BULGARIAN SOCIETY
OF BIOMEDICAL PHYSICS AND ENGINEERING

PROCEEDINGS

12-th NATIONAL MEDICAL PHYSICS
AND BIOMEDICAL ENGINEERING CONFERENCE
NMPEC-2016
with international participation

3-5 November 2016
Inter Expo Center-IEC
Sofia, Bulgaria
ADDRESS OF THE ORGANIZING COMMITTEE

Chair of the Organizing Committee- NMPEC-2016
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Assistant professor Silvia Abarova, PhD.

Editorial Note:
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BULGARIAN SOCIETY OF BIOMEDICAL PHYSICS AND ENGINEERING
Welcome address

Dear Participants,

At the 12-th NATIONAL MEDICAL PHYSICS AND BIOMEDICAL ENGINEERING CONFERENCE-NMPEC-2016 with international participation, on behalf of the Organizing Committee and the BSBPE Board, we are pleased to cordially welcome you to this scientific forum which will be held at the Inter Expo Center, Sofia.

Co-organizers of this significant event are the Nuclear Regulatory Agency of Bulgaria, the Union of Physicists in Bulgaria, the Bulgarian Association of Radiology and the Bulgarian Biochemical, Biophysical and Molecular Biology Society.

The NMPEC-2016 conference is endorsed and supported by the International Organization for Medical Physics (IOMP) and the European Federation of Organizations for Medical Physics (EFOMP). Support letters from the Croatian Medical and Biological Engineering Society (CROMBES), the Hungarian Society of Medical Physicists (HSMP) and the Hellenic Association of Medical Physicists (HAMP) were received as well.

This is the major conference in the area of Medical Physics and Biomedical Engineering in Bulgaria and we received more than 70 abstracts from participants from our and other countries. Through the exchange of ideas and discussions on state-of-the-art knowledge, on topics of education and training of specialists in this branch of science and technology, as well as on problems to be solved, the NMPEC-2016 is expected to stimulate further research in this dynamic field.

NMPEC-2016 will feature plenary talks by world-renowned experts, a variety of sessions focused on the most pressing issues in medical and non-medical use of sophisticated technology, health care concerns and regulations associated with ionizing and non-ionizing radiation, biophysical phenomena and engineering progress and applications.

NMPEC-2016 will assist the development and strengthening of international scientific and personal contacts and cooperation. During the Conference, students will have the opportunity to present their work and to demonstrate their skills in communicating and networking with the Medical Physics, Biophysics and Biomedical Engineering communities.

The NMPEC-2016 conference marks the 45th Anniversary of BSBPE, a remarkable event that will be noted in a special session at the conference dinner.

We would like to express our sincere thanks to all sponsors and collaborators who supported the conference and helped its organization.

The Organizing Committee and the BSBPE Board are particularly honored to welcome all of you to NMPEC-2016 and the wonderful city of Sofia, and to wish you pleasant stay and fruitful work.

Professor Boris Tenchov, PhD, DSc
Member of the Bulgarian Acad Sci
President of BSBPE

Assistant Professor Lubomir Traikov, PhD
Chair of Organizing Committee of NMPEC-2016
ORGANIZED BY: Bulgarian Society of Biomedical Physics and Engineering

ENDORSED BY: International Organization for Medical Physics

And:

European Federation of Organizations for Medical Physics

CO-ORGANIZERS: Nuclear Regulatory Agency

Union of Physicists in Bulgaria Bulgarian Association of Radiology

And:

Bulgarian Biochemical, Biophysical and Molecular Biology Society

SUPPORTED BY:

Croatian Medical and Biological Engineering Society

Hungarian Society of Medical Physicists

Hellenic Association of Medical Physicists
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Name</th>
<th>Place/Hall</th>
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<tbody>
<tr>
<td>10:00-18:00</td>
<td>Registration</td>
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<tr>
<td>13:30-14:00</td>
<td>Opening ceremony</td>
<td></td>
<td>Musala</td>
</tr>
<tr>
<td>14:00-15:30</td>
<td>Session Radiology and Roentgenology 1</td>
<td>Chairman Professor Boris Tenchov, President of BSBPE</td>
<td>Musala</td>
</tr>
<tr>
<td>14:00-14:45</td>
<td>PL1</td>
<td>Slavik Tabakov “Medical Imaging Equipment – 50 years of progress, related education, impact on medicine and current trends”</td>
<td>Musala</td>
</tr>
<tr>
<td>14:45-15:00</td>
<td>O1-1</td>
<td>Peter Trindev “60 years from the invention of Anger gamma camera”</td>
<td>Musala</td>
</tr>
<tr>
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<td>O1-2</td>
<td>Simona Avramova-Cholakova, D. Kostova-Lefterova “Pilot study of patient doses from digital breast tomosynthesis in Bulgaria”</td>
<td>Musala</td>
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<td>15:30-16:00</td>
<td>Coffee Break</td>
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<td>Vihren</td>
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<tr>
<td>16:00-17:30</td>
<td>Session Biomedical Engineering</td>
<td>Chair Professor Virginia Tsapaki, Secretary General of IOMP</td>
<td>Musala</td>
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<td>O2-1</td>
<td>J. Przondzio, W. Walke, J. Sza, J. Wieczorek “Ultrasound imaging: signal acquisition, new advanced processing for biomedical and industrial applications”</td>
<td>Musala</td>
</tr>
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<td>Ivan Tsanev, A. Dimov, G. Kasparyan “National Database for Patient Dose Registration and Analysis in Diagnostic Radiology”</td>
<td>Musala</td>
</tr>
<tr>
<td>17:15-17:30</td>
<td>O2-4</td>
<td>Sergey Podtaye, N. Zabareva, A. Parshakov, E. Smirnova “Detection of Endothelial Dysfunction Using Skin Temperature Oscillations Analysis”</td>
<td>Musala</td>
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<tr>
<td>09:00-10:30</td>
<td>Session Radiology and Roentgenology 2</td>
<td>Chair Professor Slavik Tabakov, President of IOMP</td>
<td>Musala</td>
</tr>
<tr>
<td>09:00-09:30</td>
<td>PL3</td>
<td>Virginia Tsapaki “Incidents and accidents in imaging departments. What’s next?”</td>
<td>Musala</td>
</tr>
<tr>
<td>09:30-09:45</td>
<td>O3-1</td>
<td>Pavlina Pike, L. Johnson “Strategies for minimizing patient radiation dose in interventional fluoroscopy”</td>
<td>Musala</td>
</tr>
<tr>
<td>09:45-10:00</td>
<td>O3-2</td>
<td>M. Dimcheva, Peter Trindev “National survey on the accuracy of dose calibrations”</td>
<td>Musala</td>
</tr>
<tr>
<td>10:00-10:15</td>
<td>O3-3</td>
<td>Kristina Bliznakova, I. Buliev, Z. Bliznakov “Education and training related to anthropomorphic phantoms for medical physics experts”</td>
<td>Musala</td>
</tr>
<tr>
<td>10:15-10:30</td>
<td>O3-4</td>
<td>Desislava Kostova-Lefterova, F. Simeonov, D. Ivanova “Tracking the effect of optimisation in a paediatric radiology department”</td>
<td>Musala</td>
</tr>
<tr>
<td>10:30-11:00</td>
<td>Coffee Break</td>
<td>- Coffee Break</td>
<td>Vihren</td>
</tr>
<tr>
<td>11:00-13:00</td>
<td>Session Biophysics</td>
<td>Chair Assist. Prof. Lubomir Traikov, Chairman of Organizing Committee</td>
<td>Musala</td>
</tr>
<tr>
<td>11:00-11:30</td>
<td>PL4</td>
<td>Boris Tencho “Nanotechnologies in gene therapy – non-viral vectors for nucleic acid delivery”</td>
<td>Musala</td>
</tr>
<tr>
<td>11:30-11:45</td>
<td>O4-1</td>
<td>Roxana Popescu, A.I. Apostol, E. A. Andronescu, M. Grumezescu, D. Savu, “Fabrication of functionalized magnetite nanoparticles with applications in drug delivery systems”</td>
<td>Musala</td>
</tr>
<tr>
<td>11:45-12:00</td>
<td>O4-2</td>
<td>Miroslav Karabaliev, B. Tacheva “Electrochemical approach to investigate drug-nanoparticles interactions”</td>
<td>Musala</td>
</tr>
<tr>
<td>12:00-12:15</td>
<td>O4-3</td>
<td>Rositsa Marina, P. Petkov, L. Litov “CG molecular dynamics study of indolicidin in water solution”</td>
<td>Musala</td>
</tr>
<tr>
<td>12:15-12:30</td>
<td>O4-4</td>
<td>Virgina Dolchinkova, P. Angelova, S. Petrova “Vipoxin effects on the surface electrical properties and membrane transport of protons in human erythrocytes”</td>
<td>Musala</td>
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</tbody>
</table>
### Saturday, November 5

<table>
<thead>
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<th>Time</th>
<th>Session</th>
<th>Name</th>
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</thead>
</table>
| 09:00-11:20  | Session Radiology and Roentgenology 3  
Chair Assoc. Prof. Peter Trindev, Past President of BSBPE | Musala     |
<p>| 09:00-09:15  | PL5     | Slavik Tabakov “MTF and Contrast Inversion”                           | Musala     |
| 09:15-09:30  | O5-1    | Pavlina Pike, L. Johnson “CT dose optimization and tracking across multiple facilities” | Musala     |
| 09:30-09:45  | O5-2    | Simona Avramova-Cholakova, E. Petrova, S. Shalamanov, I. Dyakov  “Radiation exposure of patients from whole body examinations on new PET-CT system” | Musala     |
| 09:45-10:00  | O5-3    | Simona Avramova-Cholakova, E. Petrova, S. Shalamanov, I. Dyakov  “Radiation exposure of patients from two procedures on new SPECT-CT system” | Musala     |
| 10:00-10:15  | O5-4    | Desislava Kostova-Lefterova, V. Hadzhiyska, Sh. Masso, F. Vasilova “Survey of practice and dose optimisation strategies in paediatric PET/CT procedures” | Musala     |
| 10:15-10:30  | O5-5    | Nely Gesheva-Atanasova, D. Stoeva “Comparison of 3D conformal radiotherapy and helical tomotherapy for irradiation of the breast and regional lymphatic” | Musala     |
| 10:30-11:00  | O5-6    | Dr. Küçük, Medipol clinic “Evaluation of SRS/SBRT Treatment using Dosimetric Metrics” | Musala     |</p>
<table>
<thead>
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<td>NMPEC-2016 Closing ceremony</td>
<td>Musala</td>
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<tr>
<td>15:30-18:30</td>
<td>Sightseeing tour Sofia and surroundings</td>
<td>Musala</td>
</tr>
</tbody>
</table>

**POSTERS**

**Poster Session Ionizing and Non-Ionizing Radiation**

**P01** Dimov A., Kasparyan G., Tsanev I., Vassileva F. “Application of EFOMP and EUREF Quality Control protocols in evaluation of a modern full field digital mammography system”

**P02** Gancheva M., Krastev B., Gesheva–Atanasova N. “Evaluation the feasibility of VMAT treatment plan verification with Octavius 4D phantom using different dosimetry criteria”

**P03** Ivanova N., Chaushev B., Ivanova S. “Life in radiation”

**P04** Ivanov L. “Dose-Area product meter with extended functionality”

**P05** Ivanov L. “Active ionizing chamber with analog and digital mode of operation”

**P06** Ivanov L. “Variable packet length protocol (VPL protocol) for real time data transmission”

**P07** Shalamanova Ts., Topalova Iv. “Analysis and evaluation of electromagnetic exposure in urban area with high density of sources”

**Poster Session Biophysics**

**P08** Abarova S., Koynova R., Traikov L., Tancheva L., Tenchev B. “Protectant Drug Efficacy Against Scopolamine-Induced Dementia In Mice, A DSC Approach”

**P09** Alexandrov S. A., Todorov R. K., Exerowa D. R. “The role of Ca2+ on stability of foam films from lysophosphatidylcholine and Curosurf”

**P10** Al Sharif M., Alov P., Tsakovska I., Pajeva I. „Pharmacophore modeling of PPARγ partial agonist”

**P11** Bangyozova M., Jordanova A., Tsanova A., Stoyanova V., Stoimenova E., Christova E., Lalchev Z. “Methods of diagnostic of neonatal respiratory distress syndrome based on gastric aspirates samples in order to appropriate therapy”
P12 Chakalov I., Ivanova P., Traikov L. “Estimation of biomechanical force of action as a function of three different chewing forces studied by image densitometry analysis”


P14 Djenev I. “Investigation of the influence of the endotoxin on the deformability of red blood cells in vitro”


P16 Ilieva D. “Temperature-dependent, spatial and temporal controlled azobenzene polymeric materials”


P19 Nikolova A., Keranov I., Michel M., Vladkova T., Kostadinova A. “Characterisation and biological response of electrospun amphiphilic poly (Dimethylsiloxane-B-acrylic Acid) fibrous scaffolds”

P20 Parvanova B. K., Ivanov I. T. “Thermal dielectroscopy study of under-membrane erytrocyte skeleton. Data processing and presentation”

P21 Popatanasov A. “A low-cost differential thermal analysis (DTA) apparatus for measuring the thermal properties and behavior of protein and carbohydrate-based hydrogels”

P22 Radeva D., Dimitrova St., Pavlova B., Paunov M., Kouzmanova M., Dankov K., Tsonev Ts., Velikova V., Goltsev V. “Plant health estimation using prompt chlorophyll a fluorescence imaging in leaves of two varieties of bean plants”

P23 Semkova S., Nikolova B., Murayama S., Stoyanova E., Tsoneva I., Zhelev Zh., Aoki I., R. Bakalova “Visualization of passive and electro-assisted delivery of quantum dot-labeled nanoparticles in vitro and in vivo using fluorescent and magnetic resonance imaging”

P24 Sezanova B., Antonova B., Naidenov E., Tenchov B. “Changes in the state of the plasma proteome and cerebrospinal fluid in Glioblastoma multiforme”

P25 Stoichev S., Andreeva T., Taneva S., Krastev R. “Optimization of polyelectrolyte multilayer coatings for biofunctionalization of cardiovascular stents by incorporation of graphene oxide”


P27 Vladkova R. “Ordering of the numerous cytochrome bc1 X-ray crystal structure in a sequence of events during substrate processing in the Qo site of the complex”

P28 Zaharinova S., Abarova S, Tancheva L, Stoeva S., Paypanova T., Koynova R., Tenchov B. "Effects of synthetic neuropeptides (neurotensins) on drug-induced neurodegenerative disorders"

P29 Zasheva A., Abarova S., Tenchov B. "Corticosteroid interactions with human serum albumin"

P30 Zaytseva E., Deperas-Kaminska M., Kutsalo P., Mitsyn G., Molokanov A., Gaevsky V., Wojcik A. “Cytogenetic radio-sensitivity of human peripheral blood lymphocytes to protons and gamma rays”

P31 Dimov A., I. Tsanev, D. Ivanova, F. Simeonov. Third National Patient Dose Survey in Diagnostic Radiology for Establishing of New National DRLs in Bulgaria, First Results
## CONTENT

| Session Radiology and Roentgenology 1  
Chair Professor Boris Tenchov, President of BSBPE |
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td><strong>PL1</strong> Slavik Tabakov “Medical Imaging Equipment – 50 years of progress, related education, impact on medicine and current trends”</td>
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</tr>
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Chair Professor Virginia Tsapaki, Secretary General of IOMP |
<table>
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</tr>
<tr>
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<td>Session Radiology and Roentgenology 2</td>
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<td>Session Biophysics</td>
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<td>Posting Session</td>
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</tr>
<tr>
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</tr>
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<td>P01</td>
<td><strong>Dimov A.</strong>, Kasparyan G., Tsanev I., Vassileva F. “Application of EFOMP and EUREF Quality Control protocols in evaluation of a modern full field digital mammography system”</td>
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<td></td>
</tr>
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</tr>
<tr>
<td>P09</td>
<td><strong>Alexandrov S. A.</strong>, Todorov R. K., Exerowa D. R. “The role of Ca^{2+} on stability of foam films from lysophosphatidylcholine and Curosurf”</td>
</tr>
<tr>
<td>P10</td>
<td><strong>Al Sharif M.</strong>, Alov P., Tsakovska I., Pajeva I. „Pharmacophore modeling of PPARγ partial agonist”</td>
</tr>
<tr>
<td>P11</td>
<td><strong>Bangyozova M., Jordanova A.</strong>, Tsanova A., Stoyanova V., Stojmenova E., Christova E., Lalchev Z. “Methods of diagnostic of neonatal respiratory distress syndrome based on gastric aspirates samples in order to appropriate therapy”</td>
</tr>
<tr>
<td>P12</td>
<td><strong>Chakalov I.</strong>, Ivanova P., Traikov L. “Estimation of biomechanical force of action as a function of three different chewing forces studied by image densitometry analysis”</td>
</tr>
<tr>
<td>P14</td>
<td><strong>Djenev I.</strong> “Investigation of the influence of the endotoxin on the deformability of red blood cells in vitro”</td>
</tr>
<tr>
<td>Page</td>
<td>Title</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>P16</td>
<td>Temperature-dependent, spatial and temporal controlled azobenzene polymeric materials</td>
</tr>
<tr>
<td>P17</td>
<td>The effect of co-activation of antagonist muscles on recruitment curve during transcranial magnetic stimulation</td>
</tr>
<tr>
<td>P18</td>
<td>High frequency electromagnetic field induced hyperthermia for treatment of artificially induced breast cancer in rats</td>
</tr>
<tr>
<td>P19</td>
<td>Characterisation and biological response of electrospun amphiphilic poly (Dimethylsiloxane-B-acrylic Acid) fibrous scaffolds</td>
</tr>
<tr>
<td>P20</td>
<td>Thermal dielectroscopy study of under-membrane erythrocyte skeleton. Data processing and presentation</td>
</tr>
<tr>
<td>P21</td>
<td>A low-cost differential thermal analysis (DTA) apparatus for measuring the thermal properties and behavior of protein and carbohydrate-based hydrogels</td>
</tr>
<tr>
<td>P22</td>
<td>Plant health estimation using prompt chlorophyll a fluorescence imaging in leaves of two varieties of bean plants</td>
</tr>
<tr>
<td>P23</td>
<td>Visualization of passive and electro-assisted delivery of quantum dot-labeled nanoparticles in vitro and in vivo using fluorescent and magnetic resonance imaging</td>
</tr>
<tr>
<td>P24</td>
<td>Changes in the state of the plasma proteome and cerebrospinal fluid in Glioblastoma multiforme</td>
</tr>
<tr>
<td>P25</td>
<td>Optimization of polyelectrolyte multilayer coatings for biofunctionalization of cardiovascular stents by incorporation of graphene oxide</td>
</tr>
<tr>
<td>Page</td>
<td>Authors</td>
</tr>
<tr>
<td>------</td>
<td>---------</td>
</tr>
<tr>
<td>P27</td>
<td>Vladkova R.</td>
</tr>
<tr>
<td>P29</td>
<td>Zasheva A., Abarova S., Tenchov B.</td>
</tr>
<tr>
<td>P30</td>
<td>Zaytseva E., Deperas-Kaminska M., Kutsalo P., Mitsyn G., Molokanov A., Gaevsky V., Wojcik A.</td>
</tr>
<tr>
<td>P31</td>
<td>Dimov A., I. Tsanev, D. Ivanova, F. Simeonov.</td>
</tr>
</tbody>
</table>
SESSION
RADIOLOGY AND ROENTGENOLOGY 1
MEDICAL IMAGING EQUIPMENT – 50 YEARS OF PROGRESS, RELATED EDUCATION, IMPACT ON MEDICINE AND CURRENT TRENDS

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The rapid development of medical imaging equipment in the past 50 years led to significant changes in contemporary medical diagnostics. The presentation gives an overview of the invention of the new medical imaging equipment and their implementation in clinical practice. Special place is devoted to the medical physicists, engineers and other specialists, who invented various imaging modalities in the field of X-ray imaging, CT scanning, Nuclear Medicine, Magnetic Resonance and Ultrasound Imaging. Their pioneering work is given in chronological order, showing the development of ideas and their evolution as knowledge transfer from one field to another.

The need of new type of medical physics/engineering education, related to this revolutionary equipment development, is underlined. This includes the introduction of e-learning in the profession, the pioneering of the first e-Encyclopaedia and Dictionary of Medical Physics and other innovative educational development. Their role is highlighted as some of the main supporters for the professional growth in the past 20 years.

The digitalisation of medical imaging is described as contributor to both the increased image quality and the introduction of quantitative imaging. Methods based on extraction of new information from medical imaging, as well as mathematical modelling, based on imaging, are shown as some of the future trends in the progress in medical imaging.

The presentation emphasizes medical physics and engineering as a significant driving force for contemporary imaging diagnostics.

Key words: medical physics professional issues, medical physics education
60 YEARS FROM THE INVENTION OF ANGER GAMMA CAMERA

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It is not possible to predict what would look like today the nuclear medicine diagnostic without the genius invention of Hal Anger 60 years ago. Development of modern detectors of ionizing radiation aimed to replace the low sensitivity GM-counter starts after WW II. The commercial introduction of artificial radionuclides starts in 1946 following the development of cyclotron in 1930 and the nuclear reactor in 1942. $^{131}$I is the first reactor product introduced into medicine for diagnostic and treatment of thyroid gland. In-vivo distribution of $^{131}$I in thyroid gland is mapped by hand held GM-counter. The scintillation crystal NaI[Tl] with a better detection sensitivity replaces the GM-counter in 1954 and become a detector of choice in nuclear medicine instruments for the years to come. The first automated instrument to depict in-vivo distribution of $^{131}$I – rectilinear scanner is built by B.Cassen in 1955. The main limitation of this instrument is its long acquisition duration because of sequential data acquisition. On that time the direct imaging by a large area scintillation crystal is considered impossible until 1956 when Hal Anger invents his genius circuit that makes possible to define the coordinates of a interaction of a gamma photon within a scintillation crystal. This is a milestone of the epoch of nuclear medicine. The real growth in nuclear medicine dates from 1962 when $^{99m}$Tc is commercially available. Interfacing dedicated computers to gamma cameras leads to avalanche like improvement of its performance and offer the opportunity for processing of images and the generation of numerical and graphical presentation of the data. SPECT gamma cameras in the 1980’s cause the change from circular to rectangular field of view of the detector. And finally after long period of investigation and experiments PET appears in the routine clinical practice.

Key words: Anger, gamma camera
PILOT STUDY OF PATIENT DOSES FROM DIGITAL BREAST TOMOSYNTHESIS IN BULGARIA

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The first systems for Digital Breast Tomosynthesis (DBT) were installed in 2015 in Bulgaria. This study aims to perform pilot estimation of patient doses from examinations on two DBT systems – GE Senograph Essential and IMS Giotto Tomo.

Incident air kerma (IAK) and mean glandular dose (MGD) were determined applying the method proposed in the Protocol for the Quality Control of the Physical and Technical Aspects of Digital Breast Tomosynthesis Systems (PQCDBT). Patient data for 76 patients on the first system and 92 patients on the second system were retrospectively collected from the picture archiving and communication systems (PACS) of the hospitals. The total number of exposures considered of both breasts and all projections on the first system was 300, and the respective number on the second system was 259, in both planar and DBT modes of operation. IAK and MGD were also estimated for standard PMMA phantom with thicknesses from 20 to 60 mm.

The mean compressed breast thickness (CBT) for patients’ samples for both modes of operation on the first system was 51 mm, mean IAK and MGD per exposure for the planar mode were 6.3 mGy and 1.5 mGy respectively. For the DBT mode these data were 7.5 mGy and 1.8 mGy. For the second system mean CBT, IAK and MGD for planar and DBT modes were, as follows: 62 mm, 3.6 mGy and 1 mGy; 52 mm, 6.5 mGy and 2 mGy.

Patient doses in the planar mode were about 43% higher on the first system in comparison to the second one, but in the DBT mode MGD was almost equal for both systems, within the uncertainty of the estimations. IAK and MGD were very similar for both modes of operation on the first system and about two times higher in the DBT mode compared to the planar mode on the second system. For both systems MGD for PMMA were below the published in the PQCDBT reference values. Comparison with previous studies for film-screen mammography in the country showed decrease in the dose levels with the introduction of the new digital technology. Our results are similar to published in literature.

Key words: digital breast tomosynthesis, mean glandular dose
DEVELOPMENT OF A NEW TLD HOLDER FOR EYE LENS DOSIMETRY IN INTERVENTIONAL RADIOLOGY

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\textbf{Purpose} In interventional radiology, dedicated eye lens dosimetry is the only suitable method for correct determination of the dose to the lens of the eye. There is no agreed standardized dosimetry methods for routine use. The aim of this study is to design, produce and calibrate a new ergonomic thermoluminescence (TLD) dosimeter to be used in photon fields. The main goal is to optimize the energy response of the dosimeter in order to increase the accuracy of the measurement method.

\textbf{Materials and methods} Two types of TLD detectors (MTS-N and MCP-N) were used. The energy characteristics behind different thicknesses of aluminium and copper filters were studied using the narrow spectrum series described in ISO 4037-3 standard as well as \textsuperscript{137}Cs. The irradiations were performed at the Secondary Standard Dosimetry Laboratory – Sofia. Water filled cylindrical phantom were used to represent the head.

\textbf{Results} The relative response of the detectors for the various beam qualities compared to the response at the \textsuperscript{137}Cs-photon energy was calculated for the new holder. The preliminary results indicated that the optimum configuration of the dosimeter is a PMMA filter of 2 mm thickness and aluminium filter of 1.5 mm thickness. The relative response of the TLDs behind each filter is +15 \% /-25 \% for PMMA and up to -20 \% for aluminum. The new holder was designed with two hemisphere capsules, one of 1 mm PMMA, and second of 1.2 mm aluminum. The energy compensation method was applied and the weighted response of the detectors was calculated. The results show energy dependence of the new dosimeter ± 10 \%.

\textbf{Conclusions} The energy response of TLD detectors was flattened by appropriate combination of filters in a dosimeter holder. A new eye lens dosimeter „XRayM“ was developed within Project 19D/2016 of the Medical University – Sofia. The test measurements for energy response produced the satisfactory results of ± 10 \% with respect to \textsuperscript{137}Cs.

\textbf{Key words:} eye lens dosimetry, TLD holder, energy dependence
SESSION
BIOMEDICAL ENGINEERING
COMPARATIVE STUDY OF T-WAVE ALTERNANS IN ELDERLY AND YOUNG COMPETITIVE ATHLETES

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**Objective:** Alternans of T-wave (TWA) could predict arrhythmic and sudden cardiac death risk at various clinical settings. Our aim was to evaluate the presence of T-wave alternans in two different populations: young athletes performing cardiopulmonary stress test and elderly people who performed diagnostic stress electrocardiographic (ECG) test.

**Methods:** We studied 414 young competitive athletes, 24±8 years, 395 (95.5%) males, and 107 elderly patients 62.8 ± 10.3 years (42% males). Cardiopulmonary exercise test was performed as part of the annual work-up of competitive athletes. The median duration of the exercise test was 15.27 minutes (14.8 and 16.9 the 25% and 75% percentiles), with a mean heart rate of 148 bpm. In the elderly group, digital 12-lead electrocardiograms (ECG) were acquired during stress ECG test using veloergometer (GE Marquette Stress PC ECG Application) – 2-min stages 25W incremental workload. The median duration of the exercise test was 7.08 minutes, and the mean Heart rate was 96 bpm.

**Results:** We detected and considered continuous occurrence (packets of more than 30 s) of TWA alternans. In the athletes, there was at least one packet in 329 subjects (79.5%), while in the elderly group we observed at least one TWA packet in 10 (9.3%) patients, and this results may seem surprising. These differences can be better described considering the different test conditions of the two groups, mainly the duration and the heart rate observed during the test. It is known that the TWA occurrence depends on the heart rate – as higher is the heart rate (HR), as greater is the possibility for TWA.

In order to obtain a more meaningful comparison, the evaluation of number of packets has been performed considering equal mean HR rate for the two populations. The range of HR in which there were TWA detection in both groups of patients was from 80 bpm to 125 bpm. The elderly group has a TWA presence higher that the same measure in athletes. For example the presence of TWA of the young group were 2.0%, 1.5% and 5.1% of cases in the HR range [80-95bpm], [95-110bpm], and [110-125bpm] respectively; while the TWA in elderly were 1.0%, 3.4% and 9.8% in the same HR intervals.

**Conclusions:** The quantitative analysis of TWA in two different populations has permitted a comparative study of the behaviour of an arrhythmic risk factor.

**Key words:** T wave alternans; biomedical signal processing; ECG; stress test
ULTRASOUND IMAGING: SIGNAL ACQUISITION, NEW ADVANCED PROCESSING FOR BIOMEDICAL AND INDUSTRIAL APPLICATIONS

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Use of ultrasound, namely in the biomedical diagnosis and industrial fields, pioneered in 1950s, is today particularly widespread. In the last decades, ultrasound imaging has benefited from advances in numerical technologies such as signal processing. On the other hand, the use of ultrasound imaging has increased the need for signal processing techniques. This paper presents a review and the up-to-date developments in ultrasound imaging techniques, including elementary principles, signal acquisition and processing, from one dimensional to multidimensional systems. This paper also deals with typical relevant applications.

There is a problem connected with application of steel guide wire, namely creation of microthrombi on their surface. It is caused by haemostatic activity of metallic materials. Therefore, proper modification of wire surface after drawing is essential. The purpose of this study is to analyse changes of structure and geometrical features of the surface of wires made of stainless steel X10CrNi18-8 after drawing and after further steps of surface modification. The surface of drawn wire was subject to mechanical grinding, electrochemical polishing and chemical passivation, consecutively. Such treatment is aimed at increasing wire resistance to electrochemical corrosion.

There is a problem connected with application of steel guide wire, namely creation of microthrombi on their surface. It is caused by haemostatic activity of metallic materials. Therefore, proper modification of wire surface after drawing is essential.

Key words: acoustic signal detection; biomedical ultrasonics; medical image processing; multidimensional signal processing
COMPLEX ANALYSIS OF ASYMPTOMATIC CAROTID STENOSIS AND
RESTENOSIS-ACCORDING PARAMETERS- ABI, FMD AND IMT OF
HUMAN CAROTID ARTERIES-RESTENOSIS AS A CONSEQUENCE OF
THE LOCAL STRAIN STRESS

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Impaired endothelial function and increased carotid intima-media thickness are key events in the atherosclerotic process and predict future cardiovascular events in subjects with and without coronary artery disease. The purpose of this study was to investigate whether the vasodilator response to increased flow in the brachial artery and the presence of carotid lesions may have a prognostic significance for in-stent restenosis in patients undergoing carotid angioplasty.

Arterial endothelial dysfunction is one of the key early events in atherogenesis, preceding structural atherosclerotic changes. It is also important in the late stages of obstructive atherosclerosis, predisposing to constriction and/or thrombosis.

The study population included 32 patients with carotid artery stenosis, 5 healthy volunteers, and 18 with restenosis within 6 years after stenting. All patients underwent ultrasound detection of brachial artery reactivity. Flow mediated dilatation (FMD) was investigated after 5 minutes of occlusion of the artery and nitroglycerin mediated dilation (NMD).

Working hypothesis is influence of the power of action of the stend over carotid arterial wall, as a main reason for depositing of alpha fibrils and prompt restenosis within few years.

Endothelial function can be measured in corotide arteries and in the periphery by measuring vasomotor function after intra-arterial infusion of pharmacologic substances which enhance the release of endothelial nitric oxide and with combination with Doppler ultrasound (Hitachi, Aloka-Alpha-6; Japan). Advantage of these methods is their non-invasive nature, which generally makes them suitable for studies involving asymptomatic subjects. For this reason, noninvasive tests of endothelial function have been developed. In the most widely used of these, an ultrasound-based method, arterial diameter is measured in response to an increase in shear stress, which causes endothelium-dependent dilatation (flow mediated dilation-FMD). Endothelial function assessed by this method
correlates with invasive testing of coronary endothelial function, as well as with the severity and extent of corotide atherosclerosis and stenosis.

Ankle brachial index -ABI is a noninvasive vascular screening test to identify large vessel peripheral arterial disease by comparing systolic blood pressures in the ankle to the higher of the brachial systolic blood pressures, which is the best estimate of central systolic blood pressure (Sacks et al., 2002; Vowden & Vowden, 1996, 2001).

Intima media thickness (IMT) of large artery walls, especially carotid, can be assessed by B-Mode ultrasound in a relatively simple way and represents a safe, inexpensive, precise and reproducible measure. IMT is used to detect the presence of atherosclerotic disease in humans and, more contentiously, to track the regression, arrest or progression of atherosclerosis. Ultrasound IMT measurements have been first proposed and in vitro validated in Milan by Paolo Pignoli in 1984 and later publicized in a most cited article. The use of IMT as a non-invasive tool to track changes in arterial walls has increased substantially since the mid-1990s. Although IMT is predictive of future cardiovascular events

This noninvasive endothelial function testing has provided valuable insights into early atherogenesis, as well as into the potential reversibility of endothelial dysfunction by various strategies, including pharmacological agents (lipid lowering, ACE inhibition), L-arginine, nicardipine, antioxidants and hormones.

Vascular echography was performed to measure intima media thickness (IMT) of carotid arteries. At baseline we evaluated all the established traditional cardiovascular risk factors. We also subdivided our study cohort according to values of FMD in patients with FMD above and patients below the median value.

Evaluation of ABI, FMD and carotid IMT may provide important prognostic information in patients with stenosis in order to prevent restenosis with regulation of power of action of the stent on vascular wall.

**Keywords:** endothelial function, Flow-mediated dilatation, ultrasound, Ankle brachial pressure index-ABI
A new MS Access software database was developed in order to facilitate the Third National Patient Dose Survey in diagnostic radiology in Bulgaria. It is based on an earlier database elaborated for the First Bulgarian National Patient Dose Survey performed in frame of Bulgaria-Germany EC Phare program Twinning project (2002-2004). The database contains information on different X-ray systems and procedures included in the survey. A set of different tables, queries and reports are created to facilitate the calculation of main mathematical properties for examinations included in the survey.

Results are presented for min, max, average, median, etc. values for patient anthropomorphic data and X-ray exposure parameters. Typical doses for each room and projection are derived.

Analysis of data collected from the survey up to the moment is presented and discussed.

Conclusions are drawn regarding applicability of the presented system in the process of establishing updated National Diagnostic Reference Levels (DRLs) for the purposes of the Third National Survey.

Key words: software; database; data analysis; patient dose; typical dose; National Survey, DRLs
DETECTION OF ENDOTHELIAL DYSFUNCTION USING SKIN TEMPERATURE OSCILLATIONS ANALYSIS

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Objective: The purpose of this study was to examine correlations between laboratory markers of endothelial dysfunction (ED) and the degree of endothelium dependent vasodilation using wavelet analysis of skin temperature (WAST) during a local heating test in patients with peripheral arterial disease (PAD).

Materials and methods: The study population consisted of 17 healthy subjects and 38 patients with PAD and chronic limb ischemia (CLI) at stage II (12 patients), III (8 patients) and IV (18 patients). The skin temperature (ST) on the plantar surface of the first toe was measured during the test, and the inverse wavelet transform was applied to reconstruct the ST oscillations in three frequency bands corresponding to myogenic (0.052–0.145 Hz), neurogenic (0.021–0.052 Hz), and endothelial (0.0095–0.021 Hz) mechanisms of vascular tone regulation. The patency of the major blood vessels of the lower extremities was estimated based on the results of Ro-contrast agent aortic arteriography. Doppler ultrasound was performed to allow estimations of the type of blood circulation, the state of the vascular walls, the existence of atherosclerotic plaques, the value of the regional blood pressure, and the ankle–brachial index.

Results: In the healthy subjects, a local increase in temperature up to 42°C caused a >threefold increase in the amplitudes of foot ST oscillations. Among the patients with PAD, the response to the test was much weaker in all frequency ranges, which suggests the presence of dysfunction in the myogenic, neurogenic, and endothelial regulation mechanisms. Vasodilation indices (relative changes in temperature oscillations during local heating) in the endothelial range are well correlated with the laboratory markers of ED: endothelin, homocysteine, and vWF. Increased vWF levels in PAD patients indicate arterial endothelial cell damage by atherosclerotic and revascularization processes. The level of vasodilation dysfunction, described by vasodilation indices, correlated with the level of artery stenosis in the lower extremities and with the progression of CLI.

Conclusion: WAST can be considered as a low cost, portable, and easy to use technique for the noninvasive assessment of ED.

The work was supported by the Russian Foundation for Basic Research under project R-Ural-a 14-04-96027.

Key words: endothelial dysfunction, skin temperature oscillations, local heating test, wavelet transform
SESSION
RADIOLOGY AND ROENTGENOLOGY 2
INCIDENTS AND ACCIDENTS IN IMAGING DEPARTMENTS. WHAT’S NEXT?

Virginia Tsapaki
Secretary General of IOMP

X-rays are classified as a carcinogen by the World Health Organization’s International Agency for Research on Cancer. Currently, experimental and epidemiological data do not support the proposition that there is a threshold radiation dose below which there is no increased risk of cancer. It is estimated that 0.4% of current cancers in the United States are due to computed tomography (CT) scanning performed in the past and that this may increase to as high as 1.5-2% with 2007 rates of CT usage. US Food and Drug Administration (FDA) estimates that exposure to 10 mSv from an imaging test would be expected to increase the risk of death from cancer by about 1 chance in 2000.

At the same time high dose X-ray imaging such as CT have boosted clinical applications of X-ray use. CT is currently considered as one of the most important imaging techniques of modern times. The introduction of multi-detector CT (MDCT) and CT fluoroscopy have further advanced CT applications by enabling interventional radiological (IR) procedures, which were traditionally performed using C-arm X-ray systems. However, a growing number of adverse events related to excessive CT radiation exposure, as well as its relation to elevated risk of radiation induced cancer are reported in the media.

Modern X-ray equipment together with improvement in techniques and devices have facilitated cardiologists and other physicians, pledging more successful clinical outcome, thus resulting in a profound change in the treatment of coronary heart disease and various periphery vessels. High radiation doses can very easily accumulate due to extended fluoroscopy times needed to monitor the devices to the area of interest and the large recording, there is also high risk of skin injuries to patients and occupational overexposure of the staff involved. Interventionalists are often unaware of the high radiation doses to which a patient’s skin may be subjected, even with the use of modern, state of the art equipment. It continues to surprise many cardiologists, that radiation burns can occur that can be chronically and severely painful, not to mention that both operators and hospitals are often subjected to the legal action that follows such events. All these will be discussed and recommendations will be given to avoid such events.
STRATEGIES FOR MINIMIZING PATIENT RADIATION DOSE IN INTERVENTIONAL FLUOROSCOPY

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Interventional procedures are performed in different clinical areas, such as surgery, cardiology and radiology. They are performed under the guidance of interventional fluoroscopic devices. These devices have numerous protocol setting, image processing and acquisition options. Complex procedures can often last a very long time and thus increase the probability of inducing damage to the skin. Many factors can contribute to the radiation exposure to patients and the possible effects from it, such as patient weight, age, gender, prior interventional exams, and certain medical conditions. It is important to plan each procedure carefully and to consider modifying it in order to minimize skin damage. Patient education and follow-up is very important when certain thresholds are reached and adverse effects are possible. This paper presents several strategies that help reduce patient exposure during interventional procedures.

Key words: interventional fluoroscopy
NATIONAL SURVEY ON THE ACCURACY OF DOSE CALIBRATORS

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The main objective of this national survey was to provide data on the accuracy of activity measurements of the dose calibrators (DC) used in nuclear medicine departments in Bulgaria. Materials and Methods: In 2015 over a period of two months, measurements were performed on 24 DC in all 18 nuclear medicine departments in Bulgaria. The dose calibrators included in the survey were delivered in the last 37 years by 7 manufacturers as follows: Picker – 2 pcs, Robotron – 1 pc, Isomed – 3 pcs, Atomlab – 1 pc, Capintec CRC-15R – 1 pc, Curientor – 12 pcs, Comecer – 4 pcs. The tests were focused mainly on accuracy and were carried out using two certified reference sources: Cs-137 (662 keV), with manufacturer serial number LB 165 and Ba-133 (356 keV) with manufacturer serial number KF 951 for. The last one was imposed by the fact that half of all 18 departments do not have certificates for the reference source (Cs -137) attached to each dose calibrator. The results of the accuracy test for Cs-137 show deviations from the expected value in a wide range –20.5 % to +21.3 %, while for Ba-133, deviation are in the range from –5.2% to +16.6%. According to the National Regulation only 4 DC were in the range ± 5 %.

Results: Bulgarian State Regulations require that deviation between measured and expected values must be within ±5 percent of the average. Errors greater than ±5 % in the interval from –20.5 % to +21.3 % were found in 18 of the 24 DC. It is interesting to note that the result which was close to the expected one (error of 0.3 %) was delivered by the oldest dose calibrator – Picker.

Conclusion: According to the results of this survey, the accuracy of dose calibrators does not meet the requirements of National regulations and need special attention and immediate reaction.

Key words: dose calibrators, accuracy of activity measurement, calibration factors
EDUCATION AND TRAINING RELATED TO ANTHROPOMORPHIC PHANTOMS FOR MEDICAL PHYSICS EXPERTS

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In 2013, a new ambitious unique project related to qualification of Medical Physics Experts in Diagnostic and Interventional Radiology started. The EU-TEMPE-RX project aimed at development of a course composed of 12 educational modules, providing a training scheme that allows the medical physicists in Diagnostic and Interventional Radiology to reach a high level of knowledge, skills and competences.

The EUTEMPE-RX project has been completed but its sustainability plan assured the continuation of the education of Medical Physics Experts. One of the 12 modules, the “Physical and virtual anthropomorphic phantoms for image quality and patient dose optimization”, is developed and taught at the Technical University of Varna. It provides education and training in the field of design, implementation and use of anthropomorphic phantoms in virtual clinical trials including existing and new Diagnostic and Interventional Radiology technologies.

The current paper presents the content of the module – the e-learning and face-to-face parts, and its impact on the participants. The online e-learning part includes 10 chapters with state of the art reviews in the field of physical and computational anthropomorphic phantoms, introduction and tutorials to software applications for design of phantoms and their use with x-ray imaging techniques with examples. The material for the online part was initially developed and stored on the SEKOIA platform and then migrated and made available on a MOODLE platform. The next face-to-face part of the “Anthropomorphic Phantoms” module will start on 22 May 2017 at the Technical University of Varna, and will last for 5 days. This part is organized in a blended format that includes lectures, computer-based exercises, a visit to the hospital for experimental work, discussion sessions and project work. The focus is given on practical work and development of a work project. Module assessment includes implementation of a work project on a case study from Diagnostic and Interventional Radiology, combined with a short written exam.

All lectures are led by worldwide recognized researchers in the field of anthropomorphic phantoms and their use in the research and clinical practice.

Key words: medical physics education, anthropomorphic phantoms, computer simulations, virtual clinical studies
TRACKING THE EFFECT OF OPTIMISATION IN A PAEDIATRIC RADIOLOGY DEPARTMENT

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The purpose of this work is to evaluate the effect of optimisation of paediatric chest radiography applied in a paediatric radiology department.

Optimised protocols including optimal exposure parameters and radiography technique were developed and recommended in 2011, based on patient size for different age groups. Data were analysed in four age groups: 1–12 months, 1–4, 5–9 and 10–15 y. The first survey performed immediately after the implementation of the optimised protocols showed decrease in KAP values in a factor of between 2 (from 8.06 to 3.65 μGy.m\textsuperscript{2}) and 5 (from 3.59 to 0.70 μGy.m\textsuperscript{2}) for different age groups. A new survey implemented 5 years after the utilisation of the optimised protocols showed 100 % utilization of the proposed optimized protocols. Additional decrease in KAP values was found by a factor of between 1.4 (from 0.7 to 0.5 μGy.m\textsuperscript{2}) and 3.6 (from 5.53 to 1.53 μGy.m\textsuperscript{2}) for different age groups. Image quality was assessed to be of sufficient diagnostic quality.

The survey demonstrated that the implementation of the proposed optimised protocols and radiography technique in the routine radiography practice lead to sustainable low doses to paediatric patients at good diagnostic quality.
SESSION
BIOPHYSICS
NANOTECHNOLOGIES IN GENE THERAPY – NON-VIRAL VECTORS FOR NUCLEIC ACID DELIVERY

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Synthetic cationic lipids, which form stable complexes (lipoplexes) with polyanionic nucleic acids, are presently the most widely used constituents of non-viral gene carriers. Examined here is a particularly attractive cationic lipid class, triester phosphatidylcholines (PCs). These phospholipids are biodegradable, exhibit low toxicities and good transfection efficiency. A summary of studies on a set of over 30 cationic PCs revealed the existence of a strong, systematic dependence of their transfection efficiency on the lipid hydrocarbon chain structure. Transfection activity increases with increase of chain unsaturation from 0 to 2 double bonds per lipid and decreases with increase of chain length in the range ~28–50 total number of chain carbon atoms. Maximum transfection was observed for ethyl phosphate PCs (ePCs) with monounsaturated 14:1 chains (total of 2 double bonds and 28 carbon atoms in both chains). Because lipid phase behavior is known to depend strongly on the chain molecular structure, the described above quantitative structure-activity relationship (QSAR) substantiates a view that cationic PC phase propensities are an important determinant of their transfection efficiency. Indeed, X-ray structural studies showed that the rate of DNA release from lipoplexes as well as their transfection activity well correlate with non-lamellar phase progressions observed in cationic PC mixtures with membrane lipids. These findings appear to be of substantial interest because, according to current views, key processes in lipid-mediated transfection such as lipoplex disassembly and DNA release within the cells are believed to take place upon cationic lipid mixing with cellular lipids.

Key words: X-ray diffraction, gene therapy, non-viral vectors, cationic lipids
FABRICATION OF FUNCTIONALIZED MAGNETITE NANOPARTICLES WITH APPLICATIONS IN DRUG DELIVERY SYSTEMS

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The use of nanotechnology in the fabrication of targeted carriers for anti-tumor substances is one solution for exceeding the tumor cells resistance to classical treatment schemes. We propose the obtaining of different drug delivery systems based on magnetite nanoparticles cores and organic shells to be used in the specific delivery of doxorubicin chemotherapeutic, in order to obtain specific toxic responses in human cancer cells.

The nanoparticles characterizing was done in terms of crystallinity, chemical composition and structure. Regarding the biological effects, the in vitro cytotoxic potential was proved for different tumor models using both quantitative and qualitative estimations, correlated with the determinations of nanoparticles cellular entrapment efficiency using particle induced X-ray emission technique.

Key words: magnetite nanoparticles, cancer, drug delivery systems;
ELECTROCHEMICAL APPROACH TO INVESTIGATE DRUG-NANOPARTICLES INTERACTIONS

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Albumin (bovine serum albumin (BSA) and human serum albumin (HSA)), is universally employed as a drug carrier for cancer diagnostics and therapy, due to its excellent biocompatibility and biodegradability. Previously, it was shown that albumin nanoparticles are suitable for encapsulation of hydrophobic drugs in solution.

The interaction of three different drugs with Bovine serum albumin nanoparticles (BSA-NPs) is investigated in this work by means of an electrochemical technique – cyclic voltammetry. Two phenothiazine drugs, chlorpromazine and thioridazine, and a spin-labeled nitosourea drug (SLCNUgly) are used to investigate the loading efficiency of BSA-NPs.

The method is useful for electroactive drugs and consists of investigation of the electron-transfer characteristics of the drugs which are directly related to the drug concentration in solution.

The results indicate decrease of the free drugs in solution in the presence of nanoparticles, implying a penetration of the drugs in the BSA-NPs, according to their hydrophobicity. The comparison with a hydrophilic electroactive species, potassium ferri/ferrocyanide, indicates that the latter interacts in much lesser extend with the BSA-NPs.

It is proposed that this method could be useful for in situ evaluation of drug-nanoparticle interactions.

**Key words:** protein nanoparticles, amphiphilic drugs, bovine serum albumin, electrochemistry, cyclic voltammetry
Antimicrobial peptides (AMPs) are small cationic membrane-active peptides with simple structure - from few to several tenth of amino acid residues. They are found in most living organisms and play an essential part in innate immunity. AMPs exhibit broad-spectrum antimicrobial activity against bacteria, fungi and viruses and can be potential candidates for alternative drugs.

The behavior of AMPs before interaction with the membrane and possible formation of clusters can influence significantly their activity. We investigated the behavior of three systems with different concentrations of antimicrobial peptide - Indolicidin in water solution, namely (10.9mM, 21.9mM and 99.4mM) by means of Coarse-Grained Molecular Dynamics simulations. This cationic antimicrobial agent is the shortest natural AMPs – only 13 amino acids with a largest proportion of tryptophan (Trp) residues of any known protein.

At 99.4mM, we observed saturated solution of Indolicidin. Under certain threshold concentration value (between 99.4mM and 21.9mM) globular amphipathic clusters are formed with average diameter of 4.5 nm. The form and the structure of these globular clusters are in agreement with experimental data published by [Hsu, C.. 2005]. These clusters are with a central hydrophobic core composed of proline and tryptophan, which is bracketed by positively charged regions near the peptide termini. This form of the cluster appears to be ideal for intercalation between the lipid molecules of a bacterial bilayer. Our results shed light on the behavior of the antimicrobial agent Indolicidin I solution and will be used in a virtual assessment of interaction between AMPs with bacterial membrane.


Key words: antimicrobial peptides, AMPs, indolicidin, molecular dynamics, coarse grained (CG)
VIPOXIN EFFECTS ON THE SURFACE ELECTRICAL PROPERTIES AND MEMBRANE TRANSPORT OF PROTONS IN HUMAN ERYTHROCYTES

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The main and most toxic component isolated from the venom of Bulgaria Viper ammollites meridionalis is the neurotoxin Vipoxin - a heterodimeric post-synaptic ionic complex composed of two protein subunits – a basic and strongly toxic His48 sPLA2 (secretory phosphatide sn-2 acylhydrolase, Phospholipase A2, EC 3.1.1.4) enzyme (PLA2) and an acidic, enzymatically inactive and non-toxic component (VAC). Both subunits have the same polypeptide length (122 amino acids) and are closely related sharing 62% sequence identity.

The effects of sPLA2, VAC and Vipoxin on the electrophoretic mobility (EPM), zeta potential (ζ) and surface charge density on erythrocyte membranes were presented. EPM was measured by microscopic (visual) microelectrophoresis with an OPTON Cytopherometer (Austria). There was a strong enhancement in the negative charge on the erythrocyte membrane in the presence of VAC at concentration of 1.3 μM.

Extracellular proton concentration (H⁺ex) as a function of time (s), calculated from the recorded extracellular ΔpH changes induced by sudden jumps of the extracellular proton concentration upon the treatment of erythrocytes by venom components, was measured by the modified method of Glaser (1984). VAC induced an increase in the slope of the curve and the maximum value of the proton concentration in extracellular space in erythrocytes in norm due to the lower pi of the acidic subunit - VAC. Vipoxin and its components altered the slope and maximal value of ΔpH changes in erythrocytes in patients possessing lower value of ζ potential.

The correlation between the zeta potential and the effect of vipoxin potential shows that electrokinetic parameters could be used for the assay of toxic activity of different venom components.

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TRANSFORMATION OF PHENOMENOLOGICAL MODELS OF SODIUM-POTASSIUM PUMP INTO BIOPHYSICALLY BASED ONES

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Production of action potential in any excitable cell is accompanied by ion movements along their concentration gradients across the membrane. To support the cell ready for a prolonged activity, the ions must be returned back against their gradients. The sodium-potassium pump is the main player in performing this task. It is widely studied both experimentally and mathematically. Mathematical model is not only a measure of our understanding of the processes but also a way to estimate the weight or importance of different parameters in the process. There are a few phenomenological models of sodium-potassium pump of different complexity that are used in models of activity of excitable structures, such as heart, muscle or nerve cells. They are relatively simple and characterize the steady state pump current through few affinities. The aim of the present study is to show a way for expansion the phenomenological models.

To obtain biophysically based model of sodium-potassium pump, the pump activity is represented by a cycle of chemical reactions between different pump states. That approach could give analytic expression for the steady state current, but require providing all the involved rate constants. By assuming sufficient number of states so that each transition reflects an elementary process, a linear dependence of rate constants on concentrations is expected according to the mass action law. This allows expression for the steady state pump current to be transformed from a function of rate constants into a function of concentrations. Then, the steady state pump current would depend on the available free energy and on a set of properly defined pump affinities. The general expression for the steady state pump current provides a way to estimate the quality of the phenomenological models and gives a framework for their expansion over much wider parameter set. To include dependence on the available free energy in phenomenological models, one needs no additional experimental information. Such models would predict correct direction of the current not only for forward but also for backward regime of the pump. Further refining of the phenomenological models would require information on the neglected affinities.

Key words: transporter; Na-K-ATPase; affinity; free energy
MODIFICATION WITH SHORT LASER PULSES OF COLLAGEN DERIVED MATRICES CROSSLINKED WITH D-FRUCTOSE FOR POTENTIAL USE IN THE REGENERATIVE MEDICINE

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New biomaterials with improved functionality, biocompatibility and low immunogenicity are a major focus of research in the regenerative medicine and the related interdisciplinary fields. The collagen family proteins comprise the largest percentage of the total protein mass in the body therefore they are among the first choices as compound of such biomaterials. Unfortunately the collagen processing increases its solubility and decreases its mechanical strength. To overcome these issues often crosslinking is applied. However many crosslinking agents impact negatively the toxicity and immunogenicity of the biomaterial. Therefore as a prerequisite of better biocompatibility the biomaterials studied here are made of naturally occurring compounds. Further the appropriate biomaterial texture and patterning emerges in the recent years as important aspect of the successful cell compatibility and regeneration and lasers offer the advantage of high precision and minimization of chemical contaminations in such biomaterial processing.

Purpose of the study is to evaluate the laser surface modification and micropatterning of newly synthesized collagen-derived crosslinked biomaterials for potential use in the regenerative medicine.

Methods: The matrices were casted in the form of thin films with smooth surface and prepared from collagen derived from tendon and crosslinked with D-fructose in custom crosslinker. Quantronix Integra C ultrafast laser system was used to generate the short laser pulses with 790nm center wavelength and different varied parameters sets (such as duration and number of pulses, fluence, etc.) which were applied on the samples. The resulted surface modifications were examined with optical and SEM microscopy.

Results: In surface modifications with regimes and parameters of the beam where photomechanical effects were achieved the shape of the resulting cavities and degree of foaming is influenced by the level of the crosslinking agent in the matrix, in regimes of operation above the threshold of plasma formation such relation is less pronounced. The systematic exploration in the study of the relations between the parameters of the laser beam, the matrix composition and the resulted surface modifications and microstructures give us more control in producing biomaterial with specific surface microgeometry favoured by particular cell line or tissue that needs to be regenerated.

Key words: 1,3,4,5,6-Pentahydroxy-2-hexanone, ablation, biocompatibility, scaffold, surface patterning, tissue engineering
SESSION
RADIOLOGY AND ROENTGENOLOGY 3
Assessment of the limiting spatial resolution with a test object is a routine operation during Quality Control (QC) in X-ray diagnostic radiology. During this operation one can observe inversion of contrast of the images of test patterns (visual “negative” structures). These “negative” structures are visible for objects with spatial frequency above the limiting spatial resolution.

The presentation explains the geometry and mathematics of contrast inversion in these cases. The mathematical extraction of the Modulation Transfer Function (MTF) from the image geometry is shown. The result, MTF as a decaying sine function, makes clear the contrast inversion. While for the QC purposes only the modulus of MTF is taken into consideration, the remaining parts of the decaying sine function lead to contrast inversion of the still visible small patterns. In some cases one can observe even two consecutive contrast inversions.

The contrast inversion effect can confuse the assessment of the limiting spatial resolution through the observation of the test object patterns (with increasing spatial frequency). However, and more importantly, this effect can confuse the visualisation of small objects in the medical image during digital X-ray radiography. This effect is also one of the reasons for increased noise in digital radiography images.

Understanding the contrast inversion mechanism can be useful for the development and assessment of software for automatic QC.

**Key words:** modulation transfer function, image quality assessment, quality control
CT DOSE OPTIMIZATION AND TRACKING ACROSS MULTIPLE FACILITIES

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Computed Tomography (CT) is an essential and widely utilized tool for diagnosis in today’s medicine, and there are variety of tools we can employ in order to optimize and track dose among multiple scanners and multiple facilities. CT is also a complex tool that requires proper understanding of its capabilities and knowledge of image formation, image processing, and radiation dose, and as a result, training of technologists is crucial. Ensuring consistency across multiple facilities can be challenging because of differences in equipment, staff culture and radiologist preferences. This paper intends to present our efforts to track CTDIvol across facilities, to benchmark, and to optimize the CT protocols. With the help of the Radimetrics dose tracking software, we are able to find suboptimal practices and correct them, compare CTDIvol among facilities, track and analyze protocol changes. Another tool that assists in preventing too high a patient dose is Dose Check, which informs the technologist if the planned scan would exceed established reference levels, allowing the technologist to judge whether this is appropriate. Employing Dose Check has been helpful in keeping the patient exposure below the established reference levels. Since the CT technologists are on the front lines of controlling patient dose, training requirements have been updated to better reflect the need of expertise in the field. All of these activities are under the guidance of the hospital CT Task Group, which includes radiologists, technologists, administrators and the physicist. Optimizing dose to patients from CT is a multidisciplinary problem and our team approach has lead to positive results.

Key words: computed tomography; CT dose tracking; CT dose optimization
RADIATION EXPOSURE OF PATIENTS FROM WHOLE BODY EXAMINATIONS ON NEW PET-CT SYSTEM

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First national survey of patient doses from hybrid imaging in nuclear medicine in Bulgaria was performed in 2013-2014. Afterwards new equipment was installed in the country. The purpose of this study is to supplement the information from the national survey by estimating radiation exposure of patients from the new positron emission tomography-computed tomography (PET-CT) system functioning in Bulgaria.

The system is GE Discovery IQ Clarity 5 ring. Patient data from whole body examinations with 18F-2-fluoro-2-deoxy-D-glucose (FDG) for 105 patients were retrospectively collected from the picture archiving and communication system (PACS) of the hospital. Same methods for calculation of effective dose from the PET and CT parts of the examination as in the national survey were used for consistency. The radiopharmaceutical contribution to patient exposure was determined by multiplying the averaged over all patients activity applied and the International Commission on Radiological Protection (ICRP) Publication 80/106 conversion coefficients. For the CT contribution CT Expo software was used.

The mean effective dose from the PET part of the examination for the whole cohort of patients was 4.4 mSv, 25% and 10% lower in comparison to the other two systems functioning in the country. The mean effective dose from the CT part was 3.6 mSv, applying the ICRP 103 tissue weighting factors, for the standard whole body examination. This value is about 53% lower than CT exposures calculated for the other two systems. Taking into account the overall exposure from the hybrid imaging, the mean effective dose was 8 mSv.

Patient doses from the new PET-CT system are 41% and 32% lower compared to the other systems in Bulgaria. Potential for further optimization is the use of iterative reconstruction algorithm of the CT scanner, expected to be introduced soon.

Key words: PET-CT, patient exposure
RADIATION EXPOSURE OF PATIENTS FROM TWO PROCEDURES ON NEW SPECT-CT SYSTEM

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After the first national survey of patient exposures from hybrid imaging in nuclear medicine was performed in Bulgaria, a new single-photon emission computed tomography and computed tomography (SPECT-CT) system was installed. The purpose of this study is to estimate patient doses received from procedures on this system and to supplement data from the national survey.

The study was performed on a system GE Discovery NM/CT 670 Pro. Patient data were retrospectively collected from the picture archiving and communication system (PACS) of the hospital. Since this is a new hospital, enough statistics was available only for two types of examinations – bone imaging and myocardial perfusion, the latter including stress or stress and rest examination. Also it was performed with two different radiopharmaceuticals. Separate calculations for myocardial perfusion were made based on both criteria. Data were collected for 40 patients for the first and 42 patients for the second procedure.

The methods for calculation of effective dose were the same as in the national survey for consistency, with some minor changes. The CT contribution to the dose was estimated with CT Expo software, applying the International Commission on Radiological Protection (ICRP) Publication 103 tissue weighting factors. The radiopharmaceutical contribution was estimated by multiplying the ICRP 53/80/106 corresponding conversion coefficients by the averaged on the whole patients’ sample administered activity.

For bone imaging the mean effective dose was 4.5 mSv from CT and 3.4 mSv from the radiopharmaceutical, hence the total effective dose was 7.9 mSv. For myocardial perfusion the CT contribution was 0.2 mSv for only stress, doubled for stress and rest. The 99mTc-tetrofosmin contribution to stress examination was 3.1 mSv and to stress and rest it was 6.7 mSv. The contribution of 99mTc-MIBI to the stress exam was 3.2 mSv and to the stress and rest exam the value was 8.7 mSv. The total weighted effective dose for myocardial perfusion was 4.6 mSv.

Doses from bone imaging are approximately in the middle of the range from the national survey. Doses from myocardial perfusion from the CT scanner are much lower compared to the other systems in the country and the exposure from the radiopharmaceutical is similar to them. Potential was found for optimization of bone imaging.

Key words: SPECT-CT, patient exposure
SURVEY OF PRACTICE AND DOSE OPTIMISATION STRATEGIES IN PAEDIATRIC PET/CT PROCEDURES

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The use of hybrid imaging technologies such as PET/CT systems is rapidly expanding. The radiation doses delivered to patients undergoing PET/CT are relatively low, but repeated procedures may lead to significant cumulative doses. This is of a particular concern for paediatric patients, because of their higher tissue radiosensitivity and longer life expectancy.

The purpose of this survey was to estimate paediatric patient doses from PET-CT procedures and to explore potential for optimisation. Data were retrospectively collected for 123 paediatric patients examined with the system GE Discovery 600 during the period 2014-2016. The following parameters were recorded for all patients: indication and type of examination; patient’s age, weight, height and gender; tube voltage (kV); tube current (mA); pitch; rotation time; slice width and number of frames; CTDIvol; administered activity of the radiopharmaceutical 18F-2-fluoro-2-deoxy-D-glucose (FDG). Two types of examinations were performed: whole-body or head. Large variations in CTDIvol, up to 5, were found within one of the age groups. For head examinations the average CTDIvol varied between 1.8 and 2.2 mGy for different age groups. The average administered activity for the same type of examination varied between 123 and 187 MBq (the lowest value was observed in the age group 5-9 y). Pure correlation was found between administered activity and patient weight.

The average CTDIvol for the whole body PET/CT varied between 1.2 and 3.8 mGy. The average administered activity varied between 90 and 279 MBq. For this examination strong correlation was found between administered activity and patient weight for all age groups except 1-4 y. Automatic exposure control was used for all the patients, types of examinations and body regions. Good practice for patient dose registration and records has been observed.

A potential for optimisation of procedures was found. Recommendation was given for proper selection and registration of the exposure parameters for the CT part of examination according to patient age and weight. Low dose paediatric head CT protocols should be developed. A protocol for appropriate manipulation and administration of radiopharmaceuticals according to patient’s weight should be implemented and reduction of total exposure time in the department.

Key words: paediatric PET/CT, patient exposure, optimisation
COMPARISON OF 3D CONFORMAL RADIOTHERAPY AND HELICAL TOMOTHERAPY FOR IRRADIATION OF THE BREAST AND THE REGIONAL LYMPHATICS

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**Purpose:** To compare new treatment planning protocol for left and right site breast irradiation, when the planning target volume has to include the involved breast (PTV) and supraclavicular lymph nodes (PTV_SCLN) using helical tomotherapy and routine “one isocenter” 3D conformal radiotherapy technique.

**Methods and Materials:** Ten left and ten right breast patients were planned for prescribed dose 50 Gy. 3D CRT plan was created with an isocenter situated at the lower edge of the supraclavicular part of the target volume, and asymmetrical MLC collimated beams. Tomo Helical plan was developed with field width – 5.048 cm, pitch 0.22 cm and modulation Factor 3. The fall-off of the dose was controlled by help contours at a distance of 1.5 cm from PTV and PTV_SCLN. Directional blocking was applied to heart and contralateral breast and lung.

**Results:** The outcomes for Tomo helical vs. 3D CRT are listed below: For PTV: Dmin (2ccm) 39.7 ± 1 Gy vs. 25.9 ± 6 Gy, Dmax (2ccm) 52.7 ± 0.4 Gy vs. 54.51 ± 0.6 Gy, V95% 48 ± 1 Gy vs. 44 ± 1.5 Gy; For PTV_SCLN: Dmin (2ccm) 45.4 ± 0.6 Gy vs. 37.8 ± 1.6 Gy, Dmax (2ccm) 51.8 ± 0.2 Gy vs. 55.2 ± 0.6 Gy, V95% 48.9 ± 1 Gy vs. 45.2 ± 1.5 Gy. With both techniques ipsilateral lung received the same middle dose -13 Gy (+0.3 Gy; -0.7 Gy), the volume received 30 Gy is 8.5% higher in the CRT plans but the dose received in 65% of the lung volume is 3 Gy more for Tommo helical. The middle dose for contralateral lung was 3.5 Gy lower for 3D CRT (1.2 Gy vs. 4.8 Gy). Heart’s average dose for left breast cases was 5 Gy higher for helical plans, but in 3D CRT plans Dmax was 10 Gy more and V30 Gy was 3.6% vs 0.6%. The average dose in contralateral breast was 2.5 Gy more in tomo helical plans. The liver in right breast cases with 3D CRT plans got 5 Gy less average dose but 7 Gy more for Dmax.

**Conclusion:** The conformity and homogeneity of PTVs were better for helical tomotherapy plans than the 3D CRT for both left and right breast tumor with regional lymph node involvement. The organs at risk: ipsilateral lung, contralateral lung, contralateral breast, heart and liver received higher average dose in tomo helical plans, but lower maximum dose.

**Key words:** Treatment planning, Breast cancer, 3D conformal radiotherapy (3D CRT), Helical tomotherapy
SESSION
NON-IONIZING RADIATION
BULGARIAN CONTRIBUTION IN
ELECTROMAGNETIC BIOLOGY AND MEDICINE

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Immediately after World War II research of effects of both magnetic and electromagnetic fields on living systems started in Japan, and then quickly moved to Europe, first in Romania and the former Soviet Union. Next country was Bulgaria with research that started in the late 1960’s in the Medical Universities in Sofia, Plovdiv and Varna, as well as Sofia University.

Starting with the first National conference of Biomedical Physics and Engineering, papers have been presented on the subjects of electromagnetic biology and medicine. Our Society continuously supported organizations of seminars and conferences on the topics related to radiation biology and medicine, most of which took place at the University mountain house Gyuletchiza.

Due to increased recognition of Bulgarian contribution in electromagnetic biology and medicine, two International Schools ELECTROMAGNETIC FIELDS AND BIOMEMBRANES have been organized by the Sofia University and Bulgarian National Society of Biomedical Physics and Engineering in close cooperation with Bulgarian society of physiotherapy. In both meetings more than 30 of the best experts in these areas arrived to lecture participants in the school (around 200 scientists) from Europe, North America and Asia. Our society was a founder of the European Society of Biomagnetism.

During the period 1960-1985 nearly all European countries designed and manufactured their own magnetotherapeutic systems. Such systems was created in Bulgaria by Ivan Daskalov and Nentcho Todorov. The unique union of future Academician of BAN and future professor on physiotherapy placed Bulgaria in leading positions in the electromagnetic biology and medicine in Europe. The first book in the world on practical use of this device was published by Nentcho Todorov in Bulgaria.
EVALUATION OF A MICROWAVE SYSTEM FOR HYPERTHERMIA TREATMENT OF CANCER IN ANIMALS

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The research about interactions between electromagnetic field and biological tissues for cancer treatment assumes every day a growing interest. The goal of this paper is to describe main characteristics and experimental evaluation of microwave system for hyperthermia treatment of cancer in animals. The evaluation of the temperature distribution on phantom was accomplished. Computer simulations were used for the analysis of spatial SAR distribution on the phantom.

The results show that this microwave system can be used on hyperthermia treatment of small animals according to the tumour size and deepness.

Key words: hyperthermia; SAR; microwave system; temperature distribution
DATA FROM THE NATIONAL NOISE MESURING SYSTEM IN THE URBANISED REGIONS OF BULGARIA

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The analysis of the acoustic environment in the cities of the country is carried out based on data collected by the National noise monitoring system.

The National noise monitoring system covers all agglomerations and passing through them stretches of major roads, major railways and airports, and industrial noise sources. Reliable information on the state of noise pollution in urban areas was provided by measurements, analytical and informational activities. Data on noise level measurements has been collected since the 1980’s.

Measurements of urban noise were conducted at about 727 points within the framework of the national noise monitoring system. Along with acoustic measurements the intensity and structure of traffic flows, the type of road surface, the rate of construction and the landscaping were took into account.

The assessment of noise pollution in urban areas is carried out by the indicator $L_{\text{day}}$ - (averaged equivalent) daily noise level, defining the degree of discomfort during the daytime period of the day (7:00 to 19:00 pm)

The activities in the frame of the national noise monitoring system were organized and managed by the National Center for Public Health and Analysis (NCPHA) and include training of the specialists of regional health inspectorates (RHI), performing noise measurements, and verifying their competence in terms of implementation of monitoring methods.

In 2015, in 519 out of the 727 checkpoints in the country were measured to indicate values above the limit, which represents 71.39% of the total controlled areas. 191 of the points are located in areas with intense noise protection, which constitutes 37% of the points with recorded levels above the limit. Even though our research shows that noise exposure in the country has been decreasing slightly over the past five years, noise levels generally remain high.

The analysis of the noise in several of the municipalities allowed the development of appropriate action plans that have led to the reduced noise exposure of the population.

Key words: noise; monitoring; traffic; data; discomfort
Purpose: To design a magnetic resonance phantom for assessment and analysis of reproducible tissue-selective techniques with nulling signal from silicone, fat and water component.

Method: A breast phantom with separate cavities contained silicone, fat and water was developed. In the cavity of the inner section was placed saline while the outer cavity was placed lard in a liquid state. The medical silicone implant has been glued to the cover of the inner box in order to avoid the presence of air in the core of the phantom. The constructed phantom measures 17x17x8 cm (length x width x height). All images were obtained with a 1.5-T MRI system (Signa HDxt, GE Healthcare) using 8-channel breast coil. Initially it was conducted a trial for a week using standard sequences - axial T1 SE and axial T2 FRFSE to assess the structure and further application of the phantom.

Then were acquired series of more than twenty datasets phantom images. Sequences performed included axial STIR with silicone suppression and axial STIR with water suppression using manual pre scan and adjustment of the resonant peak. The resulting images were processed and analyzed with software available on a workstation (Advantage Workstation 4.6, GE Medical Systems). Regions of interests with the same area of 78.8 mm² were defined for each of the two provided techniques for silicone and saline respectively. Signal intensities were recorded at the central section of the volume avoiding the border areas of the phantom.

Results: The mean (±SD) silicone signal intensities were found to be 817.55 (±60.64), 829.25 (±60.95), 834.95 (±66.17), 830.20 (±49.47) and 835.13 (±51.06) for axial STIR with water suppression. Saline signal intensities were found to be 29.95 (±1.62), 29.10 (±0.57), 26.05 (±0.50), 29.70 (±1.27) and 28.4 (±2.12).

On axial STIR images with silicone suppression, the mean signal intensities for silicone were found to be 30.80 (±5.69), 29.28 (±3.96), 25.80 (±1.19), 27.42 (±1.71) and 27.90 (±3.04) and 1746.05 (±159.74), 1767.70 (±178.33), 1744.45 (±164.12), 1749.10 (±165.04) and 1775.20 (±190.21) for saline.

Conclusions: The proposed breast MRI phantom has produced exciting and interesting early results. It was found that the phantom is well designed and constructed for tissue selective use. All measurements with manual water and
silicone suppression were stable and reproducible over 20 sessions. Future research may include inter observer study, imaging phantom at different centres and platforms to confirm the reliability and reproducibility of measurements from the phantom. In addition to future research should include evaluation of image quality changing image parameters in a certain sequence. The development of the phantom will permit implementation of quality control measures and technician training. However this study was limited because only one MRI unit in one location was used to image the phantom.

**Key words:** magnetic resonance, phantom, tissue-selective techniques
POSTERS
POSTER SESSION IONIZING AND NON-IONIZING RADIATION
APPLICATION OF EFOMP AND EUREF QUALITY CONTROL PROTOCOLS IN EVALUATION OF A MODERN FULL FIELD DIGITAL MAMMOGRAPHY SYSTEM

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EFOMP and EUREF Protocols for quality assurance in breast cancer screening give guidelines for Quality Control (QC) of digital mammography X-ray systems. Contrast detail analysis is a key method for assessment of image quality (IQ) of these systems. It is recommended as a tool in testing of screening mammography units according to European guideline and it is also applicable to general assessment of equipment performance as part of optimization process.

In this study main QC testing procedures recommended by above mentioned protocols were applied on a Siemens full field flat panel digital mammography system. Pictures of CDMAM and ACR phantoms were analyzed as part of image quality assessment along with assessment of LCS, HCR, MGD, SDNR compensation, detector response function, noise evaluation, image uniformity, etc.

Based on conclusions obtained, recommendations on practical application of explored methods for meeting of Bulgarian regulatory requirements for physics quality control testing of digital mammography systems as part of process for approval of mammography screening programmes are discussed.

**Key words:** digital mammography; screening; contrast detail analysis; CDMAM images
EVALUATION THE FEASIBILITY OF VMAT TREATMENT PLAN VERIFICATION WITH OCTAVIUS 4D PHANTOM USING DIFFERENT DOSIMETRY CRITERIA

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Purpose: The aim of the study is to evaluate and compare relevance VMAT treatment plan verification of 3 groups of patients with different tumor localization and using different dosimetry criteria.

Materials and methods: For the purpose of the study 3 groups of patients were selected – 10 patients respectively for prostate cases, lungs cases and head & neck cases. For all cases were prepare VMAT treatment plans. Measurements were performed by dint of: Varian Clinac, Octavius 4D phantom with a diameter of 16cm and a length of 32cm, Octavius 729 is a liquid filled ionization chamber array consisting of 729 detectors with VeriSoft 6.0. The pilot measurements were performed with the following evaluation criteria: 3 mm Distance to agreement and 3% dose difference with reference to Local dose and also for 3.5mm and 2.5% respectively.

Results: The pilot results with 3%/3mm criteria and for the prostate group, the Gamma pass rate range between 95.8% and 96.7% for voxels with more than 50% of the maximum dose, for the lung cases – 95.2% -96.5% and 94.9% - 97.2% for the head & neck cases respectively. Range between 93.3% - 96% for prostate group, 89.2% - 92.2% for lungs and 93.3% - 96% for H&N, for voxels with 50% of maximum dose and lower. Measurements performed with 2.5%/ 3.5mm criteria for voxels with more than 50% of the maximum dose, the Gamma pass rate are between 93.6% - 96.8% for prostates, 94.4% - 96.1% for lungs and for H&N – 94.4%- 96.1% and for voxels with 50% of maximum dose and lower, 95%- 97% for prostate, 91.8% - 93.7% for lungs and 95.3% - 97% for H&N.

Conclusion: For all groups of VMAT treatment plan, Octavius 4D, provide good verification results. The choice of dosimetry criteria, showing greater influence of organ at risk region - area with 50% of maximum dose and lower, than of a tumor volume region - with more than 50% of the maximum dose.

Key words: Volumetric modulated arc therapy (VMAT), Verification, dosimetry criteria, Gamma pass rate
LIFE IN RADIATION

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Well known is the so-called Radio-biological paradox:
One sip of hot tea (heat) increases the body temperature by about 0.02°. In order to raise the temperature of the human body again by 0.02° but by the energy of the ionizing radiation, it takes about 7 to 10 Gy (absorbed dose in air) and this leads to certain death.

This paradox is a visual indicator of the effects of ionizing radiation on human body and on the whole life on Earth.

Life on our planet is occurring at a specific set of conditions. Abrupt change of even just one of these conditions leads to death. The radiation limits are part of this complex.

The radiation field due to the natural radiation sources in which we live is called natural background radiation (NBR). Under the term “unchanged natural background radiation” is understood the natural background radiation, which is not affected by human activities. As a result of human activity, however, the background radiation increases. The use of radioactive minerals, building materials and other such materials leads to the so-called technogenic amplification of the background radiation. The use of radioactive isotopes and X-ray sources in industry, agriculture and especially in medicine accumulates additional so-called man-made supplements to the natural background radiation.

Natural background radiation is derived from three main sources:
• Secondary cosmic rays;
• Gamma radiation background - emitted by radionuclides contained in the earth’s crust, and especially in its surface layer and building materials;
• Internal exposure of people - emitted from the natural radionuclides contained in air, water, food and the human body itself.

This article provides information on the natural background radiation and components from which it is formed. Shown is the impact of the various components of the natural background radiation on the human body. They are given statistics obtained from human radiation exposure of various components of the natural background radiation. A comparison is made of average annual individual dose received in Bulgaria and worldwide.

\textbf{Key words:} natural background radiation, secondary cosmic rays, gamma radiation background, internal exposure of people, radio-biological paradox
DOSE AREA PRODUCT METER WITH EXTENDED FUNCTIONALITY

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**Purpose:** This article describes development of new generation Dose-Area product meter (DAP meter) which measures both standard cumulative and single-shot readings, along with environmental parameters and tube position.

**Implementation:** First, a new electronic technology called Direct Digital Processing (DDP) was developed. It has a number of advantages versus widely used solution with integrator in the input:

- Eliminates resolution degradation caused by the reset and restart time, inherently needed for integrator topology.
- Allows for simultaneous metering of instant and cumulative parameters.
- Electronics is protected against electromagnetic interference and electrostatic discharges, which often cause malfunction in equipment with input integrator.

A block for metering of environmental parameters was added. It measures temperature, barometric pressure and humidity. Another block measures tube position and vibration.

All readings are visualized on a dedicated TFT terminal with touch panel and remote control features. It also transmits information to a thermal printer or computer.

Power supply can be connected to the DAP meter, to the terminal or they can be powered separately with different voltages. Dedicated Bi-directional Serial Line (BSL) interface needs only one wire for data transmission.

Basic parameters include single shot and cumulative values of DAP, Exposure time and DAP Rate. A new parameter Pulsed Fluoro Timeout is introduced to determine beginning of the next single exposure in X-ray equipment working in Pulsed fluoro mode.

Temperature and barometric pressure measurement allows for automatic correction of dosimetric readings.

The kilovolt correction option improves accuracy when DAP meter receives data for applied tube voltage from the X-ray generator.

The color indicator and the sound indicator can signalize instant power, alarm or tube alignment.
Three groups of alarm levels are available – adult, children and neonatal.

Tube position is used for image interpretation and also facilitates tube alignment, required for some kinds of examinations. Tube vibration metering is a useful service option.

**Conclusion:** Extended functionality helps interpretation, improves dose tracking accuracy and facilitates medical and service staff.

**Key words:** DAP meter; dose-area product meter; medical dosimetry; X-ray dosimetry, medical radiology
ACTIVE IONIZING CHAMBER
WITH ANALOG AND DIGITAL MODE OF OPERATION

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Purpose: This paper describes development of new type active ionizing chamber for measurement of exposure energy in X-ray machines. It works in both analog and digital mode and has reach set of additional features like simulator, local and remote control etc.

Implementation: The sensor comprises of air-filled metering chamber with selectable fields, input converter, simulator, environmental and position measurement units, output analog converter, control panel, interface and power supply.

In analog mode sensor generates output signal, proportional to the dose passed through selected metering fields. In digital mode the output signal changes its’ logic level upon reaching desired dose. Active logic levels of all digital signals and polarity of analog output signal can be set independently.

The control panel provides access to all available settings as well to the test signal generation. Same functions are available over the serial interface.

The input converter has optional correction for field’s inequality and for pressure and temperature dependence. When generator can transmit data for tube voltage, kilovolt correction option could be applied.

The simulator generates calibrated signals for automatic system check. Their magnitude can be set either in-situ by the control panel or remotely with commands over the serial interface. When put in simulator mode, sensor accepts commands and generates output analog or digital signals as it is exposed to a real emission.

Results: Effective exposure time range is from 1ms to 5s. Simulator can generate test signal equal to exposures from 1ms to 5.5s. Fields inequality is below 5% with field correction disabled and below 2% with correction enabled. Digital operating mode reduces interface lines and simplifies generator control.

Conclusion: A new type active ionizing chamber with analog and digital operating mode has been designed. It covers full range of exposure times used in radiography. Optional corrections improve stability and accuracy. The built-in simulator allows for fast and safe testing of generator’s automatic system. The sensor can be embedded in X-ray machines with film, with CR system and with DR system.

Key words: Ionizing chamber; AEC sensor; automatic exposure sensor; medical radiology
VARIABLE PACKET LENGTH PROTOCOL (VPL PROTOCOL) FOR REAL-TIME DATA TRANSMISSIN

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**Purpose:** In modern medical equipment different amount of data is often transmitted over single interface. It may vary from one byte for commands without parameters to megabytes for images.

This paper describes definition of new type protocol for data exchange and algorithm for its implementation. They have to meet the following requirements:

- Packet length should follow the amount of data for every single packet;
- Packet format should have minimal count of service and formatting bytes and should not include filling or alignment bytes that do not carry information;
- Control field should allow for using of reliable data verification techniques;
- Algorithm should work seamlessly in real-time applications;
- Data processing associated with transmission and receiving of the packets should engage minimum processor resources.

**Implementation:** A new format with variable packet length has been defined. It has only 2 service bytes. The length of formatting field is kept to the minimum, needed to represent the size of current data field. Control field length can be dynamically selected between 1 and 32 bytes. Data field can vary from 1 byte to 18446 terabytes in one packet. Practical limits should be applied.

The algorithm for implementation synchronizes receiving without intervention of the main data processing functions.

This definition assumes that connection is made between one transmitter and one receiver.

To work with multiple transmitters and receivers, the packet should be preceded by address field, specific for the particular interface.

**Results:** The protocol described herein provides dynamic optimization of real-time data exchange. The receiver gets parameters of the incoming packet before data field and updates only one pointer at the end of each packet. The algorithm supports asynchronous connection of transmitter or receiver and automatic recovery of communication. Control field can carry polynomial values, calculated according to CRC8, CRC16, CRC32, MD4, MD5, SHA-1. It is suitable both for half-duplex and full-duplex modes of communication.

**Conclusion:** Although first announced at the NMPEC 2016 conference, VPL protocol is used for 15 years in various types of equipment. The proven reliability makes it a good choice for demanding real-time applications.

**Key words:** VPL Protocol; Variable packet length protocol; Real-time data transmission; Dynamic optimization; Information technology
ANALYSIS AND EVALUATION OF ELECTROMAGNETIC EXPOSURE IN URBAN AREA WITH HIGH DENSITY OF SOURCES

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In the last years there has been a significant increase in the use of mobile communication services and this growth is expected to continue with the introduction of new generation technology standards such as LTE (Long Term Evolution). This leads to the increasing number of base stations or optimization and reconstruction of the old ones, with the installation of new antennas and connecting new transmitting channels.

The results of studies related to the measurement of electromagnetic field from base stations show that despite of the increasing number of base stations and deployment of additional mobile technology, the levels of electromagnetic exposure remain almost the same in public areas.

The report presents the results of measurement and exposure assessment of electromagnetic fields in a central district of the capital of the country, with greater density of communication sources.

For the purpose of this study the available information concerning sources in the area, including location and technical characteristics was processed. The places with the greatest number of sources were analyzed. The points at which the measurements were performed are selected to meet the criterion of “worst” case of exposure of the population. The values of power density measured in a wide frequency range at the selected points are in the range from 0,1 to 5,3 μW/cm². A detailed assessment of the contribution of different emitters in the respective frequency ranges is made by spectrum analysis.

The measured values of the electromagnetic field are in compliance with national legislation for public protection. The measured values do not exceed the exposure limit values according to the international regulations.

Key words: EMF sources; measurement; exposure
POSTER SESSION BIOPHYSICS
PROTECTANT DRUG EFFICACY AGAINST SCOPOLAMINE-INDUCED DEMENTIA IN MICE, A DSC APPROACH

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In this study, we employed a new approach based on differential scanning calorimetry (DSC) to detect and characterize at a molecular level the changes in brain tissues associated with drug-induced neurodegenerative disorder of Alzheimer’s disease (AD) type and to evaluate the efficacy of preventive treatments with various biologically active compounds expected to hinder the AD development. We used an experimental animal (mouse) model of scopolamine-induced dementia. The DSC measurements performed on supernatants of brain tissue homogenates revealed large differences between the heat capacity profiles for healthy animals and for mice with scopolamine-induced dementia. The heat capacity profiles of brain tissue supernatants from healthy animals displayed well expressed low-temperature exothermic transitions peaking in the range 35-45ºC, thus preceding in temperature the endothermic denaturational transitions. The exothermic transitions were only observed in supernatants of brain tissue homogenates, and not in other samples from the same animals, e.g., centrifugation sediments of brain tissue homogenates, liver homogenates, blood plasma. Remarkably, the low-temperature exotherms were completely eliminated by the scopolamine treatment and replaced with high-temperature exothermic transitions. Preventive treatments with various substances (myrtenal, ellagic acid, lipoic acid) and their combinations (including also ascorbic acid and galantamine), applied simultaneously with the scopolamine treatment, were found to neutralize the scopolamine effect, i.e., to result in partial or complete preservation of the low-temperature exothermic transitions. In principle, exothermic transitions might result from processes of protein aggregation or fibrillization, or from reversal of protein cold denaturation processes. The enthalpy (area) of the exothermic transitions is similar in magnitude to that of the endothermic denaturational transitions, thus suggesting that a substantial portion of the brain proteins are involved in the exothermic processes. These experiments demonstrate that DSC is an appropriate method with great potential for detection and characterization of brain proteome changes taking place in brain tissues affected by neurodegeneration.

Key words: DSC; Neurodegenerative diseases
THE ROLE OF Ca2+ ON STABILITY OF FOAM FILMS FROM LYSO-PHOSPHATIDYLCHOLINE AND CUROSURF

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Pulmonary surfactant is a complex mixture of lipids and specific proteins and its main function is to reduce the surface tension at the air–liquid interface of the alveolus, avoiding the alveolar collapse and reducing the work of breathing. Pulmonary surfactant (PS) is absolutely necessary, being its absence, deficiency or inactivation, associated with severe pulmonary diseases. Phospholipase-mediated surfactant hydrolysis may disrupt PS function by generation of lysophospholipids and free fatty acids and/or depletion of native phospholipids. Thin liquid foam films are an adequate structure–functional experimental model to study the surfactant layers on the alveolar surface. The conditions of in vitro formation of foam films are in close correlation to the in vivo situation and propose numbers of new parameters and dependences for description of PS behavior and functions. For instance, critical concentrations for stable black film formation, film stability in terms of probability for film formation and film lifetime, film thickness etc. Black foam film model has been applied not only for fundamental studies of PS but also for diagnosis of respiratory distress syndrome (RDS) and more recently for evaluating the exogenous surfactant preparations for replacement therapy of RDS.

The aim of paper is to investigate the effect of Ca2+ on the stability of thin liquid films from palmitoyl lysophosphatidylcholine (lyso PC) and therapeutic lung surfactant Curosurf, studied by the black foam film method. It has been obtained the minimum concentration (Cc) required for black film formation and the concentration (Ct) rendering 100% black films. We have measured also the thickness of black foam films obtained. Interestingly, the thickness of these films in the presence of Ca2+ is different from previously measured in the presence of Na+. For the films with lyso PC in the presence of Ca2+ the thickness of measured black foam films is higher and for the films with Curosurf the thickness is lower.

Key words: liquid films, surfactant
PHARMACOPHORE MODELLING OF PPARγ PARTIAL AGONISTS

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The peroxisome proliferator-activated receptor gamma (PPARγ) is central in the regulation of crucial cellular pathways related to adipogenesis, lipid and glucose homeostasis, inflammatory responses, vascular biology and placental development. It is an attractive therapeutic target in the context of metabolic diseases, cardiovascular disorders, inflammatory and auto-immune diseases, Alzheimer’s disease, age-related macular degeneration, skin related disorders, addiction control etc. The current tendency in the development of novel PPARγ selective modulators is focused on partial agonists that do not present the adverse side effects induced by PPARγ full agonists (e.g. thiazolidinediones) and are simultaneously based on natural product-derived scaffolds.

In this study we present an initial pharmacophore modelling of PPARγ partial agonists as a first step in the development of a natural product-based drug discovery pipeline.

A comprehensive analysis was performed on the crystal structures of human PPARγ protein-ligand complexes available in the Protein Data Bank (http://www.rcsb.org). The generated modelling set consisted of about 40 complexes selected to contain only ligands with maximal relative efficacy ≤ 65% as evidence for the ligands’ partial agonistic effect.

The analysis of the collected complexes and the application of structure-based modelling methods allowed us to: (i) characterise the PPARγ binding pocket and the ligands’ interactions; (ii) elucidate different binding modes and (iii) outline key pharmacophoric features typical for the PPARγ partial agonists.

The collected structural and experimental data are stored in a continuously developing virtual library of PPARγ ligands (http://biomed.bas.bg/qsarmm/), thus supplying the publicly available resources useful in modelling studies. The results of the pharmacophore modelling could be used for identification of novel PPARγ partial agonists including those of natural origin.

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Key words: PPARγ; partial agonism; pharmacophore modelling; drug discovery
METHODS OF DIAGNOSTIC OF NEONATAL RESPIRATORY DISTRESS SYNDROME BASED ON GASTRIC ASPIRATES SAMPLES IN ORDER TO APPROPRIATE THERAPY

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The neonatal respiratory distress syndrome (NRDS) is a leading cause of the mortality in premature infants. For this reason different biophysical methods for analyses of the alveolar surfactant’s (AS) structure and properties have been widely developed in the recent decades. The neonatal lung maturity was diagnosed by tests using amniotic fluid, tracheal and nasopharyngeal aspirates, etc., but these methods are traumatic and invasive, and provide samples with small quantities. Therefore, it is necessary to look for new fast and noninvasive diagnostic methods for surfactant maturity assessment at birth. In the present study we performed biophysical analyses of gastric aspirates (GAs) collected from newborns with an aim to find a correlation between the parameters tested and the surfactant maturity.

The biophysical analysis of GAs, by using of Axisymmetric drop shape analysis (ADSA), has demonstrated that the values of minimal surface tension were significantly higher in premature infants with NRDS, compared to the healthy term babies, and were statistically reliable. This parameter could be successfully used for fast evaluation of surfactant deficiency in the premature infants in regard to administration of exogenous surfactant treatment. In addition, by Brewster angle microscopy (BAM), the GAs monolayers morphology showed significant differences between the samples from both groups studied. The images of the GAs monolayers from the control group of GAs (full term babies) showed thick and dense films with surface domains as a result of the higher phospholipid concentration and the enhanced adsorption at the air-water interface. In contrast, the monolayers from NRDS group were thin, homogenous without surface aggregates due to the surfactant insufficiency with these samples.

In conclusion, for the first time we used a combination of modern, innovative techniques, ADSA and BAM, for analyses of gastric aspirates from healthy full term and prematurely born infants for fast, reliable and precise surfactant’s assessment for diagnostic of respiratory disorders associated with surfactant deficiency.
Key words: Gastric Aspirates, Neonatal Respiratory Distress Syndrome, Surface tension, Axisymmetric Drop Shape Analysis, Brewster angle microscopy

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ESTIMATION OF BIOMECHANICAL FORCE OF ACTION AS A FUNCTION OF THREE DIFFERENT CHEWING FORCES STUDIED BY IMAGE DENSITOMETRY ANALYSIS

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Articulating paper is the most widely used method to visualize occlusion. A belief exists among dentists that articulating paper marks intensity is indicative of occlusal contact force. Our research aims to test the correlation between articulating paper marks intensity and chewing forces.

A novel chewing simulator - “Sofia”, designed to reproduce the dynamic masticatory forces according to the functional occlusal concept of Le Gall and Lauret (1996) (1) was used to produce different controlled forces through articulating paper on composite samples. Thirty composite samples (Herculite HRV, Enamel A1, item No 7722859, Kerr Italia Srl.) were divided in three groups according to the three different articulating papers tested (80 μm straight – Dori-Dent Dr.Hirschberg GmbH; Austria; 200 μm , Dr.Jean Bausch Gmbh; carbon paper handifilm 205; Pelikan Vertriebsgesellschaft mbH&Co.KG). Three different loads (10N, 20N and 40N) were applied on each sample and marked through the articulating paper. The resultant marks were subjected to image densitometric analysis in order to determine the correlation between color intensity and corresponding loads.

Under the condition of the present study out of the three articulating papers tested only the carbon paper (which is not actually designed for a dental use) presented correlation between marking’s intensity and force. For the other two articulating paper no steady increase in the marking’s intensity was observed with force’s increase.

The results of the study suggest that higher force can be related to lower intensity markings and therefore the accuracy of articulating paper analysis should be questioned and possibly double-checked by alternative methods.
EFFECT OF CHANGES IN MUSCLE FIBRE GEOMETRY ON EXTRACELLULAR POTENTIALS

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M-response induced by stimulation of the corresponding nerve is often used to provoke synchronized muscle activity. The shape of M-response is simple because EMG signals produced by activity of different motor units are synchronized. M-response contains a few phases that provide clues for understanding the mechanisms of electrophysiological processes.

However, even changes in M-response are sometimes difficult for explanation. One of such recent findings is related to changes in the main and terminal phases of the M-response observed 1 second after maximal voluntary contractions of relatively short durations. The main phase remained unchanged in amplitude and duration, while the terminal phase experienced an increase in amplitude, shortening and earlier appearance. Such changes could not be explained on the basis of the existing models. These models provide theoretical fundamentals of generation of extracellular potentials produced by straight uniform striated muscle fibres.

However, actual muscle fibres are not straight; they are curved. Changes in M-response 1 second after short contraction reported in literature could be due to changes in muscle geometry. The purpose of the present study is clarifying the effects of changes in fibre geometry on extracellular potentials.

The method used is mathematical modeling. We simulated the effects of changes in (a) angle of the fibre curvature, (b) length of the curved fibre portion, (c) decrease or increase in diameter of the fibre end portion. The results of the study show that changes in the fibre curvature or/and diameter induce appearance of an additional transmembrane dipole source at the region of geometrical changes. This results in production of an additional terminal phase in the extracellular potential. This phase arises before the traditional terminal phase. Relation between amplitudes of these additional and traditional terminal phases depends on the fibre curvature. This makes us conclude that the terminal phase of the M-response and the phase characteristics could be related not only to the finite length of striated muscle fibre but also to the fibre curvature and changes in fibre diameter.

Key words: extracellular potentials; single muscle fibre; geometry of fibre end; fibre curvature; terminal phase of M-response
INVESTIGATION OF THE INFLUENCE OF THE ENDOTOXIN ON THE DEFORMABILITY OF RED BLOOD CELLS. (IN VITRO)

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Experiments with endotoxin is an interesting direction for medical applications. It is known that certain abnormalities in the microcirculation in endotoxin shock are related to a reduction of the surface electrical charge and the deformability of red blood cells. Response to these characteristics could be explained by the binding of endotoxin macromolecules with erythrocyte membranes. The intravascular coagulation of blood cells or "sludge phenomenon" has been observed in endotoxin and other types of shock. To gain a more comprehensive insight into the effect of endotoxin on erythrocyte membranes an assessment was made of the light dispersion in electric field by erythrocyte suspension. This method is more sophisticated than the electrophoretic one, but its adoption enables to investigate better the dynamics of endotoxin interaction with erythrocyte membranes, changes in deformability and the like.

The observed biphasic effect of endotoxin, recorded by the electrooptic effect, is a phenomenon noted during the assessment of the properties of erythrocyte and other membranes, treated with a variety of substances. Upon treatment with drugs a biphasic pattern is disclosed in the stability of erythrocyte membranes.

In electrooptic assessment, the incubation and measurement of cells were made in water solution of sucrose of low ionic strength. In the latter case the biphasic effect became manifest at the very beginning. It was rather marked in the first minute, while ten minutes later it was no longer noted. The development of intravascular coagulation in shock is related to a lower deformability of the cell membranes.

A decrease of deformability has been established, although only qualitatively. The electrooptical method enables to estimate the quantitative alterations in deformability. From the values of the disorientation time for five of the samples was measured, a 37 per cent average reduction of deformability was obtained. It is furthermore presumed that deformability modification is proportional to the change in relaxation time of disarrangement. This is a mean value of measurements performed during the first minute, when the changes in electrooptic effect are most significant. The reduction of deformability in individual subjects varies in the 30 to 50 per cent range.

Key words: electro-optical technique, deformability of red blood cells, endotoxin shock
INTRAVITAL MICROSCOPY STUDY OF STRUCTURAL AND FUNCTIONAL INTEGRITY OF BLOOD BRAIN BARRIER (BBB) DURING EXPERIMENTAL INDUCED TUMOR GROWTH IN VIVO- INFLUENCE OF ENDOTHELIAL S1PR1 REGULATION

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Vascular leakage in the brain is a major complication associated with brain injuries and certain pathological conditions due to disruption of the blood-brain barrier (BBB).

The process of angiogenesis is complex but generally involves pericyte detachment; increased vascular permeability; extravasation of plasma proteins, such as fibronectin, which set the scaffold for further angiogenesis followed by vessel maturation; recruitment of pericytes; and finally, tightening of vessel walls. Currently, antiangiogenic therapies are used for cancer treatment and affect cancer progression with variable outcomes.

The blood–brain barrier (BBB) is a diffusion barrier, which impedes influx of most compounds from blood to brain. Three cellular elements of the brain microvasculature compose the BBB-endothelial cells, astrocyte end-feet, and pericytes (PCs). Tight junctions (TJs), present between the cerebral endothelial cells, form a diffusion barrier, which selectively excludes most blood-borne substances from entering the brain. Astrocytic end-feet tightly ensheath the vessel wall and appear to be critical for the induction and maintenance of the TJ barrier in the mammalian brain.

The laboratory animals that were used and monitored are preliminary operated and implanted open cranial window (CW) in order to observe directly microvasculatory bed of the brain. 30 Male Wister rats (n=30). Twenty of them
were treated with glioma tumor cells. Ten were treated with inhibitor of VEGF (experiment). The other ten were treated with carrageenan to induce inflammation, and 10 were left as pure control.

Preparation of cranial window. Rats were anesthetized with 40 mg/kg body wt i.p. ketamine.

A catheter was placed into a tail vein for injection of the intravascular tracer, fluorescein iso-thiocyanate albumin (FITC-albumin; molecular weight=69,000 Da and Evans Blue).

Permeability of the blood-brain barrier was evaluated using two methods. First, extravasation of FITC-albumin was indicated by measurement of tissue fluorescence through fluorescent microscopy and by means spectrophotofluorometer (Shimadzu FL-260) for evaluation of the ratio of FITC-albumin fluorescence between blood plasma and cerebrospinal fluid-CSF.

**Key words:** CSF, BBB, Endothelial tight junctions, tumour neo-angiogenesis
TEMPERATURE–DEPENDENT, SPATIAL AND TEMPORAL CONTROLLED AZOBENZENE POLYMERIC MATERIALS

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Azobenzene polymers are azodyes which are covalently attached in the side chains. They have been extensively investigated for the last three decades because of the high photoinduced birefringence in them and its potential use for optical storage and other photonic applications. It is known that linearly polarized light induces reorientation of the azobenzene groups through multiple trans–cis–trans isomerization cycles and induces large optical anisotropy into them. The value of the anisotropy achieved in different azopolymers depends on the concentration of the azochromophores and the polymer structure that influences the mobility of the azobenzenes and the rate constants of the isomerization processes. In our publications, we are stated that the photoinduced optical birefringence in a low-molecular liquid-crystalline cyanazopolymer obtained by radical-type polymerization of 4-[4-(6-hydroxy–hexyloxyd) phenylazo] benzonitrile can be controlled by combined optical and thermal treatments. At elevated (44-56oC) temperatures, the value of the anisotropic phase difference can be switched between two values by alternating the pump beam intensity. The effect can be used for light-intensity controlled optical switching. Azobenzenes are good candidates for holographic memories as they are highly efficient, stable in the dark, and reversible.

Ten years ago another interesting effect was observed in azobenzene, and has been reported by Gorostiza et al. In biological research azobenzene has been attached to ligands (drug) to photo-modulate their affinity for proteins. Azobenzenes has been employed as a photoswitchable tether between a ligand and the protein: one end of the azobenzene is substituted with a reactive group that attaches to the target protein. The other end displays a ligand for the protein. Depending on where the azobenzene is attached to, either the cis or trans isomer will present the ligand to the ligand-binding site, while the other isomer prevents the drug from reaching the site. Again, photoswitching between isomers turns the protein on and off. When applied to ion channels in the nervous system, this approach affords optical control of electrical activity in neurons. Light-sensitive channels are particularly attractive because optical manipulation offers a high degree of spatial and temporal control protein activity with light and has been reported Banghart et al.

Azobenzenes were screened for their antibacterial activity against Staphilococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, and Proteus vulgaris and for their antifungal activity against Candida albicans by disk diffusion method.

Key words: azobenzene, trans–cis–trans isomerization, photo induced optical birefringence, light-sensitive channels
THE EFFECT OF CO-ACTIVATION OF ANTAGONIST MUSCLES ON RECRUITMENT CURVE DURING TRANSCRANIAL MAGNETIC STIMULATION

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Transcranial magnetic stimulation (TMS) is a useful method to study motor cortex excitability. When motor cortex of the target muscle is stimulated at given brain hemisphere, a motor evoked potential (MEP) is recorded from the correspondent contralateral muscle, if the intensity of the stimulation is equal or higher than a given value called motor threshold (MT). Different stimulation protocols are used to study different intracortical neuronal mechanisms involved in the control of muscle activity. Generally, the intensity of the stimulation pulses are assessed as a percentage from the value of MT. It is well known that at tonic muscle activity the value of MT (active MT) is lower compare to the corresponding value at rest (relax muscle). In the literature very often the experimentalist are using active motor thresholds measured at not standard muscle activity. The last fact is a problem when compare the results reported by different authors.

The aim of the present study was to compare the effect of the different types of tonic muscle activity (with and without co-activation of antagonist muscles) on the recruitment curve during TMS. Ten right handed subjects were investigated. MEPs were recorded from the first dorsal interosseous muscle of dominant right hand in response to TMS of left motor cortex using figure of eight coil. The excitability of motor cortex was assessed by the amplitude of MEPs recorded in responses to increasing stimulation intensity – 100, 110, 120, 130 and 140% of MT measured at rest. The recruitment curves were investigated at relax muscle, isometric index finger abduction and antagonistic co-activation. In contrast to the linear recruitment curve at rest, during tonic isometric muscle activity, the recruitment curve was essentially not linear and different for both types of used activity. The amplitudes of MEPs recorded during isometric index finger abduction were significantly higher than the corresponding values during co-activation of the antagonists.

Acknowledgement: The study was supported by the Grant № ДФНП-125/12.05.2016 to Dr. Mancheva within the frames of a program supporting young scientists in the Bulgarian Academy of Sciences.

Key words: TMS – transcranial magnetic stimulation; motor threshold MT
HIGH FREQUENCY ELECTROMAGNETIC FIELD INDUCED HYPER-THERMIA FOR TREATMENT OF ARTIFICIALLY INDUCED BREAST CANCER IN RATS

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Ferromagnetic-nanoparticles offer many potential benefits for therapy by making specific drugs more efficacious and by decreasing their adverse side-effects.

The basic idea of our study is use of glucose-conjugate Fe-MNP (Glc-Fe-MNPs) for targeting and more accurate focusing in order to increase the effect of high-frequency electromagnetic fields induced hyperthermia in solid breast tumors. Tumors demonstrate high metabolic activity for glucose in comparison with other cells.

Assisted RF-EMF hyperthermia induced by glucose conjugated magnetic nanoparticles in high-frequency oscillating magnetic fields, commonly termed magnetic fluid hyperthermia, is a promising form of heat delivery in which thermal energy is supplied at the nanoscale to the tumor.

The laboratory animals that were used and monitored are 30 female Wister rats (MU-line-6171). Ten of them were treated with N-methyl-N-nitrosourea to induce breast cancer. The other ten were treated with carrageenan to induce inflammation, and 10 were left as control.

Glc-Fe-MNPs can offer a solution to increase hyperthermia effect to the desired areas in the body by accumulation and increasing local concentration due to high tissue metabolic assimilation.

In this condition, it is considered that the magnetization of the nanoparticles is a single-giant magnetic moment, the sum of all the individual magnetic moments and is proportional to the concentration of Glc-Fe-MNPs.

Glucose conjugated Ferro-Magnetic nanoparticles (Glc-Fe-MNPs) being subjected to a magnetic AC field may show remarkable heating effects related to losses during the magnetization reversal process of the particles.

Glucose is one of the fastest and easy catabolize metabolite, breast tumors has initially high levels of metabolic activity. As a result of high metabolic rate
Glc-Fe-MNPs are selectively deposited in tumor tissue.

Glc-Fe-MNPs can offer a solution to increase hyperthermia effect to the desired areas in the body by accumulation and increasing local concentration due to high tissue metabolic assimilation.

In this condition, it is considered that the magnetization of the nanoparticles is a single-giant magnetic moment, the sum of all the individual magnetic moments and is proportional to the concentration of Glc-Fe-MNP.
CHARACTERIZATION AND BIOLOGICAL RESPONSE OF ELECTROSPUN AMPHIPHILIC POLY (DIMETHYLSILOXANE-B-ACRYLIC ACID) FIBROUS SCAFFOLDS

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Silicones and silicone-containing block copolymers are suitable for application in the biomedical field. A large number of different silicone block copolymers have been reported to date. The present study demonstrates the possibility to fabricate new porous fibrous materials by controlled of extremely rarely used in electrospinning domain poly (dimethylsiloxane-b-acrylic acid) (PDMS-b-PAA). The aim of this investigation was to demonstrate how this new (PDMS-b-PAA) electrospun nanofibers mat interact with eukaryotic cells. The fiber morphology was studied by scanning electron microscopy (SEM) and the thermal properties were studied by DSC. The surface chemical composition of the electrospun fibers was determined by X-ray photoelectron spectroscopy (XPS) and hydrophilicity by water contact angle (WCA). The in-vitro biocompatibility of the fibrous scaffolds was investigated by culturing of human epithelial cells on the scaffolds and MTT assay. The observed good cells attachment and proliferation indicated a good biocompatibility of the amphiphilic PDMS-b-PAA scaffolds which could be a promising biomaterial for tissue engineering and biomedical applications.

Key words: electrospinning, block copolymers, PDMS-b-PAA, epithelial cells, biocompatibility
THERMAL DIELECTROSCOPY STUDY OF UNDER-MEMBRANE ERYTHROCYTE SKELETON. DATA PROCESSING AND PRESENTATION

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Plenty of serious diseases are associated with worsening of rheological properties of human blood, which largely depend on the deformability and elasticity of erythrocytes. In this regard most important are the erythrocyte plasma membrane and its under-membrane skeleton of peripheral proteins, predominantly spectrin and actin. Important data on the molecular mobility of erythrocyte under-membrane skeleton and its attachment to major integral proteins can be obtained studying high density erythrocyte suspensions by the method of dielectroscopy, combined with thermal denaturation of spectrin. This thermal dielectroscopy method has revealed two dielectric relaxations on the under-membrane skeleton.

The first dielectric relaxation is detected at low frequencies (0.05 - 1.0 MHz) not allowing penetration of the field into cytosole. Its amplitude was strongly subdued after severance of the attachment sites between spectrin skeleton and integral proteins. This relaxation was assumed to involve a direct piezoeffect on flexible spectrin filaments.

The second dielectric relaxation is detected at higher frequencies (1.0 - 10 MHz) which allowed the field to penetrate through the lipid bilayer and interact directly with spectrin dipoles.

This communication explains a new variant for processing and presentation of obtained impedance data. As previously described, the erythrocyte suspension was heated and the sigmoid change in its complex impedance, $\Delta Z^* = \Delta Z_{re} + j\Delta Z_{im}$, at the spectrin denaturation temperature of 49.5°C was determined at 16 frequencies from 0.04 to 10 MHz. The low and high frequency borders were preordained by the polarization of electrodes and methodological limitations, respectively. Next, the dependencies of $\Delta Z_{re}$ and $\Delta Z_{im}$ on frequency were plotted. The obtained curves leveled off at both their endings allowing the extrapolation of data to 0.01 MHz and 20 MHz. Finally, the complex plane (Nyquist) plot of $-\Delta Z_{im}$ against $\Delta Z_{re}$ and the frequency (Bode) plot of $|Z| = (\Delta Z_{re}^2 + \Delta Z_{im}^2)^{1/2}$ were obtained. The two dielectric relaxations were well distinguished on Nyquist and Bode plots. The result obtained indicates that $\Delta Z^*$ represents a separate complex impedance, inherent to the spectrin skeleton of human erythrocytes. The frequency dependence of this complex quantity is highly informative for the dynamic state of spectrin skeleton and erythrocyte plasma membrane.

Key words: erythrocyte membrane; spectrin skeleton attachment; dielectric impedance spectroscopy; impedance data processing
A LOW-COST DIFFERENTIAL THERMAL ANALYSIS (DTA) APPARATUS FOR MEASURING THE THERMAL PROPERTIES AND BEHAVIOR OF PROTEIN AND CARBOHYDRATE-BASED HYDROGELS

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Since the first laboratory usage of commercial differential thermal analysis (DTA) apparatus more than half century ago the DTA analyzers evolved into multifunctional systems providing various thermal analysis that can cost up to hundreds thousands dollars. Here is proposed a device for differential thermal analysis that can cost a fraction of the price of the commercial ones with intended implementation and requirements for the field of hydrogel biomaterials research.

**Purpose:** A low cost model of DTA device for research and teaching purposes is described, and its utility is demonstrated by measurements on protein and carbohydrate-based hydrogels such as gelatin, pectin, alginate.

**Methods:** The built system is comprised to several parts such as heating and heat control module, sample holder module, computer Graphic User Interface (GUI) and hardware interface module. Samples of gelatin, pectin and alginate hydrogels were prepared with various ratio of water content and analyzed with the built set up.

The results of calibration and testing of thermal behavior of protein and carbohydrate-based hydrogels show the usefulness of such device in precision measurements needed for determination of processes such as protein denaturation, water loss etc. in matrices with different compositions and compounds ratios.

In conclusion this DTA setup has sufficient sensitivity to be used for the proposed research purposes. Also this device is more affordable compared to commercial instruments, which makes the DTA measurements accessible to more laboratories in the developing countries working in the field of biomaterials and pharmaceutical research.

**Key words:** biomaterial, DTA, hydrogel, scaffold, tissue engineering.
“PLANT HEALTH” ESTIMATION USING PROMPT CHLOROPHYLL a FLUORESCENCE IMAGING IN LEAVES OF TWO VARIETIES OF BEAN PLANTS

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Photosynthetic apparatus (PSA) of plants transform sunlight energy into chemical energy stored in carbohydrates. Plants utilize part of the absorbed by chlorophyll a molecules energy for photochemical work but other part they don’t use, and it can be emitted as fluorescence. The intensity of the emitted light is in reciprocal proportion to photochemical activity of plants. Physiological state of the photosynthetic apparatus, as well as of the whole plant, can be assessed by analyzing the fluorescence.

In this work we have investigated effects of four herbicides-modifiers of photosynthetic process analyzing photoinduced dynamics in fluorescence images of primary leaves of two bean varieties: Cheren Starozagorski and Egyptian Alubia beans.

Imaging PAM fluorometer allows observing images of a whole leaf. Spatial heterogeneity of the photochemical activity in different areas of the leaf disc and the distribution of herbicides are visualized by fluorescence images.

Different parameters characterizing photochemical and non-photochemical quenching of fluorescence and the real quantum yield of PSA were calculated for each point of the leaf surface by analyzing photoinduced changes in chlorophyll fluorescence during the transition from dark adapted state to light adapted state.

The fluorescence images allow visualization and analysis of the herbicide distribution in the leaf, of the differences in their effectiveness, as well as of the differences in the plant response of the two investigated varieties beans.

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Key words: chlorophyll a fluorescence, bean plants, photosynthetic herbicides, fluorescence imaging
VISUALIZATION OF PASSIVE AND ELECTRO-ASSISTED DELIVERY OF QUANTUM DOT-LABELED NANOPARTICLES IN VITRO AND IN VIVO USING FLUORESCENT AND MAGNETIC RESONANCE IMAGING

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The growing number of studies in the field of biomedical applications suggests that theranostic strategy becomes an important element of cancer treatment as it helps to develop the anticancer therapeutics based on combination of imaging and therapy. The selective disposition of nano-carriers into the target cells and tissues is an essential issue in drug delivery. In this context, hydrophilic and biocompatible nano-hydrogels are appropriate matrices for development of multimodal and multifunctional theranostic substances.

The present study describes a development of nanohydrogel, loaded with QD705 and manganese (QD705@Nanogel & QD705@Mn@Nanogel), and its passive and electro-assisted delivery in Colon 26 cancer cells and in solid tumours, visualized by fluorescence imaging and magnetic resonance imaging (MRI) on colon-cancer grafted mice as a model.

QD705@Nanogel was delivered passively predominantly into the tumor cells, which was visualized both in vitro, in vivo and ex vivo using fluorescent imaging. The applied electric field benefits the internalization of the nanosomes without significant cells viability reduction. The fluorescence intensity in the tumor area was about 2.5 times higher than the background fluorescence. A very weak fluorescent signal was detected in the liver area, but not in the areas of kidneys or bladder. We found that the embedding of a hard material (as QD) in nanohydrogel changes the physical properties of the soft material. QDs decreased the size and negative charge and changed the shape of nanohydrogel, which altered its pharmacodynamics.
Electroporation facilitated the delivery of the nanohydrogel in the tumor tissue, visualized by fluorescent imaging and MRI. Strong signal intensity was recorded in the tumor area shortly after the combined treatment (QD@Mn@Nanogel + electroporation) and it was observed even 48 hours after the electroporation. The data demonstrate more effective penetration of the nanoparticles in the tumor due to the increased permeability of blood vessels at the electroporated area. There was no rupture of blood vessels after electroporation and there were no artifacts in the images due to a bleeding.

Acknowledgment: Project 133/12.05.2016 - Program for career development of young scientists, BAS for S.A. and B.N.

Key words: quantum dots; nanohydrogel; cancer; electroporation; fluorescence imaging; magnetic resonance imaging
CHANGES IN THE STATE OF THE PLASMA PROTEOME AND CEREBROSPINAL FLUID IN GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is the most common and aggressive primary tumor with an extremely poor prognosis in spite of multimodal treatment approaches. Statistical summaries point out that patients with GBM have a post operation life expectancy of about 8 months.

Differential scanning calorimetry (DSC) is a new, recently introduced method for diagnosing and monitoring various pathologies, as well as for gathering information regarding their molecular mechanisms. DSC is a highly sensitive technique, measuring temperature-induced protein conformation changes. DSC is capable of detecting variations in concentration, conformation and the interaction of proteins with other molecules, and allows to observe changes in the plasma proteome related to pathological processes. In this way, plasma proteins can serve as biomarkers for diagnosing and monitoring of diseases.

Blood plasma and cerebrospinal fluid (CSF) of nine patients (2 cases diagnosed as high-grade gliomas - WHO grade III, and 7 as Glioblastoma multiforme - WHO grade IV), have been analyzed using DSC. The blood plasma thermograms of these patients have been compared with those of healthy individuals. The results point to significant deviations in the denaturation profiles of the plasma proteome of patients with GBM as compared to those of healthy individuals. The registered shifts in the denaturation temperatures reflect significant alterations in the state of the plasma proteins. At least three major plasma protein components (albumin, immunoglobulins and transferrin denaturing at ~ 62°C, ~70°C and ~ 82°C, respectively) are affected by the disease, while fibrinogen, which denaturates at ~ 53°C, remains unaffected.

The denaturation profiles of CSF of patients with GBM were found to differ significantly from those of healthy individuals. Some thermograms of patients with GBM displayed marked exothermic shifts at low temperatures (20°C - 50°C), prior to the protein denaturation.

The differences found between the plasma and CSF denaturational profiles of the individual patients indicate that the denaturation profiles of patients with GBM also contain information pertaining to the gravity of the pathology.

In summary, the DSC approach used in these experiments displayed a significant potential for clinical applications. It is suitable for rapid diagnosing and monitoring, requires a small quantity of blood plasma and CSF and is noninvasive for the patient.

Key words: Differential scanning calorimetry, Glioblastoma multiforme
OPTIMIZATION OF POLYELECTROLYTE MULTILAYER COATINGS FOR BIOFUNCTIONALIZATION OF CARDIOVASCULAR STENTS BY INCORPORATION OF GRAPHENE OXIDE

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The use of polyelectrolyte multilayers (PEMs) fabricated by layer-by-layer (LbL) self-assembly of polycations and polyanions has emerged as a powerful and versatile strategy to engineer bioactive films for biofunctionalization of implantable biomedical devices and drug delivery. A wide variety of polyelectrolytes ranging from designed synthetic polymers to naturally derived biopolymers may be employed for film build-up and the resulting biomimetic PEM films are of special interest for coating medical implants.

The main challenge is the construction of coatings with composition, thickness, and physicochemical properties that can be varied or tailored precisely on nanometer and micrometer scales to meet different medical requirements.

This study addresses the optimization of both the physicochemical properties and the surface biocompatibility of polyelectrolyte multilayers built from the natural, biodegradable, linear polysaccharides hyaluronan (HA) and chitosan (Chi) by incorporation of graphene oxide (GO) sheets into the polymer matrix. Owing to its remarkable electrical, thermal and mechanical properties GO (the most important derivative of graphene) has rapidly become one of the most widely studied materials in the last years. GO is a novel nanomaterial with two-dimensional structure (single sheets of carbon atoms packed in a perfect honeycomb structure) and a very large surface area, which has been considered as excellent nanoscale filler for improving the barrier properties of polymer films.

We constructed diverse hybrid HA/Chi/GO multilayers with different number and position of GO-layers inserted in the polymeric matrix. We applied ellipsometry, quartz crystal microbalance (QCM), atomic force microscopy (AFM), Raman spectroscopy and contact angle technique, and demonstrated that GO sheets act as a strong polyelectrolyte and due to their negative charge adsorb successfully on positively charged Chi-layers. The insertion of GO into the polymer matrix influences the growth, thickness, morphology, macro- and micro-roughness, stiffness, and hydrophilicity of HA/Chi films and thus acts as a key to modulate the PEMs interaction with biomolecules and cells.

Key words: layer-by-layer assembly, polyelectrolyte multilayers, hyaluronan, chitosan, Hofmeister anions, biocompatibility
The aim of the study was to combine *in-vivo* and *in vitro* biophysical methods for registering potential changes in the diameter of the brain’s superficial blood vessels and the contractility of isolated blood vessels taken from rats, exposed to artificially increased intra abdominal pressure. The elevated intra abdominal pressure produces a decrease of perfusion pressure in all organs and systems thus affecting the systems hemodynamics. The acute form of abdominal hypertension (AH) has a critical effect in compartments which do not have a sufficient elastic compliance and thus they are the main target for hypoxic changes. Modified “open skull” method was used in order to detect probable in vivo changes in small brain vessels in rats with AH. For this purpose was constructed specially designed equipment: a non-traumatic head fixator and a surgical stent allowing immobilization of the animal after the air inflation; the standard system of perfusion was combined with ventilation one aiming to ventilate the work field with cooled air so that the temperature remains low enough to avoid tissues coagulation. The photographs were made with and without a cranial window (covering glass fixed by cement Adhesor) - allowing possibility to observe the effects during an open and closed compartment. The contractile activity and reactivity of isolated blood vessels was registered isometrically in tissue baths washed with Krebs solution. The expected changes in the contractility were tested with the exogenous Norepinephrine and 5-TH. Using both of the above said methods provides: lower traumatic levels in the investigated area, diminishing of coagulation changes, detection of changes in vessel diameters and differ it in opened and closed skull compartment, as well as the changes in the contractile activity of the vessel’s smooth muscles responsible for blood supply of the investigated area. Technological drawbacks of the methods are: due to the animal’s breathing movements after the inflation of the abdominal cavity the observation field moves too; impaired optical sharpness of the image when using a cranial window, which need to be tempered; as well as the impossibility to differentiate the effects of all modulating the contractility mediators referred to the *in vitro* part of the experiments.

**Key words:** abdominal hypertension, rat, haemodynamics.
ORDERING OF THE NUMEROUS CYTOCHROME bc₁ X-RAY CRYSTAL STRUCTURES IN A SEQUENCE OF EVENTS DURING SUBSTRATE PROCESSING IN THE Qo SITE OF THE COMPLEX

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The cytochrome bc₁ complex (cyt bc₁, complex III) of mitochondria and bacteria is essential for the respiratory energy transduction system. It is also the main target for antibacterial drugs and for drugs against mitochondrial diseases. Understanding the exact sequence of events at atomic resolution during substrate processing in this complex is believed to provide insight not only into processes of aging, mitochondrial diseases, apoptosis, etc, but also for drug discovery and therapy. I will present an original approach for revealing the dynamic structural changes in the cyt bc₁ during turnover at the Qo-site. It is based on the width of the identified-using-Chlorophyll a lateral gate within the Qo-site of the photosynthetic cyt b₆f (analysed 9 crystal structures from the Protein Data Bank) because of the high sequence conservation of the Qo-site in both cyt bc₁ and cyt b₆f. The gate-width is the distance between a residue from the cd-helix and a residue from the ef-loop of the cyt b subunit in the cyt bc₁. By using this gate-width as an ordering parameter, the great variety of cyt bc₁ X-ray crystal structures from various sources and with the binding of diverse inhibitors (a total of 47 structures from PDB) were perfectly ordered without mixing the structures that are bound to different types of Qo-site inhibitor. This is the first identified gate between a pair of cyt b residues that possesses inhibitor sensitivity: The stigmatellin-like inhibitors produce maximal gate widening, followed by the famoxadone-like inhibitors. The gate-width further decreased in structures with MOA-type inhibitors or triazolone, and minimal width is observed in the empty Qo-site structures. The gate-width also depended on the Qo-site occupant position and the closeness of the [Fe2-S2] cluster to the membrane plane, and correlated with the effect of the different types of Qo-site inhibitors on the dynamics of the Rieske soluble domain movement. Our understanding of the dynamic structural changes in cyt bc₁ during turnover at the Qo-site is augmented by the time-sequence ordering of a total of 47 atomic resolution cyt bc₁ crystal structures during this process.

This work was supported by the Bulgarian Academy of Sciences.

Key words: biomolecular dynamics; X-ray crystal structures of cytochrome bc-complexes; ubiquinol oxidation; redox sensor gate; chlorophyll a
EFFECTS OF SYNTHETIC NEUROPEPTIDES (NEUROTENSINS) ON DRUG-INDUCED NEURODEGENERATIVE DISORDERS

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Introduction: Neurotensin (NT) is a neuropeptide and putative neurotransmitter expected to hold up the development of some neurodegenerative diseases (NDD). Differential scanning calorimetry (DSC) is a method for thermal analysis used to characterize the stability of the native conformations of biological macromolecules. Isothermal titration calorimetry (ITC) is used to characterize the interactions between proteins and their ligands, and to determine the thermodynamic parameters of these interactions. In this work, the thermodynamic properties of brain supernatants from rodents with drug-induced neurodegenerative disorders (dementia and Parkinson’s disease) were examined using DSC and ITC.

Aim: To evaluate the impact of NT on animals with scopolamine-induced dementia and 6-hydroxydopamine-induced Parkinson’s disease (PD) using DSC and ITC.

Methods: Experimental models of scopolamine-induced dementia in male Albino mice (scopolamine 1 mg/kg, i.p., 11 days) and PD, induced in male Wi-star rats via 6-OHDA i.c. injection, were verified by cognitive and biochemical tests. NT and its modifications NT2 and NT4 were applied i.p. for 11 days simultaneously with scopolamine. Animals were treated with NT4 i.p. for 5 days in dose 5 mg/kg before induction of PD. DSC and ITC measurements were performed on brain tissue supernatants isolated from healthy (controls) and treated with drugs animals.

Results: The DSC measurements revealed significant differences between the brain proteome denaturational profiles of healthy animals and animals with drug-induced neurodegenerative disorders. They also demonstrate the recovery effect of NT4 on drug-induced NDD. The ITC measurements show high binding affinity of NT4 to human serum albumin (HSA) and suggest that HSA is a NT carrier in the blood stream.
Conclusion: Neurotensins have clearly expressed recovery effect on drug-induced neurodegenerative diseases, as demonstrated by the DSC and ITC. These methods provide information on the disease mechanisms at molecular level. They are appropriate for detection and characterization of compositional changes taking place in affected by NDD brain tissues and can be helpful in the future for further studies of these diseases.

Key words: DSC, ITC, Neurotensin, Parkinson’s disease, Alzheimer’s disease
CORTICOSTEROID INTERACTIONS WITH HUMAN SERUM ALBUMIN

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Introduction: Characterization of the thermodynamics of drug binding with blood plasma (BP) proteins is of essential importance for a better understanding of drug absorption, distribution and turnover in the circulation. Human serum albumin (HSA), the most prominent protein in plasma, plays a fundamental role in the transport of drugs.

Synthetic corticosteroids are a group of drugs used for treatment of various diseases, including lupus, inflammation, cerebral edema, etc. In the present study we investigated the interactions of betamethasone, methylprednisolone and dexamethasone with HSA.

To characterize the energetics of corticosteroid binding to HSA, we used Isothermal Titration Calorimetry (ITC) and Differential Scanning Calorimetry (DSC). DSC is a sensitive method for characterization of the stability and denaturational profiles of biological macromolecules in-solution. The ITC provides high sensitivity and flexibility in studies of protein-ligand binding, including the binding of drugs to their targets.

Aim: To characterize the mechanism of synthetic corticosteroid interactions with HSA.

Materials and Methods: The thermal effects of betamethasone, methylprednisolone and dexamethasone binding to HSA (Sigma Aldrich) was examined by ITC (Nano ITC, TA Instruments). ITC measurements were conducted in PBS (pH 7.4). The 1 ml sample cell was filled with 38 μM HSA. In the first experiment we loaded the 250 μl injection syringe with 0.5 mM solutions of dexamethasone, in the second – with methylprednisolone and, lastly, with betamethasone. The drug solutions were injected into the ITC cell in 10 μl increments in a total of 25 injections with 600 s intervals. The titration process was computer-controlled. The stirring speed was set at 250 rpm and the cell temperature was kept at 37 °C. The experimental results were processed using the calorimeter software.

Immediately after the ITC measurements the samples were degassed and loaded into the measuring cell of a Nano DSC (Nano DSC, TA Instruments), equipped with 300 μl measuring cells. PBS (pH 7.4) was used for the reference
cell. Two subsequent scans were performed at a scanning rate of 1°C/min in the range from 20 °C to 110°C, under a pressure of 3 atm.

**Results:** The ITC measurements demonstrated high binding affinity of the three glucocorticoids to HSA, as evidenced by the exothermic thermal effects. The thermograms of blood plasma recorded by DSC showed that betamethasone and methylprednisolone have higher binding affinity to HSA in comparison to dexamethasone. To see if there is binding between the glucocorticosteroids and other BP proteins, we also studied the interaction of methylprednisolone with the \(\gamma\)-globulin BP fraction. The results showed no interaction of the \(\gamma\)-globulins with methylprednisolone.

**Conclusions:** The present study demonstrates high binding affinity of synthetic glucocorticosteroids to HSA and helps to better understand the binding mechanism.

**Key words:** ITC, DSC, albumin, corticosteroids
CYTOGENETIC RADIOSENSITIVITY OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES TO PROTONS AND GAMMA RAYS

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The aim of this study was to compare sensitivity of human peripheral blood lymphocytes (PBL) to gamma radiation and protons radiation in the G0 and G2 phases of the cell cycle. Chromosome aberrations were analyzed in PBL of healthy volunteers by the classical metaphase assay and by premature chromosome condensation analysis (PCC). The samples from 7 donors were irradiated with Go⁶₀ gamma rays or protons in the Bragg peak region E ≈ 0-30 MeV and LET ≈ 0.5-100 keV/μm (initial E=170 MeV). The exposed whole blood samples (G0- lymphocytes) were used for cultures set up. Cells were harvested after 48 h following 3h of colcemid block and 1.5 h of calyculin A treatment. For G2-exposure whole blood cultures prepared from the same blood samples were irradiated after 72 h of incubation. For G2 cells PCC analysis calyculin A was added immediately after exposure for 1.5 h. Slides were stained by the FPG technique. Chromosome aberrations in normal M cells and fragments in PCC cells were analyzed in MI cells - aberrations were scored in cells with homogenously dark chromosomes showing no signs of replication bands.

The results show a high correlation between the G2-sensitivity to gamma rays and protons assessed in normal M cells, whereas no correlation was found in PCC cells. No correlation was observed between G2 and G0 sensitivity for the both gamma rays and protons. It can be concluded that the relative G2 sensitivity to gamma rays correlates with the G2 sensitivity to protons and the radiosensitivity of G2 phase differs from G0 sensitivity both for gamma rays and protons.

Key words: peripheral blood lymphocytes; radiosensitivity; gamma rays; protons; chromosome aberrations
THIRD NATIONAL PATIENT DOSE SURVEY IN DIAGNOSTIC RADIOLGY FOR ESTABLISHING OF NEW NATIONAL DRLS IN BULGARIA, FIRST RESULTS

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National Centre of Radiobiology and Radiation Protection, Sofia, Bulgaria

The Third National Patient Dose Survey in diagnostic radiology in Bulgaria started in June 2016. Its goal is to update existing National Diagnostic Reference Levels (DRLs) and to establish DRLs for projections and examinations not included in the National Patient Dose Surveys so far.

All relevant forms as well as instructions for patient dose data collection are available on the website of the National Centre of Radiobiology and Radiation Protection (NCRRP) at: http://www.ncrrp.org/new/bg/DRL2016-c437. Patient dose information is being submitted to NCRRP via any of following ways: by e-mail; via special internet based software platform; by post. A special software database is developed on MS Access to facilitate the analysis of data in addition to internet based software platform. Third quartile value of typical patient doses is used to derive DRL for each examination. The typical dose of each facility is calculated as a mean value of patient doses for standard sized adults and as a median value of patient doses for children, as children are distributed in following age groups: 0-1 months; 1-12 months; 1-5 years; 5-10 years; 10-15 years.

Results for typical patient doses acquired by the survey so far as well as first results for National DRLs (NDRLs) are presented and discussed.

Conclusions based on experience accumulated and hints for future work are communicated.

Key words: DRLs, NDRLs, National Survey, Typical dose, paediatric dose, database, data analysis.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abarova S.</td>
<td>79,87,105, 103</td>
</tr>
<tr>
<td>Achilles Gerou-Christov,</td>
<td>91</td>
</tr>
<tr>
<td>Al Sharif M.</td>
<td>81</td>
</tr>
<tr>
<td>Alexandrov S. A.</td>
<td>80</td>
</tr>
<tr>
<td>Alov P.</td>
<td>81</td>
</tr>
<tr>
<td>Andreeva T.</td>
<td>100</td>
</tr>
<tr>
<td>Andronescu E. A.</td>
<td>42</td>
</tr>
<tr>
<td>Angelova P.</td>
<td>45</td>
</tr>
<tr>
<td>Antonov I.</td>
<td>91</td>
</tr>
<tr>
<td>Antonova B.</td>
<td>99</td>
</tr>
<tr>
<td>Aoki I.</td>
<td>97</td>
</tr>
<tr>
<td>Apostol A.I.</td>
<td>42</td>
</tr>
<tr>
<td>Arabadzhiev T.I.</td>
<td>85</td>
</tr>
<tr>
<td>Ardasheva R.</td>
<td>101</td>
</tr>
<tr>
<td>Arsova P.</td>
<td>22</td>
</tr>
<tr>
<td>Atanasov N.</td>
<td>60</td>
</tr>
<tr>
<td>Atanasov N.</td>
<td>91</td>
</tr>
<tr>
<td>Atanasova G.</td>
<td>60</td>
</tr>
<tr>
<td>Atanasova G.</td>
<td>91</td>
</tr>
<tr>
<td>Avramova-Cholakova S.</td>
<td>21, 53, 54</td>
</tr>
<tr>
<td>Bakalova R.</td>
<td>97</td>
</tr>
<tr>
<td>Bangyozova M.</td>
<td>82</td>
</tr>
<tr>
<td>Bliznakov Z.</td>
<td>36</td>
</tr>
<tr>
<td>Bliznakova K.</td>
<td>36</td>
</tr>
<tr>
<td>Bortolan G.</td>
<td>25</td>
</tr>
<tr>
<td>Buchacliev Z.</td>
<td>22</td>
</tr>
<tr>
<td>Buliev I.</td>
<td>36</td>
</tr>
<tr>
<td>Chakalov I.</td>
<td>84</td>
</tr>
<tr>
<td>Chaushev B.</td>
<td>71</td>
</tr>
<tr>
<td>Christov I.</td>
<td>25</td>
</tr>
<tr>
<td>Christova E.</td>
<td>82</td>
</tr>
<tr>
<td>Dankov K.</td>
<td>96</td>
</tr>
<tr>
<td>Deperas-Kaminska M.</td>
<td>107</td>
</tr>
<tr>
<td>Dimcheva M.</td>
<td>35</td>
</tr>
<tr>
<td>Dimitrov A. G.</td>
<td>46, 85</td>
</tr>
<tr>
<td>Dimitrov V.G.</td>
<td>85</td>
</tr>
<tr>
<td>Dimitrova N.A.</td>
<td>85</td>
</tr>
<tr>
<td>Dimitrova St.</td>
<td>96</td>
</tr>
<tr>
<td>Dimov A.</td>
<td>29, 69, 86, 108</td>
</tr>
<tr>
<td>Djenev I.</td>
<td>86</td>
</tr>
<tr>
<td>Doltchinkova V.</td>
<td>45</td>
</tr>
<tr>
<td>Dyakov I.</td>
<td>53, 54</td>
</tr>
<tr>
<td>Dzambazova A.</td>
<td>27, 87</td>
</tr>
<tr>
<td>Exerova D. R.</td>
<td>80</td>
</tr>
<tr>
<td>Gaevsky V.</td>
<td>107</td>
</tr>
<tr>
<td>Gancheva M.</td>
<td>70</td>
</tr>
<tr>
<td>Georgiev E.</td>
<td>62</td>
</tr>
<tr>
<td>Georgieva S.</td>
<td>25</td>
</tr>
<tr>
<td>Gesheva–Atanasova N.</td>
<td>56, 70</td>
</tr>
<tr>
<td>Goltsev V.</td>
<td>96</td>
</tr>
<tr>
<td>Gruev I.</td>
<td>25</td>
</tr>
<tr>
<td>Grumezescu M.</td>
<td>42</td>
</tr>
<tr>
<td>Hadjiolova R.</td>
<td>27, 91</td>
</tr>
<tr>
<td>Hadzhiyska V.</td>
<td>55</td>
</tr>
<tr>
<td>Ilieva D.</td>
<td>89</td>
</tr>
<tr>
<td>Israel M.</td>
<td>61</td>
</tr>
<tr>
<td>Ivanov I. T.</td>
<td>94</td>
</tr>
<tr>
<td>Ivanov L.</td>
<td>72, 74, 75</td>
</tr>
<tr>
<td>Ivanov V.</td>
<td>62</td>
</tr>
<tr>
<td>Ivanova D.</td>
<td>37</td>
</tr>
<tr>
<td>Ivanova N.</td>
<td>71</td>
</tr>
<tr>
<td>Ivanova P.</td>
<td>61, 84</td>
</tr>
<tr>
<td>Ivanova S.</td>
<td>71</td>
</tr>
<tr>
<td>Johnson L.</td>
<td>34, 52</td>
</tr>
<tr>
<td>Name</td>
<td>Pages</td>
</tr>
<tr>
<td>---------------</td>
<td>------</td>
</tr>
<tr>
<td>Tancheva L.</td>
<td>79,103</td>
</tr>
<tr>
<td>Taneva S.</td>
<td>100</td>
</tr>
<tr>
<td>Tashev R.</td>
<td>101</td>
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<tr>
<td>Tashev R.</td>
<td>87</td>
</tr>
<tr>
<td>Tenchov B.</td>
<td>41,79,99,103,105</td>
</tr>
<tr>
<td>Todorov R. K.</td>
<td>80</td>
</tr>
<tr>
<td>Topalova Iv.</td>
<td>76</td>
</tr>
<tr>
<td>Traikov L.</td>
<td>27,60,79,84,87,91</td>
</tr>
<tr>
<td>Trifonov A.</td>
<td>47</td>
</tr>
<tr>
<td>Trindev P.</td>
<td>20,35</td>
</tr>
<tr>
<td>Tsakovska I.</td>
<td>81</td>
</tr>
<tr>
<td>Tsanev I.</td>
<td>29,69</td>
</tr>
<tr>
<td>Tsanova A.</td>
<td>82</td>
</tr>
<tr>
<td>Tsapaki V.</td>
<td>33</td>
</tr>
<tr>
<td>Tsonov Ts.</td>
<td>96</td>
</tr>
<tr>
<td>Tsonova I.</td>
<td>97</td>
</tr>
<tr>
<td>Tsrunchev Ts.</td>
<td>22</td>
</tr>
<tr>
<td>Turiyki V.</td>
<td>101</td>
</tr>
<tr>
<td>Vasilev D.</td>
<td>27</td>
</tr>
<tr>
<td>Vasileva F.</td>
<td>55,69</td>
</tr>
<tr>
<td>Vassilev P.</td>
<td>101</td>
</tr>
<tr>
<td>Vassileva J.</td>
<td>22</td>
</tr>
<tr>
<td>Velchev V.</td>
<td>27</td>
</tr>
<tr>
<td>Velikova V.</td>
<td>96</td>
</tr>
<tr>
<td>Veselinova L.</td>
<td>60</td>
</tr>
<tr>
<td>Vladkova R.</td>
<td>102</td>
</tr>
<tr>
<td>Vladkova T.</td>
<td>93</td>
</tr>
<tr>
<td>Walke W.</td>
<td>26</td>
</tr>
<tr>
<td>Wieczorek J.</td>
<td>26</td>
</tr>
<tr>
<td>Wojcik A.</td>
<td>107</td>
</tr>
<tr>
<td>Wolf W.</td>
<td>90</td>
</tr>
<tr>
<td>Zagorska A.</td>
<td>22</td>
</tr>
<tr>
<td>Zaharino S.</td>
<td>103</td>
</tr>
<tr>
<td>Zasheva A.</td>
<td>105</td>
</tr>
<tr>
<td>Zaytseva E.</td>
<td>107</td>
</tr>
<tr>
<td>Zhelev Zh.</td>
<td>97</td>
</tr>
<tr>
<td>Zubareva N.</td>
<td>30</td>
</tr>
</tbody>
</table>
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