Russian-Chinese Workshop on Biophotonics and Biomedical Optics

*September 26-28, 2012*

*Saratov State University Saratov, Russia*

**Organized by**

International Research-Educational Center of Optical Technologies for Industry and Medicine “Photonics”, Saratov State University, Russia

&

Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, P.R. China

**Sponsored by**

Russian Foundation for Basic Research

&

National Natural Science Foundation of P.R. China
Russian-Chinese Workshop on Biophotonics and Biomedical Optics

Organized by

Saratov State University named after N.G. Chernyshevsky, Russia
Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, P.R. China
Biomedical Photonics Committee of Chinese Optical Society
International Research-Educational Center of Optical Technologies for Industry and Medicine
“Photonics” at Saratov State University, Russia
Research-Educational Institute of Optics and Biophotonics at Saratov State University, Russia
Institute of Precision Mechanics and Control, Russian Academy of Sciences, Russia
Volga Region Center of New Information Technologies, Russia
Saratov State Medical University, Russia
SPIE Student Chapter at SSU
OSA Student Chapter at SSU

In cooperation with

Academy of Natural Sciences, Saratov Regional Division
Russian Society for Photobiology
Saratov Science Center of the Russian Academy of Sciences
Sponsored by

Russian Foundation for Basic Research
National Natural Science Foundation of P.R. China
SpectrAcoustic LLC

Russian Academy of Sciences
SPIE - The International Society of Photo-Optical Instrumentation Engineers
SPE “Nanostructed Glass Technology” Ltd., Saratov
SSU Site Map
Workshop Co-chairs

Valery V. Tuchin, Saratov State University, Russia

Qingming Luo, Huazhong University of Science and Technology, P.R. China

Secretaries

Dan Zhu, Huazhong University of Science and Technology, China

Natalia A. Trunina, Saratov State University, Russia
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<thead>
<tr>
<th>Name</th>
<th>Institution</th>
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<tbody>
<tr>
<td>Elina A. Genina</td>
<td>Saratov State University, Russia</td>
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<td>Alexey N. Bashkatov</td>
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<td>Natalia A. Trunina</td>
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<td>Irina Yu. Yanina</td>
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<td>Elena K. Volkova</td>
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<td>Maxim A. Vilensky</td>
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<td>Iliya V. Smirnov</td>
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<td>Dmitry N. Agafonov</td>
<td>Saratov State University</td>
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<td>Daria K. Tuchina</td>
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<td>Anton A. Grebenyuk</td>
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<td>Yana V. Tarakanchikova</td>
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<td>Sergey A. Savonin</td>
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<td>Ekaterina A. Kolesnikova</td>
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Welcome

Dear Participants,

We are delighted to welcome you to the 5th Russian-Chinese Workshop on Biophotonics and Biomedical Optics-2012.

We are pleased and honoured to host the Russian-Chinese Workshop on Biophotonics and Biomedical Optics-2012 and hope that you will have an exciting and inspiring time. The goal of the Workshop is to encourage and bring together both Russian and Chinese scientists, engineers and clinical researchers from variety of disciplines engaged in applying optical science, photonics and imaging technologies to problems in biology and medicine. The scope of this bilateral workshop ranges from basic research to instrumentation engineering, to biological and clinical studies. Browsing through this book of abstracts, you will find an impressive body of work in the talks and poster sessions.

Actually, Russian-Chinese/Chinese-Russian Workshops on Biophotonics and Biomedical Optics has been hosted in Saratov, Russia and in Wuhan, P.R. China. The Workshops achieved a big success with comprehensive and professional lectures from Chinese and Russian scientists. Some feedbacks from the attendees and invited experts confirmed they have learnt a lot with fundamental knowledge, frontier techniques and current applications. After that, some Chinese-Russian cooperation research projects were approved by NSFC and RFRB, more and more papers from the cooperation have been published.

Science in general and a workshop in particular is all about communicating and exchanging ideas. In this spirit of scientific exchange, we would like to thank you in advance for you active contribution to making the Workshop a success. We hope that while you are experiencing the exciting atmosphere of this event, you will gain new insights and learn about the fascinating new developments in Biophotonics and Biomedical Optics.

With best wishes from the organizers,

Prof. Valery V. Tuchin
Russian Co-Chair of the Organizing Committee

Prof. Qingming Luo
Chinese Co-Chair of the Organizing Committee
# Program and schedule of events
## 26-28 September, 2012, Saratov, Russia

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<tr>
<td><strong>9.30-21.30</strong></td>
<td>Registration, OSA Short Course, SFM-12 sessions, welcome party</td>
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<td><strong>Building 10, SSU campus</strong></td>
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<tr>
<th>September 26, Wednesday</th>
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| **9.00-9.10** | Opening of the Workshop  
**Valery Tuchin**, Saratov State University, Russia and  
**Lin Lin**, Huazhong University of Science and Technology, P.R. China |
| **Building 10 Main Conference Hall** |

## PLENARY SESSION
**Chairs:**  
Alexander V. Priezzhev  
Moscow State University, Moscow, Russia  
**Hui Ma**  
Tsinghua University, Shenzhen, P.R. China

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>9.10-9.45</td>
<td>Progress in optical clearing of tissue <em>in vivo</em></td>
<td><strong>Dan Zhu</strong>, Huazhong University of Science and Technology, Wuhan, Hubei, P.R. China</td>
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<tr>
<td>9.45-10.20</td>
<td>Fluorescence molecular bioimaging in drug design and screening</td>
<td><strong>Alexander P. Savitsky</strong>, A.N. Bach Institute of Biochemistry, Moscow, Russia</td>
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<tr>
<td>10.20-10.40</td>
<td>Coffee break</td>
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## INVITED LECTURE SESSION I
**Chairs:**  
**Da Xing**  
South China Normal University, Guangzhou, P.R. China  
**Alexander P. Savitsky**  
A.N. Bach Institute of Biochemistry, Moscow, Russia

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<th>Time</th>
<th>Topic</th>
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<tr>
<td>10.40-11.00</td>
<td>Real-time monitoring of rare circulating liver cancer cells in an orthotopic model by <em>in vivo</em> flow cytometry assesses resection on metastasis</td>
<td><strong>Xunbin Wei</strong>, Shanghai Jiaotong University, Shanghai, P.R. China</td>
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<tr>
<td>11.00-11.20</td>
<td>Laser nanotechnologies for diagnosis and therapy of cancer and infections</td>
<td><strong>Valery Tuchin</strong>, Saratov State University, Saratov, Russia</td>
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<tr>
<td>11.20-11.40</td>
<td>Peptide-based nanoparticles for the targeted delivery of cancer diagnostic and therapeutic agents</td>
<td><strong>Zhihong Zhang</strong>, Huazhong University of Science and Technology, Wuhan, Hubei, P.R. China</td>
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<td>Time</td>
<td>Invited Lecture Session II</td>
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<td>11.40-12.00</td>
<td>In vivo multi-scale photoacoustic imaging for biomedical applications</td>
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<td></td>
<td>Da Xing, South China Normal University, Guangzhou, P.R. China</td>
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<tr>
<td>12.00-12.20</td>
<td>Optical assessment of biocompatibility and biosafety issues in interaction of nanoparticles with blood</td>
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<td>Alexander V. Priezzhev, Moscow State University, Moscow, Russia</td>
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<td>12.20-12.40</td>
<td>Photobiomodulation-mediated pathway diagnostics</td>
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<td>Timon Cheng-Yi Liu, South China Normal University, Guangzhou, P.R. China</td>
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<tr>
<td>12.40-13.00</td>
<td>Photo of Russian and Chinese Delegations together</td>
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<td>13.00-14.00</td>
<td>Lunch</td>
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<td>15.00-17.00</td>
<td>Social Event (Volga Boat Trip)</td>
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September 27, Thursday

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<th>Time</th>
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<tr>
<td>14.00-14.20</td>
<td>Polarization imaging: techniques, applications and the physics insights</td>
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<td>Hui Ma, Tsinghua University, Shenzhen, P.R. China</td>
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<tr>
<td>14.20-14.40</td>
<td>Spectroscopy analyses and detection of skin cancer</td>
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<td>Valery P. Zakharov, Samara State Aerospace University, Russia</td>
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<tr>
<td>14.40-15.00</td>
<td>Imaging-based quantification of glottic opening by fiber optical nasopharyngoscopy in healthy and asthmatic subjects</td>
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<td>Linhong Deng, Changzhou University, Changzhou, P.R. China</td>
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<td>15.00-15.20</td>
<td>Low-dimensional structures: sparse coding for neural activity</td>
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<td>Xin Tian, Tianjin Medical University, Tianjin, P.R. China</td>
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<td>15.20-15.40</td>
<td>Dynamic investigation of breast tumor response to the targeted therapy by using gold nanoparticle based molecular beacons</td>
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<td>Yueqing Gu, China Pharmaceutical University, P.R. China</td>
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<tr>
<th>Time</th>
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<tr>
<td>15.40-15.55</td>
<td>Microwave-induced thermoacoustic tomography for biomedical applications</td>
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Building 10 Main Conference Hall
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<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker(s)</th>
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<tbody>
<tr>
<td>15.55-16.10</td>
<td>Laser diffraction analysis of shear deformability of human red blood cells incubated with nanodiamonds</td>
<td>Andrei E. Lugovtsov, Moscow State University, Moscow, Russia</td>
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<td>16.10-16.25</td>
<td>Molecular imaging of small animals using combined system of fluorescence molecular imaging and micro-CT</td>
<td>Xiaoquan Yang, Huazhong University of Science and Technology, Wuhan, Hubei, P.R. China</td>
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<td>16.25-16.40</td>
<td>Application of confocal laser microscopy for mesh explants control</td>
<td>Ivan A. Bratchenko, Samara State Aerospace University, Russia</td>
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<td>16.40-17.10</td>
<td>Coffee break</td>
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<td>17.10-18.30</td>
<td>BBO PLENARY INTERNET SESSION</td>
<td>Valery Tuchin, Saratov State University, Saratov, Russia</td>
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<td>Chairs:</td>
<td>Dan Zhu, Huazhong University of Science and Technology, Wuhan, Hubei, P.R. China</td>
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<td>18.30-21.00</td>
<td>BBO POSTER SESSION AND INTERNET DISSUSION</td>
<td>Dmitry Agafonov, Ivan Fedosov, Saratov State University, Saratov, Russia</td>
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<td>Moderators:</td>
<td>Xiaoquan Yang, Huazhong University of Science and Technology, Wuhan, P.R. China</td>
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**September 28, Friday**

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<th>Session</th>
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<td>9.00-12.00</td>
<td>Round-table discussions, visiting of research and student training laboratories, and closing of the Workshop</td>
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<tr>
<td>12.00-14.00</td>
<td>Social program (Open-air-Museum)</td>
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<td>14.00-17.00</td>
<td>Social program (Barbecue)</td>
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Poster Session

Chair: Ivan V. Fedosov, Saratov State University, Saratov, Russia

September 27, Thursday
18.30-21.00

1. **The role of water in human tissue with certain compression: Studied with reflectance spectroscopy** Jingying Jiang, College of Precision Instrument and Optic Electronic Engineering, Tianjin University, Tianjin, P.R. China

2. **Second-harmonic generation imaging of elastic cartilage repair in a rabbit ear model** Xiaoqin Zhu, Fujian Normal University, Fujian, P.R. China

3. **Establishment of visible animal metastasis models for human nasopharyngeal carcinoma based on a far-red fluorescent protein** Min Chen, Hospital of Huazhong University of Science & Technology, Wuhan, P.R. China.

4. **Noise reduction methods for OCT images** Oleg O. Myakinin, D.V.Kornilin, I.A. Bratchenko, V.P. Zakharov, and A.G. Khramov, Samara State Aerospace University and Image Processing Systems Institute, Samara, Russia

5. **The non-linear dynamic study on epilepsy ictal EEG** Tao Huaying, Xin Tian, Lab of Neurophysiology, Tianjin Medical University General Hospital, Tianjin, P.R. China

6. **Microwave therapy via bronchoscopes for severe tracheal stenosis** Liu Wei Min, Dai Li, and Lin Yu Hui, Respiratory Department of Zhongnan Hospital, Wuhan University, Wuhan, P.R. China

7. **Evaluation of the red blood cells shape parameter variance from the data of laser ektacytometry** Sergey Yu. Nikitin, Maria A. Kormacheva, Alexander V. Priezzhev, and Andrey E. Lugovtsov, Lomonosov Moscow State University, Moscow, Russia

8. **In vitro OCT study of alterations of adipose tissue structure induced by PDT treatment** Irina Yu. Yanina, Natalia A. Trunina, and Valery V. Tuchin, Saratov State University, Saratov State Medical University, and Institute of Precise Mechanics and Control RAS, Russia; University of Oulu, Finland

9. **Skin optical clearing by glucose and quantification of glucose diffusivity at its impact on skin tissues** Daria K.Tuchina, Alexey N. Bashkatov, Elina A. Genina, Vyacheslav I. Kochubey, and Valery V. Tuchin, Saratov State University and Institute of Precise Mechanics and Control RAS, Russia; University of Oulu, Finland

10. **TiO2 and ZnO nanoparticles as disinfection compounds** Natalia A. Trunina, Alexey P. Popov, Jürgen Lademann, Valery V. Tuchin, Risto Myllylä, and Maxim E. Darwin, Saratov State University and Institute of Precise Mechanics and Control RAS, Russia; University of Oulu, Finland; Charité – Universitätsmedizin Berlin, Germany
11. **Relationship between the inflection point of anaerobic threshold and muscle oxygenation measured by NIRS during incremental exercises** Li Zhang, Wuhan Institute of Physical Education, P.R. China

12. **Reveal of using artificial sweetener in the process of natural juices and other drinks manufacturing** Anton Malinin, Anastasia Zanishevskaya, Julia Skibina, Valery Tuchin, and Igor Silokhin, Saratov State University, LLC SPE Nanostructured Glass Technology, and Institute of Precise Mechanics and Control RAS, Russia; University of Oulu, Finland

13. **Full field laser speckle contrast imaging technique application for visualization of rat's pancreas micro capillary blood flow** Polina A. Timoshina, Maxim A. Vilensky, Denis A. Alexandrov, Valery V. Tuchin, and Victor A. Kuleshov, Saratov State University, Saratov State Medical University, and Institute of Precise Mechanics and Control RAS, Russia; University of Oulu, Finland
Internet Session

1. **Multi-Modality Molecular Imaging for Nanomedicine and Cancer Research** Qiushi Ren, Department of Biomedical Engineering Scholar, College of Engineering, Peking University, P.R. China (Internet Plenary Lecture)

2. **Newly developed NIR contrast agents for cancer targeted imaging** Chunmeng Shi, Institute of Combined Injury, State Key Laboratory of Trauma, Burns and Combined Injury, Department of Preventive Medicine, Third Military Medical University, Chongqing, P.R. China (Internet Invited Lecture)

3. **Singlet oxygen dosimetry for photodynamic therapy** Buhong Li, Key Laboratory of OptoElectronic Science and Technology for Medicine of Ministry of Education, Fujian Provincial Key Laboratory for Photonics Technology, Fujian Normal University, Fuzhou, Fujian, P.R. China (Internet Invited Lecture)

4. **Investigation of red blood cells aggregation in plasma and in proteins solutions by optical trapping** Kisung Lee, A.V. Priezzhev, A.Yu. Maclygin, I.O. Obolenskii, M. Kinnunen, R. Myllylä, Lomonosov Moscow State University, Moscow, Russia; University of Oulu, Oulu, Finland

5. **Optical properties of the human nasal polyps in the spectral range from 300 to 2500 nm** Ekaterina A. Kolesnikova, Alia A. Muldasheva, Julia P. Ireneva, Darya N. Zmeeva, Alexey N. Bashkatov, Elina A. Genina, Vyacheslav I. Kochubey, Anatoly B. Knyazev, and Valery V. Tuchin, Saratov State University, Saratov State Medical University, and Institute of Precise Mechanics and Control RAS, Saratov, Russia; University of Oulu, Oulu, Finland
Abstracts
Professor Dan Zhu, Ph.D. in Physics Electronics in 2001, and completed a postdoctoral fellowship in Biomedical Engineering at Huazhong University of Science and Technology (HUST), rising to the position of Associate Professor in Biomedical Engineering in 2003, and professor in 2007. She has authored more than 90 papers in the field of Biomedical Photonics. During the recent years, she has been focused on optical clearing of tissue in vivo. She is also the Secretary General of Biomedical Photonics Committee of Chinese Optical Society.

Progress in Optical Clearing of Tissue in vivo

Dan Zhu

Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Key Laboratory of Biomedical Photonics of Ministry of Education, Huazhong University of Science and Technology, Wuhan 430074, P.R. China

E-mail: dawnzh@mail.hust.edu.cn

The tissue Optical clearing technique based on immersion of tissues into optical clearing agents (OCAs) allows one to effectively control optical properties of tissues, leads to essential reduction of scattering, and therefore enhance the depth to which light penetrates in tissue. During the last several years, we have been focusing on optical clearing of tissue in vivo, such as skin and skull. In order to develop some safe, effective and reliable optical clearing methods to perform in vivo, non-invasive optical imaging, we firstly paid attention to mechanisms of tissue optical clearing and biocompatibility of OCAs. Secondly, we paid attention to the dynamical function and morphology of blood vessels caused by OCAs, the negative effect on blood vessel or skin depends on the dose of OCAs and type. Thirdly, some optical clearing were developed to make skin or skull transparent by topical application, and realize the visualization of subcutaneous and cortical microvessels. Combining optical clearing technique of tissue in vivo with laser speckle temporal contrast analysis, the blood flow at high resolution can be obtained. With transgenosis technique, it is possible for fluorescence imaging to observe the neural activity in context or the immune response.

References

Bioimaging is a new direction of the scientific research methods, allowing us to receive the information about molecular and cellular events in a living organism in a real-time manner. Detection of caspase which are responsible for programmed cell death and metalloproteinase responsible for metastasis of cancer cells was performed on tumoral cells A549 and mice melanoma B16, which were transduced with the genes of fluorescent substrate TagRFP-X-KFP. Fluorescent proteins TagRFP and KFP form a FRET-pair, and the FRET efficiency is controlled by measuring the donor fluorescence lifetime. 3D structures of the TagRFP-DEVD-KFP biosensor and its FRET parameters have been performed by methods of modern computer modeling including molecular docking and molecular dynamics. The resulting biosensor structure was found as tetrameric, consisting in tetrameric KFP structure and four TagRFP molecules on periphery. The geometrical arrangement of molecules of the donor and acceptor explains the double-exponential character of biosensor fluorescence decay. The induction of caspase-dependent apoptosis leads to the substrate cleavage on the DEVD site, therefore growth of a fluorescent signal and increase of fluorescence lifetime of the donor is observed. The images received by means of FLIM method allowed us to distinguish apoptotic and non-apoptotic cells. As the majority of modern anticancer drugs induce apoptosis, the given approach can be used to estimate their specificity and antitumoral efficiency in vivo.
Figure 1. a) FLIM of the cell line B16-R23K in 24 h after the treatment by 10 mg/ml cis-platin; b) Life-time distribution over the frame; c) 3D structure of the R23K substrate.

References

Real-time monitoring of rare circulating liver cancer cells in an orthotopic model by in vivo flow cytometry assesses resection on metastasis
Xunbin Wei
School of Biomedical Engineering, Shanghai Jiao Tong University, P.R. China
E-mail: xwei01@sjtu.edu.cn

The fate of circulating tumor cells (CTC) is an important determinant of metastasis and recurrence, which leads to most deaths in hepatocellular carcinoma (HCC). Therefore, quantification of CTCs proves to be an emerging tool for diagnosing, stratifying, and monitoring patients with metastatic diseases. In vivo flow cytometry has the capability to monitor the dynamics of fluorescently labeled CTCs continuously and noninvasively. Here, we combine in vivo flow cytometry technique and a GFP-transfected HCC orthotopic metastatic tumor model to monitor CTC dynamics. Our in vivo flow cytometry has approximately 1.8-fold higher sensitivity than whole blood analysis by conventional flow cytometry. We found a significant difference in CTC dynamics between orthotopic and subcutaneous tumor models. We also investigated whether liver resection promotes or restricts hematogenous metastasis in advanced HCC. Our results show that the number of CTCs and early metastases decreases significantly after the resection. The resection prominently restricts hematogenous metastasis and distant metastases. CTC dynamics is correlated with tumor growth in our orthotopic tumor model. The number and size of distant metastases correspond to CTC dynamics. The novel in vivo flow cytometry technique combined with orthotopic tumor models might provide insights to tumor hematogenous metastasis and guidance to cancer therapy.

References
Several promising diagnostic and therapeutic technologies rely on nanoparticle delivery into a tissue and their usage as contrasting agents or mediators of cell laser heating and killing are overviewed in this paper [1-5]. The main advantage of transcutaneous administration of nanoparticles is that the delivery is targeted directly to the pathologically modified areas of the tissue. Erbium laser fractional microablation (ELFMA) of tissues is one of the promising technologies for nanoparticle delivery [2]. Titanium dioxide, zinc oxide, and gold nanoparticles are often used as contrasting agents for optical coherence tomography (OCT) of skin and other tissues [1], therefore nanoparticle delivery and control of their distribution in tissues and organs are of research interest. Gold nanoparticles are used for photothermal treatment of tumors and killing of metastasis [5]. Photocatalytic properties of TiO$_2$ nanoparticles, as well as gold nanoparticle laser-induced heating alone and in combination with photodynamic dyes [4] provide phototoxic action on pathogenic microorganisms.

On the cellular level a novel thermal effect – hoop-shaped hot zone on the nanoshell surface, can be used for precise laser-induced optoporation and nanosurgery of cells [3]. Figure illustrates calculations of the electrical field distribution at pulsed laser irradiation of nanoparticles attached to a cell membrane.
The basic principles, recent results, advantages and limitations of laser-induced nanoparticle delivery in application to medicine will be discussed.

Author is thankful to many collaborators for their input in this work, especially to G.G.Akchurin, G.B. Altshuler, Yu.A. Avetisyan, A.N. Bashkatov, E.I. Galanzha, E.A. Genina, L.E. Dolotov, B.N. Khlebtsov, N.G. Khlebtsov, K.V. Larin, G.S. Terentyuk, N.A. Trunina, E.S. Tuchina, A.N.Yakunin, I.V. Yaroslavsky, and V.P. Zharov.

This work was supported by grants 224014 PHOTONICS4LIFE of FP7-ICT-2007-2, RF Governmental contracts 02.740.11.0770, 02.740.11.0879, and 11.519.11.2035; FiDiPro TEKES Program (40111/11), Finland.

Peptide-based Nanoparticles for the Targeted Delivery of Cancer Diagnostic and Therapeutic Agents

Haiming Luo1,2, Honglin Jin1,2, Chuan Huang1,2, Zhihong Zhang1,2,*

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*Natural occurring lipoproteins are attractive as nanocarrier due to their high payload capacity, exquisite nano-size control and receptor recognition. However, the need to isolate apolipoproteins from fresh donor plasma has limited its application. By adopting a lipoprotein-mimicking peptide self-assembly principle, we created the high density lipoprotein (HDL)-liked peptide-phospholipid scaffold (HPPS), a sub-30 nm core-shell lipid nanoparticle stabilized by apoA-1 mimetic peptides(1, 2). HPPS size is tunable between 10-30 nm depending on cargo payloads, which are shielded from leakage at physiological conditions until released intracellularly. Biomimetic HPPS design provides its ideal surface chemistry and intrinsic/coordinated targeting capabilities. Therefore, compared to conventional lipid carriers (e.g., liposomes), HPPS nanoparticles are equally biocompatible, biodegradable but are advantageous with the stabilized ultra small size range (10-25 nm) for the prolonged circulation time, reduced RES uptake and more importantly, the improved ability to navigate through interfibrillar spacing (20-42 nm). The HPPS nanocarrier adds a new dimension to the core-shell nanoparticle families particularly valuable for the intracellular delivery of molecular therapeutics (e.g., siRNA(3)) in solid tumors.

Figure 1. Schematic diagram of HDL-liked peptide-phospholipid scaffold (HPPS) nanoparticle.

References
Da Xing received the Doctor degree in Engineering from Harbin Institute of Technology, China, and PhD in Physics from University of Electro-Communications, Japan, in 1989 and 1991. He joined the faculty of UEC from 1991 to 1995 with the Department of Electrical Engineering. He became the Director and professor of Institute of Laser Life Science, South China Normal University since 1996. He got Chinese Prime Minister’s Fund for Distinguished Young Scholars in 1997. He has published more than 300 peer-reviewed papers, and over 30 major invited talks of international conferences. His research activities include Biomolecular Spectroscopy, Noninvasive Functional Imaging and Biomolecular Sensing.

In vivo Multi-scale Photoacoustic Imaging for Biomedical Applications

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Based on the measurement of ultrasonic waves induced by electromagnetic pulses, photoacoustic imaging can reveal optical properties of tissues that are closely related to the physiological and pathological status of tissues and they have become the promising clinical imaging modality. Photoacoustic imaging is designed to overcome the poor spatial resolution of pure optical yet to retain the high electromagnetic contrasts and have become the promising clinical imaging technique. In this report, Different implementations of photoacoustic imaging allow the spatial resolution to be scaled with the desired imaging depth. New progress of in vivo photoacoustic microscopy and imaging with functionalized nanoparticles for biomedical applications were presented and experimental demonstrated. 2D and 3D microvascular imaging were provided, as well as hemoglobin oxygen saturation (SO₂) and carboxyhemoglobin saturation (SCO) were detection by photoacoustics. The uptake of Intracellular label-free gold nanorods were monitoring with photoacoustic microscopy. Intravascular photoacoustic tomography was used to localize and quantify the lipids in early atherosclerosis. Functional nanoparticles as PA contrast agent were achieved for noninvasive multimodality imaging.
References:
Optical assessment of biocompatibility and biosafety issues in interaction of nanoparticles with blood

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This paper will focus on possible effects of nanoparticles (NP) on microrheologic properties of red blood cells (RBC), in particular, their deformability and aggregation, which largely determine blood viscosity and microcirculation. All NP designed for \textit{in vivo} biomedical applications are carefully tested as for their biocompatibility and toxicity. In particular, this concerns the NP designed for intravascular administration into the organism for diagnostic or therapeutic purposes. However the interactions of these NP with blood components (plasma proteins and formed elements) are still poorly investigated, whereas, in principle, the adsorption of plasma proteins on NP surfaces and interaction on NP with and penetration through RBC membranes can change the blood microrheology and impair its microcirculation.

State-of-the-art optical techniques used to study the \textit{in vitro} effects will be discussed with particular emphasis on laser diffractometry [1] and diffuse scattering aggregometry [2]. These techniques were applied to investigate the effect of nanodiamonds (ND) and fullerenes (FN) on deformability and aggregation of human and rat RBC after 1 hour-long incubation of fresh whole blood samples with NP \textit{in vitro} at room temperature. Our results show that the NP do stick to RBC membranes and the smallest ones (5 nm sized) penetrate inside the cells. This interaction results in a decrease in RBC shear induced deformability and alteration of several important parameters of the spontaneous aggregation kinetics. The effect largely depends on NP size, concentration and surface functionalization. The effects come out differently in the case of ND and FN. Experiments performed with the suspensions of ND and major blood plasma proteins show considerable adsorption of the protein molecules on ND surfaces. All these results prove the importance of microrheologic tests in assessing the biosafety and biocompatibility issues in interaction of NP with blood.

References

Photobiomodulation-mediated pathway diagnostics

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Photobiomodulation (PBM) on a biosystem function depends on whether the biosystem is in its function-specific homeostasis (FSH). A FSH, a negative feedback response for the function to be performed perfectly, is maintained by its FSH-essential subfunctions (FESs) and its FSH-non-essential subfunctions (FNSs). A function in its FSH or far from its FSH is called a normal or dysfunctional function. The quality of a FSH is also called the functional fitness of normal function. All the FESs are normal, but only some of FNSs are normal. The more the normal FNSs of a normal function and the higher the FNS fitness, the higher the functional fitness of the normal function. A normal function in its FSH can resist external/internal disturbance, but the FSH can be disrupted by a FSH-specific stress (FSS). A normal/dysfunctional FSS is called a successful/chronic stress. A stress disrupting the normal FES/FNS of a normal function is called an extraordinary/ordinary stress of the normal function.

Direct PBM (dPBM) may self-adaptively modulate a chronic stress until the stress is successful so that it can be used to discover the optimum pathways for a FSH to be established. Indirect PBM (iPBM) may self-adaptively modulate an ordinary stress of a normal function until the FNSH is established and the normal function is then upgraded so that it can be used to discover the redundant pathways for a normal function to be upgraded.

Figure 1 Direct & indirect photobiomodulation (dPBM & iPBM)
Professor Hui Ma, obtained Ph.D degree from Department of Physics, Imperial College, London, majoring in atomic physics and laser spectroscopy. He worked as post doctors in Imperial College and Dalian Institute of Chemical Physics before joining Department of Physics, Tsinghua University in 1991. In 2003, he moved to the newly established Graduate School at Shenzhen, Tsinghua University and set up the Laboratory of Optical Imaging and Sensing. His current research interests include optical techniques and their biomedical applications, particularly polarized photon scattering in anisotropic media and polarization imaging techniques.

Polarization imaging: techniques, applications and the physics insights

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There have been increasing interests in polarization based optical imaging techniques for the potential applications in biomedical studies, clinical diagnosis and even material characterization. Using different polarization characteristics of the photons, one may discriminate the background of diffusive photons to improve the contrasts of optical imaging. More importantly, polarization measurements also serve as powerful tools for understanding the complicated behaviors of polarized photons as they pass through the turbid media, such as biological tissues and for extracting information on the structural and optical properties of the media. Detailed studies on the behavior of polarized photon scattering will lead to deeper physics insight of scattering and better understanding of contract mechanism behind polarization imaging techniques.

In this talk, we summarize our recent progresses in the studies on polarized photon scattering in anisotropic media, including the sphere cylinder scattering model (SCSM) and sphere cylinder birefringence model (SCBM), the Monte Carlo simulation program, the characteristic features of Mueller matrix elements and their decomposition, and a new set of parameters which are based on Mueller matrix elements but display more explicit relationships to the optical and microstructural properties of the scattering media.

**Spectroscopy analysis and detection of skin cancer**

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Problem of different skin formations types determining by rapid non-invasive methods requires an integrated approach and today can only be solved on the basis of optical methods. This research focuses on numerical and experimental studies of pathological formations optical diagnostics possibility.

The main method used in this study for formations control was differential backscattering. Due to the fact that the nature skin tumors is primarily determined by blood filling in vessels and melanin content, we have introduced the differential backscattering coefficient \( K = \frac{S_{760}}{S_{560}} \), which takes into account the boundaries of neoplasms, where \( S_{760} \) is backscattering coefficient, which takes into account content of melanin, and \( S_{560} \) is backscattering coefficient at the absorption maximum of hemoglobin. Experimental results shows that proposed differential coefficient allows one to perform a confident diagnosis of formations type and localization in the skin, that is important in the preoperative period.

Model experiments based on Monte Carlo method were carried out for different types of human skin. In the simulation experiments backscattered radiation spectra in the visible and NIR are obtained for the skin formations with known optical parameters. For the model backscattering spectra possibility of formation type determining based on the spectrum characteristics is shown.

Model experiments for the case of Raman backscattering from tissue formations are carried out. For different formations types values of radiation intensity corresponding to the Raman shift are defined; possibility of formation type determining with Raman shift detection in backscattered radiation is shown.

This study was supported by Federal Targeted program «Scientific and scientific-pedagogical staff of innovative Russia».

*Figure 1. Distribution of melanin index in normal tissues (1) and tumor (2).*
Imaging-based quantification of glottic opening by fiber optical nasopharyngoscopy in healthy and asthmatic subjects

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Quantitative measurement of glottis opening is important in diagnosing and understanding the mechanisms of vocal cord dysfunction. Although glottis opening can be directly observed and video recorded using optical pharyngoscope, quantitative analysis of the recorded video images has been technically challenging. Here, we present an automatic image processing method that allows for batch analysis of the glottic images, thus facilitates image-based quantification of the glottic opening. Video images of the glottis of volunteer subject were acquired using a fiber optical nasopharyngoscope, and subsequently processed using Gaussian smoothing filter, threshold segmentation, differentiation, and Canny image edge detection, respectively. Thus the glottis opening area was identified and calibrated using a predetermined converter. We used this method to measure the glottic opening of either healthy or asthmatic subjects during tidal breathing with or without making cough or ‘Hee’ sound. The results indicate that the image-based method can accurately quantify the glottic area change waveform as observed by the optical nasopharyngoscopy. Importantly, the change of glottic opening correlated with that of airway resistance, and could differentiate asthmatic and healthy subjects. Such quantitative assessment of glottic opening fluctuation may provide a useful tool to assist clinical diagnosis of vocal cord dysfunction and other airway pathologies.
Low-dimensional structures: sparse coding for neural activity

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Neural ensemble activity codes working memory. In this work, we developed a neural ensemble sparse coding method to reduce the dimension of neural ensemble during rat working memory task, so that the description of neural coding is more effective with low-dimension.

Spike time-spatial series were recorded at rat prefrontal cortex during work memory tasks in Y-maze. As discrete signal, spike was translated into continuous by estimating entropy. Then the normalized continuous signal is decomposed by non-negative sparse method, and non-negative components are extracted to reconstruct a low-dimensional ensemble, while none of the feature components are missed, as the number of the source components is larger than that of neurons. In well-trained rats, neural ensemble activities in the prefrontal cortex changes dynamically during the working memory task. And the neural ensemble is more explicit both in time and space after using non-negative sparse coding. These results indicate that the neural ensemble sparse coding for working memory effectively with low-dimension.

![Figure 1](image_url)

**Figure 1** Low-dimensional structures in sparse coding during rat working memory task
(a) Neural ensemble rate coding (b) Neural ensemble sparse coding

References
Professor Yueqing Gu is the director of Biomedical Engineering Laboratory in China Pharmaceutical University. Her research is focused on the optical molecular imaging, especially on the synthesis of different optical probes for early tumor diagnosis and nanomedicine for targeted tumor therapy. Different nano drug carriers and activated nano probes allows the tumor targeting drug delivery and in vivo investigation of the therapeutical efficacy. Her research team consists of a multidisciplinary investigators with expertise in a wide range of fields, including chemistry, molecular and cell biology, pharmacy, physics.

Dynamic investigation of breast tumor response to the targeted therapy by using gold nanoparticle based molecular beacons

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In this study, the folate acid modified chitosan micelles were used to encapsulate the fluorescent dyes and anticancer drugs for the localization of the tumor site and targeted chemotherapy on breast tumor bearing mice. The therapeutic efficacy was evaluated by the changes of the tumor volume. Meanwhile, two gold nanoparticles based molecular beacons were constituted for the measurements of key proteins related to tumor progression according to the theory of fluorescence resonance energy transfer (FRET). The quenching and recovery properties of the two molecular beacons were characterized.

The folate modified chitosan micelle was demonstrated as an excellent targeted drug carrier for folate receptor over-expressed breast tumors. The targeted chemotherapy displayed better therapeutic efficacy. 2) The gold nanoparticle is an idea quencher for the formation of the molecular beacons with good quenching and recovery properties. The #1 beacon can be endocytosed into the cytoplasm of the tumor cells and detect the mRNA of STAT5b proteins in living cells. Meanwhile, the #2 beacon was successfully applied on the in vivo measurement of the MMP2 enzyme secreted by the breast tumor cells before and after the targeted chemotherapy.

Conclusion: Gold nanoparticle based molecular beacons are promising agents for the dynamic evaluation of the tumor response to therapy. The correlation between the key protein expression and the therapeutic efficacy needs to be further investigated.
Sihua Yang received his PhD in Optics from South China Normal University, China. He joined College of Biophotonics in SCNU in July 2009, and became the Assistant Director of Institute of Laser Life Science, South China Normal University since 2011. His current research interests focus on the developments of new noninvasive biological imaging methods and apparatus, photoacoustic/thermoacoustic imaging with functional nanoparticles.

Microwave-induced Thermoacoustic Tomography for Biomedical Applications

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Microwave-induced thermoacoustic tomography is a nonionizing imaging modality based on the difference in microwave absorption of various biological tissues. The advantage of this imaging over traditional optical imaging is that it retains intrinsic microwave contrast characteristics while benefiting from the diffraction-limited high spatial resolution of ultrasound. Microwave-induced thermoacoustic imaging may find unique applications because microwave radiation provides a deeper penetration depth in biological tissues than optical radiation. Thermoacoustic images of radiolucent objects including glass fiber, wood, and bamboo hidden in phantom and residual in living mice were compared with radiography and ultrasonography. Iron oxide nanoparticles conjugated with tumor ligands was fabricated for targeted TAT tumor detection at the molecular level on the first time. Experimental results demonstrate that thermoacoustic tomography may be used for radiolucent foreign body detection and tumor imaging in animals and human.

References:

Laser diffraction analysis of shear deformability of human red blood cells incubated with nanodiamonds

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Nanodiamonds have been proposed to be biocompatible and promising for various biological and bio-medical applications. The nanodiamonds can affect the red blood cells (RBCs) properties such as their ability to change their shape under shear stresses when propagating along blood vessels and capillaries.

In this work, the analysis of nanodiamonds influence on human RBCs deformability was performed by means of the laser diffractometry. The essence of the method is in obtaining and processing the diffraction images from a cell suspension moving in a shear flow in a Couette chamber. The measurements result in the calculation of the index of deformability of RBCs (IDE) as a function of shear stress. To obtain the diffraction images a 1-mm thick layer of the suspension was illuminated with a collimated beam of a He-Ne laser (633 nm, 0.5 W/cm²). In the experiments with human RBCs we investigated blood samples from 10 volunteers. The each sample of whole blood was incubated with nanodiamonds with sizes 5 nm, 100 nm, 200 nm and 500 nm. Two different concentrations (33 and 100 µg/ml) of each nanodiamond type were used for incubation. Samples of RBC suspension with and without nanodiamonds were investigated by means of diffractometry technique. We have obtained dependences of deformability index on shear stress for each sample.

It was shown, that IDE of RBCs incubated with nanodiamonds was in average 7 – 15 % lower than this for pure RBCs. The decrease in deformability is seen to be more significant for the higher nanodiamond concentration. Basing on these results one can conclude that the nanodiamonds can be used as an effective bio-labeling tool in ambient conditions, without complicating the blood’s physiological conditions. However, controlling the deformability properties of RBCs and rheological properties of blood is necessary during treatment.
Xiaquan Yang, received Ph.D. degrees in biomedical engineering in 2010 from the Huazhong University of Science and Technology, China. Dr. Yang is now a post-doc fellow in Britton Chance Center for Biomedical Photonics, Wuhan National laboratory for Optoelectronics. His main research area is multimodality small animal imaging with fluorescence molecular imaging and micro-CT.

Molecular imaging of small animals using combined system of fluorescence molecular imaging and micro-CT

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Fluorescence imaging has been wildly used in the biomedical research for many years. Using FPs in small animal imaging remains a challenge, due to the biological tissue will highly diffuse and attenuate the fluorescence, especially when the fluophors are located deep in the tissue. In small animals, fluorescence molecular imaging (FMT) could potentially address this difficulty. But the ill-posedness of the inverse problem and the heterogeneity of the tissue in small animals make the FMT usually unpractical in biomecial research. We combined micro-CT with FMT to overcome this issue [1]. We found that the micro-CT can provide high-resolution anatomical localizations of fluophors for FMT. Furthermore, the anatomical information can be used as prior information in the reconstruction of FMT to effectively improve the performance of FMT [2]. The small animal imaging also validates our dual-modality imaging system.

Figure 1 Results of imaging a nude mouse with the multimodality imaging system. (a) A photo of the nude mouse. (b) The result of combining FMT and micro-CT. An arrow indicates the tumour in (a) and (b)

References:
Ivan A. Bratchenko, graduated from Samara State Aerospace University (SSAU) in 2009 with Master’s degree in Applied Mathematics and Physics; Assistant Professor of Radio-Engineering Department of SSAU, collaborator of Laboratory «Photonics», received Ph.D. degree in Optics in 2012. Scientific interests: optics, biophotonics, spectroscopy, mathematical modeling.

Application of confocal laser microscopy for mesh explants control

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Usage of different chemical composition tissues substitutes defines life time of implanted explants and gives an opportunity to vary prosthetic material acclimation rate in different tissues. The main aim of this study is analysis of confocal laser microscopy usage in herniology, detection of regularities, which appear in the course of different shape and chemical composition explants splicing with tissues, identifying opportunities of various wound healing pathologies detection, appearing in the explant area.

Significant impact on the relapse probability in hernioplasty provide a state of mesh implants surface and defects in their weaving. Moreover, it is possible qualitative change in the microtrauma with increasing of size, number and density of microdefects – formation of free play area in the encapsulation zone. To eliminate these effects and growth of pathologies preoperative microscopic control of implants should be carried out. Confocal laser microscopy allows one to carry out comprehensive control of mesh explants, determining the presence of weaving heterogeneities in explant. To assess the ability of explant visualization in tissues by optical methods model experiments based on Monte-Carlo technique were carried out. Mathematical simulations showed that postoperative control and monitoring of wound healing process and encapsulation of the implant are possible by the instrumentality of

Figure 1. Typical heterogeneities on explant surface.
differential backscattering methods with the depth of visualization up to five millimeters. The developed technique allows comprehensive monitoring of transplant surgery and postoperative control of tissues and explant in encapsulation area. Control of mesh explants microscopic structure allows screening of explant samples that do not meet the requirements for the transplant surgery.

This study was supported by supreme program for 2012-2014 years "Visualization and control of mesh explants encapsulation process".

References


The role of water in human tissue with certain compression:

**Studied with reflectance spectroscopy**

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In the research of spectrum measurement, external pressure brought by fiber optic probe may influence properties and microcirculation of detecting tissue¹-⁴. Under certain local pressure, the spacing between tissue components and the refractive index mismatch decrease due to water displacement, while scatters’ concentration increased as the tissue is compressed. In this paper, a systematic study is focused on how probe contact pressure change the intrinsic volume of water and scattering particles in tissue. The objectives of the currently study were: (1) quantify the short and long term effects of probe contact pressure on diffuse reflectance; (2) study the mechanism of water transports under the compressed area; (3) simulate the deformation and reflectance spectrum changes.

**Reference**

Second-harmonic generation imaging of elastic cartilage repair in a rabbit ear model
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Collagen deposition plays an important role in elastic cartilage repair. Label-free imaging and characterizing the collagen of repairing cartilages is critical toward understanding the mechanism of cartilage repair and efficacy of potential therapeutics. In this study, elastic cartilage repair in a rabbit ear model was examined with second-harmonic generation (SHG) microscopy without using labeling agents. High resolution SHG images of repairing tissues were obtained and image texture was quantitatively analyzed showing significant alteration in collagen content and distribution associated with cartilage repair. Our results suggested that SHG microscopy has the potential in label-freely imaging the repairing cartilage dynamic with time, thus monitoring the cartilage repair progression at a molecular level, and guiding the establishment of novel efficacious therapeutic strategies based on the better understanding of response mechanism.

References
Establishment of visible animal metastasis models for human nasopharyngeal carcinoma based on a far-red fluorescent protein

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The spectral properties of enhanced green fluorescent protein (EGFP) used in current visualizable animal models for nasopharyngeal carcinoma result in a limited imaging depth. Far-red fluorescent proteins have optimally spectral wavelengths that allow deep tissue penetration, thus are well-suited for the imaging of tumor growth and metastases in live animals. This study aims to establish an imageable animal model of nasopharyngeal carcinoma using far-red fluorescent proteins. Eukaryotic expression vectors of far-red fluorescent proteins, mLumin1 and Katushka S158A2, were separately transfected into 5-8F nasopharyngeal carcinoma cells, and cell lines stably expressing the far-red fluorescent proteins were obtained. These cells were intraperitoneally or intravenously injected into mice, and their tumorigenicity and metastatic potential were examined through fluorescence imaging. Finally, factors affecting their tumorigenic ability were further assessed through testing side population (SP) cells proportion by flow cytometry. Nasopharyngeal carcinoma cell line with high tumorigenicity and metastasis (5-8F-mL2) was screened out, which stably expressed far-red fluorescent protein. Intraperitoneal and intravenous injection of 5-8F-mL2 cells resulted in an abdomen metastasis model and a lung metastasis model. In addition, nasopharyngeal carcinoma cell line without tumorigenicity (5-8F-Katushka S158A) was screened out. The percentage of SP cells between 5-8F-mL2 and 5-8F-Katushka S158A was found different, suggesting that the SP cell proportion may play a key role in the determination of cell tumorigenic ability. We

Figure 1 Whole-body optical imaging for the abdominal cavity metastases (left) and lung metastases (right) of Nasopharyngeal carcinoma cells 5-8F-mL2 in vivo.
successfully established animal models for nasopharyngeal carcinoma with high tumorigenicity and metastasis using a super-bright far-red fluorescent protein. Owing to the super brightness and excellent wavelength parameters, these models may be applied as useful tools for intuitive and efficient monitoring of tumor growth and metastasis, as well as assessing the efficacy of nasopharyngeal cancer drugs.

References


Oleg O. Myakinin received his Bachelor (2009) and Master of Science (2011) degrees on Applied Mathematics and Informatics from Samara State Aerospace University named after academician S.P. Korolyov (SSAU). He is a postgraduate student in SSAU. His research interests are computer vision, interferometry, biomedical image processing.

Noise reduction methods for OCT images

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Optical Coherence Tomography (OCT) is a rapidly developing imaging technology which allows noninvasive cross-sectional imaging of weakly scattering samples with high sensitivity. It has been successfully applied as an imaging tool in medicine [1]. SD-OCT (OCT with spectral domain detection) technology can reach imaging speeds up to several hundred thousand lines per second.

Speckle pattern (as in every coherent imaging technology) significantly reduces axial and lateral resolution of OCT-setup. Most techniques for reduction of speckle noise uses classical algorithms [2] or require hardware (and maybe also software) modification of scanning and imaging system [3]. Sometimes such modifications are undesirable or even impossible.

We propose and demonstrate the new two-stage technique for noise suppression of OCT-images. First stage is a low-level processing of A-scans and second stage is a high-level denoising of B-scans (sometimes maybe C-scans). Low-level signal processing may be embedded in DSP-module for SD-OCT. High-level image processing shall be introduced in the software for post-processing procedures. In both stages we used the space-frequency analysis methods. That's Discrete Wavelet

Figure 1. C-scans of SD OCT and its middle y-line of a) raw b) EMD-filtered image of implant under the layer of mouse skin.
Transform (DWT) and Hilbert-Huang Transform (HHT) also known as Empirical Mode Decomposition (EMD). Using of DWT and EMD helps to avoid Fast Fourier Transform drawbacks. Besides EMD allows to construct an adaptive non-linear basis and to carry out the localization in the spatial-frequency domain [5]. We also propose two threshold functions (separate for each stage).

References


The non-linear dynamic study on epilepsy ictal EEG

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This paper will study the non-linear dynamic character of EEG changes in patient with epilepsy in the whole seizure process, including non-ictal, pre-ictal, ictal and post-ictal periods. The EEG in the whole seizure process of the patient with epilepsy was recorded. His convulsion was regarded as grand-mal seizure with consciousness lost at the beginning. The most abnormal EEG in FP1 and FP2 channel were chosen to calculate the correlation dimension (Dc). The EEG were digitized at 256 Hz. The filtering was 0.1-45 Hz. 2048 points were calculated on Matlab 7.0¹², with 512 points overlapped. In the whole seizure process, Dc declined (from 4-5 to 3-4) periodically from pre-ictal to ictal period and returned the slightly lower level of the non-ictal period after seizure. In fact, Dc had began to fluctuate at 100 seconds before the clinical seizure. This suggests that the non-linear dynamic analysis of the whole seizure made it possible to carry out the short-time clinical seizure prediction.

References

Microwave therapy via bronchoscopes for severe tracheal stenosis

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To evaluate the efficacy of microwave therapy via bronchoscopes for treatment of severe tracheal stenosis. METHODS: 37 patients with severe tracheal stenosis caused by tumor or other diseases, accepted microwave thermotherapy, include microwave tissue coagulation (MTC) and diathermy (MD) therapy via bronchoscopes. RESULTS: All of them had accepted 2 or more treatments of microwave thermotherapy via bronchoscopes. The effective rate after treatment is 100%, and the effective rate 1 month later: 67% to malignant tumors, 100% to benign diseases. CONCLUSIONS: We can take microwave thermotherapy via bronchoscopes to treat those patients with benign trachea blockage avoiding operation, and those whose in the afternoon of carcinoma with trachea soakage, stenosis, blockage for prolong their life time and alleviate their agony at suitable time.

Figure 1 Microwave therapy for tracheal stenosis.
(1) before treatment, (2) during fourth treatment, (3) before fifth treatment, (4) after fifth treatment.
Evaluation of the red blood cells shape parameter variance from the data of laser ektacytometry

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Impairment of blood microcirculation in human body is caused, in particular, by the reduction of the RBCs deformability. This results in the obstruction of capillary blood flow, which leads to the development of many diseases. Therefore, quantitative characterization and measurement of RBCs deformability in a population of cells is an important and actual issue of biomedical diagnostics.

By now, several techniques have been designed for evaluating the deformability of RBCs using various methods, in particular, the micropipette aspiration, passage of RBCs through a porous filter, laser tweezers, laser diffractometry. One of the variants of the latter, also referred to as ektacytometry, allows for estimating the deformability of blood cells by processing the diffraction pattern arising as a result of laser beam scattering on a suspension of RBCs deformed by the forces of viscous friction in a shear fluid flow. Schematic layout of ektacytometer is shown in Fig.1. Conventionally, this method is used to measure the average deformability of RBCs in a blood sample. In principle, this method should allow for evaluating more delicate characteristics of the RBCs ensemble, for example, the variance of particle sizes [1] or the fraction of cells with reduced deformability [2]. However, the realization of these possibilities requires further development of the measurement procedure and data processing.

This paper presents the results of theoretical analysis of laser beam scattering on a nonhomogeneous ensemble of elliptical disks mimicking the RBCs in ektacytometer. We model the RBCs as elliptical disks with the semi-axes $a$ and $b$ given by the following equations:

$$a = a_0 \cdot (1 + \varepsilon),$$
$$b = b_0 \cdot (1 - \varepsilon)$$

Here $a_0$ and $b_0$ are the average values of the semi-axes, $\varepsilon$ is a random shape parameter of a particle with zero mean value and variance $\mu^2 << 1$. Thus, the shape nonhomogeneity of the
ensemble of particles is comparatively weak. Note that in our model \( ab \approx a_b b_0 \), i.e., the area of the base of the elliptic disk does not differ much from its average value.

Scattering of light by an ensemble of RBCs is described in the anomalous diffraction approximation. From the mathematical point of view, this problem is equivalent to the problem of diffraction of a plane light wave on an elliptical aperture with fixed area and a random eccentricity. The solution of this problem is expressed using the Bessel function of first order. In the vicinity of the first minimum of the diffraction pattern, we approximate the Bessel function by a linear function. This allows for averaging the diffraction pattern over the random parameter of shape of the particles. As a result, we obtained an approximate analytical expression for the distribution of light intensity on the screen near the central maximum of the diffraction pattern [3].

Analysis of this expression leads to the following conclusions. While in the case of laser beam scattering on an ensemble of RBCs homogeneous in particle shapes the lines of constant intensities on the diffraction pattern (isointensity lines) take a characteristic elliptical form, in the case of non homogeneous ensemble of RBCs the isointensity lines acquire a rhomboid shape. This conclusion is confirmed by our observations (Fig. 2) and the data given in Ref. [2]. One of the criteria of the RBCs ensemble nonhomogeneity in particle shapes can also be the existence on the observation plane of four dark dots forming the vertex of a rectangle.

In order to interpret the experimental data of laser ektacytometry, we have introduced several new notions. They include the polar and characteristic points, which can be found in the diffraction pattern. Determining the peculiarities of the nonelliptical isointensity curves, in particular, the coordinates of these points allows for estimating the variance of the particles shape parameter. Evaluations of the experimentally obtained diffraction patterns performed in this way prove that the proposed algorithm allows for obtaining reliable results.

References
In vitro OCT study of alterations of adipose tissue structure induced by PDT treatment

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Temporal changes in structure and refractive index distribution of adipose tissue at photodynamic treatment were studied with OCT. The 100-150 µm fat tissues slices were used in in vitro experiments. Water-ethanol solutions of indocyanine green (ICG) and brilliant green (BG) of 1 mg/ml and 6 mg/ml concentration, respectively, were used for fat tissue staining. CW laser diode (ACCULASER, 810 nm) and dental diode irradiator Ultra Lume Led 5 (442 and 597 nm) were used for irradiation of tissue slices. Light exposure of laser was 1 min, and the diode lamp – 5 min. Power densities were 250 mW/cm² and 75 mW/cm², respectively. The studies were conducted at room temperature.

As the geometric thickness of the probed layer was known, the effective refractive index \( n \) can be calculated using

\[
 n = \frac{z}{l}
\]

where \( l \) is the geometric path length (true thickness of the sample, µm) and \( z \) is the OCT-observed depth, i.e., the optical path length, µm [1-3].

Fig. 1. Experimental setup: Spectral Radar OCT with Handheld Probe: schematic diagram of OCT imaging (left) and general view (right)

Fig. 2. Example of OCT images of fat tissue stained by BG after 300-min incubation. The concentration of BG was 6 mg/ml. The Dental diode irradiator Ultra Lume Led 5 (442 and 597 nm) was used as a light source for PDT.
It was found that relative refractive index of the scatterers decreased with time elapsed after treatment that indicated the immersion optical clearing. These data support the hypothesis that photodynamic treatment induces fat cell lipolysis during some period of time after treatment.

References
Skin optical clearing by glucose and quantification of glucose diffusivity at its impact on skin tissues

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We present results of in vitro skin optical clearing by 40%-glucose solution. Ten samples of intact rat skin were measured to obtain spectra of skin optical properties before and after incubation in 40%-glucose solution. Diffuse reflectance and total and collimated transmittance spectra were measured by LAMBDA 950 (Perkin Elmer, USA) spectrophotometer with an integrating sphere in the spectral range 350 – 2500 nm. All measurements have been performed during 24 hours after obtaining the samples. Then the samples were incubated during 24 hours in 40%-glucose solution and all of the spectra were measured again. The last stage was incubating the samples during 24 hours in physiological solution and measuring spectra after that. Inverse Monte Carlo technique has been used for processing the experimentally measured spectra of the skin samples; and wavelength dependence of absorption and scattering coefficients, and anisotropy factor has been obtained.

Glucose diffusion coefficient was estimated from the measurement of collimated transmittance. The collimated transmittance was measured in the spectral range 400-1000 nm for ten rat skin samples using USB4000 spectrometer (Ocean Optics, USA) concurrently with administration of 40%-glucose solution and diffusion coefficient was evaluated using a specially developed computer program. The average value of glucose diffusion coefficient was estimated as (1.52±1.62)×10⁻⁶ cm²/sec. The presented results can be useful for the development of the optical imaging technologies and diagnostics and therapy of diabetes mellitus.
MSc Natalia A. Trunina received the Graduate degree (Hons.) from Saratov State University (SSU), Saratov, Russia, in 2009. She received the Master degree in physics in 2011. She is currently working toward the Ph.D. degree as a postgraduate student and an engineer of the Department of Optics and Biophotonics, SSU. She is a specialist in biochemical physics. Her research interests include optical coherence tomography, two-photon microscopy, and laser optics.

**TiO\(_2\) and ZnO nanoparticles as disinfection compounds**

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Penetration of nanoparticles into tooth tissues is of significant interest in solving the problems related to the reduction of tooth sensitivity, enamel strengthening and restoration and cosmetic bleaching. Particles of TiO\(_2\) and ZnO are known for their photoactive properties and can be used as bacteria inhibitors.\(^1\),\(^2\) Monitoring of particles penetration into tooth is, however, a challenging task.

Keeping in mind the potential application of nanoparticles as an antibacterial agent and a free-radical producer when embedded into the tooth dentine tissue, in the present paper we dwell on two key issues relevant to the problem. The first aim is to study the process of nanoparticle penetration into tooth dentine samples by using a nonlinear optical technique. The second is to investigate the influence of the same nanoparticles on the generation of free radicals using the EPR technique.

To visualize the penetration of TiO\(_2\) and ZnO nanoparticles into tooth tissues we used two-photon autofluorescent (AF) and second-harmonic generation (SHG) microscopy.\(^3\) Tooth tissue slices containing dentin and enamel sections were used as samples. The samples submerged in a suspension of nanoparticles were placed in an ultrasonic bath to enhance the penetration before imaging. Evidence of TiO\(_2\) and ZnO nanoparticles penetration into dentin and enamel of human tooth was observed using multiphoton tomography (MPM) operating in the superficial tissue area down to 200 µm. In this study, AF and SHG images of the enamel and dentin were obtained.

We have found that the enamel produces a strong AF signal, clearly revealing the structure of the enamel rods. Dentin produces both AF and SHG signals. The collagen of the dentin tubules produces a strong SHG signal, while the peritubular dentin response contains both the SHG and AF signals.

It was found that ZnO nanoparticles penetrated up to a depth of 12 µm and 45 µm in human tooth enamel and dentine correspondingly, while the maximum detectable penetration depth of TiO\(_2\) nanoparticles was 5 µm in tooth dentine. The size and shape of nanoparticles as well as their aggregation ability play a significant role in the penetration process. ZnO nanoparticles in contrast to TiO\(_2\) produce strong SHG signal, because they possess considerable second-order nonlinear optical coefficients (d\(_{333}\) and d\(_{311}\)) due to their crystalline symmetry.
Our results demonstrate the efficiency of using MPM for imaging the tooth structure and nanoparticles penetration.

Figure 1. TPEF (a, c) and SHG/HRS (b, d) images of tooth dentine after 30 min of ultrasound treatment in TiO$_2$ suspension at a depth $z = 0$ µm (a, b) and $z = 5$ µm (c, d). Bright white spots indicate TiO$_2$ nanoparticles.

In addition to penetration studies, we performed phototoxicity assessment of the used TiO$_2$ particles. Original (undoped) TiO$_2$ (anatase) nanoparticles (mean size 30 nm) were fabricated by Sigma-Aldrich. Treated TiO$_2$ were obtained when TiO$_2$ was heated in either 2% NH$_3$ in an N$_2$ atmosphere at 600 °C for 4 hours (TiO$_2$/NH$_3$) or in a pure N$_2$ atmosphere at 600 °C for 4 hours (TiO$_2$/N$_2$). The former becomes doped with nitrogen. The temperature was ramped at 2 °C/min. Figure 2 illustrate the time dependence of the electron paramagnetic resonance (EPR) signal intensity obtained from an EPR marker (stable radical). In the presence of short-lived radicals generated subsequent to TiO$_2$ irradiation with light (UV or UV in combination with visible), the EPR signal of the marker decreased. Presence of the UV fraction in the irradiating light causes a sufficiently stronger signal decrease (the lowest curves on each plot), i.e., radical production, than in presence of visible part only. Undoped TiO$_2$ (treated with N$_2$ and pristine) showed no radical production in presence of visible light; only doped TiO$_2$ nanoparticles were photoactive in this spectral range (the “visible” curve is lower than the “no light” curve).

Figure 2. Dynamics of the EPR signal in presence of (a) TiO$_2$/N$_2$ (undoped) and (b) TiO$_2$/NH$_3$ (N-doped) samples.

References
Polina A. Timoshina was graduated from Saratov State University in 2012. Her research interest is application of full field laser speckle contrast imaging technique to the study of the dynamics of blood flow.

Full field laser speckle contrast imaging technique application for visualization of rat's pancreas micro capillary blood flow

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The study of blood microcirculation is one of the most important problems for the modern medicine. Full field speckle contrast technique is a noninvasive contactless method that allows for visualization of capillary blood flow in a real time without scanning of the laser beam. The full field speckle imaging based on the analysis of contrast of time-averaged modulated speckle images. Depending on velocity of red blood cells (RBC) in the imaged area, the blurring of the speckle pattern is differed. The speckle contrast is defined as the ratio between the standard deviation of the intensity/If velocity of RBC increases, the blurring will also increase and the standard deviation of the intensity will decrease, and consequently the speckle contrast will be lower. On the contrary, if there is no RBC movement, the speckle contrast will be larger since the blurring will decrease and the standard deviation will increase. The mean intensity is remain unchanged[1-6].

Fig.1 Speckle images of blood flow of the pancreas recorded by CMOS camera.

For monitoring of blood microcirculation, laboratory model of the speckle-correlometer was developed. With the help of this model the study of blood flow can be conducted at coherent...
irradiation (He-Ne laser~630nm) (speckle technique) and at incoherent irradiation (digital microscopy technique) without mechanical reconfiguration. The developed software allowed for real time calculation of the contrast spatial distribution from the experimental data obtained in the study of the dynamics of blood microcirculation in pancreas after local ischemia. In laboratory animals hemorrhagic pancreatitis was modeled. Altogether 20 experimental animals were studied. Contrast of time-averaged speckle for different regimes of the microcirculation in the normal state and at ischemia and reperfusion was measured.

It was found that the dynamic behavior of blood flow and vascular network studied by speckle imaging technique and digital microscopy is well match to physiological response to induced pathologies.

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Multi-Modality Molecular Imaging for Nanomedecine and Cancer Research

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Development of new imaging technology that is capable of non-invasive or minimal invasive, in vivo, in situ, real-time observation of pharmacological interactions between new drug compounds and diseased cells at the molecular level on the animal model plays a critical role in the translational research activities in new drug discovery.

For the past decade or so, many molecular imaging modalities have been separately developed for such a purpose. These imaging modality includes: optical spectroscopy imaging with single or multi-photon stimulation modalities, optical confocal imaging with single or multi-photon stimulation and optical or spectroscopic reflectance analysis, optical coherence tomography (OCT), photo-acoustic microscopy (PAM) or tomography (PAT), combined with animal sized X-ray CT, animal-sized positron emission tomography (PET), or single positron emission tomography (SPET).

Recently, our group has been devoted a great deal of research efforts in the development of multi-modality molecular imaging technologies that combines many of CT, PET, SPECT, and Fluorescence Molecular Tomography (FMT) into one imaging platform. These integrated four modality imaging technology allows researchers to effectively obtain many critical biochemical information in vivo from the same site by a single or multiple scan through out different disease-treating stages on the same animal. These imaging modalities will greatly enhance the research activities in biomedical research.
Newly developed NIR contrast agents for cancer targeted imaging

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The development of multifunctional agents for simultaneous tumor targeting, imaging and treatment is expected to have significant impact on future personalized oncology. Current most common strategies are based on the chemical conjugation of chemical agents or nanoparticles with tumor specific ligands, such as small molecules, peptides, proteins and antibodies. However, these strategies have proven to be challenging and are still at an early or proof-of-concept stage due to several fundamental problems and technical barriers. Our recent studies have developed an alternative strategy to overcome these limitations by providing natively multifunctional heptamethine cyanine dyes with simultaneous tumor targeting and imaging capabilities without the need of chemical conjugation to targeting ligands. These dyes have unique optical and biocompatible properties and selectively accumulate in the viable tumor cells of a variety of human cancer types. We believe these natively multifunctional heptamethine cyanine dyes will broaden current concept and have promise in cancer targeted imaging and therapy.

References


Singlet oxygen dosimetry for photodynamic therapy

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Singlet oxygen (1O₂) is believed to be the major cytotoxic species generated during Type-II photochemical reaction of photodynamic therapy (PDT), and the direct detection of 1O₂ luminescence has received considerable attention during the past two decades. The most attractive advantage of this photo-physics-based technique for direct PDT dosimetry is that it can circumvent the complicated interactions between photosensitizer, treatment light and molecular oxygen. It has been previously demonstrated that PDT efficiency is correlated well with cumulative 1O₂ luminescence counts detected during PDT treatment. In this talk, the mechanisms for 1O₂ generation and the detection techniques of 1O₂ luminescence during photosensitization will be presented. In particular, the 1O₂ lifetimes in different solvents are summarized. Furthermore, the most recent advances in PDT-1O₂ dosimetry for in vitro cell model, in vivo animal model, and pre-clinical studies are introduced in details. Finally, the challenges and trends in developing PDT-1O₂ dosimetry for clinical applications are briefly discussed.

References
Investigation of red blood cells aggregation in plasma and in proteins solutions by optical trapping

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Aggregation of red blood cells (RBCs) is an intrinsic property of blood, which has direct effect on blood viscosity and blood circulation throughout the body. Up to date there is no clear understanding of RBC aggregation mechanism. Measuring interaction forces between RBCs within a single aggregate and/or between two aggregates in the presence of various macromolecules in the solution that induce or inhibit aggregation with an optical trap allows for assessing the features of aggregation, and better understanding the mechanism of this important process.

In this work, we used a double-channel optical trap to measure the interaction forces between red cells in the single aggregate. The measurements were performed with RBCs from freshly drawn human blood samples in autologous plasma and in the solutions of dextran and fibrinogen molecules of different concentrations. In the latter case the RBCs were preliminarily washed.

In the presence of macromolecules, as well as in autologous plasma we observed three different processes of disaggregation when trying to take apart the RBCs with the optical trap [1].

In the first case, at the final stage of disaggregation the RBCs were connected with thread-like strand presumably consisting of the macromolecules, which was very hard to break. In the second case, before total disaggregation a small contact area remained that held the RBCs. In the third case, the RBCs disaggregated easily without change of the shape of the membrane. In the first two cases, the interaction forces exceeded 50 ± 5 pN for all types of macromolecules and plasma.

For dextran, by measuring the interaction forces between RBCs when one of them was attached to the bottom of the couvette without fully disaggregating...
RBCs, we found that the forces increase with concentration. The force was measured by decomposing the aggregate without breaking the final connection and was in range from 18 to 38 pN for different cells. In the case of fibrinogen, we could observe changes in aggregation rate at higher concentrations. At higher concentrations RBCs aggregated instantly as they came into first point contact, while at lower concentrations it was required to hold RBCs intact for a few seconds to form an aggregate. Visually, the number of RBC aggregates in the unit area of observation was higher in the presence of fibrinogen than in the presence of dextran at equal concentrations of macromolecules at given concentration of RBCs. In the third case, the interaction force was about $8.4 \pm 1.1$ pN. The experiments were performed with RBC having different morphological shapes, e.g., echynocytes.

The same optical trap was used to measure the elastic modulus of red blood cells, an important characteristic of the deformability of red blood cells while passing through the microcapillaries [2].

References


Optical properties of the human nasal polyps in the spectral range from 300 to 2500 nm

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The results of the study of optical properties of the human nasal polyps in the spectral range from 300 to 2500 nm are presented. The experiments were performed with 20 samples of the human nasal polyps, obtained from different patients in the course of elective surgery. The samples were kept in saline at temperature 4-5°C during 48 hours until spectral measurements. The dimensions of the samples were approximately 15×15 mm, the average thickness was 1.0±0.5 mm.

The total and collimated transmittance and total reflectance spectra were measured using the LAMBDA 950 spectrometer (PerkinElmer, USA) with an integrated sphere. The halogen lamp was used as a light source for the NIR and visible measurements and deuterium lamp for UV measurements.

Inverse Monte Carlo technique has been used for processing the experimentally measured spectra of the tissue samples; wavelength dependence of absorption and scattering coefficients, and anisotropy factor has been obtained.

The analysis of the results has shown that investigated spectra are depend on scattering coefficient of collagen fibers and on absorption bands of interstitial matrix water. The absorption bands of oxyhemoglobin at the wavelengths 415, 540 and 570 nm are well seen.
Our results can be used for the development of new methods and optimization of the existing ones of therapy of rhinological diseases.

Wavelength dependence of scattering (a) and absorption (b) coefficients, and anisotropy factor (c) of the nasal polyps.
Leading experts from P.R. China are invited to attend the Workshop - Prof. Gong Hui, Prof. Lei, Dr. Lin Lin, Dr. Zhiyu Qian, and Dr. Luo Xudong. They will be involved in the Round Table discussions on the development of education and research in the field of biophotonics and biomedical optics.

In the framework of the Workshop they will also visit student and research laboratories of Research-Educational Institute of Optics and Biophotonics and International Research-Educational Center on Optical Technologies in Industry and Medicine. During these visits novel educational programs for bachelor, MS, and candidate of science (PhD) in Medical Photonics, Medical Physics, Biophysics and new educational standard “Physics of Living Systems” in SSU will be discussed.

Prof. Gong Hui is one of the founding members of Britton Chance Center for Biomedical Photonics, Huazhong University of Science and Technology and Wuhan National Laboratory for Optoelectronics. Her research interests include micro-optical sectioning tomography for obtaining high resolution mouse brain atlas and brain connectivity, near infrared functional optical imaging for brain activity and sports. As first or corresponding author, she has published papers in Science, Review of Scientific Instruments, Journal of Biomedical Optics, Medical Physics, et al.

Prof. Lei obtained his Ph.D degree from University of Manitoba, Canada. He did postdoctoral research in Dartmouth College and University of Minnesota before his return to China on 2002. He is currently the Head of the Magnetic Resonance Imaging (MRI) group in the State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences. Research interest of Dr. Lei’s group is developing novel magnetic resonance imaging (MRI) and in vivo magnetic resonance spectroscopy (MRS) technology for biomedicine applications, and applying MRI/MRS technology as tools to study such diverse areas of subject including metabolism and function the brain, animal models of neurological diseases and their treatments, development of novel contrast agent for MRI and biological effects of nanoparticles etc.
Lin Lin is Assistant President of Huazhong University of Science and Technology (HUST), Committee Chief of Wuhan National Laboratory for Optoelectronics. She obtained her Ph.D degree in Higher Education (2003), MS degree in Hydropower Engineering (1988) and Bachelor Degree in Hydropower Station Engineering (1977) from HUST, China. Her Research Area is higher education.

Dr. Zhiyu Qian is currently a full Professor and director of the Department of Biomedical Engineering in Nanjing University of Aeronautics and Astronautics (NUAA). His research is focused on the medical instruments and the corresponding software. As a principal Investigator, he got several key projects from the Ministry of Science and Technology, the Natural Science Foundation Committee (NSFC) and Jiangsu Province of China. With the financial support, Prof. Qian designed and built several therapeutic systems for in vivo guiding/monitoring/ evaluation of neurosurgery and other pathological conditions/ therapy. Up to now, he has obtained 8 Chinese patents and software copyright for all the related systems, and he published more than 100 scientific papers in domestic and international journals.

Luo Xudong, general manager. Graduated from Huazhong University of Science and Technology, Department of Computer Science and Technology (1988). The founder of NBL imaging system Co., Ltd. The company's business and research directions are optical imaging methods and its technical applications and promotion. The follow technological achievements have been completed: the spectral imaging of biological tissues, microcirculation microscopic imaging, etc. Undertake and complete the project of the Ministry of Science and Technology, PRC, the “11th Five-Year” National Science and Technology Support Program subject-spectral imaging key technologies research, and be a member of the Remote Sensing Hyperspectral Imaging Professional Committee, and also a member of the Chinese Criminal Science and Technology Association.
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