





Управление оптическими свойствами биологических тканей: новые приложения в мультимодальной визуализации и фототерапии

В.В. Тучин

Саратовский национальный исследовательский государственный университет им. Н.Г. Чернышевского, Саратов Национальный исследовательский Томский государственный университет, Томск Институт проблем точной механики и управления РАН, Саратов tuchinvv@mail.ru



- *Motivation and basics of tissue optical clearing Creation of UV window Fluorescence measurements *Combined MRI and fluorescence imaging *Practical examples, spectroscopy, imaging and treatment *Summary
- Conclusion

Motivation: Challenges of Optical Imaging and Treatment

Skin





Hard limit ~10 δ

-

MFP =
$$l_{ph} = 1/(\mu_a + \mu_s)^-$$

 $\mu_s' = \mu_s(1-g)$

OM: **Optical microscopy** SNOM: Scanning near-field optical microscopy Confocal microscopy CFM: 2PM: Two-photon microscopy SHM: Second harmonic microscopy OCT: **Optical coherence tomography** DOT: Diffuse optical tomography UOT: Ultrasound-modulated optical tomography PAT: Photoacoustic tomography



OM, SNOM

OCT

CFM, 2PM, SHM, etc.

DOT,

UOT, PAT

Fluorescence cancer cell imaging



Femtosecond Laser Treatment

Tissue 'optical windows'



Y. Zhou, et al. J. Biomed. Opt. 21(6), 061007 (2016)





Absorption (μ_a) and reduced scattering (μ_s') coefficients, and light penetration depth (δ) of peritoneum within tissue 'optical windows'
Bashkatov A. N. *et al*. *Opt. Spectrosc.* **120** (1), 1-8, 2016; JBO, **23** (9) 2018

Rat muscle treatment with 20% glucose solution

P. Peixoto, et al., *J. Biomed. Photon.Eng* **1**(4) 255, 2016



http://elte.prompt.hu/sites/default/files/tananyagok/IntroductionToPracticalBiochemistry/ch04s06.html



Ordering of collagen fibers



From Oliveira et al., SFM, 2020

Spectral measurements from deep UV to NIR and THz, and OCT of tissues *ex vivo* and *in vivo* + immersion optical clearing (OC)

Spectral measurements ex vivo/in vivo + optical clearing



Journal of Biophotonics

Shimadzu UV-3600, 350-2500 nm Shimadzu UV-2550, 200-800 nm

Collimated and total transmittance, reflection spectroscopy (fiber-optic and integrating sphere) from deep UV to NIR and THz





Colorectal muscle, OC efficiency, Glycerol 60%



Gingival mucosa, OC efficiency, Glycerol 99.5%

 $25 - 420 \text{ nm} - \frac{\text{Gehnart et al. [19]}}{\text{Honda et al. [20]}} - \frac{1}{150} - \frac{1}{150$





OCP930SR 022 (930 nm) Spectral band, 100 nm Output power, 2 mW Scanning depth, 1.6 mm (air) Axial resolution, 6.2 μm (air)



GANYMEDE, SD-OCT (930 nm) Spectral band, 150 nm Scanning rate A-scan 29 kHz Scanning depth, 2.7 mm (air) Axial resol., 5.8 /4.4 μm (air/tissue)

Spectral OCT systems (Thorlabs Inc.)



The OCT imaging of brain clearing from GNRs: A - 1) GNRs are injected into the brain parenchyma; 2) GNRs drain from the brain via the *meningeal lymphatic vessels*; 3) 20 min after injection, GNRs are accumulated in the deep cervical lymph node; **B** – transmission electron microscopy image of GNRs (92×16 nm) (1) and their extinction spectrum (2); **C** – OCT image of the deep cervical lymph node before injection of GNRs; **D** – a set of OCT images illustrating the kinetics of accumulation of GNRs in the deep cervical lymph node; **E** – OCT signal average intensity within the ROI showing linear accumulation of GNRs in the deep cervical node with slope k = 0.0007.

Optical Coherence Tomography

Illustrations are designed by Kirill Larin





Time domain OCT (TDOCT)

Swept source OCT (1325 nm)





SS-OCT (Thorlabs, SL1325) setup: MZI: Mach–Zehnder interferometer; ADC: Analog to digital converter

Creation of UV window

I. Carneiro, S. Carvalho, R. Henrique, L. M. Oliveira, V. V. Tuchin, Moving tissue spectral window to the deep-ultraviolet via optical clearing, *J. Biophotonics*. 2019;e201900181.

The sample was immersed in the **glycerol** solution and measurements (200-1000 nm) were aquired during 30 min treatment with a 5 s - time resolution



Surgical colorectal specimen









Similar behavior is observed on both sides of the DNA/Protein absorption band

The OC effect in UV improves with the increase of glycerol concentration in the solution

Tissue optical clearing using MRT or CT contrast agents

D.K. Tuchina, I.G. Meerovich, O.A. Sindeeva, V. V. Zherdeva, A. P. Savitsky, A. A. Bogdanov Jr, V. V. Tuchin, Magnetic resonance contrast agents in optical clearing: Prospects for multimodal tissue imaging. J. Biophotonics 13(11) 2020; e201960249. https://doi.org/10. 1002/jbio.201960249



Multi-wavelength measurements RI of MRI agents













	$OCE = T_c^{OC} / T_c^0$				
Wavelength, nm	Gadovist	Magnevist	Dotarem	Visipaque	
500	32.9 ± 5.5	29.7 ± 4.0	11.3 ± 7.7	7.5 ± 1.2	
600	16.0 ± 2.8	16.0 ± 5.7	5.0 ± 1.0	5.0 ± 1.1	
700	9.2 <u>+</u> 0.3	10.5 ± 0.7	3.9 <u>+</u> 0.1	2.8 <u>+</u> 0.3	
800	7.2 <u>+</u> 0.2	9.5 ± 0.7	4.4 <u>+</u> 0.6	2.7 <u>+</u> 0.2	
900	6.5 ± 0.7	9.5 <u>+</u> 2.1	4.4 ± 0.6	3.8 <u>+</u> 0.8	

MRI agent diffusion coefficients measured in mouse skin ex vivo

Agent	MR		X-rav	
Trademark	Gadovist	Magnevist	Dotarem	Visipaque
$D_{\rm a}^{\rm tissue}$, cm ² /s	$(4.29 \pm 0.39) \times 10^{-7}$	$(5.00 \pm 0.72) \times 10^{-7}$	$(3.72 \pm 0.67) \times 10^{-7}$	$(1.64 \pm 0.18) \times 10^{-7}$
$D_{\rm a}^{\rm free}$, cm ² /s	3.9×10^{-6}	_	4.5×10^{-6}	—
Tortuosity, $l_{\rm d}/L$	3.0	_	3.5	_

□ Gadovist – is the most effective OCA

Fluorescence measurements at OC

Fluorescence intensity images of mouse cancer cells in vivo

D.K. Tuchina, I.G. Meerovich, O.A. Sindeeva, V. V. Zherdeva, A. P. Savitsky, A. A. Bogdanov Jr, V. V. Tuchin, Magnetic resonance contrast agents in optical clearing: Prospects for multimodal tissue imaging. J. Biophotonics **13**(11) 2020; e201960249. https://doi.org/10. 1002/jbio.201960249

20 days after tumor cell enucleation (HEp2-TagRFP line) in BALBc/nude mice

OCA: 70% glycerol, 5% DMSO, 25% water





Cassette with animal

Instrumentation and Protocol

*DCS-120 confocal scanning system (Becker & Hickl GmbH)

*WhiteLase SC480-10 supercontinuum laser with acousto-optic tunable filter AOTF-V1-D-FDS-SM (FIANIUM)

HPM-100-40 hybrid detector (Becker & Hickl GmbH)

*Fluorescence excitation wavelength was 540 nm

*Fluorescence emission from a tumor was collected through the skin in the epi-illumination configuration

*Long- and bandpass filters were used (HQ550LP and 580BP)

SPCImage 3.2 data analysis software (Becker & Hickl GmbH).

♦NIH ImageJ 1.48v software

Animals were anesthetized by Zoletil-Rometar mixture and were put in a cassette on a mobile stage
Optical clearing was performed for 15 min using a 70% glycerol, 5% DMSO, 25% water solution
Image collection time for anesthetized mouse varied from 3.5 to 8 min depending of fluorophore expression level

Profiles for fluorescence signal







Fluorescence intensity images of mouse cancer cells in vivo D.K. Tuchina, I.G. Meerovich, O.A. Sindeeva, V. V. Zherdeva, A. P. Savitsky, A. A. Bogdanov Jr, V. V. Tuchin, Magnetic resonance contrast agents in optical clearing:

Prospects for multimodal tissue imaging. J. Biophotonics 13(11) 2020; e201960249. https://doi.org/10. 1002/jbio.201960249

20 days after tumor cell enucleation (HEp2-TagRFP line) in BALBc / nude mice

OCA: Gadobutrol (GB) Gadovist[®]







Tag-RFP fluorescence and MRI of tumor mouse xenografts after an intravenous injection of GB

N.I. Kazachkina, V.V. Zherdeva, I.G. Meerovich, A.N. Saydasheva, I.D. Solovyev, D.K. Tuchina, A.P. Savitsky, V.V. Tuchin, A.A. Bogdanov Jr., "MR and fluorescence imaging of gadobutrol-induced optical clearing of red fluorescent protein signal in an in vivo cancer model," NMR in Biomedicine, e4708-1-13 (2022).



A: Time course of FI and T1w MRI SNR measured using whole tumor ROIs after an intravenous injection of GB (0.3 mmol/kg, n = 3). Data shown as mean \pm SD. Asterisks indicate that measured means are statistically significant from the baseline values (P < 0.01). B: Tag-RFP FI imaging before intravenous injection of GB.

- C: Tag-RFP FI imaging 4 min after intravenous injection of GB
- D: Single sagittal MR slice, T1w GRE MRI of the tumor before intravenous injection of GB.
- E: Matching single sagittal slice, T1w GRE MRI of the tumor 9 min after intravenous injection of GB; bar, 1 mm

The effectiveness of human gingival tissue OC and therapy

A.A. Selifonov and V.V. Tuchin, Control of the optical properties of gums and dentin tissue of a human tooth at laser spectral lines in the range of 200 – 800 nm, Quantum Electronics, 50 (1), 47-54 (2020)

I. Carneiro, S. Carvalho, R. Henrique, A. Selifonov, L. Oliveira, V.V. Tuchin, Enhanced ultraviolet spectroscopy by optical clearing for biomedical applications, *IEEE Journal of Selected Topics in Quantum Electronics* **27** (4), 7200108-1-8 (2021)



OCA: 99.7% glycerol 3 60 min 40 min 2.5 30 min 20 min 2 $10 \min$ % 1.5 L 5 min 1 min 0 min 0.5 0 200 300 400 λ , nm



The effective diffusion coefficient in human gum mucous tissue measured *in vitro*: $D(30/70/0) = (2.3\pm0.4) \cdot 10^{-6} \text{ cm}^2/\text{s}$ $D(50/50/0) = (2.6\pm0.6) \cdot 10^{-6} \text{ cm}^2/\text{s}$ $D(55/35/10) = (3.2\pm0.8) \cdot 10^{-6} \text{ cm}^2/\text{s}$

A.A. Selifonov – PhD thesis on study of biophysics of control optical properties of biological tissue to optimize phototherapy for oral cavity diseases

The UV treatment of 120 patients with chronic stomatitis in children's clinic No. 3 in Saratov showed high efficiency for 4-6 procedures





The effectiveness of human gum OC in propylene glycol / glycerol / water mixture E-cigarette vapor liquid A.B. Bucharskaya, et al., Optical clearing and testing of lung tissue using inhalation aerosols: prospects for monitoring the action of viral infections, *Biophysical Reviews* (2022).



L.R. Oliveira, R.M. Ferreira, M.R. Pinheiro, H.F. Silva, V.V. Tuchin, L.M. Oliveira, Broadband spectral verification of optical clearing reversibility in lung tissue, *J. Biophotonics* (August. 2022)









L.R. Oliveira, R.M. Ferreira, M.R. Pinheiro, H.F. Silva, V.V. Tuchin, L.M. Oliveira, Broadband spectral verification of optical clearing reversibility in lung tissue, *J. Biophotonics* (August. 2022)





In vivo immersion optical clearing of adipose tissue Motivation: Conventional or Laser Surgery, to see and avoid dissection of blood vessels

I. Y. Yanina, et al., "Immersion optical clearing of adipose tissue in rats: ex vivo and in vivo studies," J. Biophotonics e202100393 (2022)



1 FEW: 50% Fructose, 30% Ethanol, 20% Water (*n*=1.408) 2 DDN: 21% Diatrizoic acid, 66% DMSO, 13% N-methyl-glucamine (*n*=1.511)

MDN: 30% Metrizoic acid, 58% DMSO, 4 N-methyl-glucamine (*n*=1.529)

SUD: 60% Sucrose, 40% DMSO (*n*=1.509)

Detection of Melanoma Cells in Whole Blood Samples Using Spectral Imaging and Optical Clearing

Polina A. Dyachenko[®], Leonid E. Dolotov, Ekaterina N. Lazareva, Anastasia A. Kozlova, Olga A. Inozemtseva, Roman A. Verkhovskii, Galina A. Afanaseva, Natalia A. Shushunova, Valery V. Tuchin[®], Ekaterina I. Galanzha, and Vladimir P. Zharov



7200711

Principle of spectral absorbance measurement of a whole blood sample at immersion OC



-Before OC

After OC

680

400 450 500 550 600 650 700 750 800 850

Wavelength, nm



Scheme of experimental setup for spectral study: 3 is the color video camera DCC1645C, 4 is the monochrome camera DCC1545M, 5 is the spectrometer USB4000, 6 is the illumination unit KL-1500Z, 7 is the blood slide



_ Saline

Omnipaque

Images of slides of thickness 120 μ m with a whole blood (3 μ l) of a laboratory mouse and mouse melanoma cells (B16F10) (1 μ l) mixed with 4 μ l of saline (upper image) or Omnipaque (lower image)

60

50

40

30 20 10

Amelanin/Ablood



a.u.







Increasing the penetration depth for ultrafast laser tissue ablation using Glycerol based optical clearing

Ilan Gabay^a, Kaushik G. Subramanian^a, Chris Martin^a, Murat Yildirim^a, Valery V. Tuchin^{c,d,e}, and Adela Ben-Yakar^{a,b}

- ^a Department of Mechanical Engineering, The University of Texas at Austin, Austin, TX, USA
- ^b Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX, USA ^c Research-Education Institute of Optics and Biophotonics, NG Chernyshevsky Saratov National Research State University, Russian Federation
- ^d Interdisciplinary Laboratory of Biophotonics, Tomsk National Research State Universit ^e Laboratory of Laser Diagnostics of Technical and Living Systems, Institute of Precise N
- **Russian Federation**



Scarred Vocal Fold affecting 2-6 M Americans *Overuse Surgery Scar affect the elasticity of the collagenous layer – Lamina Propria (LP) *The scar might extend hundreds of microns deep *Treatment: Injection of biomaterial to subsurface voids created by ultrashort laser pulses

Handheld, flexible device



Y. Alexandrovskaya et al., Controlling the near infrared transparency of costal cartilage by impregnation with clearing agents and magnetite nanoparticles, *J. Biophotonics* **11** (2), e201700105 (2018)

31

The role of optical clearing to enhance the applications of in vivo OCT and photodynamic therapy: Towards PDT of pigmented melanomas and beyond

Layla Pires, Michelle Barreto Requena, Valentin Demidov, Ana Gabriela Salvio, I. Alex Vitkin, Brian C. Wilson, and Cristina Kurachi



OCT imaging of melanoma before and after OC (t = 4 h). Murine B16- F10 tumors in nude mice. Tissue microvasculature images were obtained in speckle-variance OCT (green = top tissue layers, black = deepest). Scale bars are 1 mm.

L. Pires, *et al.*, *Cancers* **12**(7), 1956 (2020). L.P. Martinelli, *et al.*, *Biomedical Optics Express* **11**(11), 6516–6527 (2020).

The effect of OC on the PDT outcome in melanotic melanoma



Top row: Histology images (H&E and Ki-67 marker staining viable melanoma cells).

Middle row: Tumor cell counts at t = 10 days post-PDT using either the single vascular-targeted photosensitizer Visudyne (VIZ) or tumor cell-targeted photosensitizer Photodithazine (PDZ) or both (means ± SD: n = 12).

Bottom row: Raman

microspectroscopy shows that PDZ - mediated PDT damaged the tumor up to ${\sim}125~\mu m$ depth as with OC PDT effectiveness of up to ${\sim}700~\mu m$ depth is observed.

Overview of effects of *in vivo* skin optical clearing on light-induced therapy



(a) Thermal response of blood vessels without or with optical clearing when irradiated with different numbers of laser pulses



and Ki67-labeled tumor slices obtained 14 days after synergistic treatment of **photothermo-chemotherapy** in a deepsited tumor model without or with OC

(c) Three pigmentation areas on rat dorsal skin before and after treatments: area 1: laser irradiation after OC

area 2: only laser irradiation after OC area 3: without treatment



Optical Clearing of Tissues and Blood



Valery V. Tuchin

SPIE.

TISSUE OPTICS

Light Scattering Methods and Instruments for Medical Diagnostics THIRD EDITION



To read:

New book

SPRINGER BRIEFS IN PRYSICS

Luis Manuel Couto Oliveira Valery Victorovich Tuchin

The Optical Clearing Method A New Tool for Clinical Practice and Biomedical Engineering

Springer

HANDBOOK OF TISSUE OPTICAL CLEARING

New Prospects in Optical Imaging



edited by Valery Tuchin Dan Zhu Elina A. Genina



Valery V. Tuchin Jürgen Popp Valery Zakharov *Editors*

Multimodal Optical Diagnostics of Cancer

🖄 Springer



Conclusion

- Optical clearing technology is beneficial for enhanced multimodal spectroscopy/imaging and PDT/PTT treatment
- The efficiency of medical lasers working on selected wavelengths in a wide wavelength range from deep-UV to IR range can be improved significantly due to this technology

Acknowledgements

SSU

Elina Genina, Irina Yanina, Polina Dyachenko, Daria Tuchina, Alexey Selifonov, Vadim Genin, Ekaterina Lazareva

National and international collaborators

Alla Bucharskaya et al., Saratov State Medical Univer. Nikolai Khlebtsov et al., Inst. of Biochemistry and Physiology of Plants and Microorganisms of the RAS Yulia Alexandrovskaya et al. Inst. Photon Technologies of the RAS Yury Kistenev, et al. Tomsk State University Dan Zhu, Qingming Luo et al. HUST Univ., Hainan Univ. China Luis Oliveira et al. ISEP-IPP, Porto, Portugal Alexander P. Savitsky, et al., FRC of Biotechnology, RAS Alexei A. Bogdanov Jr, UMass, USA Viacheslav Artyushenko, artphotonics gmbH, Germany

























Russian Science Foundation grants 23-14-00287

Editorial Board

Welcome to JBPE

Editor-In-Chief Valery V. Tuchin

Saratov State University, Institute of Precision Mechanics and Control RAS, Russia, University of Oulu, Finland

Deputy Editor-In-Chief Valery P. Zakharov Samara State Aerospace University, Russia

Editorial Board

Stefan Andersson-Engels - Lund University, Sweden Alexey N. Bashkatov - Saratov State University, Russia Elina A. Genina - Saratov State University, Russia Ekaterina Borisova - Academician Emil Djakov Institute of Electronics, Bulgarian Academy of Sciences, Bulgaria Wei Chen - University of Central Oklahoma, USA Arthur Chiou - National Yang-Mind University, Taiwan Min Gu - Swinburne University, Australia Nikolay G. Khlebtsov - Institute of Biochemistry and Physiology of Plants and Microorganisms RAS, Russia Juergen Lademann - Charite University Clinic, Germany Kirill V. Larin - University of Houston, USA Martin Leahy - University of Galway, Ireland Qingming Luo - Huazhong University of Science and Technology, China Stephen J Matcher - University of Sheffield, Great Britain

Igor Meglinski – University of Ótago, New Zealand Vladimir S. Pavelyev - Image Processing Systems Institute RAS, Russia

Francesco Pavone - University of Florence, Italy Roberto Pini - Institute of Applied Physics, Italy Igor A. Platonov - Samara State Aerospace University, Russia Juergen Popp - Institute of Photonic Technology, Germany Alexander V. Priezzhev - Moscow State University, Russia David Sampson - The University of Western Australia, Australia Alexander Savitsky - Institute of Biochemistry RAS, Russia Alexander P. Shkurinov - Moscow State University, Russia Peter So - Massachusetts Institute of Technologies, USA Ilya V.Turchin - Institute of Applied Physics RAS, Russia Vladimir G. Volostnikov - N.P.Lebedev Physical Institute, Russia Lihong V. Wang - Washington University, USA Ruikang Wang - University of Washington, USA Xunbin Wei - Shanghai Jiao Tong University, China Dan Zhu - Huazhong University of Science and Technology, China

I am very pleased to introduce the Journal of Biomedical Photonics & Engineering (JBPE). This new, online-only, open-access journal, published quarterly, is aimed at the rapid dissemination of high-impact results in all areas of biomedical engineering and photonics, from fundamental studies to applied technology.

JBPE will publish original research letters (3-4 pages), research articles (6–12 pages), and reviews (12–20 pages), as well as special issues. All submissions will undergo rigorous reviewing in order to ensure high-quality publications. Papers will be refereed by at least 2 experts as suggested by the Editorial Board. The accepted manuscripts will be published online first in the "Online Ready" section before the whole issue is available.

The Editorial Board is comprised of a fantastic group of renowned researchers and has a diversity of expertise that covers all areas of biophotonics and biomedical engineering. They will work hard to ensure that papers are given careful and quick consideration to maintain the spirit of rapid dissemination.

Starting a journal with such lofty goals is challenging. I am highly encouraged by superb articles that have already been submitted. I wish to express my gratitude to many individuals who have contributed to the successful startup of the JBPE. I thank all members of the Editorial Board for their efforts in soliciting manuscripts and seeing them through the review process.

Many strategic aspects of JBPE were developed in the course of extensive discussions that I had with many colleagues, and I continue to welcome your thoughts and suggestions on how we can further improve the journal.

Valery V. Tuchin Editor-in-Chief Journal of Biomedical Photonics and Engineering



The Samara State Aerospace University publishing

presents an Open Access

Issue 1, 2014

SSAU

Journal of Biomedical Photonics & Engineering Editor-In-Chief Valery V. Tuchin

Journal of Biomedical Photonics & Engineering (JBPE)

http://journals.ssau.ru/JBPE

Welcome to SEVE24 September 23 – 27, 2024

https://sfmconference.org/