miRNAs serve as regulating proto-oncogenes. Back in 2005, our team was one of the first to develop new software for identifying miRNA target sites, which has since been used for discovering new cancer-related genes that can be regulated by miRNAs.

As well as human health topics, our team is also doing extensive work in the field of plant and food genomics. Three different groups of grasses, represented by corn, rice and wheat, provide most of the grains that support human health nutrition. The genomes of two of the three groups have been sequenced. *Brachypodium distachyon*, which contains key food and fodder crops such as wheat, barley and forage grasses, is the first member of the third group to be sequenced. Since the genome of Brachypodiym is relatively new, our group is focused on better annotation concerning miRNA genes as well as identifying monocot specific and abiotic stress miRNA genes.

Present activities connected with implementation of National and European Projects

In this period the University coordinated or was taking part in several large national and European projects:

• Project from the Program Capacities of FP7 - BioSupport (2.2 mil Euro). The BioSUPPORT project aims to support the development and increasing the visibility of the research excellence of the University of Plovdiv - Faculty of Biology (UoP-FoB) in the areas of **plant systems biology, food testing** and **biotechnology**. The faculty is very well **recognised** within the Bulgarian scientific society as due to the high number of research projects and high level of education it provides. The University has also established strong links with European research groups and important European programs and initiatives. The



The new laboratory of Plant Microspore Cultures established by the project

number of scientists has increased mostly on the basis of research grants and today the Faculty of Biology employs around 50 people working in the field of Genomics, Systems Biology and Bioinformatics and affiliates around 40 more researchers working in the field of food analysis from **the Faculty of Chemistry** and F**aculty of Physics**.

• Project from Bulgarian National Science Fund (BNSF) - "Developing a regional university centre for teaching and research in the field of genomics and bioinformatics" - coordination.

• Project from BNSF "Developing a Laboratory for Food Analysis"- participant; coordinated by University of Food Technology.

• PLAPROVA - Plant Produced Vaccines - FP7 Integrated Project - participant.

• BIONET - CSA FP7 project - KBBE NCPs Network - participant. Good influence for BioSupport dissemination.

Experience of the Genomics Research Center Ltd. Spin-off

Green biotechnologies facilities

Creation of new varieties of vegetable crops with a better nutritional value and content of substances beneficial to health is the goal of this lab. This technology is based on using Microspore cultures, which can then be used for the production of dihaploid plants which can serve as the basis for breeding hybrid plants with desirable qualities. For this, purpose a new laboratory for ***Plant Microspore Cultures*** was built. The Laboratory is equipped with 2 laminar cabinets, an inverted microscope, a fluorescent microscope, centrifuges, several thermostats, as well as other lab necessities and consumables (supplemented by another project). The laboratory is now fully functional and is producing microspore cultures from tomato, pepper and red beet. The primary purpose of the laboratory is to produce haploid and double haploid plants from agriculturally important species to be used by biotech companies for plant breeding. With this lab, the Project will help Bulgarian Plant Biotechnology and its *per se* implementation of the Systems Biology in agricultural practice.

Table 1. Areas of research Excellence where the GRC can connect to the industry

Areas of Research Excellence	***Collaborative Entities***		***Generated Marketing Leads (per Research Area of Interests)***
	Research/Academic	***Corporative***	
Plant Breeding Technologies for Drought Resistance	University of Plovdiv, Dept. Plant Physiology and Molecular Biology; Agrarian University, Plovdiv	Genomics Research Center Ltd. (Spin-off - SME)	Monsanto, Pioneer, Beyer Crops Science
Doubled Haploid and Hybrid seeds Production for Crop Plant Breeding	University of Plovdiv, Dept. Plant Physiology and Molecular Biology	Genomics Research Center Ltd. (Spin-off - SME)	Bayer Crop Science, Group Limagrain, KWS AG, Monsanto, DuPont, Syngenta
Plant Breeding for Biomass and Biofuel (*Paulownia* and *Brachypodium* Breeding)	Genomics Research Center Ltd. (Spin-off - SME), Fruit Tree Institute	Velboy Ltd., Bulgaria	Biomass, Biofuel and Timber Industry; Agricultural Research Service (ARS)
Novel Biodiversity Sources for Improving Food Crops Quality	University of Plovdiv, Vegetable Institute, Plovdiv		Agriculture Practice, FAO
Development of Transient Expression Systems for Rapid Production of Biopharmaceuticals (Vaccines and Antibodies)	University of Plovdiv, Dept. Plant Physiology and Molecular Biology; Agrarian University, Plovdiv	Genomics Research Center Ltd. (Spin-off - SME)	World Pharmacy Companies
De Novo Sequencing of Drought Tolerant Plants Genomes	University of Plovdiv, Genomics Research Center	Genomics Research Center Ltd. (Spin-off - SME)	LGC, ServiceXS, BGI, 1000 Genomes Project, Welcome Trust Sanger Institute
Cancer Research (Oncogenes Regulation)	University of Plovdiv, Genomics Research Center, Medical University, Plovdiv	Genomics Research Center Ltd. (Spin-off - SME)	Rosetta Genomics
Molecular Basis of Schizophrenia and Autism	University of Plovdiv, Genomics Research Center, Medical University, Plovdiv		Medical Practice, Individual Medicine
Animal Cell Cultures Models for Medical Use	University of Plovdiv, Dept. Biology of Development; Medical University, Plovdiv		SIGMA Life Science
Analytical Methods for Food Quality and Safety Testing	University of Plovdiv, GRC, Food Technology University	Komihris Ltd. (SME)	Food Industry
Monitoring of Bioactive Components in Functional Foods	University of Plovdiv, Dept. of Biochemistry and Microbiology, Dept. of Experimental Physics	Tsvetelina Ltd, Bulgaria, LB Bulgaricum	Nestlé Europe, Unilever Bulgaria, DMV Nutrition, DANONE Bulgaria,
Biological Pest Control	University of Plovdiv , Dept. Plant Physiology and Molecular Biology	Beneficial Insects Co.	Symbiont
Bioinformatics	University of Plovdiv, Genomics Research Center	Genomics Research Center Ltd. Spin-off	Rosetta Genomics

MOLECULAR PHOTONICS LABORATORY FOR ULTRAFAST SCIENCE AND TECHNOLOGY AT SOFIA UNIVERSITY "ST. KLIMENT OHRIDSKI"

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BASIC CONCEPTS AND MISSION

Molecular Photonics is an emerging field of research located at a strategic crossroad where physics, chemistry, engineering and life science meet. These four closely interacting areas provide a relevant multi-disciplinary background as well as timely motivations for the development of molecular photonics at ultimate time and space scales. Applied and technological as well as exploratory and fundamental issues related to light matter interaction in organic or inorganic-organic hybrid systems at micro and nanoscale are now increasingly at the focus of research in molecular photonics.

The Molecular Photonics Laboratory for Ultrafast Science and Technology (MPLUST) at Sofia University is designed first and foremost to promote contemporary scientific research and education. We implement photon-based methods with femtosecond timing to record molecular dynamics, which fully resolve the primary processes. A focus of the experimental and theoretical work is electron, proton and energy transfer, which have fundamental importance for the building blocks of life profound impact on the development of new photonics technologies. With the laboratory equipment we are able to excite coherently multiple electronic states and to probe the evolution of the resulting electronic wave packet with femtosecond resolution. Additionally, the laboratory tools allow high degree of coherent control over the outcome of chemical reactions.

The MPLUST's mission is to perform multidisciplinary research in the basic science and technological applications of ultrashort laser pulses, to educate students from a wide variety of backgrounds in the field, and to spur the development of new technologies. Furthermore, its goal is to advance bio-research and photonics engineering - by focusing its intellectual, scientific, educational and industrial outreach efforts on the rapid and directed development of molecular photonics technology. In doing so, the first strategic roadmap for molecular photonics research in Bulgaria will be formulated. MPLUST will coordinate activities within three Sofia University departments and a number of foreign partners, and will devote efforts in building an extensive network of relationships with other universities, industrial partners, and other molecular photonics research groups abroad. While the majority of MPLUST investigators are distinguished for their substantial individual contributions to organic photochemistry, laser physics, molecular photonics, ultrafast spectroscopy, the establishment of this new research lab is expected to greatly enhance their research potential by placing an emphasis on the vertical integration of knowledge.

IMPORTANCE OF THE LABORATORY RESEARCH FIELD

The speed of the molecular dynamics is given by the speed of elementary chemical processes within the molecules. Dynamics is often classified according to time scale. The focus of the effort described here is on the ultrafast time scale of femtoseconds (1 fs = 10^{-15} s) to picoseconds (1 ps = 10^{-12} s). This is a typical time scale of elementary chemical reactions and of electronic and nuclear motions in molecules. Although there is an assumption that practical chemistry and biology occur on much longer time scales, these longer time scales are often reached by adding statistical probability to the short time scale dynamics of molecules. Furthermore, bio-

logical function results from the unique entanglement of structure and dynamics. By structure we mean the time-independent information about the state of a system in some form of equilibrium or quasi-equilibrium. Structure was the focus of most chemists in the past, and it is the key issue in biology. However nature is not in equilibrium and the ultimate aim in chemistry and biology nowadays is to understand dynamics - how systems move between apparently equilibrated structures, changing their chemical composition and performing biological functions on the way. Thus, the fundamental understanding of chemical and biological dynamics ultimately relies upon a thorough explanation of the ultrafast processes. With the emerging new and highly reliable techniques for femtosecond pulse generation and amplification completely new applications of ultrashort pulses come within reach. The time-resolved spectroscopy is a rapidly advancing field with applications in many areas of science and technology. The significance of the area is even more obvious after the Nobel Prize in Chemistry awarded to Ahmed H. Zewail in 1999 "for his studies of the transition states of chemical reactions using femtosecond spectroscopy".

The multidisciplinary interest in time-resolved measurement techniques is the driving force behind the rapid establishment of University centers all around the world in the recent years. They aim to promote interdisciplinary research using time-resolved spectroscopy, e.g. Physical Biology Ultrafast Laser Science and Technology at Caltech, Center for Time-Resolved Spectroscopy at the University of Durham, etc. One of the research areas in the recently established cluster of excellence Munich Center of Advanced Photonics is studding electron dynamics in atoms, molecules and solids.

HISTORICAL OVERVIEW OF THE THEMATIC RESEARCH IN BULGARIA

The first attempts for experimental studding of fast dynamics with resolution better than 10^{-9} s in Bulgaria are done in the 1980's. After successful measurements of the relaxation kinetics in the nanosecond range [1-2], other experimental studies of ultrafast processes in Bulgaria are not known to us. The reasons for this are: first, the dynamics of chemical processes is mostly in the sub- ns and fs as it is explained above; second, up to now the fs-lasers were expensive and not available. The lasers used the first time-resolved measurements are home-built laser systems at the Department of physics (Sofia University) with quite poor repeatability of the output parameters, which complicates the experiments tremendously. However, the ultrashort (10-100 fs) laser pulses that now can be generated with today's solid-state laser technologies have several very useful properties, other than the short duration, that may be exploited in experiments and technical applications, e.g. very high peak power, broad spectral band-width (due to the short time duration), very widely tunable wavelength, tunable temporal and phase characteristics. These possibilities have given rise to several new applications of ultrafast pulses, not previously possible (or practically feasible) with earlier technologies. On top of that, today's pulse sources are highly stable and reliable, often computer controlled, which further extends the applications to previously unthinkable environments.

Time resolved methodologies currently available at MPLUST: Femtosecond Broadband Pump–Probe Spectroscopy

This method is an extension of the traditional steady-state absorption spectroscopy. Here the absorbance at a particular wavelength or range of wavelengths of a sample is measured as a function of time after excitation by a short pulse of light. In this experiment both the light for excitation ('pump') and the light for measuring the absorbance ('probe') are derived by the same pulsed laser source. The sample is photoexcited ("pumped") from the ground-state to the excited state by the strong pump beam and is brought out of thermal equilibrium. The excited state will decay to other states with a characteristic decay time. By measuring the absorption of the weaker probe beam which is delayed relative to the excitation, the time evolution of the state under study (ground state, excited state, radical pair, etc.) can be monitored. Contemporary state-of-the-art femtosecond pump-probe spectrometers use a laser-generated white light for probing. This technique pro-

vides broad probing window, spanning from the UV to the NIR spectral range. The white light (or super-continuum) method is highly advantageous over conventional (single wavelength) pump-probe technique in its capability to capture and resolve reactant, intermediate and product states ***simultaneously***. By measuring the pump-probe spectra as a function of time one not only will obtain kinetic traces at multiple wavelengths but also will monitor the complex spectral evolution. Local and interchromophore transitions can be simultaneously detected, and detailed information such as spectral intensities and shifts, line shape and band width changes can be readily detected and analyzed. Induced excited state absorption (ESA) peaks are typical signals appearing in broadband pump-probe spectra; but besides these, other contributions may also be observed depending on the spectral range of interest, such as stimulated emission from excited states and ground state bleaching. On the one hand, the transient signals with varied spectroscopic origins might mix together, complicate the spectra and add difficulties to the component assignments; on the other hand, however, they offer more comprehensive spectral information and thus reveal more detailed dynamic processes.

Figure 1 is a schematic representation of the femtosecond broadband pump-probe experimental setup. The laser source is a commercial Titanium:Sapphire based pump laser that provides a pulse train of femtosecond pulses (130 fs) with energy of 3.5 mJ at 800 nm, and repetition rate of 1 kHz. The output beam is divided into several fractions by the beam splitters. A portion of laser output (250-300 μJ) feeds a commercial non-collinear optical parametric amplifier (NOPA) to generate the pump pulses while another small fraction of the laser output (5 μJ) is used to generate the white light (WL) for broadband probing. The output of the NOPA is compressed to have pulse durations as short as 30 fs. The typical tuning range of the NOPA is 480-900 nm and its output can be used directly to pump the sample. If pump pulses deeper in the UV range are desired, an optional frequency doubling of the NOPA output is used to produce pump pulses with wavelength in the ~240 - 450 nm range. In the real experiments, the generated pulses (NOPA output and its second harmonic) are optimized by additional prism compressors (not shown in the scheme) to compensate for group velocity dispersion.

The changes in optical density are measured by probing with a femtosecond white-light continuum (WLC) generated by tight focusing of a small fraction of the laser output into a thin Sapphire or CaF2 monocrystal plate. By this means, a usable probe source that covers the UV-VIS-NIR spectral range (from 300 to 900 nm) is achieved.

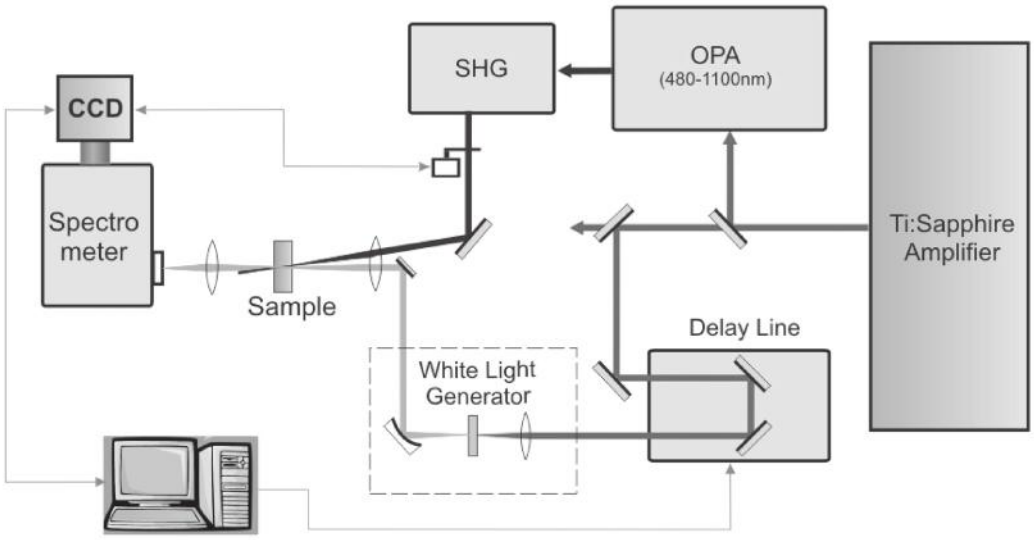

Fig. 1. Schematic representation of the UV-VIS-NIR broadband pump-probe experimental setup

To improve sensitivity of the measurement, transient absorption spectrometer implements dual-beam probe geometry and a digital lock-in technique. The WLC is split into two beams (probe and reference) and focused into the sample using reflective optics. The probe beam passes trough excited volume of the sample and the reference passes through the non-excited spot. After passing through the sample, both probe and reference are spectrally dispersed by an imaging polychromator and simultaneously detected on a TEC-cooled CCD. The signals are collected in a two-step measurement cycle. In each step probe and reference signals are recorded at certain conditions determined by the state of the pump shutter (or chopper). The change in optical density is obtained using the standard formalism for pump-probe spectroscopy [3]:

$$OD = -\log_{10}\left(\frac{I_{pr}^{exc}}{I_{ref}^{exc}} \Big/ \frac{I_{pr}^{0}}{I_{ref}^{0}} \right) \qquad (1)$$

where the lower index denotes the recorded probe ***(pr)*** and reference ***(ref)*** signal, respectively. The upper index characterizes the setting of the shutter which controls the excitation beam: ***(ex)***: pump shutter is open; ***(0)***: pump shutter is closed.

The recorded signals at each step are result from integration, typically for several 100 laser pulses. The exact number of acquired laser shots within a single two-step cycle is selectable and will depend on the specific experimental conditions.

The WLC probe pulse passes through several optical elements which introduce a chirp due to the group velocity dispersion (GVD), i.e. its different spectral components accumulate different group delays. As a result, different spectral components of the WLC pulse are interacting with the excited sample molecules at different delay times. Before entering the sample the WLC spreads in time typically from several hundred fs up to ps depending on thickness and the refraction index dispersion of the passed optical material. Independent measurements of the chirp of the WLC will be carried out to correct the pump-probe spectra for time-zero differences.

In this arrangement, the time resolution is around 100 fs, depending on the wavelength of the excitation pulses and sample optical thickness. It is practically measured by the cross-correlation function between pump and probe pulses in a liquid or solid-state transparent media. The spectral resolution of the spectrograph is 3-5 nm in the entire UV-VIS range.

Femtosecond Pump–Repump–Probe Spectroscopy

Many of the photo-induced chemical reactions occur via series of elementary steps including various short-lived intermediate species. The latter can show very similar absorption spectrum hindering the detailed understanding of complex photo-chemical reactions by pump-probe spectroscopy. The problem could be overcome using the recently developed ***pump–repump–probe (PREP)*** technique [4]. This method is similar to the pump-probe spectroscopy discussed just above, but an additional third pulse is used for excitation of the intermediates involved in the relaxation dynamics. Secondary excitation of the short-lived species could change the reaction pathway yielding novel information on the relaxation dynamics. The method also allows optical manipulation of chemical reactions [4].

The experimental set-up used for the PREP experiments includes an extension of the commercial UV-VIS-NIR femtosecond broadband pump-probe system described above. The necessary modification includes a source for secondary excitation of the sample and an additional optical delay stage for precise control of the relative delay of the secondary pulse, related to the first excitation pulse. In particular, the intermediate species involved in the electron photodetachment of neat water and aqueous electrolytes are expected to show strong absorption on the Ti:Sa laser wavelength of 800 nm. Therefore, in this experiment the secondary excitation pulse is derived directly (typical energy of about 5 uJ) of the regenerative amplifier output.

RESEARCH AREAS AND SCIENTIFIC PROJECTS

The main research areas of the MPLUST are: electron transfer reactions in solutions; photo-induced dynamics in DNA; early-stage resonant

energy transfer in lanthanide-organic complexes as well as ultra fast temperature jumps in hydrogen bonded systems.

Roadmap for the future development of the research laboratory until 2015

The schematic of the main instruments and corresponding expansion in the methods for time resolved analysis available at the Laboratory are shown on Figure 2.

Development of UV-pump-IR-probe and IR-pump-IR-probe spectroscopy will be a powerful approach to elucidate the evolution of molecular structures during ultrafast processes by monitoring vibrational marker modes in real time. This technique can monitor the site-specific interactions in hydrogen bonds and the conversion between excited electronic states by inspection of the fingerprint IR-active vibrations in conjunction with quantum chemical calculations. This spectroscopic technique is a straightforward extension of the pump-probe method developed in the first three years of the project. The fundamental arrangement of the setup, i.e. the temporal sequence of the laser pulses and the detection scheme will remain unchanged. The only components that need to be replaced are the probe light sources, the spectrograph, the CCD detection array and some of the optical components for steering the IR beams.

Time-resolved fluorescence spectroscopy (Femtosecond Fluorescence Up-Conversion Spectroscopy) provides a powerful technique for investigating electronic properties of solids and liquids. Several different techniques have been used for this purpose over the last four decades. Streak cameras have been used successfully; however, their use is limited to the visible and very near-infrared spectral regions and their usable time resolution in the synchroscan mode at high repetition rate has been limited to about several picoseconds. Optical Kerr cells can provide subpicosecond time resolution, but their low efficiency limits the technique to strong signals. The well-established techniques for growth of high quality nonlinear crystals allow measurement of fluorescence time evolution with subpicosecond time resolution using ***fluorescence upconversion technique***. The contemporary state-of-the-art femtosecond upconversion spectrometers can provide time resolution of <100 fs. However, dispersive elements in collection optics prevented measurement of spectra

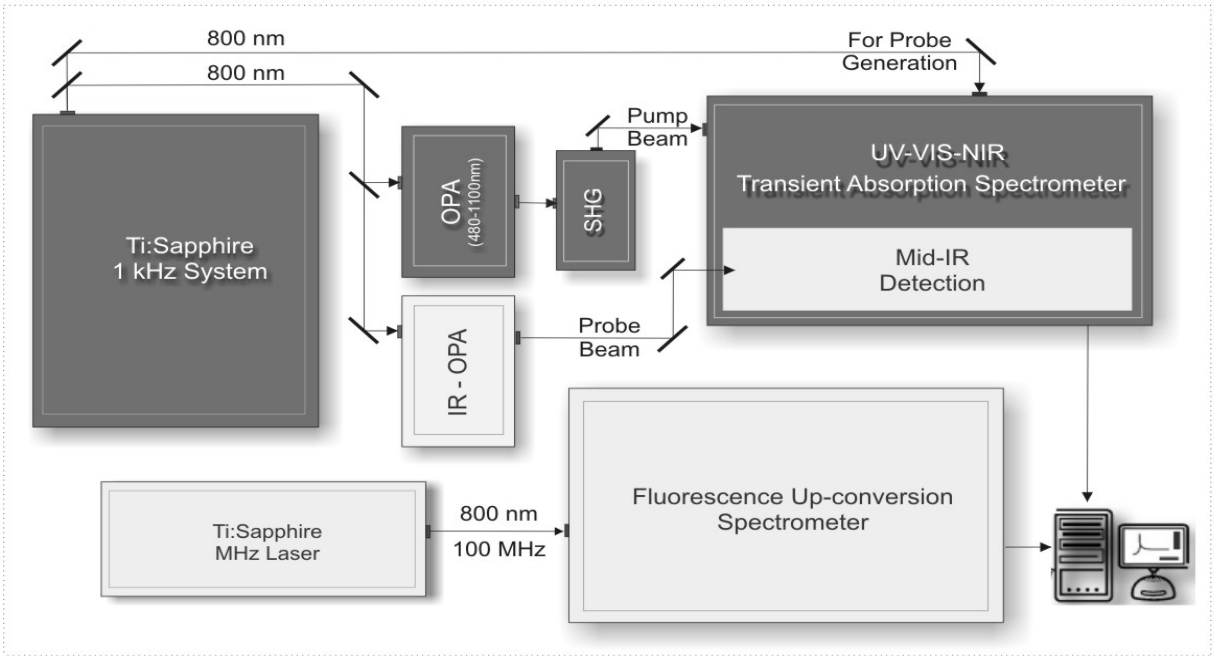

Fig. 2. Layout of the main time-resolved instruments and methodologies, available at MPLUST. The blocks are shaded in order to show the roadmap of the acquired equipments. The dark gray marks the equipment acquired during the establishing of the laboratory, light grey - instruments planned for the future.

and an accurate determination of the zero delay. The required upconversion system for time-resolved fluorescence spectroscopy will have the following desirable attributes: a time resolution of < 100 fs (limited primarily by the laser pulse width), an ability to determine the zero delay precisely, and a wide spectral range (from 430 to 1000 nm with a set of two nonlinear crystals cut at different angles). In addition, this system is extremely sensitive; one can obtain data with a dynamic range exceeding four orders of magnitude from typical chromophores with quantum yield close to unity. This combination of high sensitivity, wide dynamic and spectral ranges, and high temporal resolution is not currently available in any other system. The principle of this technique is phase-matched mixing of the fluorescence excited by an ultrashort laser pulse with a suitably delayed beam from the same laser in a nonlinear crystal.

Acknowledgment

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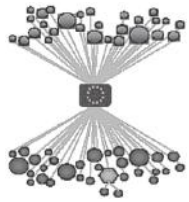

MADE IN BULGARIA WITH EUROPEAN SUPPORT

SENSOR EFFECTOR BOOT MODULE FOR A NEW TECHNOLOGY SPINAL PATIENTS REHABILITATION

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Abstract

In this paper we represent the development of a mechatronic sensor effector boot module for effective rehabilitation of patients in the early post-traumatic stage (1 month) after the occurrence of a serious spinal-cord trauma or poli-trauma. The module is optimised by size and by weight and it can apply into independent portable device with assurance for vertical position in the bed of the immovable patients.

The device includes: a module for basing and fixation of the patient's leg, a sensor "SkinTouch" for measuring bio-electrical impedance in points of the foot skin, a mechanism for acupressure, a carriage module for basing of a measuring sensor and a mechanism for acupressure, a module positioning of carriage module to various foot points for the programme performance of the procedures and PC controlled sensor and effector elements connected to the physician's monitor.

The studies are carried in Bulgarian Academy of Sciences (BAS) - Russian Academy of Sciences (RAS) collaboration advance to develop a new device for treatment of spinal patients and creating new technologies of their rehabilitation.

INTRODUCTION

Serious disability and recent increase of a number of spinal cord injuries imply a profound study of the problem and development of new methods and devices for the support-locomotion recuperation. One of the methods of restoring the support-locomotion functions is to affect the neurons of the spinal cord using the limb reception system as a natural communication channel. Foot receptor activation is applied for that purpose. The objective of this study is to complete the following tasks: (i) to affect the extra receptors, which signals stimulate the central locomotion generator located in the spinal cord; (ii) to affect the nerve endings in the biologically active zones, stimulating functioning of internal organs of a seriously disabled patient. The start of the procedure during the early post-traumatic stage (after the occurrence of a spinal cord trauma or poli-trauma) is considered to be appropriate for the decrease of atrophy speed. Receptors stimulation is proposed at the start of the recuperation procedure, only due to patient's poor physical state. A method of acupressure and multi-point trajectory of area activation is selected for application, combined with methods of reflexotherapy.

Technical solution of mechatronic sensor effector boot device

The device includes:

- a module for basing and fixation of the patient's leg,

• a sensor "SkinTouch" for measuring bio-electrical impedance in points of the foot skin,

• a mechanism for acupressure,

• a carriage module for basing of a measuring sensor and a mechanism for acupressure,

• a module for a plane motion and positioning of carriage module to various foot points for the programme performance of the procedures,

• PC controlled sensor and effector elements, connected to the physician's monitor.

The mechanical modules are designed in Institute of mechanics, BAS. The specialized sensor "SkinTouch" for measuring bio-electrical impedance in points of the foot skin is developed in the Institute of Applied Mathematics "Keldish"-RAN.

The design, modelling and experimenting of the separate modules are presented below.

Design of a module for basing and fixation of the patient's leg

Requirements to module for basing and fixation of the patient's leg

The designed module must meet the following requirements:

1. Sustainable basing the patient's leg, which is in a bed.

2. Accessibility to be ensured to maximum number of points on the foot with the aim to measure the bioelectrical resistance by means of a specialized sensor.

3. Accessibility to maximum number of points on the foot for automatic acupressure performance according to a programme assignment, individually for each patient.

4. Discomfort must not be created to the patient, and also not to influence the precision of the carried out measurements.

5. Basing to be possible at different patient's feet sizes.

6. Safety of the patients to be ensured of the device activity at arising programme or mechanical reasons.

View of the module for basing and fixation of the patient's leg

The developed module includes the following components: (Fig. 1): 1- heel support, 2 - foot fixations, 3 - regulated foot fixing of the leg.

The foot is based at support 1. The leg is fixed above the ankle joint and in the front part

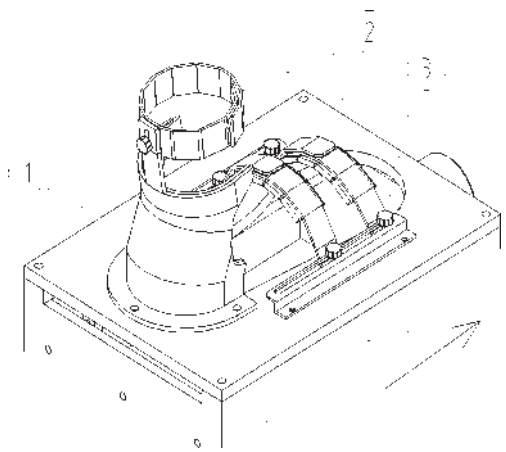

Fig. 1. General view of the module for basing of the patient's leg

of the foot are prepared fixations 3, which location is regulated longitudinally according to the foot size. The surface of the foot remains uncovered for a direct contact to each point for measurement of the skin electrical resistance and acupressure.

A carriage module for basing of a measuring sensor and a mechanism for acupressure

Combined module - carriage (Fig. 2) comprises carriage 1, to which module for acupressure 2 and a measuring module 5 are immovably attached. Two linear drives, by means of which a translation along axis Z is realised, are included respectively in the modules joints.

The respective useful contact pressure of an electrode for measurement 4 is achieved by

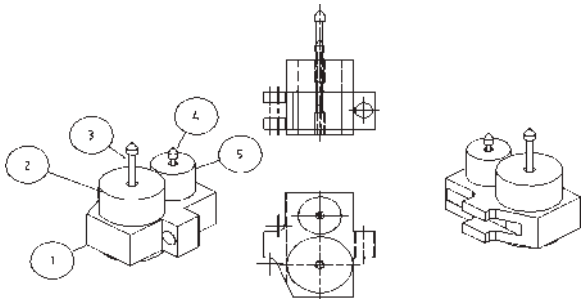

Fig. 2. A carriage module for basing sensor and effector elements of device

means of drive control and the external force (acupressure) is regulated by means of the executive mechanism 3.

A mechanism for acupressure

The mechanism for acupressure includes (Fig. 3) translation linear drive 1 and removable

instrument with different number of pins 2 (Fig. 4).

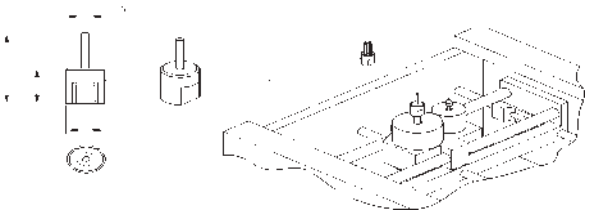

Fig. 3. A mechanism for acupressure

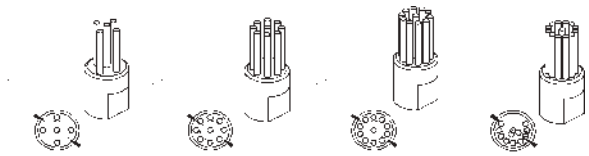

Fig. 4. Effector instruments with different number of pins

Effector instruments with different number of pins are applying for receptor activation by the method of skin-deep multi-pins acupressure combined with methods of reflexotherapy.

Design of a module for a plane motion and positioning of carriage module to various foot points

Movement along axes X and Y is realised by two mechanisms respectively (Fig. 5).

The mechanism for longitudinal translation X includes: a foundation 1, a drive 2, a leading screw 3 and a guide 4 for translation along axis X. It is necessary the run of this translation to be equal to the length of the foot $H_1 \approx$ 110 to 150mm.

The mechanism for translation along axis Y includes: a base-carriage 5, a drive 6, a gear 7 and a screw-carriage 9.

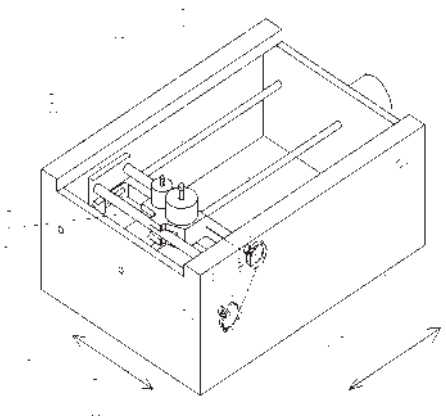

Fig. 5. A module for a plane motion and positioning of the carriage

The drive 6 is fixed on the base-carriage 5 witch by means of the gear 7 activates the screw- carriage 9. It moves by itself the carriage module 9 along axis Y lead on the guide 8. It is necessary the translation run to be equal to the width of the foot $H_2 \approx$ Lp (40 to 60mm.

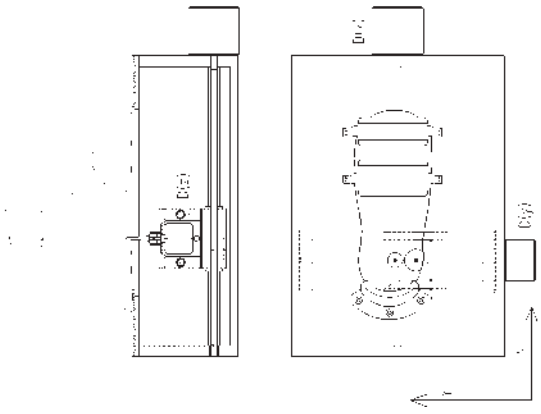

Fig. 6. Basing of the patient's leg for the programme performance of the procedures

Fig. 6 shows basing of the patient's leg on the module with possibility for connection sensor and effector instruments to various foot points for the programme performance of the procedures.

Development of the sensor "SkinTouch" for measuring bio-electrical impedance in a skin point

Proof of the design necessity of a specialized sensor for measuring bio-electrical impedance

The device measuring both the initial (pre-treatment) foot skin electro-conductivity, as well as the current one operating during the process of medical treatment, is of essential importance for the successful stimulation of patient's foot. Such a sensor is needed to design a high-spatial resolution map of acupuncture points of a patient's foot, and the map should be used to analyze the results of the treatment. The complexity of the registration needed originates in the measurement of current extremely low values. Note that current low value is due to the high ohm resistance of patient's foot skin, and to the restriction of voltage applied to patient's skin. That restriction is imposed to avoid not only injury or pain, but also data distortion due to the

effect of voltage of the measuring instrument over the physiology of controlled processes.

A mechatronic approach for the measurement of human skin electro-conductivity is developed in the Institute of Applied Mathematics "Keldish"- RAS. The measurement method proposed is based on the use of a differential operational amplifier, controlled by a microprocessor. It is capable of maintaining stable ultra-small current (not larger than several mcA) in the chain of the analogue-digital analyzer (ADA) of the skin resistance receptor. Signals coming from an analogue-digital converter (ADC) are used to apply microprocessor control of measurements. The ADC is switched to a differential operational amplifier. A special measuring instrument "SkinTouch" is designed to realize the software algorithms and analyze the capabilities of the proposed method. It is assembled using modern electronic compounds and schemes that guarantee the measurement accuracy required.

Technical description of the "SkinTouch" sensor

Sensor function

Basic function of the sensor - the "SkinTouch" instrument, is to perform point measurements of the human skin resistance characteristics. Among its technical characteristics, one should note the ultra-low current of measurement, from 0,5 to 2 мкА, and the moderate potential difference (voltage) applied to the electrodes and not exceeding 7V. These characteristics exceed twice the characteristics of the existing equipment. They enable one to significantly decrease the measurement pattern distortion and minimize the unfavourable effects over the patient.

Requirements to the measurer of resistance characteristics

Methodically, the instrument for measurement of the resistance characteristics of human skin should satisfy the following requirements:

1. Measurements should be performed using low current in the range 0,1 - 1 мкА.

2. Current value should be constant during the measurement.

3. The measurable parameter should be the difference between the electrode potentials.

4. The working voltage between the electrodes should be within the range [0,1 - 1] V.

5. The maximal admissible difference between the electrode potentials should be 15 V.

6. The measurements performed should minimally affect patient's state.

Note that the designed sensor device "SkinTouch" satisfies the outlined requirements.

Principal scheme of the "SkinTouch"

The principal scheme of the measuring device "SkinTouch" is shown in Fig. 2.

The instrument basic compounds are:

- Microcontroller;
- Measuring unit;
- Generator unit;
- Supply sources.

The microcontroller coordinates performance of all other units, transition of measurement results to other devices along the channel RS-232, as well as receives of controlling commands.

The measuring unit consists of:

- Instrumental operational amplifier, with small value of leak current and incorporated means of protection against electrostatic discharges;
- Analogue-digital converter incorporated into the microcontroller.

The generator unit consists of:

- Digital-analogue converter with current output, which provides the necessary range of the supporting current.
- Current mirror which exactly copies the supporting current of the ADC within the range of the electrodes supply voltage. The commutation unit is used to eliminate the electrode polarization.

The direction of current flowing between the measuring electrodes can be varied upon an order issued by the microprocessor. The unloading resistance is used for calibration and discharge of the electrode static charge.

Two direct current sources are used to supply the measuring instrument "SkinTouch". The first source, with voltage 3,5V, is used to supply the instrument digital section. The second source provides two voltage values: + 3,5 V and - 3,5 V, and it supplies the instrument analogue section. Batteries or accumulators are used to reduce disturbances. A prototype of the measuring device "SkinTouch" is assembled, and instrument tests

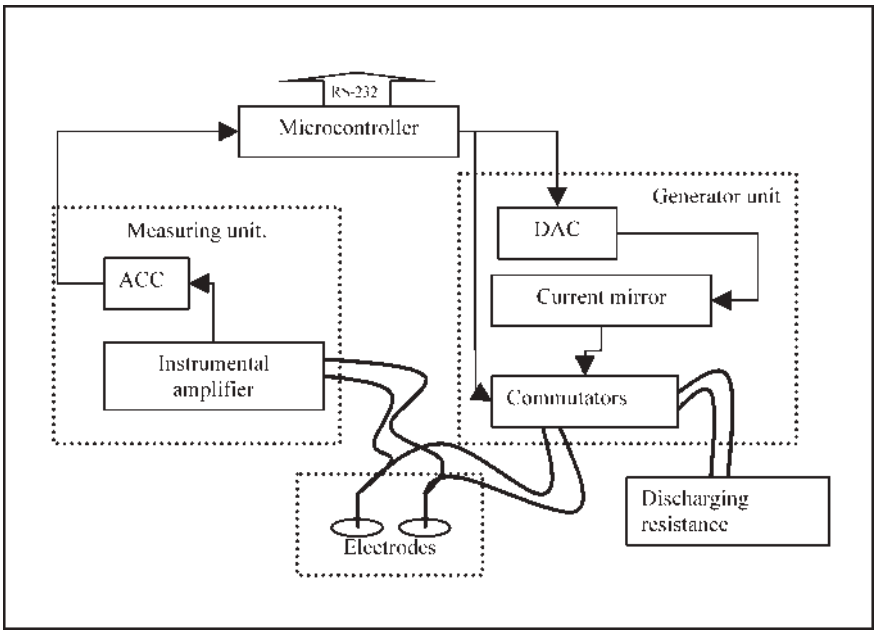

Fig. 7. Principal scheme of the measuring device "SkinTouch"

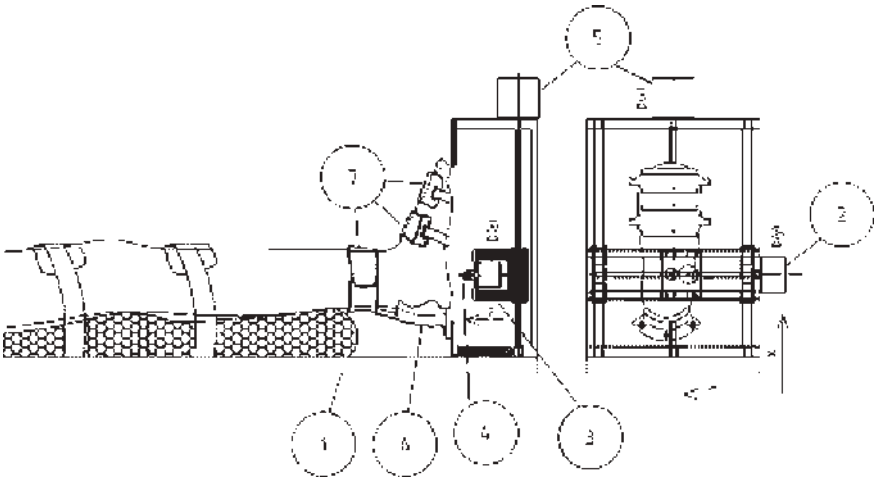

Fig. 8. General view of the device in the bed of the immovable patient

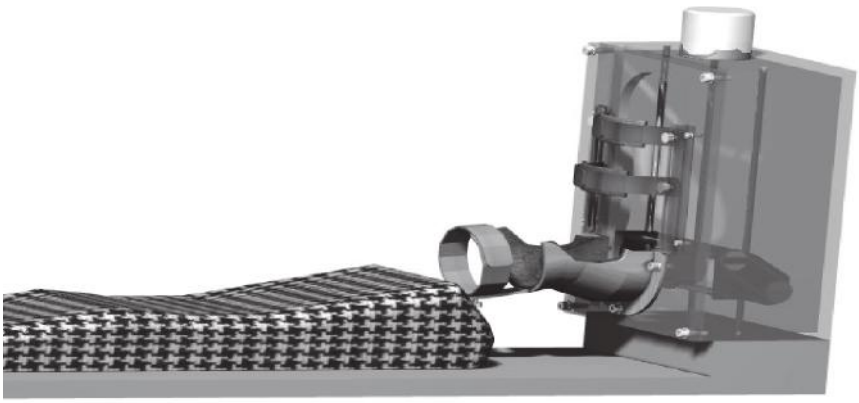

Fig. 9. 3D model of a mechatronic sensor effector boot device

are performed.

View and modelling of the sensor effector boot device

Product *Mechanical Desktop 2005* is applied for creation of a virtual 3D model of the module. The program "MSC. visual Nastran 4D 2002" for computer simulation is applied on the 3D model.

CONCLUSIONS

Conclusions derived after modelling and simulation help successful realization of the prototype of the portable module for procedures with supporting and healing effect of patients in the early stage after the occurrence of a spinal cord trauma or politrauma.

The following scientific and application results are expected:

1. Measuring of the bioelectrical resistance to be performed in the foot points for each individual patient by means of a specialized sensor ***"Skin Touch"***. An individual chart of the acupuncture foot points of the patient is built up.

2. Planning and performing of treatments (acupresura) in definite points, along a defined trajectory or an area on the foot.

3. Performing of a periodical estimation and control of the treatment results by means of measuring of the bioelectrical resistance in definite foot points. The healing programme undergoes a consecutive variation if necessary.

The mechatronic sensor effector boot device can be applied in the clinic practice after performing technical, laboratory and clinical experiments.

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СЕНЗОРНО-ВЪЗДЕЙСТВАЩ МОДУЛ-ОБУВКА ЗА НОВА ТЕХНОЛОГИЯ НА РЕХАБИЛИТАЦИЯ НА ПАЦИЕНТИ С ГРЪБНАЧНО-МОЗЪЧНА ПАТОЛОГИЯ

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Резюме

Представена е разработката на мехатронен сензорно-въздействащ модул-обувка за ефективна рехабилитация на пациенти в ранния пост-травматичен стадий (1 месец) след тежка гръбначно-мозъчна травма или политравма. Модулът е оптимизиран по размери и тегло, и може да се прилага като независим портативен уред, поставен във вертикално положение в леглото на имобилизирания пациент.

Уредът включва: модул за базиране и фиксиране крака на пациента, сензор "SkinTouch" за измерване био-електричното съпротивление в точка от повърхността на стъпалото, механизъм за акупресура, модул-каретка за базиране на измерителния сензор и механизма за акупресура, модул за позициониране на модула-каретка в различни точки на стъпалото за програмно извършване на процедурите и PC за управление на сензора и изпълнителните елементи, свързан с монитора на лекаря.

Изследванията са проведени в сътрудничество между Българска академия на науките и Руска академия на науките и са насочени за развитие на нови средства и технологии за рехабилитация на пациенти с гръбначно-мозъчна патология.

LIBRARIES WITH PLC PROGRAMMING MODULES

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Together with the rapid development of PLC technologies, nowadays, the requirements for short engineering and commissioning times are growing more and more. At the same time imposed is the conflicting requirement for lowering the ready products price. To meet these requirements, two interesting trends can be seen in the field of automation. One of them is the permanent strive for increasing the software realization of the part, controlling the motion, on the account of the mechanical part (Fig. 1). This trend makes sense, since the software can be changed and developed much easier than the hardware. Another advantage here is that this approach substantially reduces the price for development of the product, as expenses are made only for software development, but no new materials are introduced. Correcting the design errors is also performed much easier.

Many machine structures can be easily replaced by their software analogues. Such are gearbox, cam gears, position-signalling mechanisms, coupling and decoupling mechanisms and many others. Software analogues make the work easier thanks to the good connection between the servo-mechanisms (which have also a predominant software part) and the controlling system.). Comparison between the software and the mechanical implementation of a simple, but often used, mechanism - the gearbox. is shown in the table (Table 1).

The general part of the implementation is usually related to the driving and controlling part. It is therefore of great importance for the controlling part to be well developed and enough adaptive. Due to the presence of powerful microprocessors today, it is possible the controlling part to be integrated in the controller, where the logic is. This, in a great extent, re-

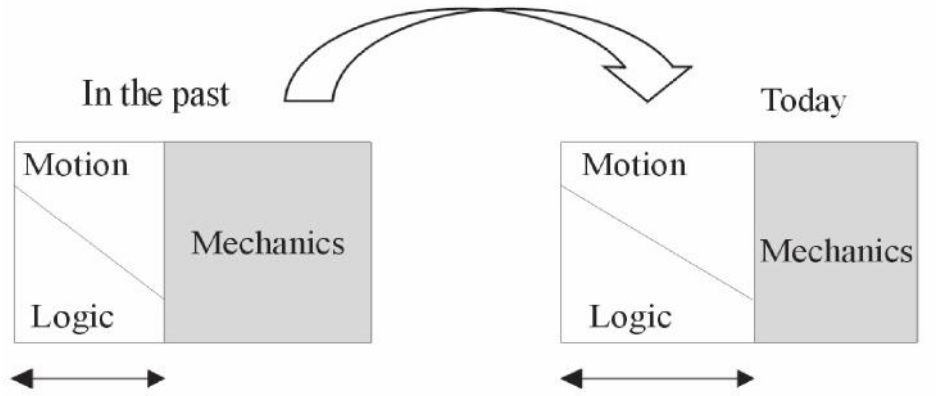

Fig. 1. Trend for decreasing the mechanical part of the automation systems

Table 1. Comparison between the software and the mechanical implementation of a gearbox

Criterion	Software implementation	Mechanical implementation
Reliability	High, constant in time	Depends on the make, decreases in time
Possibility for varying the ratio	Yes	No
Performance	High, depends on the microprocessor performance	Limited, depends on the material, make, ...
Maintenance costs	None	Usually, yes

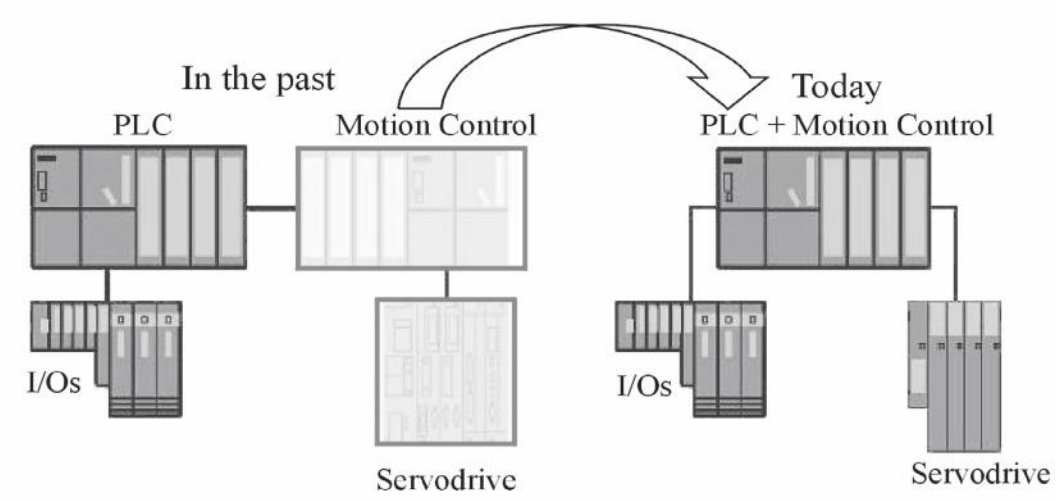

Fig. 2. The trend for integration of the motion control in the PLC

duces the overall costs of the system. It is there where the second trend in the field of automation manifests - integration of the motion control in the PLC itself (Fig. 2).

More and more the PLC manufacturers are emphasizing on developing library modules for facilitating the quick implementation of applied projects. Libraries make it possible for the applied software developers to focus their efforts on the details, specific for the particular application,using multiple ready-made units - for giving commands to the servo-mechanisms, often used algorithms and even ready-to-use technologies.

The new generation of the AMK company controllers are based on the contemporary technologies in the field and contemporary trends in the development of the contemporary automation engineering. Developed are libraries with library modules which are meant mostly for the motion control. These libraries comply with the following requirements:

• to comprise most of the basic functions and technologies - servo-mechanisms control, ratio implementation (underdrive, overdrive), positioning, synchronization of several servo-mechanisms, interpolation of complex profiles, synchronization of print marker, binary variables control, dependent on the servo-motor position, etc.;

• fast response (i.e. optimal code) of the library modules;

• clear (maximum simplified) interface of the library modules;

• ability for changing parameters even after releasing the program modules;

• ability to work both with local access (for integrated in the servo-mechanisms programmable controllers) and with external (accessible through industrial interface);

• purpose dependent organization of the library modules in library files and groups.

The AMK company offers a wide range of program modules for servo-mechanisms control. Here are examined only few of them.

Servo-mechanisms are often required to rotate with definite speed, as also the acceleration ramp, or deceleration ramp, may appear to be more important. There are also applications where this is not enough and forming an S-shaped curve of acceleration and deceleration is needed, and a jerk is set as well.

For achieving this goal three different interpolators of speed are developed - VGEN, VGEN_A and VGEN_AJ. They calculate a position set-point for the servo-mechanism (though here the position is of no importance) guaranteeing an exact set speed of rotation for the servo-motor. They have similar principles of action, thus only the most complex one shall be examined - VGEN_AJ.

The abbreviation AJ is derived from „**A**cceleration" and „**J**erk". This interpolator generates (interpolates) a speed with set by the user acceleration and jerk. Actually, this interpolator makes calculations on its output incremental position, as the increment, compared to the respective time in the positioning regulator (ID2 - SERCOS cycle

time) causes rotation of the servo-motor with a speed diVelocity. Several accelerations for a greater flexibility are also set - udAccel (spinning acceleration), udDecel (deceleration) and udQDecel (fast deceleration). Several jerks correspond there to these accelerations - udAccJerk, udDecJerk and udQDecJerk.

The library module allows to:

• generate an incremental position, according to the speed, set by the entered parameter;

• set individual values of acceleration upon reaching certain speed and stopping (reaching the speed 0);

• fast (emergency) stopping with special, separate value for deceleration (usually greater than the others);

• setting an independent value for a jerk, corresponding to each of the above mentioned accelerations;

• percentage change of the set speed,

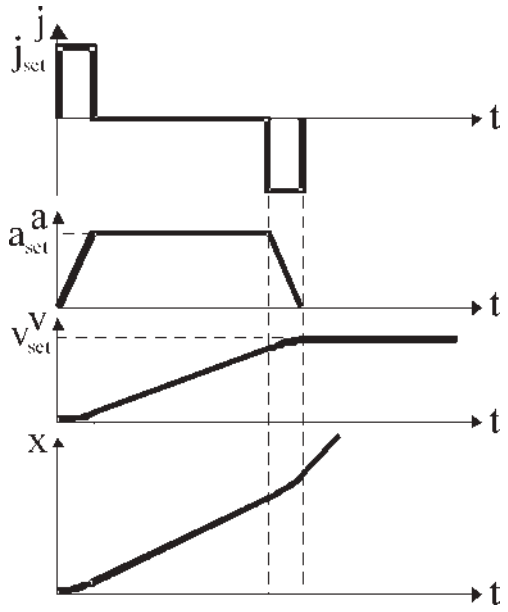

Fig. 3. Action principle of the speed generator

through a special input;

• changing parameters during motion.

The principle of action is explained on Fig. 3. Relations between the jerk, acceleration, speed and the resultant position are shown graphically.

To be able to cover multiple customer requirements, the library module VGEN_AJ offers a flexible control, dependent on the signals of several inputs, and the interpolator state is identified through proper states of a definite number of outputs. The action principle of this library module is shown in detail in Fig. 4.

All the parameters can be changed during the interpolation, as they get into effect timely, but the following limitations must be concerned:

• If the jerk value changes during a constant acceleration, providing the set speed (or speed 0 upon stopping) will be missed, a decreasing of the acceleration with proper (corrective) jerk starts immediately (Fig. 5).

• If the jerk changes during deceleration, upon reaching the set, or zero speed (the phase, when this jerk is active), it shall be neglected and the interpolator shall reach the target speed with the current jerk value.

• If the speed assignment, or the percentage change of speed is changed during the acceleration, upon reaching the set speed, this change shall be considered, on condition that boStart = TRUE, boStop = FALSE and boQStop = FALSE. If the changed speed requires reverse acceleration (i.e. to lower speed), the change of direction shall be realized dependent on the set acceleration and jerk through the parameters udAccel and udAccJerk (Fig. 6) Actually, it is deceleration, but with values for acceleration and the jerk upon reaching given speed.

If during acceleration the input boStop is activated (state TRUE), VGEN_AJ processes first a phase with a jerk, set through the parameter udAccJerk, until it reduces the acceleration to 0 and then starts a deceleration according to the values, given for the parameters udDecel and udDecJerk.

More different is the behaviour of the interpolator upon fast stop through activating the input boQStop (state TRUE). In this case VGEN_AJ reduces the current acceleration instantly, with the jerk value set by the parameter udQDecJerk. When the current acceleration reaches 0, the stopping is performed in accordance with the deceleration value, set through the parameter udQDecel and jerk - udQDecJerk.

The difference between the two ways of stopping is shown in Fig. 7.

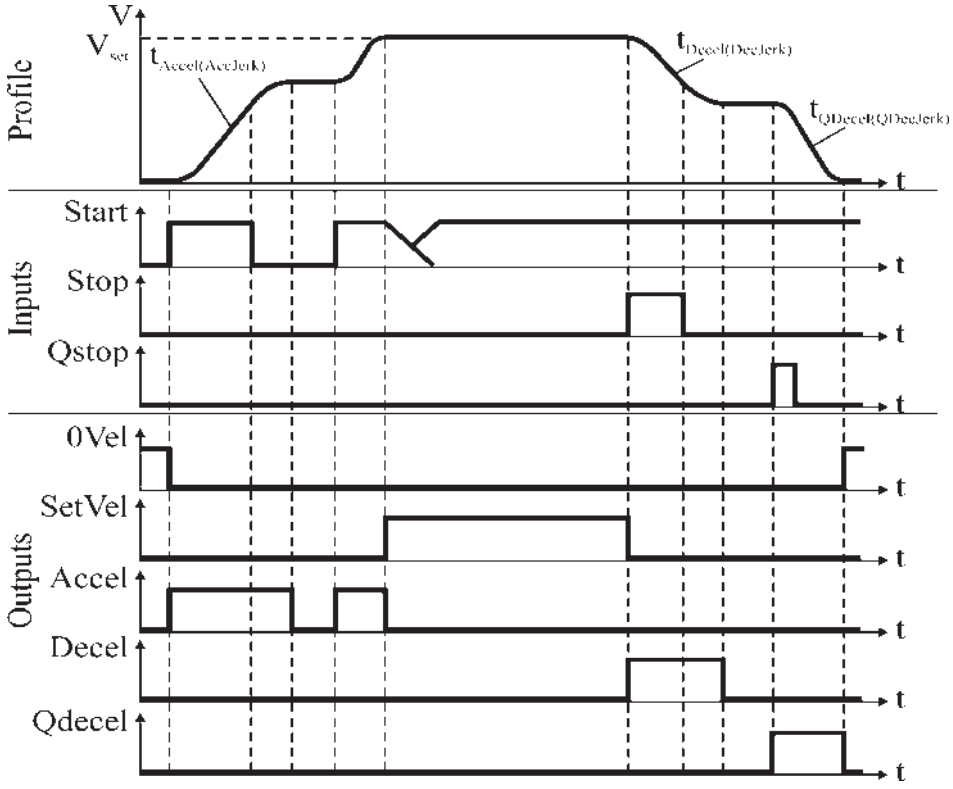

Fig. 4. The library module VGEN_AJ action principle

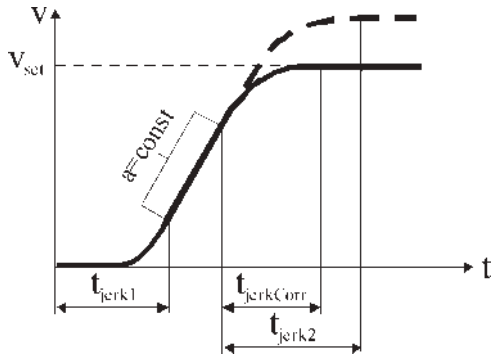

Fig. 5. Jerk correction upon reaching the set speed

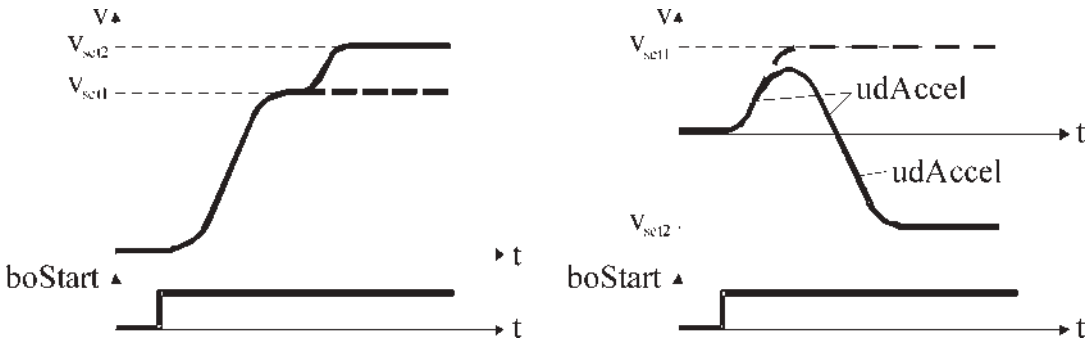

Fig. 6. Reaction of the interpolator VGEN_AJ upon changing the speed

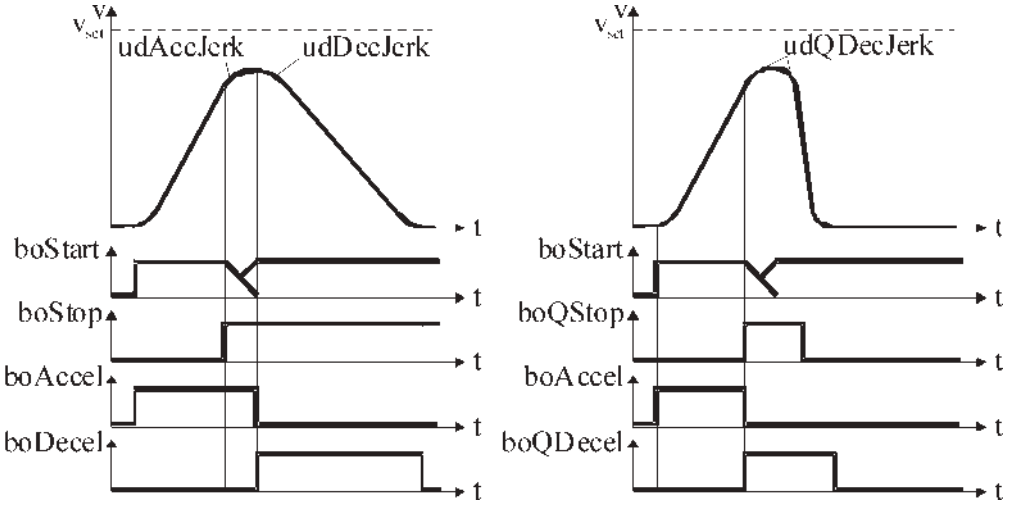

Fig. 7. Reaction upon normal and fast stopping

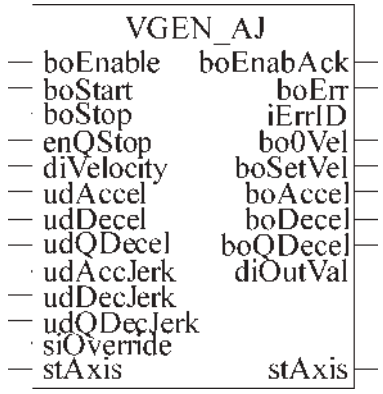

Fig. 8. Function block VGEN_AJ

In Fig. 8 shown is the functional unit VGEN_AJ.

Synchronization by print marker (**P**rint **M**ark **C**ontrol - PMC) is a task often assigned to the contemporary driving and controlling techniques. Some companies already provide ready-to-use solutions, but they are rather partial than devised to satisfy the user needs as a whole. Such applications are oriented mainly to realization on machines and production lines for the packing industry.

In print marker control there are two motions - of the transporting device, and of the tool, as the first is synchronized with the latter (Fig. 9). The PLC program, using the above described library modules can reside in one of the servo-mechanisms PLC, or in autonomous PLC. In both cases for communication with the one, or the two, external for the controller servo-mechanisms, synchronous industrial interface is used. The two servo-mechanisms work in a position-control mode and the function of the library modules is to synchronize the motions, reading and interpolating the error within a given scope. The aim is to achieve good synchronization between the transport device and the tool, so the processing of the material, moved by the transporting device, to be performed always in accordance with the image, pre-printed on it.

The reasons for marker shifting in the most cases are as follows:

- inexact printing of the marker (respectively, the whole image);
- inexact interrelation between the transport and the tool;
- external influence on the marker position (e.g. at temperature changes, stretching the material, etc.).

The library modules adapt to the specific requirements through giving input parameters, controlling the synchronization process. The main parameters and their interrelation are shown on Fig. 10.

The used parameters are named in the way they are defined in the library modules and have the following meaning:

udModulo - size of the format [inc];

udPmcDist - distance between the tool and the sensor [inc];

udSetVal - distance between the beginning of the format and the sensor [inc];

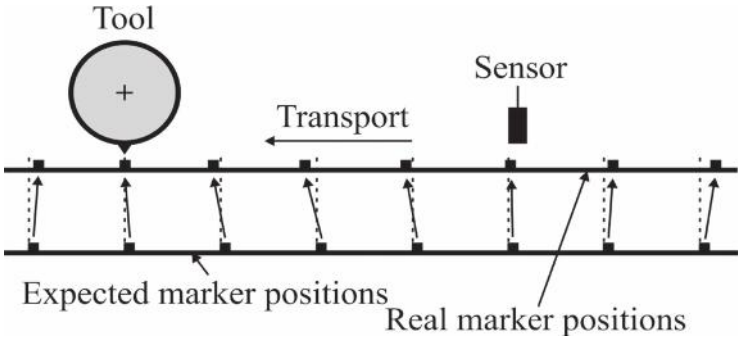

Fig. 9. Action principle of the print marker control

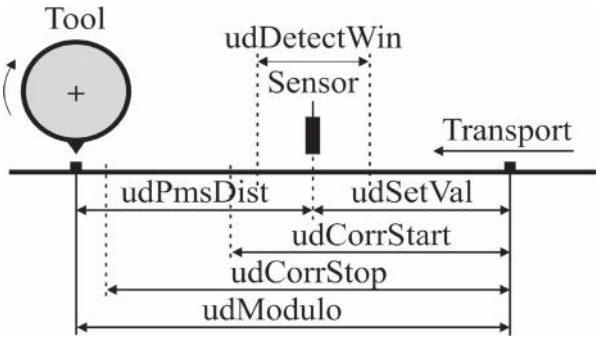

Fig. 10. Parameters, used to synchronize the print marker

udCorrStart - beginning of the correction [inc];

udCorrStop - end of the correction [inc];

udDetectWin - window (interval) in which it is expected a print marker to be found [inc].

The print marker detection is performed through a sensor. It reads the actual position of the marker on passing over it, and the error is calculated as a difference between the read value and the expected one. For better reliability, the marker reading is performed within an interval, set by the parameter *udDetectWin*. Thus the possibility for wrong reading in a section with characteristics similar to those of the print marker, which is not a print marker, is limited. The calculated difference is compensated within the interval from *udCorrStart* to *udCorrStop*.

Due to the technological specificities of the particular implementation, sometimes it is necessary to move the print marker reading sensor away by n formats. To keep the right order of corrections, in this case a FIFO-structure (**F**irst **I**n - **F**irst **O**ut) shall be used, in which they are memorized and processed in a proper moment, i.e. the calculated difference is compensated *n* formats later. This functionality is achieved by using two independent library modules - PM_DETECT (for reading the difference and determining the correction) and PM_CORRECT (for processing the correction), which communicate mutually through the structure ST_CORR_FIFO (FIFO with the corrections) (Fig. 11).

The delay, by which the PM_CORRECT block makes the corrections, is equal to the difference in the formats *n* between the tool and the print marker:

$$n = \frac{udPmsDist - 1}{udModulo}$$

$$x = udPmsDist \% udModulo$$

$$udSetVal = udModulo.(n+1) - x$$

Depending on which motion is leading and which is subordinate, two different implementations of PMC can be specified:

- leading tool, synchronization of the trans-

Fig. 11. Block diagram of a PMC

porting belt (Fig. 12);

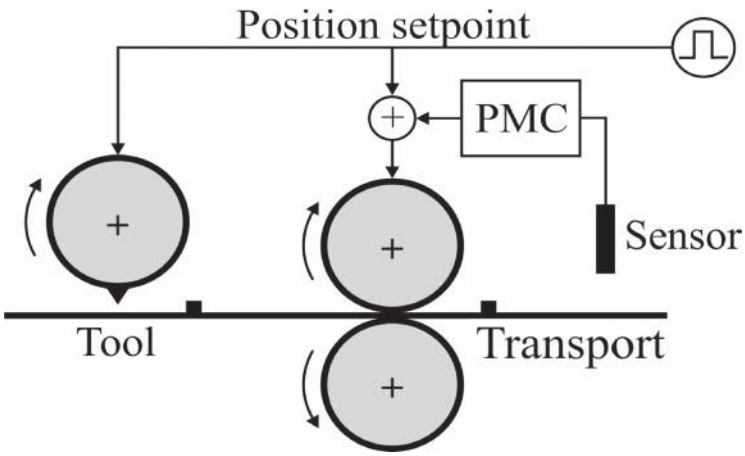

Fig. 12. PMC - synchronizing the transport

The controller synchronizes the transportation belt by the print marker. The motion of the tool is leading, as it can be performed with constant and with variable speed as well.

• leading transporting belt, synchronization of the tool (Fig. 13).

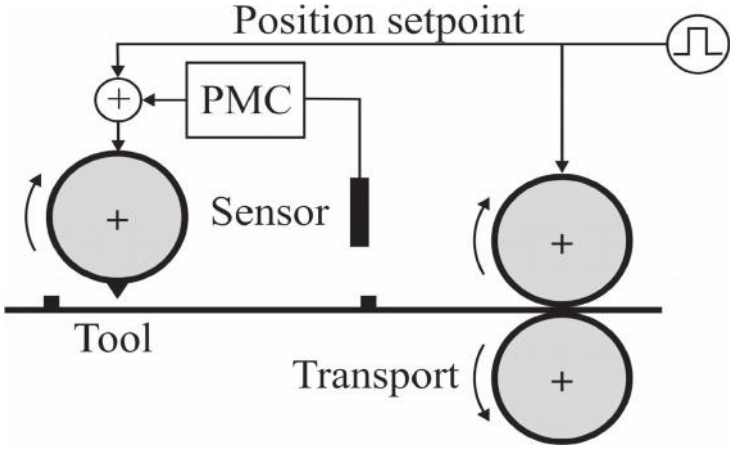

Fig. 13. PMC - synchronizing the tool

The controller synchronizes the tool by the print marker. The motion of the transporting belt is leading, as it can be performed with constant and with variable speed as well.

Sometimes, in real conditions, it happens the print marker not to be read because of a rather big shift that lets it out of the set window udDetectWin or simply because of the fact, that it was poorly printed. If the process gets unsynchronized, due to comparatively high productivity of such a type of machines and production lines, a great amount of unfit for use production shall be produced before measures are taken for removing the problem. For resolving this situation, the library module REF_RESET has been created. Its input parameters are the signals for found and missed print marker, generated by the library module PM_DETECT, as well as the maximum allowed number of consecutively missed print markers. When reaching this number, the library module initiates a sequence for a new synchronization, and then the PMC operation continues normally. This is possible, as far as the above-mentioned basic units for determining the correction and processing have additional inputs for automatic referencing. They can also be used for initial start-up of PMC. To facilitate the users, all these separate library modules are united in one module, and between the modules are established connections (Fig. 14). The result is a complex library module PMC, which comprises all the necessary functionality. The PMC_BASE module is realized on the basis of PM_DETECT, PM_CORRECT and the necessary communication FIFO-structure between them.

Information from the sensor is supplied through the input parameters *boPmSig* and *diPmOffs*. The Boolean variable *boPmSig* is directly connected to the signal from it. Through *diPmOffs* the difference between the discrete input value $diInVal(kT_0)$ is set and the actual input value $diInVal(T_{boPmSig})$ in the moment of registering an active front of the sensor signal. The relation between these values can be presented through the equation:

$$diInVal(T_{boPmSig}) = diInVal(k.T_0) + diPmOffs(k.T_0)$$

This way can be obtained precise information about the moment of reading the print marker by the sensor, though the PLC reads the input variables in precisely set cycles.

To choose the PMC type, we use the Boolean

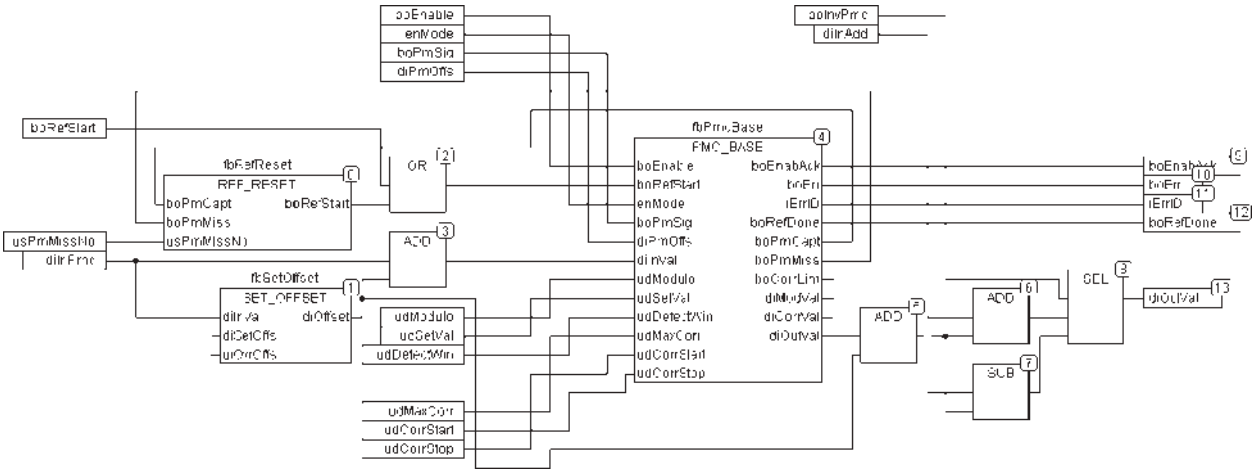

Fig. 14. Block diagram of the full PMC functionality

variable *boInvPmc*. As seen in the figure, through this variable we choose the way, in which the final value of PMC_BASE shall be corrected - through summing up or subtraction.

Another feature, to which attention shall be paid, is that through the library module SET_OFFSET an offset can be set, which can be useful during precise adjustment of the machine.

Representation of the complex library module PMC through the programming language Function Block Diagram (FBD), specified by the standard IEC61131-3, is shown on Fig. 15.

Performing complex positioning profiles through contemporary servo-mechanisms is an often assigned application task. Thanks to the rapid development of the driving techniques nowadays exact processing of these complex profiles does not present such a great problem. However, for this purpose a proper interpolator is needed which to generate the position as an assignment for the servo-mechanism, which in turn is set in a position-control mode.

The AMK company presents a tabular interpolator in the form of a library module for the programmable logic controllers (PLC). Its principle of action is shown on Fig. 16. The aim is on the output of the interpolator to be received a position, corresponding to another position, called "leading", according to the rule, described in a tabular form.

The assignment to the interpolator is obtained through one of the following ways:

• by the actual position of a servo-mechanism

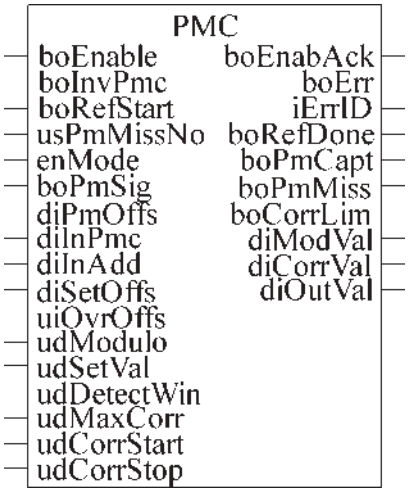

Fig. 15. Function block PMC, represented through FBD

motor;

• by the actual position, received from the encoder, coupled to some leading motion;

• virtual (fictive) generator of assignment.

The interpolator, on its part, based on data in tabular form, interpolates a position which is fed to a servo-mechanism for execution. In the practice it is common between the servo-mechanism and the output of the interpolator to be put other library modules, i.e. for realization of print marker control, for setting a ratio (electronic gear), etc.

The library module has many input and output variables for realising different modes and for satisfying multiple requirements,imposed by the practice. Due to the fact, that similar interpolators in different literature sources are called „CAM profile interpolator", the library module examined is named CAM_PROF (Fig. 17).

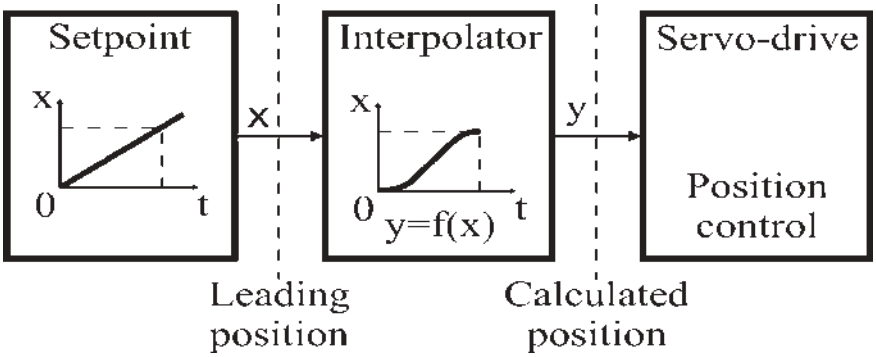

Fig. 16. Action principle of the tabular interpolator

The interpolator can work with three types of tables: Y, XY and XYVA (polynomial).

The interpolator action principle using Y-table is shown in Fig. 18.

The Y-tables have the ability to receive great amount of information, because they store only y-values; moreover, they are easy to track due to their simple structure. Along with the described above advantages, there is a major disadvantage - the points concentration, dependent on the profile of the set curve, cannot be controlled, as the x-coordinates of the points are equidistant. For instance, it is enough a linear section to be described by 2 points, but generally it shall not be, using a Y-table.

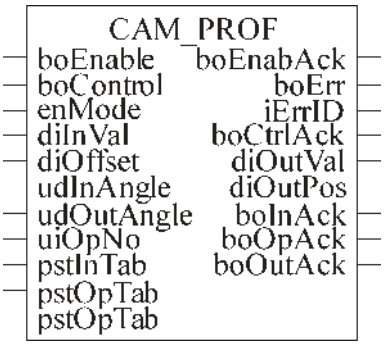

Fig. 17. Function block CAM_PROF, represented through FBD

The interpolator action principle using XY-table is shown in Fig. 19.

The XY-tables are good for describing more complex curves, because the distance between the points by the x-axis can vary. Thus, the redundant points in the linear sections are avoided and more points are concentrated in the non-linear sections. The XY-tables have disadvantages

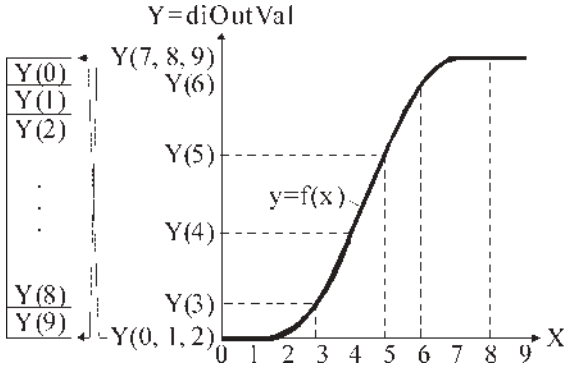

Fig. 18. Action principle of interpolation by Y-table

too - reduced number of points (the memory volumes for the XY-table and for the Y-table are the same, but the number of points in a XY-table is twice as less due to setting the full coordinates) and the values are difficult to track due to the complicated structure.

The parameters for XYVA-tables require more efforts to be determined. They are not suitable for manual generation. For this purpose integrated in the development environment of AMK editor of curves is used, called CAM Editor.

XYVA-tables have one basic advantage against the other types of tables - they describe the desired curve in the most precise manner, as interpolations are made through a polynomial of 5-th order:

$$y(x) = c_0 + c_1.x + c_2.x^2 + c_3.x^3 + c_4.x^4 + c_5.x^5$$

This, however, predetermines there great disadvantage - long calculation time. They are not proper for any type of PLC, and mostly for those which have great power for calculation, as well as for arithmetic co-processors (e.g. x86).

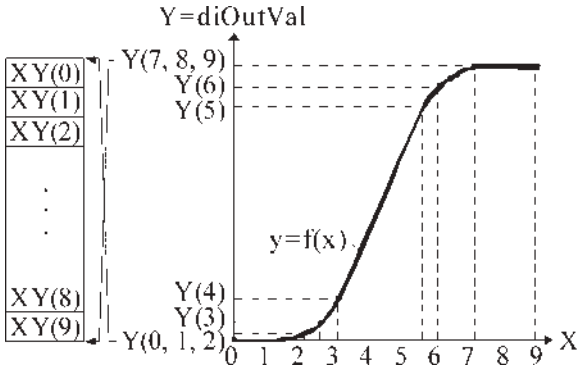

Fig. 19. Action principle of interpolation by XY-table

The tabular interpolator CAM_PROF allows for switching tables at any time of operation. This gives a lot of new possibilities for the developers of applied projects. The switch can be synchronous (upon exiting the current table) or asynchronous (at any time). Moreover, completely different tables can be switched - of different type, number of elements uiNoElement, different resolution by x or by y, etc. At switching tables with different resolution, the new position on the output of the interpolator is calculated by the formula:

$$X_{new} = X_{act} \cdot \frac{X_{\max\_new}}{X_{\max\_act}},$$

where:

X_{act} is the leading position in the current table;

X_{new} is the leading position in the new table;

X_{max_act} is the resolution in the current table;

X_{max_new} is the resolution in the new table.

At a synchronised switch of tables there is no loss of position. For this purpose a new external logic must be created which to trigger the tables switch only on value TRUE on the respective output boInAck, boOpAck or boOutAck. The output variable boOpAck (the same is true for boInAck and boOutAck) remains in TRUE for two cycles (two calls) of CAM_PROF.

The tables switch which is not agreed with the output variables boInAck, boOpAck и boOutAck is called asynchronous. In this case the interpolator takes the new leading position for a current one and continues the interpolation by the new table. This, naturally, leads to a change in the relation between the leading and the interpolated motion.

PROTOTYPE GIS FOR ANALYSIS AND PROTECTION OF THE BULGARIAN ARCHAEOLOGICAL HERITAGE – ARCHGIS

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The ***overall objective of the project "Prototype GIS for analysis and protection of Bulgarian Archaeological heritage"*** is to elaborate a prototype geographic information system (GIS) for analysis and protection of the archaeological heritage in Bulgaria. Accomplishment of this objective will fill the gap in documenting archaeological sites in the country, particularly with regard to their spatial characteristics and their environment. Development of a prototype GIS will be based on the principles of the system approach to ensure the necessary quantitative information about possible structures, behaviours and conditions of modelled sites. By doing so and in case of a possible future corporate solution of the proposed prototype GIS, the system will provide the necessary integrated information environment and relevant tools for complex sites modelling.

In relation with the overall objective, the following ***specific objectives*** of the project are defined:

• Extending the interdisciplinary relations and interinstitutional cooperation in solving complex research problems related to the development and application of geoinformation technologies

in archaeological research and protection of the archaeological heritage.

• Development of a uniform methodology for solving the existing problems, based on commonly agreed standards for identification and codification of existing archaeological information as well as on common requirements for data quality and quantity, position and attributive precision of the data included.

• Optimization of the developed prototype GIS after its testing against as many as possible multi-proxy variations relevant to a concrete modelled territory

• Demonstration of the functional capacity of the system in internet environment with the aim to communicate to the scientific community and the public with the results achieved.

The project activities are carried out by scientific consortium, in which the leading organizational role plays the Sofia University "St. Kliment Ohridski", represented by faculty, postgraduate (PhD) and undergraduate students from the Cartography and GIS Department - CGIS (Faculty of Geology and Geography). They will be directly engaged with the formal project management, coordination of all activities and the work of all participants. Apart from the leading functions in seven of the ten work packages, one PhD and one postgraduate student will also be engaged in elaboration of GIS database and field work for data verification purposes.

The second partner on behalf of the Sofia University - Department of Archaeology (DA) will take part with two faculty members, one PhD student and one postgraduate student. The engagements within the project will be in preparation of five out of ten work packages.

The National Archaeological Institute with Museum - NIAM-BAS is a partner of the project since this is the institution in charge of standardization and documentation of archaeological information in accordance with the requirements for maintenance of the archaeological map of Bulgaria in a digital form. Two researchers who are directly involved in the AMB maintenance, development and improvement are part of this project proposal. The NIAM-BAS project team will lead the development of three and participate in eight work packages.

Participants in the project also originate from the Centre for Underwater Archaeology in Sozopol - CUA, which is the only institution in the country directly involved with promotion and protection of the submerged archaeological heritage in the country.

What are the main research tasks of the project?

The proposed archaeological GIS, which is subject to development within the proposed project is actually the information fundament for the analysis and preservation of the archaeological heritage in the country. Creation of a logically consistent archaeological GIS will be of significant benefit to the archaeological community, government institutions and the wide public. The reason is that it will enable high quality visualization and most of all - analysis of the archaeological heritage in combination with its geographical environment. The link *environment - statistically significant number of archaeological sites* is new to Bulgarian archaeology and it will support the analyses in a completely new way. Such a link will enable researchers to identify patterns in the spatial configuration of sites and the existing trends in the location and geographical characteristics. Integration of data based on a geographical principle will also ease field work through integration of the existing with new data by using GPS and other surveying techniques, such as total stations, etc. Lastly, the high quality cartographic visualization along with the ability to create 3-dimensional images and physical models and mapping services through the internet are of particular interest for museums, since the produced products will ease communication with the public and will create an innovative environment for presentation of information to a broad range of users.

Research tasks which are addressed with this project proposal are related to advantages for the archaeological scientific agenda, which contemporary geographic information systems create. Research tasks in the project include the following:

1. *Registration and documentation of archaeological sites within their geographic settings.* The process of spatial registration, preservation, manipulation and visualization of ar-

chaeological sites, monuments and artefacts in a GIS database offers decisive advantages to proper investigation and preservation of archaeological heritage. As an analogy with all other types of information systems that possess analytical and visualization capacities it is necessary to create a logically well-grounded geo-spatial database that presupposes all the other research and administrative activities. The geographic information system, subject to elaboration within the project, will be based on three interrelated thematic subsystems (Figure 1).

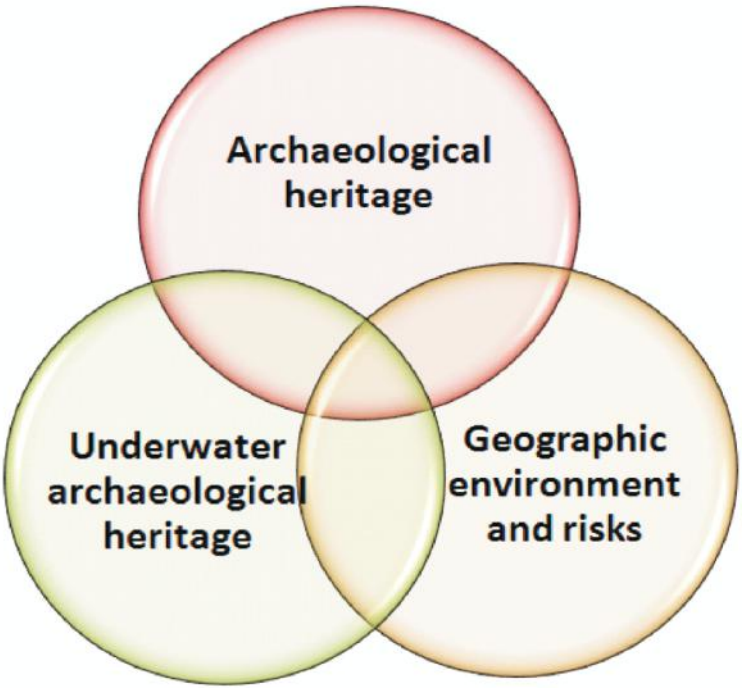

Fig. 1. Thematic subsystems - ArchGIS

2. *Spatial analyses and modelling.* This field of research concerns creation and use of the so-called predictive models for identifying potential (unknown) archaeological sites through special analytical methods. Predictive models divide the territory under study into three groups: areas with low, medium and high concentration of archaeological artefacts. These methods base their predictions on prior definition of the influence of spatial factors on actual distribution of archaeological sites (e.g. small slopes, exposure of the slope, presence of sources of fresh water, sites situated close to other open sites, good visibility, etc). Factors of this type can be identified through application of statistical and geo-statistical analyses on complexes of sites and monuments. These include logistic regression, weight of evidence, analyses of applicability, analyses of nearest neighbour (shortest distance), density analyses, etc.

3. Activities in the project, related to documentation and georeferencing of sites will produce the *scientific foundation for institutionalization* on behalf of the National Archaeological Institute at Bulgarian Academy of Sciences, of documenting the sites through the successor of the so-called "Archaeological Map of Bulgaria", which is currently a register without the functionality to represent the features in a geographical database.

What is the project methodology?

The proposed project in methodological aspect is based on a wider interdisciplinary approach towards the analysis of the archaeological heritage. The creation of an archaeological GIS will be based on the effective partnership between four units, being part of three separate institutions (Sofia University "St. Kliment Ohridski" - Department of Archaeology, Department of Cartography and GIS, Bulgarian Academy of Sciences - National Archaeological Institute, Ministry of Culture - Centre for Underwater Archaeology).

Information and communication technologies represented here by contemporary GIScience and the geographic information systems as dependent by it will play significant role in successful implementation of the project. The focus however will not be placed on technology itself. It will only be considered as means for serving the project goals. Functional requirements for the future archaeological GIS are as follows:

- Creation of a possibility to include structured information from various, mainly humanitarian sciences, but also data coming from the earth sciences;
- Appropriate management of the stored in this way information by using automatic trans-

fer of specialized knowledge;

• Use of technologies that correspond to the International and European standards for geo-information systems and exchange of spatial data, commonly known under the term 'interoperability'[1];

• Ability to do spatial analyses and high quality visualization of geographic data for archaeological sites, along with their natural environment.

Information, which will be collected and systematized within the future Geographic information system, can be separated into two subordinated levels (partitions):

1. Structured information from the previous experience and knowledge of the respective specialists. This partition may be called documental.

2. Data which correspond to the respective ontology, relations, attributes and rules for use. This way all these ontological and functional aspects of the system will conceptually define every registered event.

Part of the activities included in the project address the creation of a Geographic Information System (GIS) that exhibits better functionality in internet: easy access, registration of new sites, updating of the information. An innovative internet application will allow registration of data from a distance combined with regulated access for the end-users. An electronic form will be developed for these purposes. A new approach for data entry online will be proposed to users. All this will be accomplished through the use of the available server GIS[2] in combination with the use of open source products which will enable publishing data online. Doubtlessly the integration of spatial data into a server based GIS will improve management of the database of archaeological sites/events and will optimize the time for electronic presentation of the required information. All necessary information will be available at the NIAM-BAS website.

Using the digital data for the modelled territory as an environment will enable each site in the future to be precisely positioned through the use of GPS coordinates along with other sources of information. Using digital data for the geographic environment will allow automatic calculation of various characteristics of each point within the territory where geographic data is collected. The following thematic layers of geographical data will be elaborated for a decided part of the country in order to ensure the linkage of archaeology and their geographical environment:

- Archaeological sites
- Digital elevation model
- Slopes
- Aspects
- Geological composition
- Geomorphology
- Soils
- Land cover
- Contemporary infrastructure
- Other

Internet users will have access to the geographic location and the basic functionality of work with geographic data. The project will build up over the old version of the archaeological map of Bulgaria (AMB). The system will be created as an update of the outdated software from the late 90-s and add new types of information, minimal content management capabilities, standard GIS functionality and architecture especially designed to encompass new features and data. The successor of the AMB is to be designed to support both online and offline deployment over a single code base, including deployment as portable application (on a flash memory stick for example). This will enable it to provide both online access to the full database, and offline usage by field researchers for data entry and limited data inquiries (depending on the amount of data carried). By completing the fully automated synchronization for the offline and the online instances the IS will be able to serve all the needs for data registration and reporting of researchers working in distant regions where access to the online application may be difficult or impossible.

Tight integration with the existing own GIS server side software will enable the online version to be extended to provide additional serv-

[1] The system should be in line particularly with the requirements of the European INSPIRE directive
[2] ESRI ArchGIS server, available at NIAM-BAS.

ices such as data creation, automatic deduction of geographically dependent characteristics, registration of sites with the GIS server and variety of analytical services over the collected data. The IS will be extended to support content management of additional resources such as images, documents and others (with embedded scanning functionality) and various other types of data. All these features of the system will allow the end-users to have at their disposition the following basic functionalities:

- Interactive data manipulation and navigation;
- Enabling and disabling of layers;
- Identification of features and their descriptive (attributive) information;
- Spatial and attributive queries;
- Creation of sketches and maps;
- Spatial analyses and modelling.

The process of building up of the archaeological GIS will inevitably encounter the problem of statistical significance and representation of the registered information. This is of particular importance for the analysis which can be performed at a later stage. The question of statistical significance is predominantly related to the survey methods applied in the terrain and collecting other information as well as the quality of the specialists involved in this process. The chance sources of information also have their significance but the basic body of data comes from systematic terrain surveys that are conducted with modern methods. The same rule applies to collection of information from the other disciplines that will be integrated in the IS. This way the IS assures not only the statistical significance of the data but also makes the wide audience of end-users confident in working with reliable information. This is why the AMB turns into a reliable tool that serves the central and local authorities in the process of preservation of the rich archaeological heritage of the country. Successful implementation of the proposed project creates a chance for defining specifications for rules for data creation with respect to their further georeferencing.

Functionality of the AMB will also enable users to search according to the registration number of an event, date, attributive data and location. The opposite way will also be possible: by pointing out a point in space - related to it information will appear on the screen. The navigation on the map will have the possibility for selective search of sites/events in a chosen area, administrative region, etc.

In Bulgaria the only functioning information system dedicated to protection of the Cultural Heritage is the Archaeological Map of Bulgaria (AMB). It was created in 1990 and currently holds information about more than 15 000 archaeological sites collected from all over the country. For the time period of its existence it played a crucial role both for protection of the cultural heritage of Bulgaria and as a basic tool for proper scientific research. In the present days the AMB database takes the role of a sanctioned by law institution that operates as a basic reference account in the process of protection of cultural heritage. The references made in the AMB are used as legal argumentation for allowing or stopping investments in particular areas. The references are required by judicial processes and police investigations connected with destruction of archaeological sites and monuments. The information of AMB has useful application in Cadastral Agency, the Commission of land-use, woodland services and in other departments of the local administration. The data coming from AMB are considered as official statement having primary importance in the process of assessment of the impact on the environment caused by investment intentions for developing particular areas.

Unfortunately this system is now outdated and does not meet the increased requirements for processing information as well as establishing better relations between its users. The lack of possibility for precise localization with GPS coordinates of archaeological sites and their boundaries requires significant changes in the system. This also poses the necessity for increasing the standards of field surveys for registration of archaeological sites and monuments.

The instruction for registration of new data into the system, the Intellectual Property Rights, the relations between the different stakeholders that are established as a result of the operation of the AMB as well as the management of the

database are regulated by the statement of the Ministry of Culture[3]. The importance of the AMB was acknowledged by the Parliament. The newly accepted law for the Cultural Heritage[4] regulates the role of the AMB in the process of registration of all the information coming from systematic archaeological surveys into a single database. It is pointed out that the AMB as a national database has to be managed by the NIAM-BAS, National Institute for Preservation of Cultural Heritage and the Ministry of Culture.

The methods for the field survey, with respect to the ability of integration of data within the archaeological GIS, have to solve the following problems:

• Update of the available information of the database;

• Field visits to archaeological sites that aim their precise localization and position verification with the use of a GPS and mobile GIS;

• Boundaries of the sites with greater surfaces defined by GPS coordinates;

• Update of the state of the sites and monuments registered in the database;

• Shortlist of recommendations for preservation of each registered site.

Methodological scheme of the prototype GIS for identification and protection of the archaeological heritage, provided below, gives a clear overview of the logic in accomplishing the proposed activities (Fig. 2).

Expected outcomes

The results of the project implementation will strengthen the inter-institutional links between the Sofia University and the NIAM-BAS and will create favourable conditions for accomplishment of future interdisciplinary research in the field of archaeology based on mutual geoinformation technologies. The expected outcomes will also benefit the transfer of knowledge between specialists in different areas (geography, cartography, GIS, archaeology, archaeometry, cultural anthropology, tourism, etc.) as well as between the participants, other interested institutions and the public.

The expected outcomes can be summarized into five major groups, each with a real world application:

1. Effective organization of archaeological in-

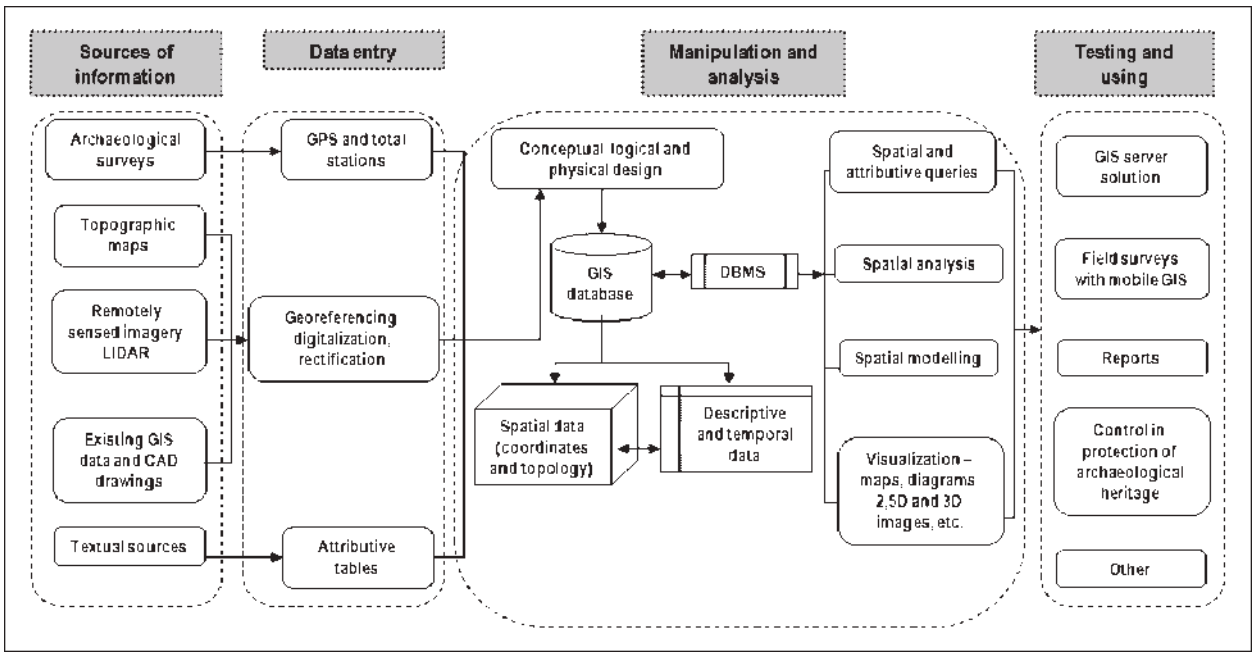

Fig. 2. Methodological scheme of the proposed prototype GIS - ArchGIS

[3] Order 26, published in the State Newspaper, issue 34, 1996
[4] Published in the State Newspaper from 13. 03. 2009

formation

• Elaborated and tested methodology for effective data storage and use of archaeological information in GIS environment based on the critical analysis of world trends and experience.

• Prepared GIS database for archaeological sites and their environment for a chosen territory. Verified information through field work with a mobile GIS and GPS.

• Support for the management of the Archaeological map of Bulgaria, which currently exists at NIAM-BAS.

• Transformation to an industrial GIS standard for the information system implementation of the Archaeological map of Bulgaria through the use of recognized GIS technologies and data interoperability.

2. Protection of the archaeological heritage

• Inventory of the condition of archaeological sites in the territory of concern in relation to the currently functioning national and European legal framework for protection of archaeological heritage.

• Identification of archaeological sites and structures, which are endangered by environmental risks and disasters.

• Identification of archaeological sites endangered by human activities.

3. Real world use of the elaborated prototype GIS

• Fulfilled possibilities for conduction of spatial analyses for characterizing the density of sites, their patterns, trends in time and space, etc.

• Effective visualization of archaeological sites, incl. ensured options for simultaneous visualization of many sites on screen and on paper (maps, 2,5D and 3D visualization, diagrams, photos, text, drawings, video, etc.).

• Real world options for predictive modeling of the archaeological sites position with respect to protection of sites in the dynamically changing conditions of the environment (construction, development, etc.).

4. Increasing the information provision concerning the archaeological heritage of Bulgaria

• Supply of authorized online access to web application of the elaborated prototype GIS for researchers as well as the public.

• Creation of conditions for effective exchange of data between NIAM-BAS and huge electronic archives such as Cadastral agency, Ministry of interior, libraries, NGOs, etc.

• Creation of conditions for unification of services and provision of services for exchange of spatial data in relation to the requirements of the INSPIRE directive.

5. Improvement of the educational process

• Practical applicability of the results in the process of educating undergraduate and postgraduate (incl. PhD) in archaeology, archaeometry at the departments of GIS and Cartography and Archaeology at Sofia University.

• Increase of the effectiveness of the educational process through provision of mapping and other information through the internet. Creation of an educational environment, stimulating the self-learning through generation of questions arising in the course of data analysis and visualization.

• Stimulation of self-learning through search based on own motives and experimenting with the information system.

EQUAL IN EUROPEAN RESEARCH AREA

BULGARIAN VIPs

Prof. STEFAN KOSTIANEV, MD, DSc

Pathophysiology Department, Medical University - Plovdiv
15A, Vassil Aprilov Blvd., 4000 Plovdiv, Bulgaria
Phone/Fax: +359 32 602 593; E-mail: s_kostianev@mail.orbitel.bg

Rector of Medical University – Plovdiv; author of more than 160 articles in peer-reviewed journals, took part in writing over 10 textbooks, educational supplementary textbooks and monographs.

Prof. DMSc Stefan Stoilov Kostianev, was born in 1952 in Sofia. He graduates in medicine "summa cum laude" in 1979 from Higher Medical Institute, Plovdiv. In the same year he becomes assistant professor in Pathophysiology Dept. at HMI, Plovdiv, where he has been working since then. Prof. Kostianev defended successfully his PhD thesis in 1987, became associated professor in 1996, acquired DMSc in 2004 and became full professor in 2006. In 1999 he was elected as Vice Dean of the Medical Faculty. He is head of Pathophysiology Dept. at the same faculty since 2004 and was elected as Rector of Medical University - Plovdiv in July 2011.

He specialized in elite scientific centers and laboratories abroad. Prof. Kostianev has taken part in the leadership of numerous scientific organizations. He is the chief editor of the highest ranked medical journal in Bulgaria - *Folia Medica*. Prof. Kostianev has participated in scientific and educational projects financed by the European Research Fund, Tempus, The World Bank, Bulgarian Ministry of Education, Youth and Science, Medical University - Plovdiv and in many clinical investigations.

His scientific interests are in the area of functional pulmonary diagnostics, pediatric respiratory physiology, blood-gas analysis and acid-base balance, functional diagnostics software development, physiology and pathophysiology of exercise and sleep.

Prof. Kostianev has published more than 160 articles in peer-reviewed journals and took part in writing over 10 textbooks, educational supplementary textbooks and monographs, and in constructing national guidelines. He has been cited more than 500 times in foreign and Bulgarian journals.

Numerous MSc and PhD theses were successfully defended under his scientific guidance. At present he is a PhD advisor of 5 PhD students.

Hobbies: chess, guitar, sport, aphoristics; Member of the Union of Independent Bulgarian Writers.

Prof. IVAN MINKOV, DSc

Department of Plant Physiology and Molecular Biology and Genomics Research Center,
Plovdiv University „Paisii Hilendarski"
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Head of the Department of Plant Physiology and Molecular Biology, and the Genomics Research Centre (GRC). Author of 12 books, a number of research papers, and 8 monographic book chapters in international editions.

Professor in the area of Plant Molecular Biology, Ivan Minkov has been the head of the Department of Plant Physiology and Molecular Biology, as well as the Genomics Research Centre (GRC), University of Plovdiv (UoP) for the last 22 years. With 18 defended PhD students, he made a major contribution to Molecular Biology and Bioinformatics research at the University. He is a leading scientist at UoP with a high publication record and strong participation in European research programs. Prof. Minkov is a member of the Program Committee and National Contact Point for the Seventh Framework Program.

Education and career

Prof. Minkov was born in 1949 in Plovdiv. He graduated from the University of Plovdiv in 1972 as a MA in General Biology. In 1977 he defended his PhD thesis at the University of Plovdiv in the area of Plant Physiology and Biochemistry of resurrection plants with high drought tolerance. Later, he specialized in the area of Chlorophyll Biosynthesis in higher plants at the University of Gothenburg, Sweden for a total period of 2 years, and later at the Institute for Photobiology, Minsk, Belorussia. After that he defended his Doctor of Sciences degree in the same field and was promoted to full professorship at the University of Plovdiv shortly thereafter, in 1996.

After 1989, Prof. Minkov started work on a research infrastructure in the field of Plant Molecular Biology and Plant Biotechnology. Over the course of this project he visited a number of European Universities and created numerous working collaborations, many of which still exist today. Many young researchers have been educated and have defended their own PhD theses over these collaborations.

Areas of teaching and research experience

The main areas of teaching and research are Molecular Basis of Plant Stress Response - oxidative Stress in Higher Plants, Chloroplast Development and Chlorophyll Synthesis, Plant Microspore Cultures, Regulation of Nitrate Reduction in Higher Plants, DNA markers in the taxonomy of parasitic plants, Plant Transformation, viroid RNA-proteins interaction, Bioinformatics - microRNA in genes regulation.

Publications

Prof. Minkov has published 12 books, a number of research papers, as well as 8 monographic book chapters in international editions. Around 40 research papers are in peer review international journals with a combined total impact factor of about 85 and more than 520 citations. Prof. Minkov also holds two patents for biologically active compounds for nitrates regulation in plants.

Teaching and research projects

In the period of 1996-2000, Prof. Minkov supervised five TEMPUS projects for enhancing the higher education in the field of Molecular Biology and Plant Biotechnology. He later participated as a local coordinator in 7 projects in Framework Program 6 (FP6) and 4 projects in FP7. Presently, Prof. Minkov is a coordinator of a large FP7 REGPOT project for developing research infrastructure in the area of Systems Biology. Under his guidance, the Department and the GRC also took part in projects from NATO scientific program and Swiss National Science

Fund. He also coordinated a number of projects from the National Science Fund - and especially one large project aimed at establishing a Genome Research Centre.

Prof. Minkov has initiated two Bachelor degrees at the Faculty of Biology - Molecular Biology and Bioinformatics - both as a concept and as a material science with human and teaching potential.

Awards

Prof. Minkov was awarded with the Pythagoras Award for the largest research financing in the period 2008-2010, and the Regional Centre headed by him was distinguished with a plaque from the Ministry of Education and Sciences as a center with the highest FP6 participation (2009).

Prof. IVAN PETKOV, DSc

Department of Organic Chemistry, Faculty of Chemistry, University of Sofia "St. Kliment Ohridski"
Phone: +359 2 8161 442, E-mail: ipetkov@chem.uni-in sofia.bg

Head of the Department of Organic chemistry at University of Sofia. Leading University Research Centre for Nanoscience and Knowledge-based Materials.

Prof. Ivan Petkov received his MSc degree in the Faculty of Chemistry at University of Sofia in 1973 and a PhD degree at the Department of Organic Chemistry, Faculty of Chemistry, University of Sofia "St. Kliment Ohridski". He defended his doctoral thesis on the topic of "On the photoinduced prototropic and metallotropic tautomerism of β-dicarbonyl compounds, their metaloderivatives and nitrogen-containing analogues" in 1981.

Prof. Ivan Petkov defended his DSc thesis "Photochemical behaviour of β-dicarbonyl compounds and derivatives of benzotiazole" in 2003. He is currently leading the Department of Organic Chemistry at University of Sofia. His scientific interests are in the fields of organic synthesis, organic photochemistry; photochemical properties of organic compounds, dyes in solution, polymer films, monolayers on different surfaces; photochromism, electrochromism, termochromism, radiochromism of organic compounds, derivatives and polymer films; photoelectrochemistry; photovoltaic cells and effects; organic materials for nonlinear optics, photonics; applied photochemistry - dosimeters, solar indicators, sensors, solar cells, photoprotectors; intelligent organic compounds.

Prof. Ivan Petkov has participated in many projects. Part of these projects is held in partnership with international organizations such as:

- University of Sofia - Pharmachim: *Photoprotector "COMBRENOL" for the cosmetics*;
- Bulgarian Science Fund: *Plastic gamma dosimeters; Composite materials for application in the HIGH-TECH: immobilization of organic components in nano- and subnano molecule sieves*;
- MAGATE: *Plastic gamma dosimeters for foods*;
- Programme Copernicus: *Sensors for chemistry, medicine, and biology*;
- CEA (France): *Photochromic polymer films with nonlinear optical properties*;
- University of Sofia: *Modification of inorganic glasses with sensitive organic compounds*;
- Leading University Research Centre for Nanoscience and Knowledge-based Materials;
- International project SENSIT EUREKA.

He has above 120 publications, 4 books, 5 patents.

Member of European Photochemical Association, Inter-American Photochemical Association; Member of the Scientific Board of *International Journal of Photoenergy*; Chair holder, UNESCO Chair in Sustainable Development and Ecological Awareness.

He specialized at: Department of Organic Chemistry, University of Amsterdam, The Netherlands; Guest Professor in the University of Tokoshima, Japan; visiting researcher in Centre D'Etudes de Saclay, Electronics and Nuclear Instrumentation Department, Group for Organic Devices, Paris, France; Guest Professor in University of Bordeaux, France, Department of Molecular Photophysic and Photochemistry; visiting researcher in Centre D'Etudes de Saclay, Electronics and Nuclear Instrumentation Department, Group for Organic Devices, Paris, France; Guest Professor in Laval University, Department of Physics, Quebec, Canada; Guest Professor in Research Institute of Electronics, Shizuoka University, Hamamatzu, Japan.

Assoc. Prof. NELLY GEORGIEVA, PhD

Department of Biotechnology, University of Chemical Technology and Metallurgy, Sofia
Phone +359 2 8163307, E-mail: nelly.georgieva@yahoo.com

Leader of research projects; author of more than 60 manuscripts in international scientific journals, two patents and more than 30 papers and published contributions to international congresses.

Nelly Georgieva is an Associate Professor at the University of Chemical Technology and Metallurgy, Sofia, Department of Biotechnology. She graduated from Sofia University "Kliment Ohridski", Faculty of Biology in 1987. In 1997 she presented PhD thesis on "Selection of methionine-enriched analogue-resistant mutants of *Trichosporon cutaneum*" at the Institute of Microbiology of the Bulgarian Academy of Science. From 1997 till 1999 she made postgraduate fellowship sponsored by DAAD Agency of Germany, Halle-Wittenberg, Germany.

Areas of scientific interest: bioremediation of waste water containing phenol products and heavy metals; immobilization and entrapment of cells; probiotic and microbial food supplement, biobleaching of flax fibers with enzymes.

Assoc. Prof. Nelly Georgieva delivered lecture courses on Microbiology for bachelor and master degree students and also Microbiology, Biochemistry and Biotechnology products in German language. She is an author of more than 60 manuscripts in international scientific journals, 2 patents and more than 30 papers and published contributions to international congresses.

Assoc. Prof. Nelly Georgieva is the leader of projects sponsored by the National Science Research Fund, Ministry of Education and Science - "Investigation on covalently bound yeast Candida for biotransformation of furfural" - 1994 and "Synthesis and application of nanomaterials by biofilm formation for waste water treatment" - 2010.

Assoc. Prof. ZHECHKO DIMITROV, PhD

LB-Bulgaricum Plc. R&D Center, 12-A, Malashevska Str., 1000 Sofia, Bulgaria
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Head of the Centre for Research and Development at LB Bulgaricum. Author of over 40 scientific publications in foreign and Bulgarian scientific journals and 5 projects with external financing.

Zhechko Panayotov Dimitrov was born in 1966 in Shumen. He graduated Master degree from the University of Food Technologies in Plovdiv and graduated as an engineer-technologist in the specialty "Microbiological and fermentation processes" in 1994. From October 1994 until now he works at the Center for Research and Development at the state company LB Bulgaricum Plc (LB). In 1996 he became a research associate in the specialty "Instrumental Analysis" and in 1997 he created laboratory "Instrumental Analysis", covering chromatographic and electrophoretic analyses. In 1998 he specialized in the Central Research Institute of the Corporation "Meiji Milk" in Japan. Between 1999 and 2002 he introduced in Bulgaria DNA-based methods for typing of microorganisms such as pulse electrophoresis, AFLP and Denaturing gradient gel electrophoresis. The first two methods make it possible to reliably identify and distinguish microbial individuals (strains), which is impossible with conventional microbiological methods. The third method allows characterizing the bacterial communities without the use of cultivation of representatives of various bacterial species. In 2007 Zhechko Dimitrov acquired educational and scientific degree "Doctor" in Molecular Biology at Bulgarian Academy of Sciences - Institute of Molecular Biology. In the same year he founded Laboratory "Molecular Biology" at LB. In 2009 he acquired the academic title of Associated Professor in Bioactive substances at the University of Food Technologies in Plovdiv. Much of the research work of Zhechko Dimitrov is aimed at studying the health (probiotic) effects of lactic acid bacteria (LAB). In connection with these studies, Zhechko Dimitrov introduces or develops methods to evaluate the probiotic effects of LAB at laboratory level, making it possible to undertake studies on probiotic properties of hundreds and thousands of strains, and thus to select those strains that possess the most prominent properties. The next stage with selected strains with beneficial human health effects are clinical trials.

Main subject of research of Zhechko Dimitrov are probiotic properties of LAB, such as: effects to human immune system, anticholesterolic effects, properties of some bioactive peptides to decrease high blood pressure and to utilize calcium from the food, and others. After testing for probiotic properties, including clinical trials, probiotic products are developed on the base of the selected probiotic bacteria. In the areas of molecular biology and probiotic effects Zhechko Dimitrov is author of over 40 scientific publications in foreign and Bulgarian scientific journals with a total impact factor over 18.5, 5 projects with external financing to total about 2.6 million Euro, and three patent applications. Zhechko Dimitrov has 4 scientific publications in Advances in Bulgarian sciences journal. Since the beginning of 2011 Zhechko Dimitrov is head of the Centre for Research and Development at LB Bulgaricum. Over the next few years under the leadership of Dr. Zhechko Dimitrov LB Bulgaricum's Research Centre will focus its efforts to introduce new research areas of technological research and health properties of LAB, as well to conduct clinical trials to definitively prove the probiotic properties. An important focus will be the development of new probiotic products. Since the beginning of 2011 the development of four new probiotic products containing specially selected probiotic LAB was completed as a result of long research and innovation. Main types of the probiotic products will be: fresh dairy products with probiotics and prebiotics, dried products, cheeses fortified with bioactive peptides, drinks, capsules, etc. It is expected that the probiotic products developed in LB Bulgaricum will be introduced on the Bulgarian market in 2012 and from 2013 on foreign markets.

AWARDS

JOHN ATANASOFF AWARD FOR THE YEAR 2011

At an official ceremony in the beginning of October 2011 President Georgi Parvanov for the ninth time bestowed **John Atanasoff Award** for achievements in the development of information society. The award is bearing the name of John Atanasoff - renowned scientist of Bulgarian origin and creator of the first digital electronic computer in the world.

The award was established by President Georgi Parvanov in 2003. It is adjudged every year to a young Bulgarian with considerable contribution to the development of computer and information technologies and information society in Bulgaria.

The carrier of the John Atanasoff award for 2011 is Dr. Kuzman Ganchev. He was born in Sofia in 1981. He graduated from Swarthmore College - Pennsylvania with bachelor degree in computer sciences, and was distinguished by special award for high performance. He got his MA from University of Pennsylvania, where in 2010 he successfully defended his PhD thesis in the field of machine learning with application of computer linguistics, on which he worked for several years.

In 2008/2009 he was a visiting fellow at the Institute for Parallel Processing of Information at the Bulgarian Academy of Sciences. He conducted a course in linear modules in machine learning for Master's program in artificial intelligence at the Faculty of Mathematics and Informatics of Sofia University.

Since autumn 2010 he works for Google.

He has publications in prestigious journals and was involved as a reviewer for authoritative publications, as well as in the international conference "Recent Advances in Natural Language Processing" held in Bulgaria in September 2009.

He is the author of a software package for machine learning which at present is used by the Ontotext joint-stock company, Sofia for analysis of biomedical texts. Other approaches and concrete systems developed by him or with his participation are used in American companies TrialPay Inc. and the Bank of America. Method for decreasing the quantity of machine memory necessary for self-education offered in his article in 2008 has been approved and used by Yahoo! Inc.

Diploma for **applied projects and developments in the field of electronic management and information society in Bulgaria** in 2011 was adjudged for the second time. The Head of State handed it to **Haralambi Haralambiev**. He was born in 1985 and in 2009 graduated from Sofia University St. Kliment Ohridski, faculty of mathematics and informatics, with bachelor degree in discrete and algebraic structures. He has a number of national and international insignias of honour in the field of informatics and computer sciences. In the recent years he heads the applied research and development centre at Musala Soft Company. Within the centre he is responsible for 4 innovative projects having contribution to the development of definite aspects of information technologies at national and international level. Owing to his energetic activity Musala SOFT Company received Pythagoras award of the Ministry of Education, Youth and Science for successful cooperation with scientific and public organizations for 2010.

The Head of State also handed **"John Atanasoff award for schoolchildren"**. This year it was adjudged to **Rumen Hristov**, born in 1993, a student at "Nancho Popovich" High School of Natural Sciences and Mathematics in the city of Shumen. Among the distinctions he has won there are: the 2007 award of the city of Shumen for the student of the year in the field of natural and mathematical sciences, golden insignia for contribution to the youth innovation and information society; gold medal at the International Olympiad in mathematics in Thailand, gold medal at the International Zhautikov Olympiad in mathematics, physics and

informatics in the city of Almati, Kazakhstan, gold medal and second place in the final ranking at the International Olympiad in informatics in Canada; gold medals at the Youth Balkan Olympiad in informatics in Shumen, Belgrade and Bistritza (Romania), where he had highest score in the final ranking; silver medal at the International Olympiad in informatics in Cairo. He is "John Atanasoff award for schoolchildren" winner for 2008 and 2009.

In his speech during the ceremony the Head of State expressed his content for validation of the initiative as a serious moral stimulus for young people who dedicated themselves to scientific and applied research in the field of computer technologies, as well as for commitment to it by Bulgarian universities, BAS, state institutions and business.

AWARDED WORKS AND SCIENTISTS IN THE COMPETITION FOR HIGH SCIENTIFIC ACHIEVEMENTS BY THE UNION OF SCIENTISTS IN BULGARIA IN 2011

FOR RESEARCH ACHIEVEMENTS IN DISSERTATION WORKS DEFENDED IN 2010 BY RESEARCHERS AGED UPTO 35 YEARS

Diploma for Research Achievements and Cash Prize

Dr. RAZVIGOR BORISLAVOV DARLENSKI from Tokuda Hospital for his dissertation "Clinical-experimental Investigations on the Role of Epidermal Barrier at Contact Skin Hypersensitivity and Irritation".

Diploma for Research Achievements

KIRIL STOYANOV SHTEREV, PhD from the Institute of Mechanics at BAS for his thesis "Digital Modeling of Micro-flows of Compressible, Viscous, Heat-conducting Gas".

SHAZIE YUMER YUSEIN–MYASHKOVA, PhD from the Institute of Molecular Biology of BAS for her dissertation "Molecular-genetic Characteristics of hcIB Gene Coding the Histamine Receptor in Drosophila".

YULIA RUSLANOVA ROMANOVA, PhD from the Chair of Physical Chemistry at the Faculty of Chemistry of Sofia University St. Kl. Ohridski for her dissertation "Influence of the Environment on Geometry, Electronic Structure and Magnetism of Polyaniline".

FOR HIGH RESEARCH ACHIEVEMENTS OF YOUNG SCIENTISTS, MEMBERS OF THE UNION, UPTO 35 YEARS OLD

Diploma and Cash Prize

Theologian **BLAGOVEST BLAGOEV VARBAKOV** from the Faculty of Theology of Sofia University St. Kl. Ohridski for his monograph "Founders of the Pchelin Church" and an essay "Spiritual Aspects of the Christian Art".

Diploma

Chief Assist. **VIOLETA VALCHEVA RUSEVA**, PhD from the Institute of Microbiology at BAS for five articles in international journals in the field of infectious microbiology

Assoc. Prof. **NATALIYA DANAILOVA NIKOLOVA**, PhD from Nikola Vaptzarov Naval Academy - Varna, for 8 publications in the field of qualitative analysis of solutions and of simulation modeling, published in prestigious referenced international journals.

FOR HIGH RESEARCH ACHIEVEMENTS OF SCIENTISTS, MEMBERS OF THE UNION, OVER 35 YEARS OLD

Diploma and Cash Prize

Prof. **ZDRAVKO IVANOV LALCHEV**, DSc Head of Department at Sofia University St. Kl. Ohridski Faculty of Biology - for his monograph "Alveolar Surfactant and Neonatal Respiratory Distress Syndrome. Physiological Aspects and Modern Treatment" and 32 scientific publications, from which 18 in journals with impact factor, connected with development and application of model systems in therapy of some types of pulmonary pathologies.

Prof. **ILZA KONSTANTINOVA PAZHEVA**, DSc from the Institute of Biophysics and Biophysical Engineering of BAS - for 16 works in the field of computer-assisted research of the transport P-

glycoprotein, with 12 of them published in journals with high impact factor and one chapter of the monograph - in specialized research publishing house Bentham.

Prof. **KIRIL VASILEV DIMCHEV** - for three monographs: "Methods of Teaching Bulgarian Language. Realities and Trends", "Fundamentals of Methods of Teaching Bulgarian Language", "Linguistics. Language Teaching. Methods of Language Teaching".

Assoc. Prof. **MITKO KONSTANTINOV GAIDAROV**, PhD from the Institute of Nuclear Research and Nuclear Energy of BAS - for a cycle of research works thematically directed to investigation of nuclear structure of exotic nuclei and their participation in nuclear reactions. Seven of them are published in international scientific journals with impact factor.

Prof. **HRISTO BOYANOV BOYADZHIEV**, DSc from the Institute of Engineering Chemistry at BAS - for his monograph "Theoretical Chemical Engineering. Modeling and Simulation" published in Germany.

Diplomas

Assoc. Prof. **ANDREI IVANOV CHORBANOV**, PhD from the Institute of Microbiology at BAS - for 12 scientific publications in the field of new generation of vaccines and new molecules for therapy of autoimmune diseases with potential application in the clinical practice.

Prof. **MONI ESHUA ALMALEH**, DSc from New Bulgaristics Department of the New Bulgarian University - for his monograph "Light in the Old Testament"

Chief Assist. **NADEZHDA VASILEVA MARKOVA**, PhD from the Institute of Organic Chemistry at BAS - for her article „Tautomeric Equilibria of 5-Fluorouracil Anionic Species in Water", published in Journal of Physical Chemistry.

Chief Assist. **STEFKA GEORGIEVA VENKOVA**, PhD from the Institute of Art Studies at BAS - for her monograph "Music of the Catholic Church of Eastern Rite in Bulgaria", adding new knowledge to the history of music, musical archival science and anthropology of music.

Prof. **STEPAN AGOP TERZIYAN**, DSc from "Angel Kanchev" University of Ruse - for 8 articles in referenced journals with total impact factor 6,059 in the field of differential equations.

Diploma for Scientific-Applied Contribution

GEORGI VLADIMIROV VLADIMIROV, PhD an expert at the Ministry of Culture - for two monographs: "The Other Bulgaria on the Volga: the Lost Civilization" and "Golden Horde and the Bulgarians".

YULIA KIRILOVA MAKSIMOVA, PhD from the University Hospital of Neurology and Psychiatry St. Naum - for a monograph "Alzheimer's Disease. Practical approaches of Communication".

AWARDS FOR WORKS CREATED BY BIG RESEARCH TEAMS ON PROJECTS OF NATIONAL AND FOREIGN PROGRAMS

Diploma for Considerable Scientific-Applied Contribution

"ATLAS OF AREAS AT RISK OF TSUNAMI ON THE NOTHERN BULGARIAN BLACK SEA COAST. BALCHIK AREA", developed by a team with editor-in-chief and compiler Assoc. Prof. Boyko Rangelov.

ENCYCLOPEDIA "BULGARIA. DISTRICTS. REGIONS. MUNICIPALITIES". The idea for writing this work belongs to Prof. Petar Slaveikov - dean of the Geology and Geography Faculty of St. Kl. Ohridski University of Sofia, who is a co-author and editor of the work. Other co-authors are: Assoc. Prof. Anton Popov, Assoc. Prof. Stilyan Dimitrov, Chief Assistant Aleksandar Kotzev, and Chief Assistant Kliment Naidenov.

NACID IS HONORED WITH DIPLOMA FOR SUCCESSFULLY REALIZED PROJECT ON THE OPERATIONAL PROGRAM "ADMINISTRATIVE CAPACITY"

At the official ceremony in the Museum of Archeology on November 29, 2011 **National Centre for Information and Documentation (NACID)** was honored with a Diploma for successfully realized project on the Operative Program "Administrative Capacity". The project *"Optimization, integration and introduction of electronic management of administrative and information services on acknowledgement of diplomas for higher education and professional qualifications obtained in the countries from the European region and third countries"* is one of the six projects distinguished by the Managing Body. It was presented as a good practice at the annual information event "OPAK - main instrument for implementation of administrative reform and electronic management. What we have achieved, what we are proud of".

The project was realized during the period of June 2009 - March 2011, and unquestionable benefits for the citizens and business are:

- Accelerated administrative service on acknowledgement through a shortened time for consideration of applications, not exceeding 2 months;
- Access to public information from the register of issued certificates and register of refusals;
- Electronic filing of documents for academic recognition of diplomas and possibility of getting the certificate electronically;
- Monitoring on the Internet of the process of processing of the filed documents by every applicant - the course of consideration of the application, the stage of the procedure, the decision taken on the application;
- Simplified checking of the academic status of a foreign higher educational institution and legitimacy of the proposed by it education before its final choice;
- Checking of the academic and qualification status of candidates for the job in the relevant organization on the part of employers;
- Access to current information on the new site of NACID on procedures of acknowledgement in Bulgaria and abroad.

ARTICLES

RECENT PUBLICATIONS OF BULGARIAN SCIENTISTS

Title: **Investigation of critical safety function "Heat sink" at low power and cold condition for Kozloduy Nuclear Power Plant WWER-1000/V320**

Authors: Andreeva, M. , Pavlova, M. P., Groudev, P. P.

Source: Annals of Nuclear Energy, In Press Available online (15 Nov. 2011)

Author Affiliations: Institute for Nuclear Research and Nuclear Energy, 72, Tzarigradsko Shaussee Blvd. 72, 1784 Sofia, Bulgaria

ISSN: 0306-4549

Title: **CFAR BI detector for mariner targets in time domain for bistatic forward scattering radar**

Authors: Kabakchiev, Chr.[1], Garvanov, I.[2], Cherniakov, M.[3], Gashinova, M.[3], Kabakchiev, A.[4], Kiovtorov, V.[4], Vladimirova, M.[4], Daskalov, P.[4]

Source: Proceedings of SPIE - The International Society for Optical Engineering, Vol. 8008, (2011), Article number 80081U

Author Affiliations: [1]Faculty of Mathematics and Informatics, Sofia University "St. Kliment Ohridski", Bulgaria;
[2]State University of Library Studies and Information Technologies, Sofia, Bulgaria;
[3]School of Electrical, Electronic and Computer Engineering, University of Birmingham, United Kingdom;
[4]Institute of Information and Communication Technologies, BAS, Sofia, Bulgaria.

ISSN: 0277-786X

Title: **Photoelectron transport ability of chloroplast thylakoid membranes treated with NO donor SNP: Changes in flash oxygen evolution and chlorophyll fluorescence**

Authors: Vladkova, Radka[1], Dobrikova, Anelia G. [1], Singh, Ranjeet[2], Misra, Amarendra N. [2], Apostolova, Emilia[1]

Source: Nitric Oxide, Vol. 24, 2, (15 Mar. 2011), 84-90

Author Affiliations: [1]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Science, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria;
[2]Department of Biosciences and Biotechnology, School of Biotechnology, Fakir Mohan University, Jnan Bigyan Vihar, Balasore 756020, India.

ISSN: 1089-8603

Title: **What have 10 years of health insurance reforms brought about in Bulgaria? Re-appraising the Health Insurance Act of 1998**
Authors: Atanasova, Elka[1,2], Pavlova, Milena[2], Velickovski, Robert[1,2], Nikov, Bogomil[1,2], Moutafova, Emanuela[1,] Groot, Wim[2,3]
Source: Health Policy, Vol. 102, 2-3, (Oct. 2011), 263-269
Author Affiliations: [1]Department of Health Economics and Management, Faculty of Public Health, Medical University - Varna, Varna, Bulgaria;
[2]Department of Health Organisation, Policy, and Economics, Faculty of Health, Medicine and Life Sciences, CAPHRI, Maastricht University Medical Center, Maastricht University, The Netherlands;
[3]Top Institute Evidence-Based Education Research (TIER), Maastricht University, The Netherlands.
ISSN: 0168-8510

Title: **Geographical situation of radio and television transmitters in Bulgaria**
Authors: Stefanova, Tereza Angelova
Source: Procedia - Social and Behavioural Sciences, Vol. 19, (2011), 81-89
Author Affiliations: New Bulgarian University, 21, "Montevideo" Str., 1618 Sofia, Bulgaria
ISSN: 1877-0428

Title: **PIN33 Economic Impact of the Antiretroviral Pharmacotherapy on Cost and HIV/AIDS Control in Bulgaria**
Authors: Dimitrova, M.[1], Manova, M.[1], Yancheva, N.[2], Tcherveniakova, T.[3], Stefanova, M.[2], Petrova, G.[1]
Source: Value in Health, Vol. 14, 7, (Nov. 2011), A271
Author Affiliations: [1]Medical University Sofia, Faculty of Pharmacy, Sofia, Bulgaria;
[2]University Hospital for active treatment of infectious and parasitic diseases, Sofia, Bulgaria;
[3]Hospital for Infectious and Parasitic Diseases, Sofia, Bulgaria.
ISSN: 1098-3015

Title: **Evaluation of mobile learning system**
Authors: Georgieva, Evgeniya S., Smrikarov, Angel S., Georgiev, Tsvetozar S.
Source: Procedia Computer Science, Vol. 3, (2011), 632-637
Author Affiliations: University of Ruse, 8, Studentska Str., 7017 Ruse, Bulgaria
ISSN: 1877-0509

Title: **Oxygen isotopic evidence of residence and migration in a Greek colonial population on the Black Sea**
Authors: Keenleyside, Anne[1], Schwarcz, Henry P.[2], Panayotova, Kristina[3]
Source: Journal of Archaeological Science, Vol. 38, 10, (Oct. 2011), 2658-2666
Author Affiliations: [1]Department of Anthropology, DNA Building Block "C", Rm 224, 2140 East Bank Drive, Trent University, Peterborough, Ontario, Canada K9J 7B8;
[2]School of Geography and Earth Sciences, General Science Building, Rm 302, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada;
[3]Institute of Archaeology, Saborna Str. 2, 1000 Sofia, Bulgaria.
ISSN: 0305-4403

Title:	**Comparison of calculation models for determination of the mesopause temperature using SATI images**
Authors:	Atanassov, Atanas Marinov
Source:	Advances in Space Research, Vol. 47, 11, (1 Jun. 2011), 1990-1998
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ISSN: 0308-8146

EVENTS

EUROPEAN RESEARCHERS' NIGHT 2011

European Researchers' Night was held in Bulgaria for the sixth consecutive year - annual event which is held on the same day in the whole Europe. It was realized with financial support of the European Commission on the 7-th Framework Programme for research and technological development, subprogram "People".

European researchers' night was held on September 23, 2011 simultaneously in the cities of Sofia, Plovdiv, Varna, Ruse, Stara Zagora and Burgas with various events.

In Bulgaria the project "European Researchers' Night 2011" - RECSES (REsearchers in Chemistry Supporting Economy and Society), was realized by a consortium with participants: Sofia University St. Kl. Ohridski (coordinator), Young Talents Club, Technical University - Sofia, Thracian University - Stara Zagora, Information and improving teacher's qualification department, "Angel Kanchev" University of Ruse, Plovdiv University "Paisii Hilendarski", Education Centre - Varna, Burgas Free University and GIS-Transfer Centre Foundation.

European Researchers' Night Program 2011 was realized with support from a number of organizations on the national and local level, such as Union of Scientists in Bulgaria, Bulgarian Academy of Sciences, National Polytechnic Museum, "Obekti" magazine, "AzBuki" newspaper, etc.

During previous years intention of the team was to direct public attention to different spheres of science: physics and technologies (2006); archeology and history (2007); biology, chemistry and ecology (2008); astronomy (2009). In 2010 the accent was made on researchers' role in science and industry for technological development in promotion of EU policies for improving economic growth and quality of life. Events organized during previous years were very successful and highly appreciated by the public, especially by young people who were given various possibilities for participation.

In the context of the International Year of Chemistry European Researchers' Night 2011 in Bulgaria was conducted under the motto: **"Researchers and chemistry in support of economy and society"**. The year 2011 was the centenary from adjudging the Nobel Prize in chemistry to Marie Sklodowska-Curie - the first woman Nobel Prize laureate and the first scientist honored twice by Nobel Prize, which was the occasion to mark women's contribution to science.

2011 г. marked the 100-th anniversary from creation of a model of the atom by Rutherford - a discovery that changed our idea of the structure of the environment and had great influence over advances in different human activities and over the development of economy and quality of life in modern society.

START OF BULGARIAN–SWISS RESEARCH PROGRAM 2011 – 2016

On October 27, 2011 Mrs. Petya Evtimova - Deputy Minister of Education, Youth and Science, and HE Mrs. Regina Escher - the ambassador of the Swiss Confederation, opened an Information Day to mark the start of the Bulgarian-Swiss Research Program 2011 - 2016.

Swiss Confederation takes part in enlargement of the EU, supporting efforts in decrease of economic and social inequities within the enlarged EU by means of measures mutually agreed upon by the parties. Bulgaria is among the countries that get the support. One of the chosen fields for cooperation with Bulgaria is research activity. For this purpose **Bulgarian–**

Swiss Research Program (BSRP) was created.

Swiss national research foundation and Research Department at the Ministry of Education, Youth and Science (MEYS) are national organizations which are responsible for the entire management and administration of the program. They are responsible for guarantying proper realization of the program in accordance with its aims, principles and procedures.

Bulgarian-Swiss Research Program meets the needs of the Bulgarian research community in better international integration and cooperation. It will be realized by means of mobilization of the research capacity in Switzerland and Bulgaria and supporting research cooperation by means of joint research projects and non-recourse financing. The projects will give chance to Bulgarian researchers together with scientists from Switzerland under the form of consortiums to perform research activities directed to particular problems in four subject areas:

- ecology: forestry, agriculture, land management, waste utilization;
- social sciences: social and human inequality and regional inequities;
- medicine: design and simulation of medicines;
- engineering: innovative construction design, methods and materials.

Projects carried out under the research program aim at strengthening the research cooperation between Bulgaria and Switzerland. Particular tasks of the Bulgarian-Swiss research Program for achieving the goal are:

- promoting integration of Bulgarian researchers into international networks;
- facilitation of knowledge and know-how exchange among scientists.

Duration of the projects is up to 3 years and the research will be carried out in the participating research centers in both countries.

FIRST SOFIA FESTIVAL OF SCIENCE

Sofia Festival of Science - a place where boundaries between culture and science do not exist - was held on May 11-15, 2011 in the Doctors' Garden in Sofia. The event is a part of the Cultural calendar of the capital.

Sofia Festival of Science is organized by the British Council and Democritus Forum under the patronage of the Ministry of Education, Youth and Science in partnership with the capital municipality.

A festival uniting different interesting events, varied publics, young and recognized scientists from the country and abroad is organized for the first time in Bulgaria. It will be held annually and will become a part of the international network.

In the era of the increasingly fast-paced technological development and continuous scientific discoveries the mankind is facing unparalleled challenges, and more and more solutions are in the hands of researchers. On the other hand, human understanding of basic notions from the field of science is both right and obligation of every member of the modern democratic society.

For the opening of the festival many foreign researchers came to Sofia - the creators of the Large Hadron Collider (particle accelerator) at CERN Institute and ideologists of the experiments in it - Lyn Evans and Jim Virdee. Dr. Spiros Katsinelis from Greece disclosed the science in dreaming - what is a sleep, what we know and what we do not know about dreams. Mark Lewney from the United Kingdom told about physics through his acoustic show "Rock Guitar in 11 Dimensions". Frank Burnet, First Professor in Science Communication in the UK, revealed an entirely unexpected cause to go out with friends for a drink. Hristo Kolev and Bozhidar Stefanov impressed with their show "Chemical Illusionism".

Partners of the Sofia Festival of Science are: Sofia University St Kl. Ohridski, Forestry University, Bulgarian Academy of Sciences, Union of Scientists in Bulgaria, Polish Cultural Institute - Sofia, Bea Solutions, Programata Journal, Obekti Journal, Dunkin' Donuts, Ciela Publishing House, Roi Publishing House, Microsoft, Go Green Communications. Media partners are: Bulgarian National Television, iNews, NetInfo, az-deteto.com, BBC Knowledge journal, Osem Journal, Bulgarian Science journal and newspaper "Az-Buki".