Spectroscopy and imaging of biological tissues at optical clearing

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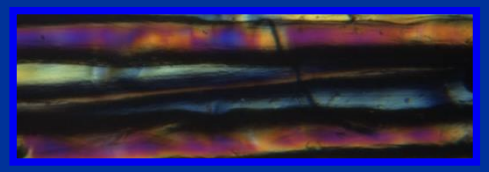
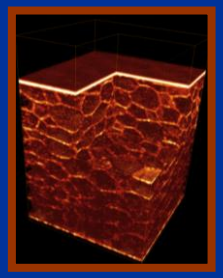
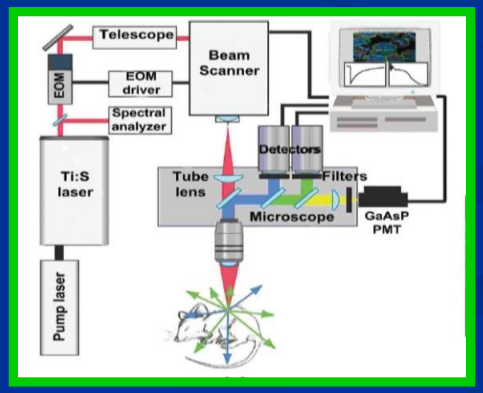
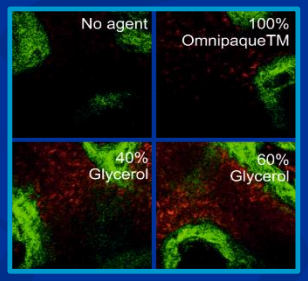
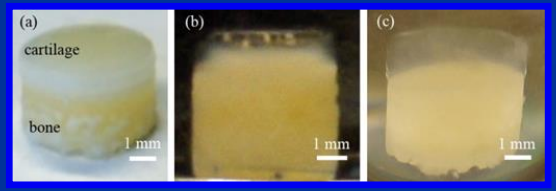
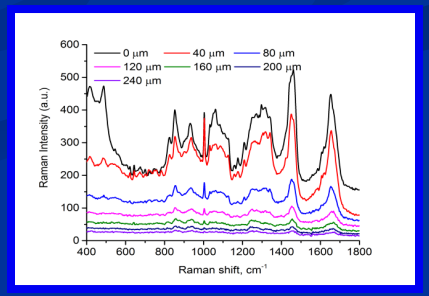












Saratov National Research State University

SSU has about 100 Departments with 23,000 students, 1700 teachers and researchers including more than 200 Full Professors, and 850 Associate Professors

Biophotonics
Research/Developments/Educational Programs

- Chair and Research-Educational Institute of Optics and Biophotonics
- International Research-Educational Center of Optical Technologies in Industry and Medicine “Photonics”

70 research and faculty members
10 Full Professors
30 PhD and MD

Outline

- Motivation
- Fundamentals of optical clearing (OC)
- Optical clearing agents (OCAs)
- Quantification of optical properties
- Collimated transmittance, free and bound water
- OC of pathological tissues
- OCT
- Speckle dynamic microscopy
- Photoacoustic microscopy and flow cytometry
- Nonlinear optical imaging
- Confocal Raman spectroscopy of skin
- Nanoparticle labeling
- Summary

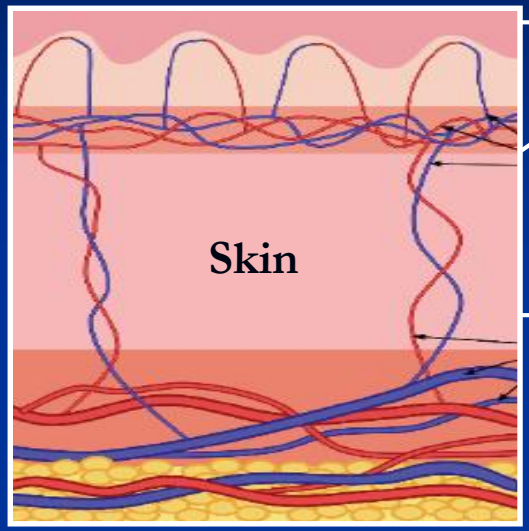
Motivation

Challenges in Optical Imaging

Soft limit ~ δ

1 mm

$$\delta = 1/\sqrt{3\mu_a(\mu_a + \mu_s')}$$



OM, SNOM

CFM, 2PM, SHM, etc.

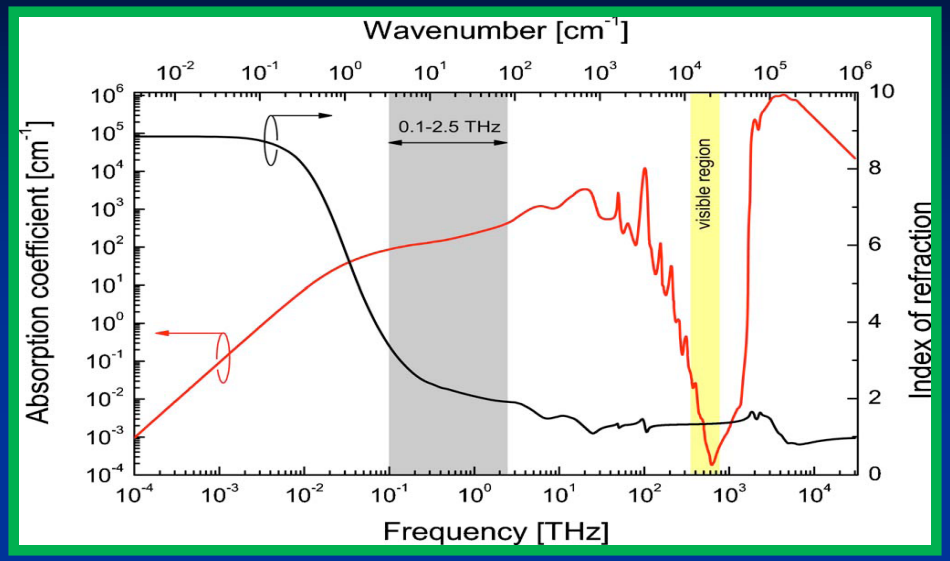
OCT

DOT,
UOT, PAT

Hard limit ~10 δ

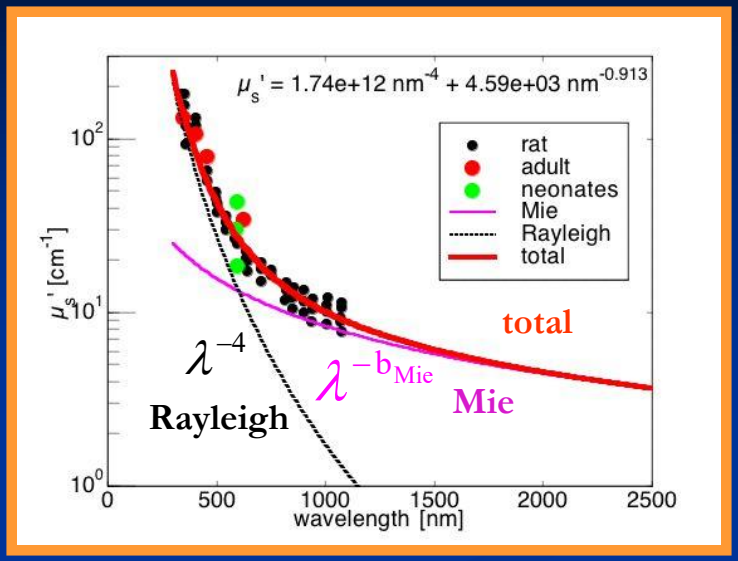
OM: Optical microscopy
SNOM: Scanning near-field optical microscopy
CFM: Confocal microscopy
2PM: Two-photon microscopy
SHM: Second harmonic microscopy
OCT: Optical coherence tomography
DOT: Diffuse optical tomography
UOT: Ultrasound-modulated optical tomography
PAT: Photoacoustic tomography

Tissue Optics: absorption & scattering



Absorption and dispersion of water

Møller et al., J. Opt. Soc. Am. B, 26 (9), 2009



Reduced scattering coefficient of skin

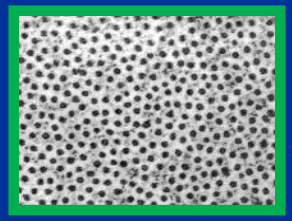
Steven L. Jacques, Ulm, LALS-2014

OCT : Examples of clear and turbid (scattering) tissues

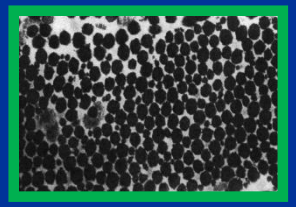
M.G. Ghosn, V. V Tuchin, K. V. Larin, IOVS, 2007

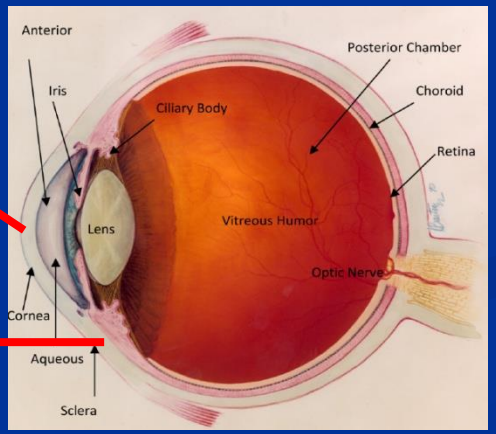
EMI :

Cornea

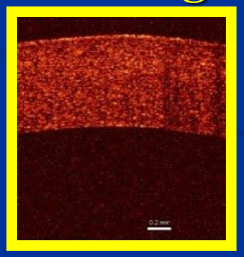


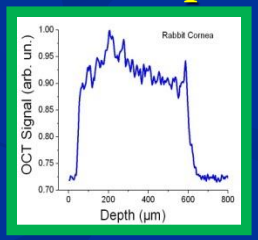
Sclera

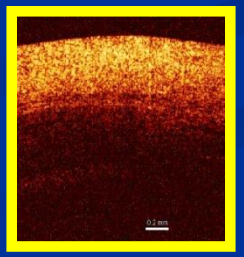


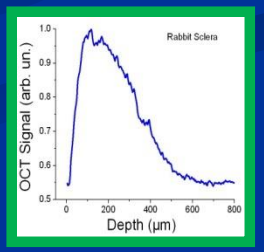


OCT images and in-depth signal

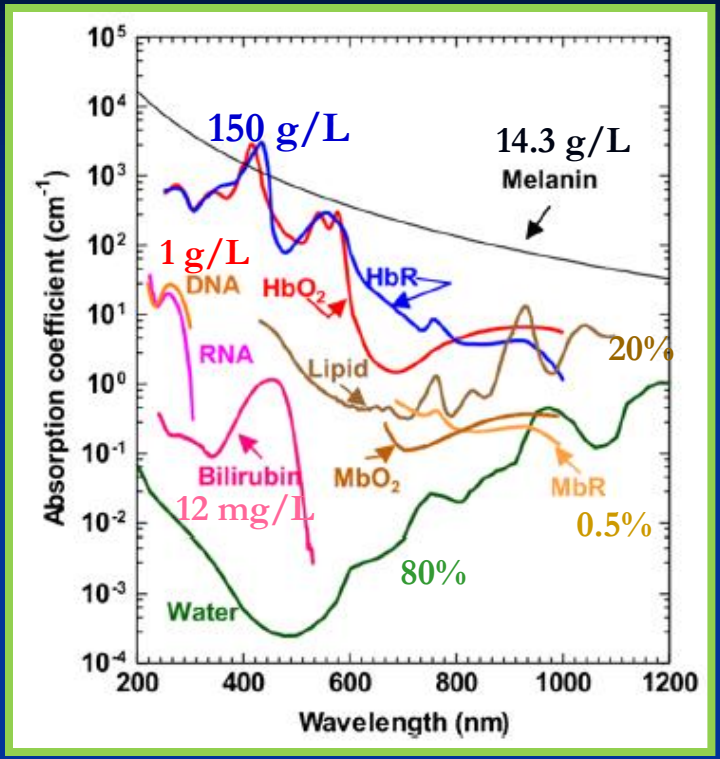




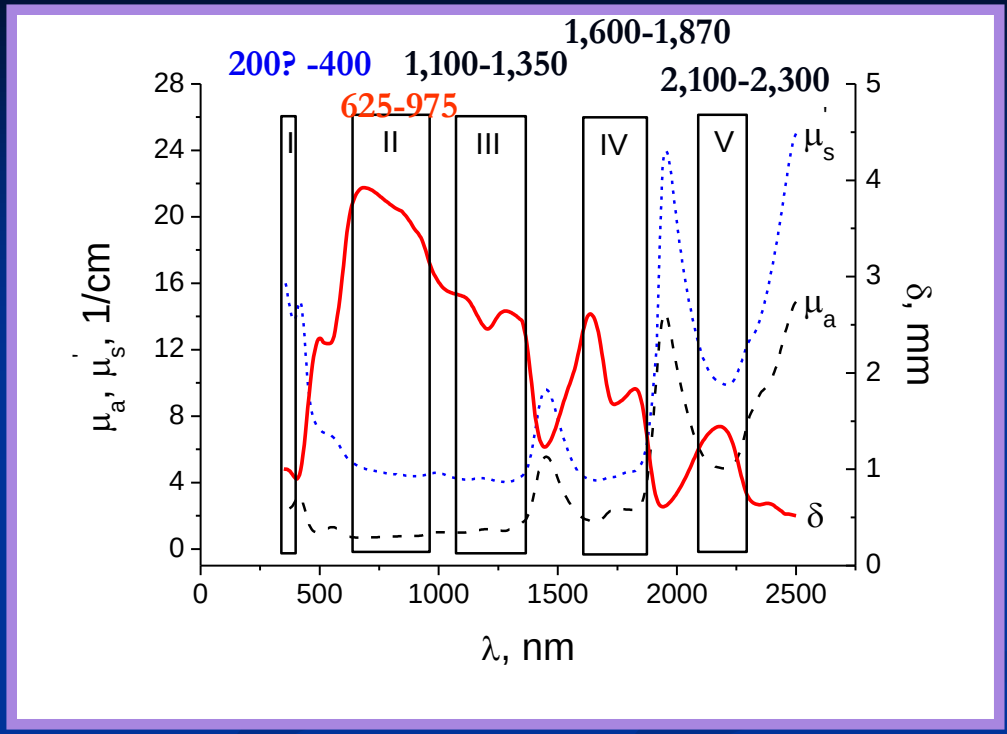




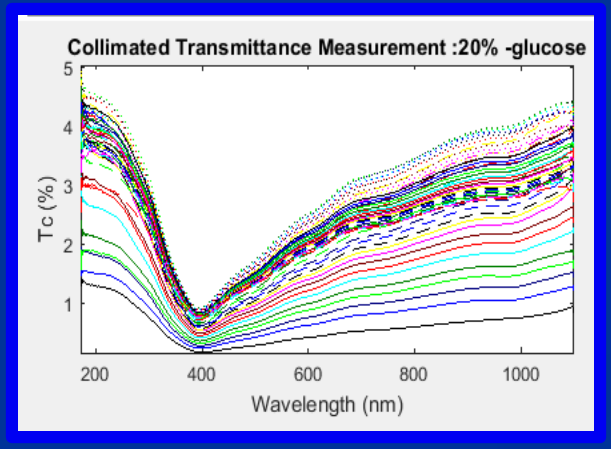
Tissue 'optical windows'



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



Experimental data for rat muscle treatment with 20% glucose solution

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

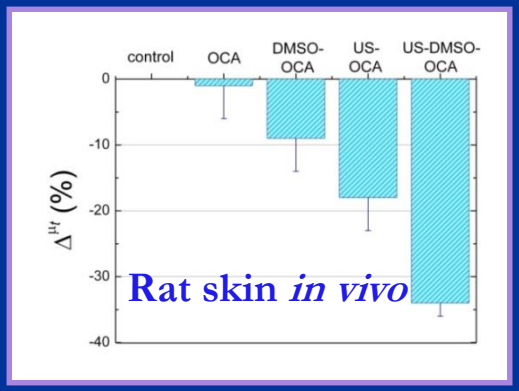
E.A. Genina et al.: Optical clearing of biological tissues: prospects of application in medical diagnostics and phototherapy [Review]

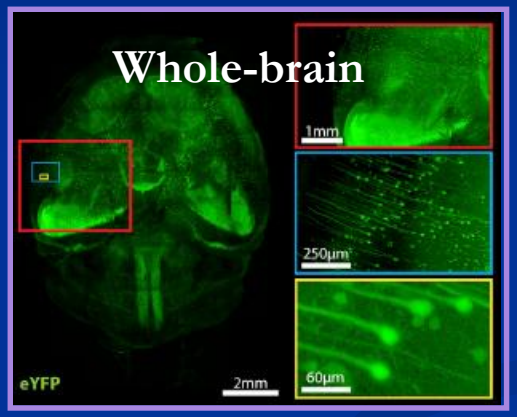
J. Biomed. Photon. Eng., 1(1), 22-58, 2015

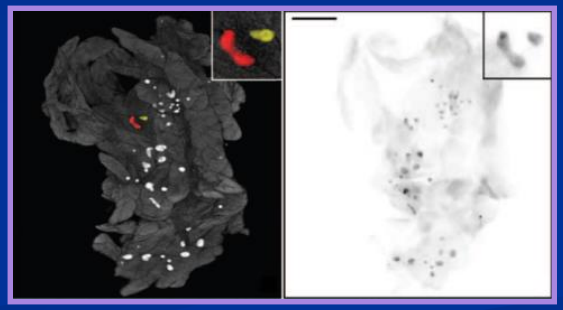
Optical clearing of biological tissues: prospects of application in medical diagnostics and phototherapy

E.A. Genina[1,2*], A.N. Bashkatov[1,2], Yu.P. Sinichkin[1], I.Yu. Yanina[3], V.V.Tuchin[1,2,4]

[1] N.G. Chernyshevsky Saratov State University, 83 Astrakhanskaya Str., Saratov, 410012, Russia





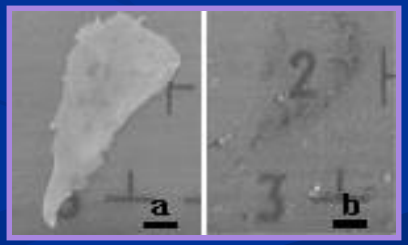


Pancreas

Scleral tissue optical clearing:

The action of *propylene glycol* on the human eye ball - dropping protocol

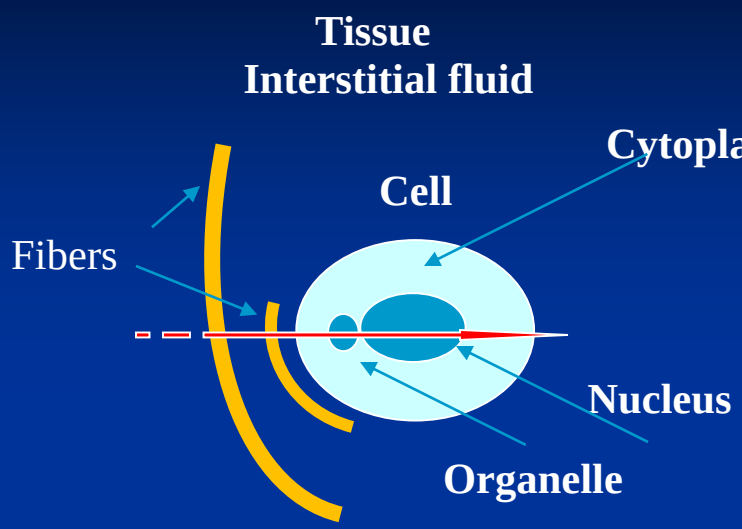
0 min **5 min** **30 min**

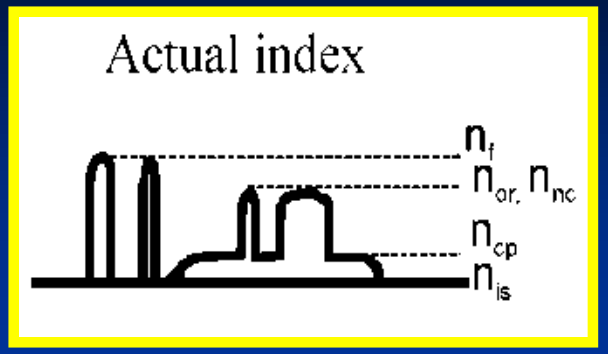


Rabbit *dura mater*, 1-min in glycerol

- Fundamentals of optical clearing

Immersion optical clearing method





$$\mu_s' = \mu_s(1-g) \sim d^2\rho(d/\lambda)^{0.37}(m-1)^2$$

$$m \equiv n_s/n_0$$

⇒ **Refractive index matching of tissue/cell components and interstitial fluid (ISF)/cytoplasm due to:**

1) agent diffusion into the tissue/cell

2) tissue/cell dehydration caused by osmotic action of an optical clearing agent (OCA)

⇒**Tissue shrinkage: less thickness and better ordering of collagen fibers with volume fraction $f_c(t)$ caused by temporal/reversible dehydration**

$$\mu_s' = \mu_s(1-g) \sim [1-f_c(t)]^3/[1+f_c(t)]$$

⇒ **Disruption of the hydration shell collagen (hydrodynamic radius)**

V.V. Tuchin, *Tissue Optics*, 3rd edition, SPIE Press, **PM 254**, 2015

Optical clearing agents (OCAs)

In vitro measured optical clearing potential (OCP) at OCA application to dermis side of human skin using a Franz diffusion chamber

B. Choi et al. "Determination of chemical agent optical clearing potential using *in vitro* human skin," *Lasers Surg. Med.* 36, 72-75, 2005

Hydroxy-terminated chemical agent	Refractive index	Osmolality (mOsm/kg)	OCP
Glycerol	1.47	14,550	2.9±0.8
50% TMP (trimethylolpropanol)	1.43	6,830	2.2±0.3
100% TMP	1.47	13,660	2.1±0.7
1,3-butanediol	1.44	22,050	2.4±0.7
1,4-butanediol	1.44	26,900	2.8±0.5
Ethylene glycol	1.43	22,640	1.9±0.6
MPDiol glycol (1,3-diol, 2-methyl-propane)	1.44	23,460	2.3±0.2
P-0062[1]	1.48	1,643	2.0±0.5

[1] P-0062 is a polyethylene glycol based prepolymer (Univ. of California, Irvine)

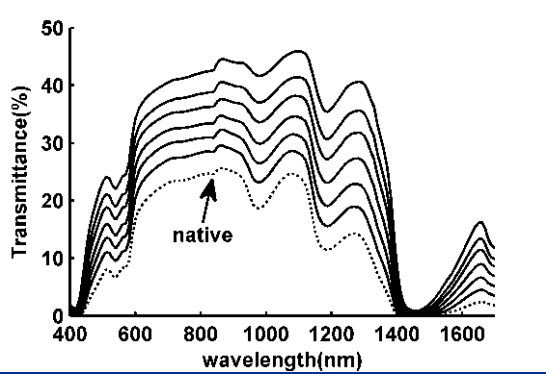
$OCP \equiv \mu_s'(\text{before})/\mu_s'(\text{in 20 min after application})$

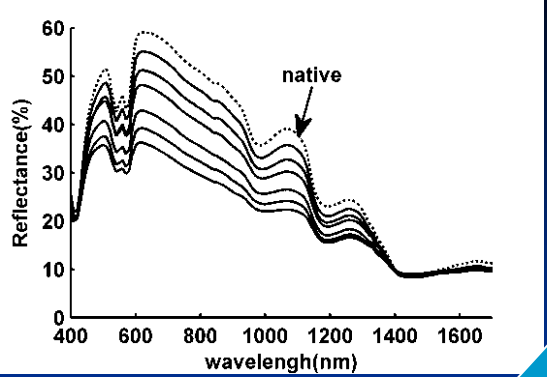
Saccharides: glucose, sucrose, maltose, fructose

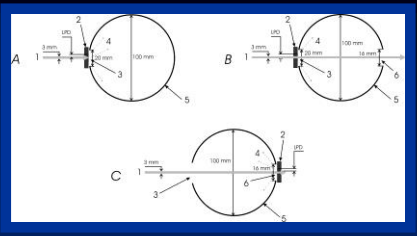
PEGs, Propylene glycol (1,2-propanediol), DMSO, et. al

Iodine based non-ionic contrast media have lower osmolarity and tend to have less side-effects: Omnipaque, Ultravist, Visipaque, etc.

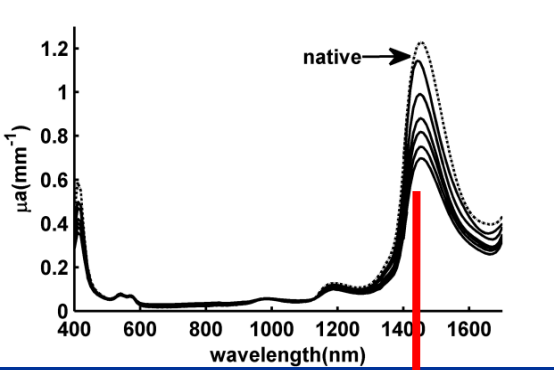
Quantification of optical properties

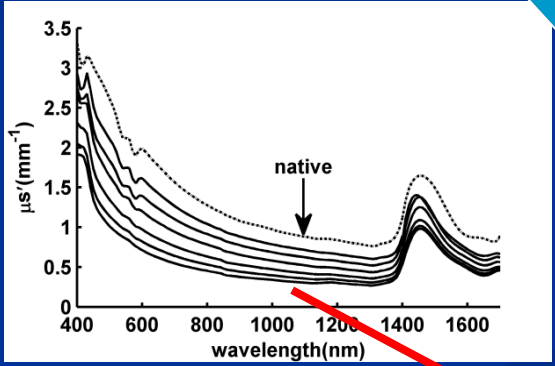




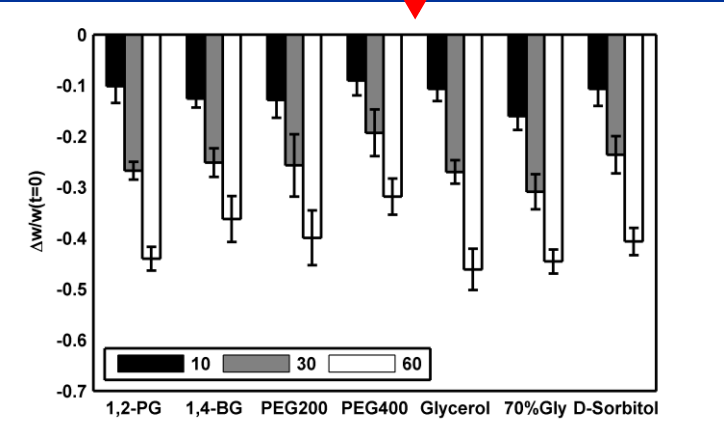


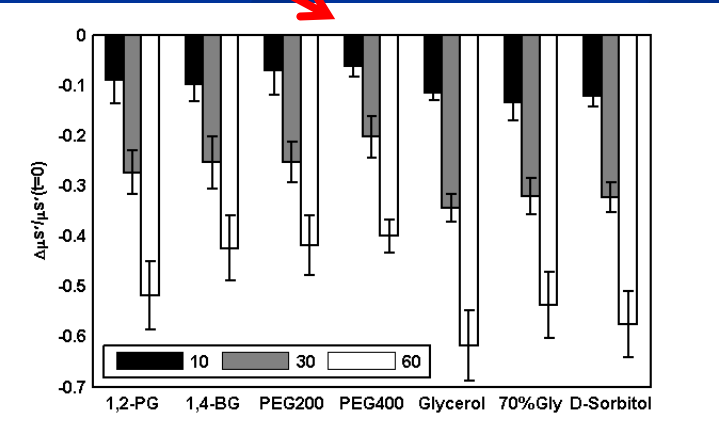
T. Yu, X. Wen, V.V .Tuchin, Q. Luo, D. Zhu, *J. Biomed. Opt.* 16 (2011)





Pig skin sample before and after treatment by 1,2-propanediol at time intervals of 0 (native), 10, 20, 30, 40, 50 and 60 min





Dehydration

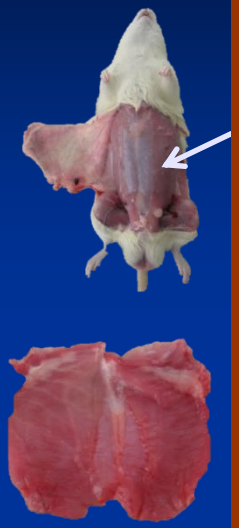
Optical clearing

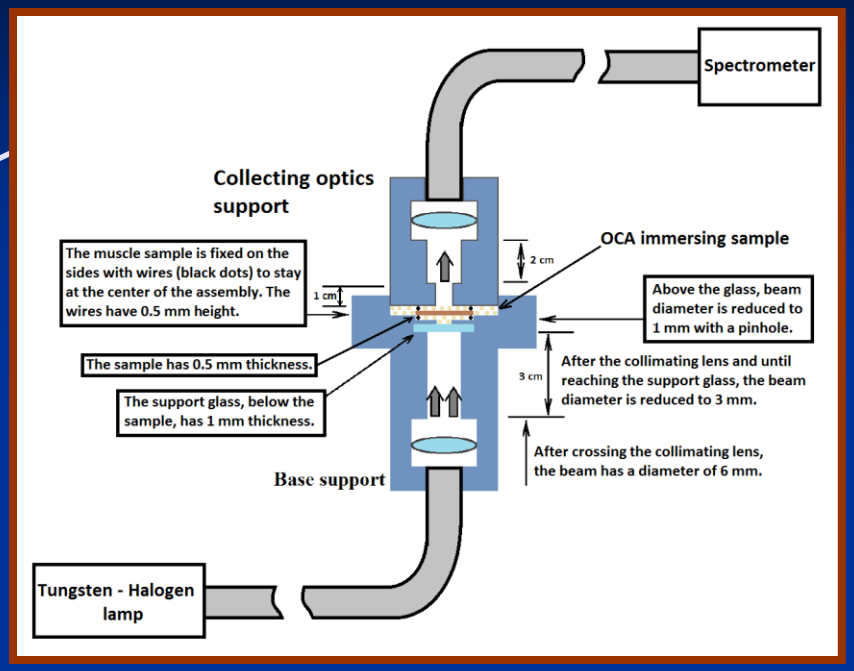
- Collimated transmittance, free and bound water

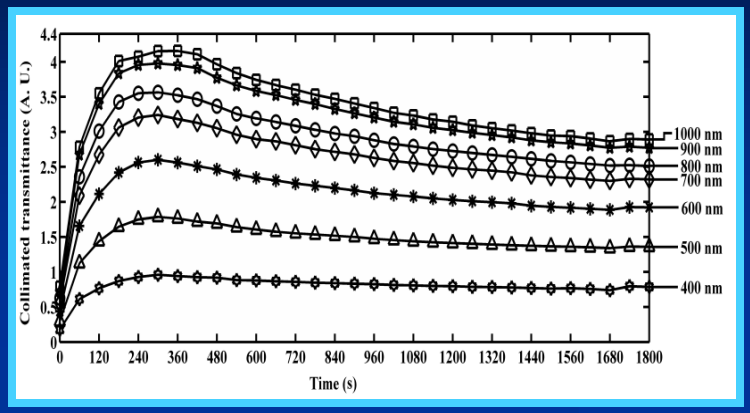
$T_c(\lambda) = 1 - \exp\left(-\frac{t}{\tau}\right)$

Time dependence for collimated transmittance for the rat muscle treatment with glucose

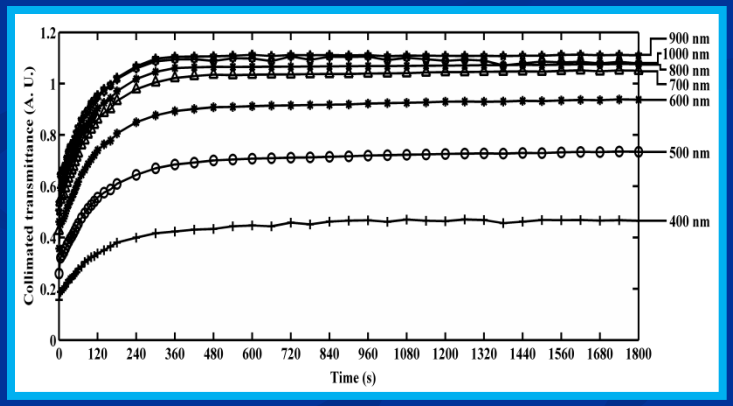
(L.Oliveira, M.I. Carvalho, E. Nogueira, V. V. Tuchin *JIOHS*, Vol. 6, No. 2, 1350012, 2013; JBO, 2015)



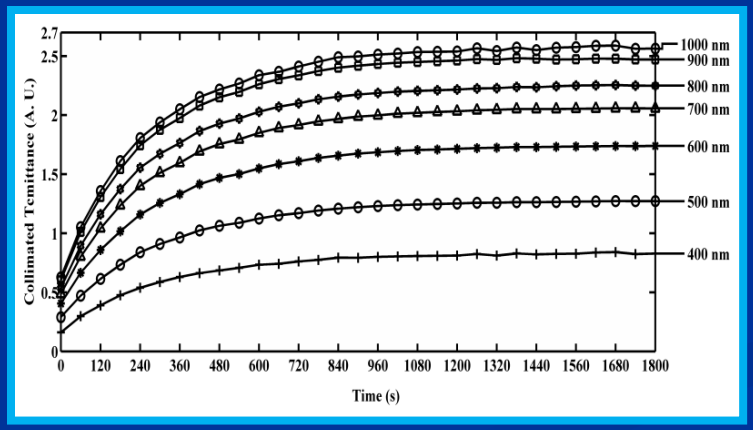




Glucose 20%



Glucose 50%



Glucose 40%

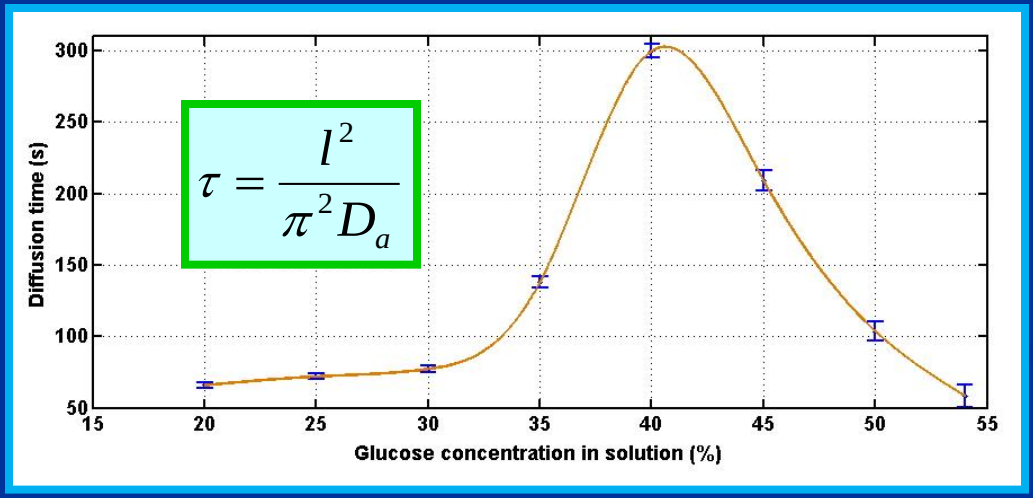
$$T_c(\lambda) \propto 1 - \exp\left(-\frac{t}{\tau}\right)$$

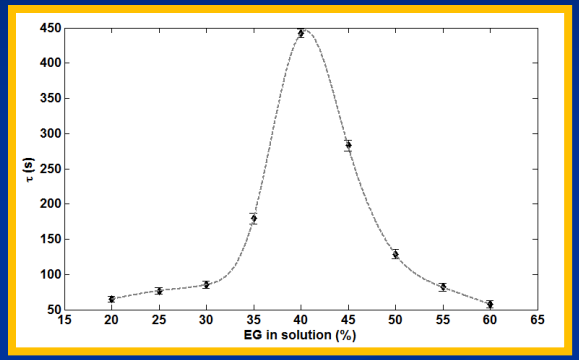
Diffusion time of Glucose and Ethylene Glycol for rat muscle on concentration in solution.

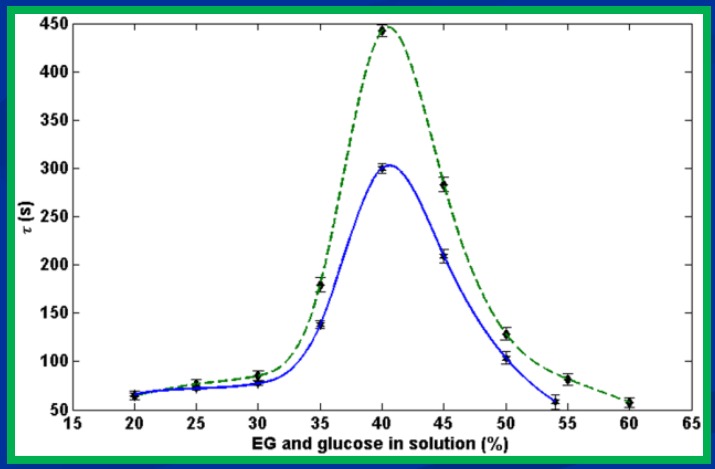
(L.Oliveira, M.I. Carvalho, E. Nogueira, V. V. Tuchin, *Laser Physics*, 2013, JBO, 2015)

$$D_a = \frac{l^2}{\pi^2 \tau} = \frac{0.05^2}{\pi^2 \times 302.9} = 8.36 \times 10^{-7}\ cm^2 / s$$

$$D_{EG} = \frac{d^2}{\pi^2 \tau_{EG}} = \frac{0.05^2}{\pi^2 \times 446.0} = 5.68 \times 10^{-7}\ cm^2 / s$$







$$f_{solid\ part} = 0.244$$

$$f_{water\ \ natural} = f_{bound\ water} + f_{free\ water} = 0.161 + 0.595 = 0.756$$

- OC of pathological tissues

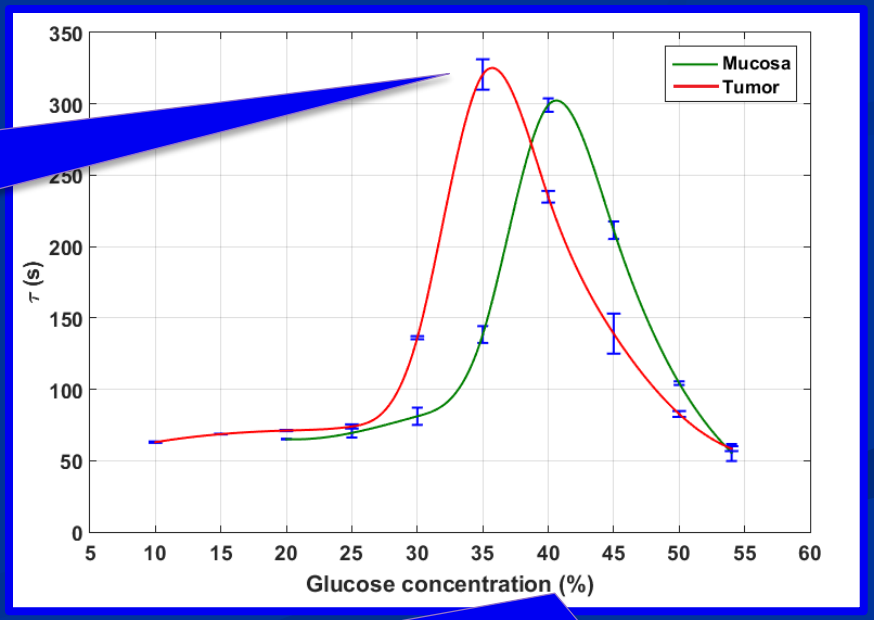
Diffusion time for healthy colon mucosa and tumor

Tissue type	Mucosa									
OCA concentration	10%	15%	20%	25%	30%	35%	40%	45%	50%	54%
Diffusion time , s			65.1	69.4	81.1	138.4	299.2	211.5	104.3	55.7
SD			0.2	3.2	6.1	5.9	4.7	6.1	1.3	5.9
Tissue type	Tumor									
Diffusion time , s	62.9	68.6	71.1	73.9	136.1	320.6	234.9	139.0	82.7	58.4
SD	0.5	0.2	0.5	1.5	1.1	10.6	4.1	14.0	2.0	1.7

(L. Oliveira *et al*, Glucose diffusion in colon mucosa – a comparative study between healthy and cancerous tissue, ALT-**16)**

Tumor has ~5% more of free-water content than mucosa

Glucose takes more time to diffuse into tumor than into mucosa



(Software from: P. Peixoto, L. Oliveira, M. I. Carvalho, E. Nogueira, and V.V. Tuchin, Software development for estimation of optical clearing agent's diffusion coefficients in biological tissues, *J. Biomed. Photonics & Eng* **1**(4) 255, 2016**)**

Normal mucosa shows similar results to muscle tissue (Oliveira *et al*, 2013)

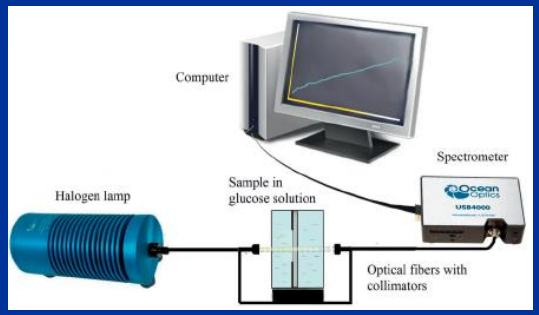
J. Biophotonics 1–15 (2015) / **DOI** 10.1002/jbio.201400138

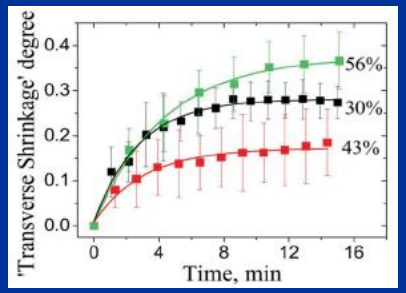
Journal of BIOPHOTONICS

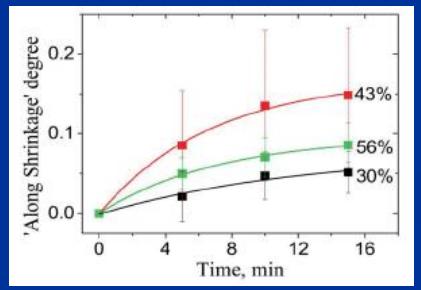
FULL ARTICLE

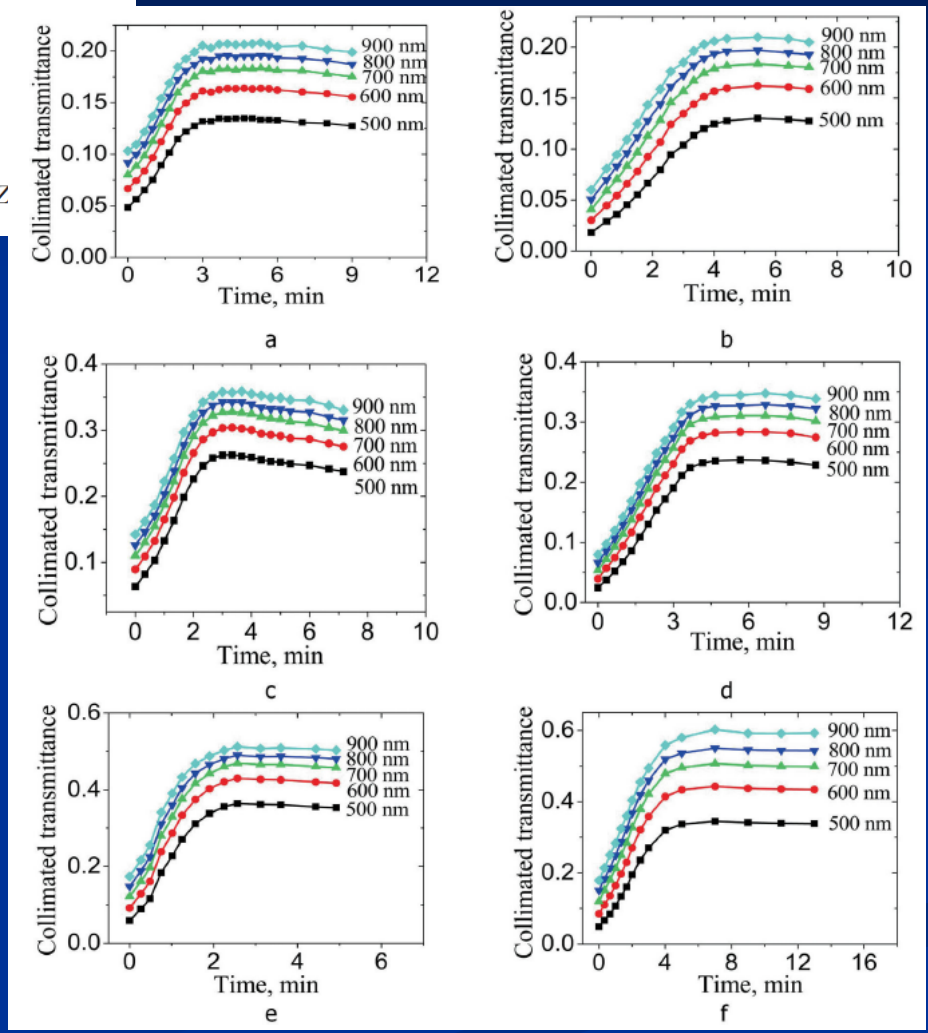
Ex vivo optical measurements of glucose diffusion kinetics in native and diabetic mouse skin

Daria K. Tuchina[*, 1, 2], *Rui Shi*[1], *Alexey N. Bashkatov*[2, 3], *Elina A. Genina*[2, 3], *Dan Zhu*[1], *Qingming Luo*[1], *and Valery V. Tuchin*[1, 2, 3]



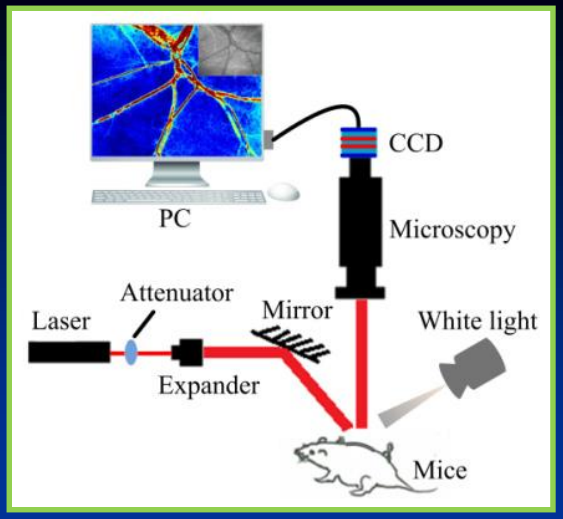






Clearing efficiency was 1.5-fold better and glucose diffusivity was 2-fold slower for diabetic skin

Speckle dynamic microscopy



Speckle contrast imaging microscopy

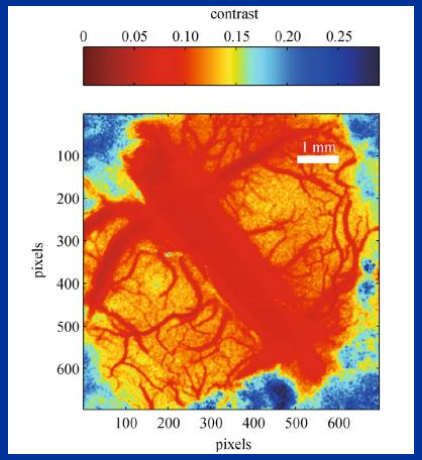
$$K = \sigma / \langle I \rangle \sim 1 / \langle V \rangle,$$

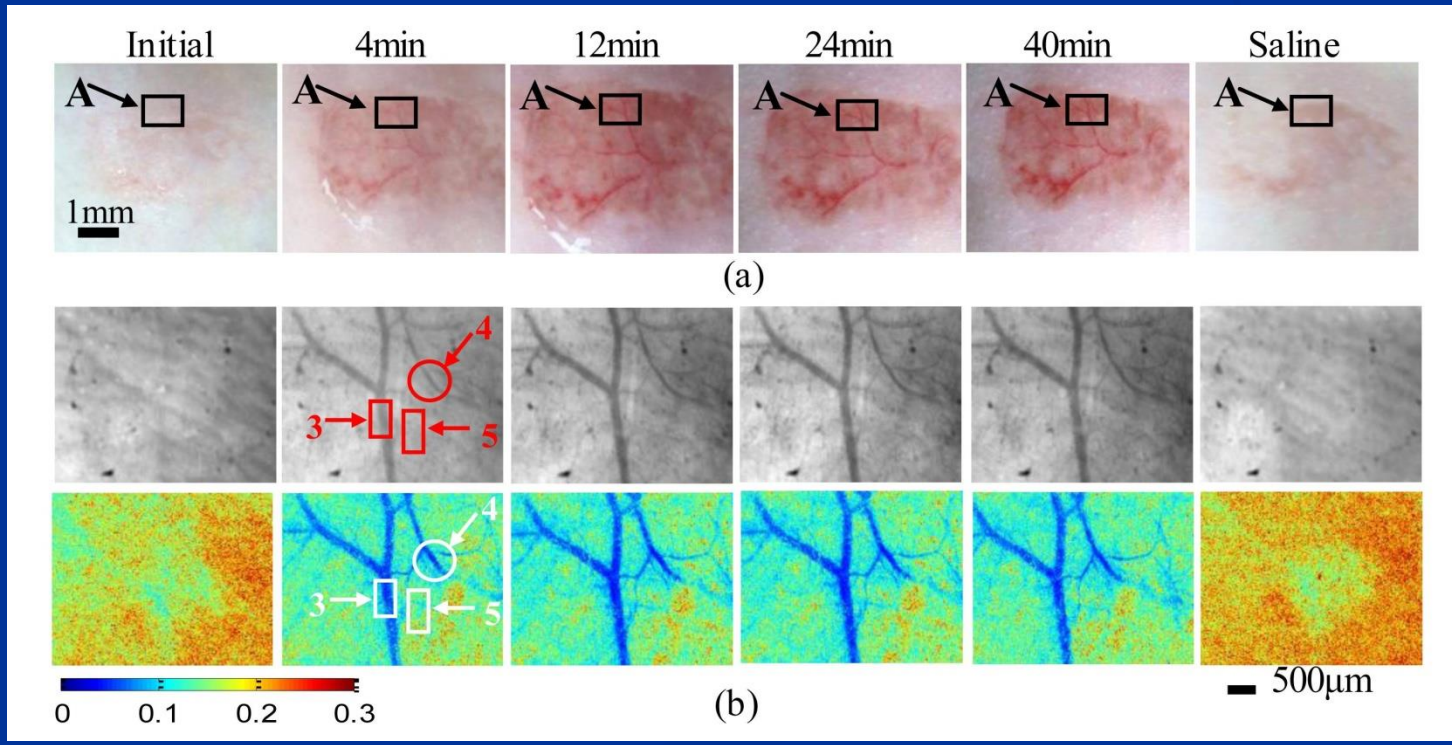
σ is the standard deviation of the intensity fluctuations

$\langle I \rangle$ is the mean intensity, and $\langle V \rangle$ is the mean velocity

Blood vessel visibility at topical treatment of rat skin *in vivo* by a mixture of PEG-400 and thiazone

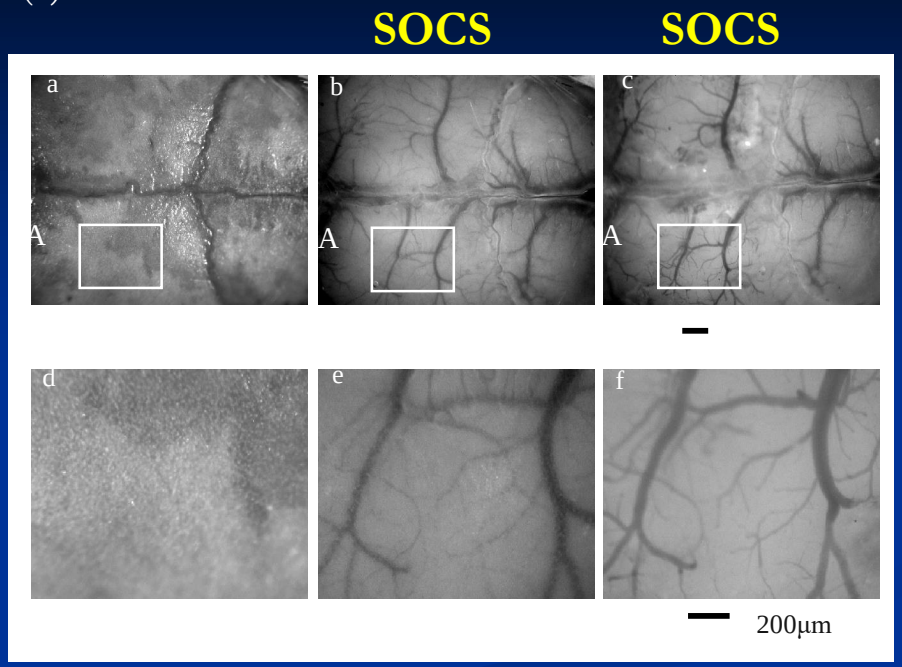
Zhu D., et al. *J. Biomed. Opt.* 15(2), 026008 (2010)





J. Wang， Y. Zhang，T. Xu，Q. Luo，D. Zhu，“An innovative transparent cranial window based on skull optical clearing，” Laser Phys. Lett., 9(5): 2012

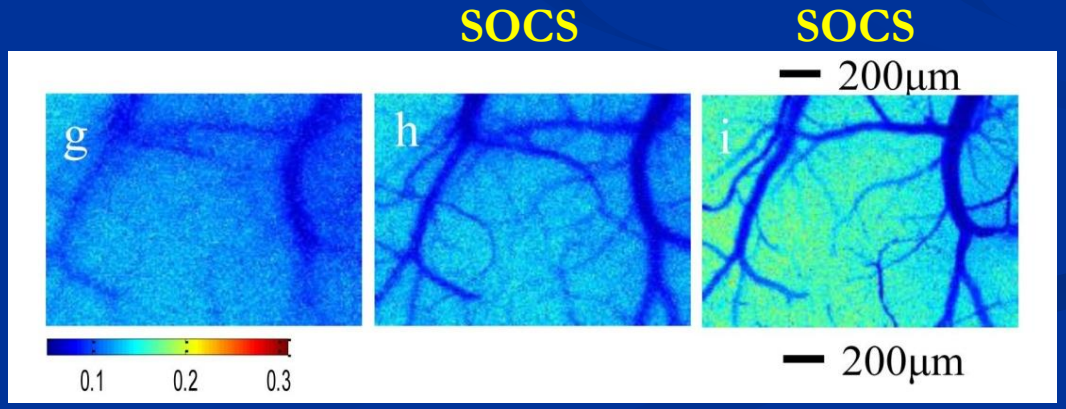
Skull optical clearing solution (SOCS):
laurinol,
weak alkaline substances,
EDTA,
dimethyl sulfoxide (DMSO),
sorbitol,
alcohol,
glucose



area A

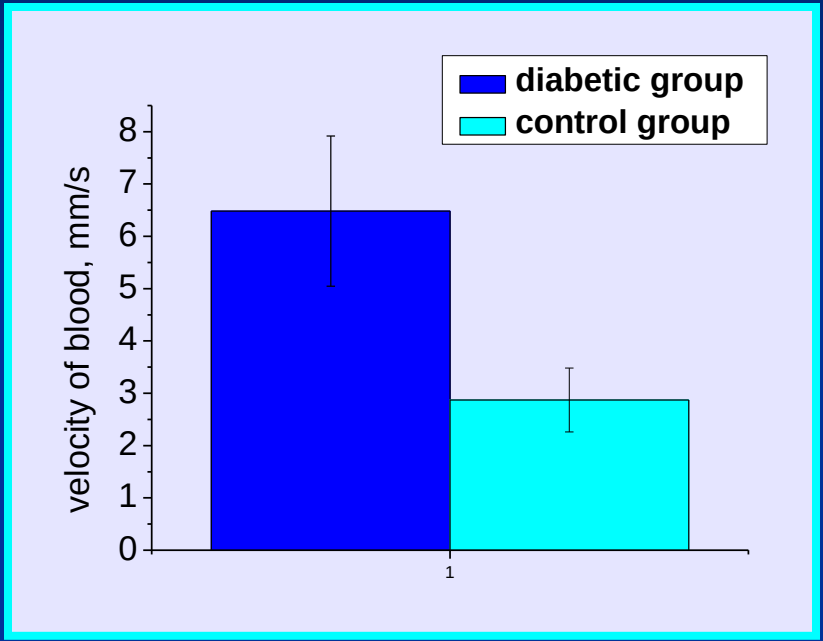
White-light images: intact skull (a), SOCS – 25 min (b), removed skull of area A (c); (d), (e), (f) – magnified images of the area A

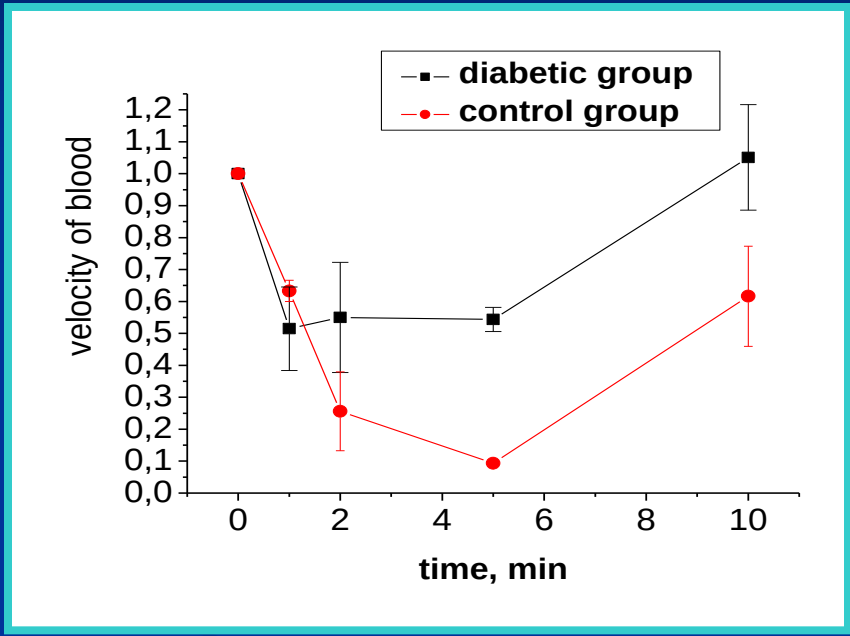
area A



Laser speckle temporal contrast images

Polina A. Timoshina et al. Study of blood microcirculation of pancreas in rats with alloxan diabetes by Laser Speckle Contrast Imaging, AD FLIM, 2017, poster





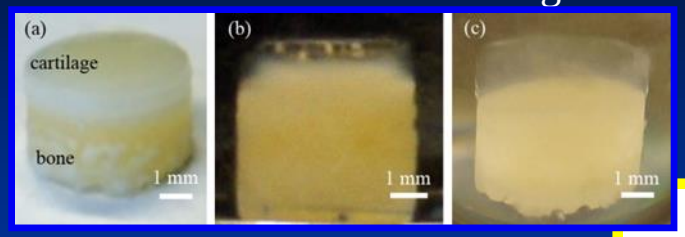
Application of 70%-aqueous glycerol solution demonstrates 50%-decrease of blood flow velocity in the group of diabetic animals, to 10th min blood flow velocity was completely restored. Blood flow in the control group almost stopped and to 10 min has not been recovered.

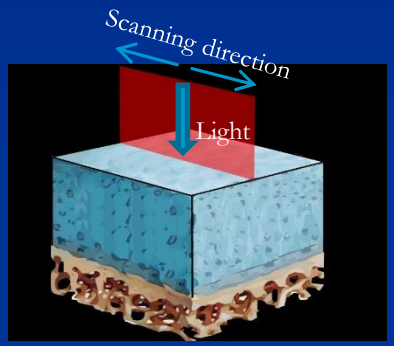
- OCT/digital microscopy

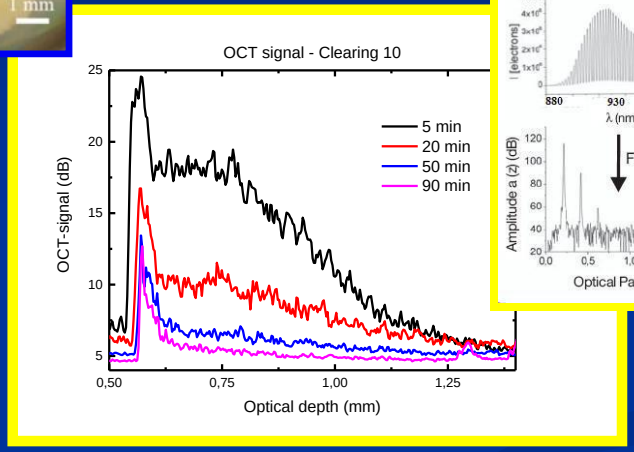
OCT/Cartilage/Omnipaque as an OCA

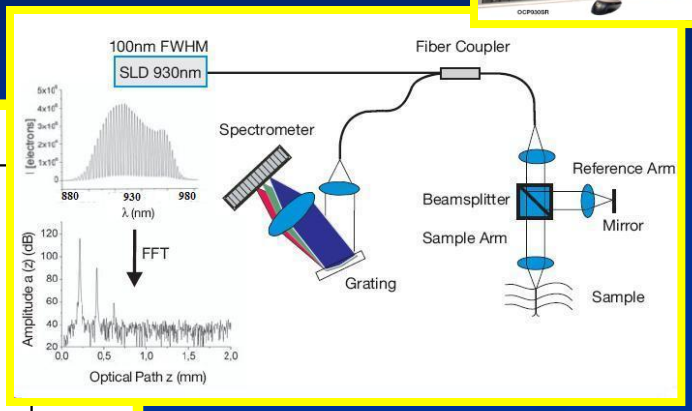
A. Bykov et.al. , **Imaging of subchondral bone by optical coherence tomography upon optical clearing of articular cartilage**, J. Biophotonics, 2015; DOI: 10.1002/jbio.201500130)

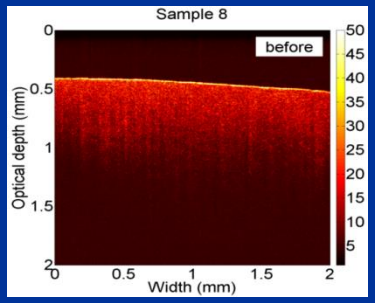
Cleared cartilage

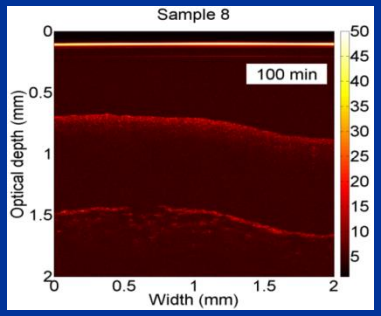




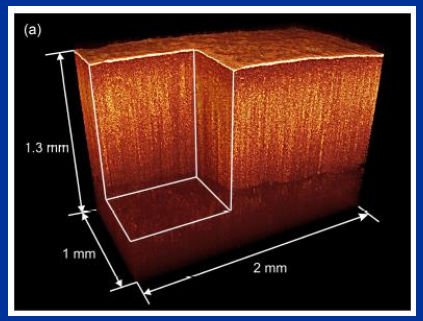




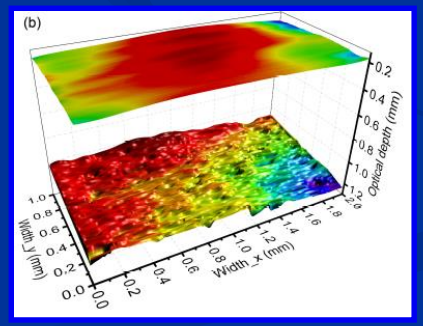




15 min of clearing by Omnipaque

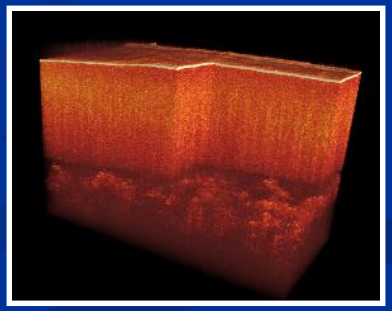


3D OCT image



Roughness of the cartilage-bone interface Sa = 10 μm

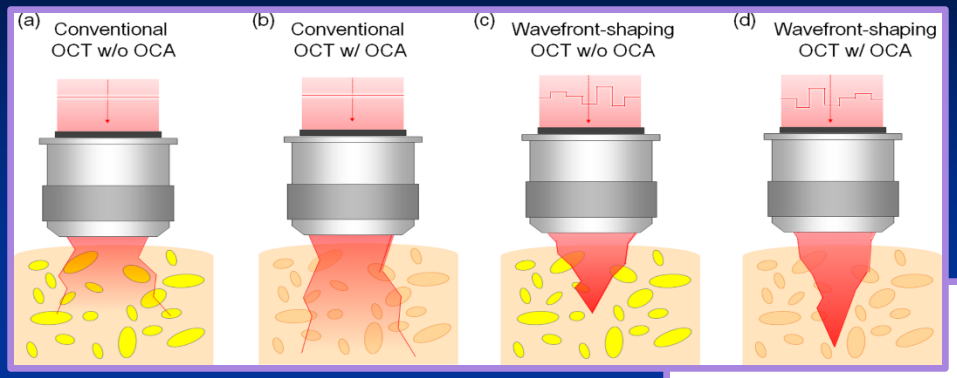
5 min of clearing by Propylene Glycol



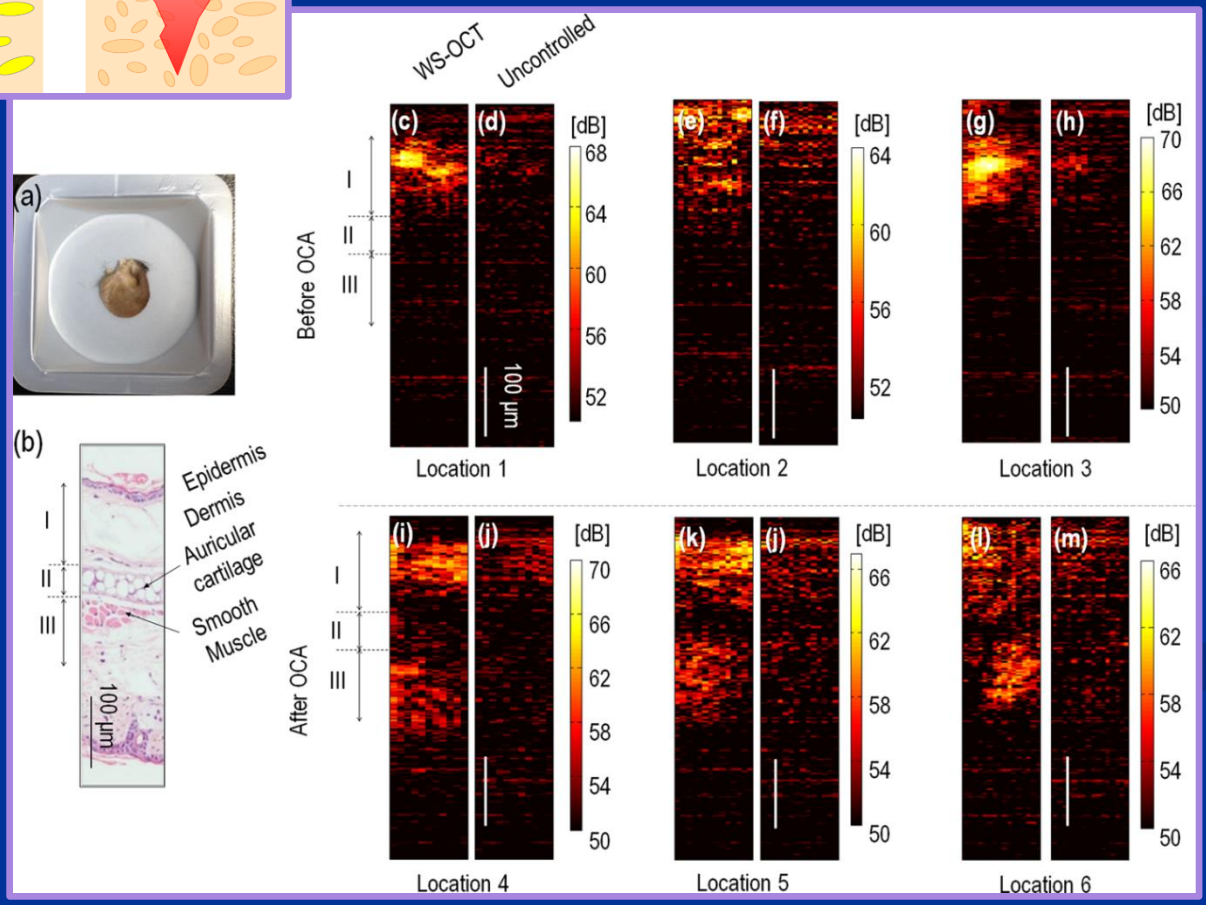
3D OCT image

Synergetic effects of OCT wavefront shaping and optical clearing

H. Yu, P. Lee, Y. Jo, K. Lee, V. Tuchin, Y. Jeong, Y. Park, Synergetic effects of wavefront shaping and optical clearing agent in optical coherence tomography, *J. Biomed. Opt.* **21** (12), 2016



Principle of **optical clearing** and **wavefront shaping** in an OCT system

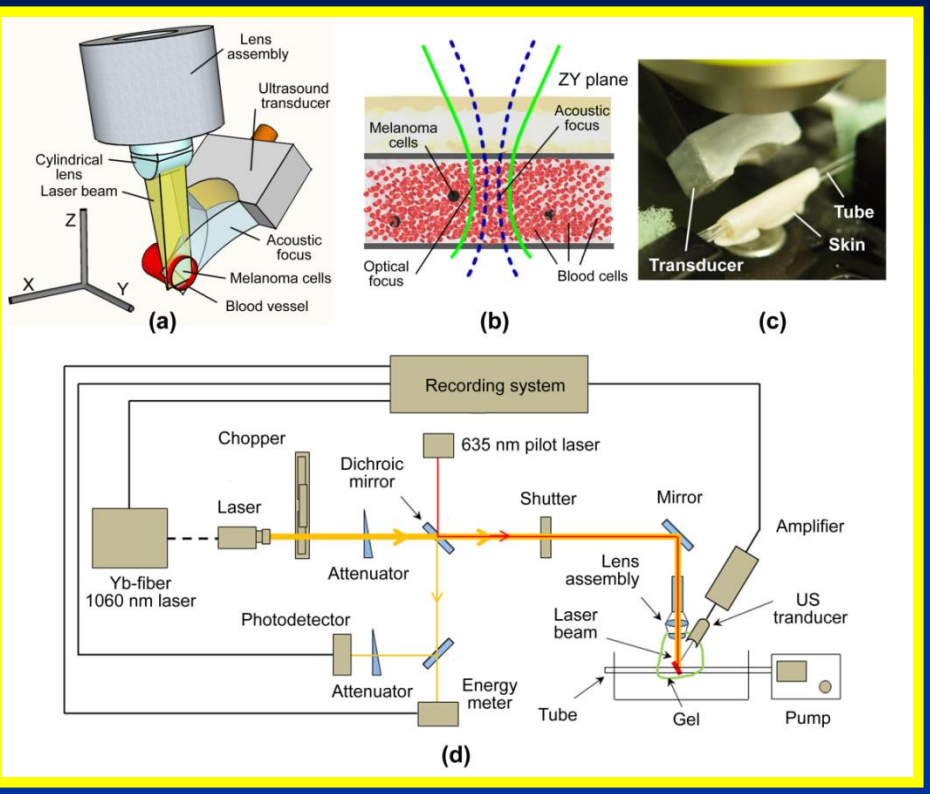


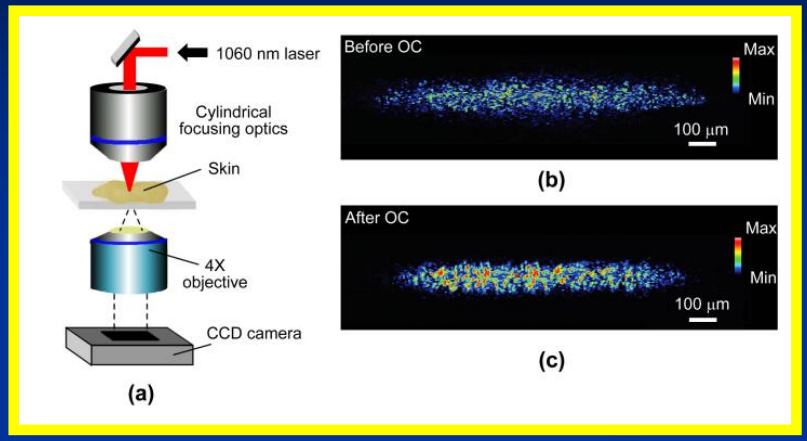
- Dissected mouse ear in glycerol 70% solution for 1 hr
- Histology of the ear
- Images acquired before the **OCA**
- Images acquired after the **OCA**
- Image optimized by **WS** with the input power of 0.55 mW
- Images acquired for uncontrolled beam of 0.55 mW

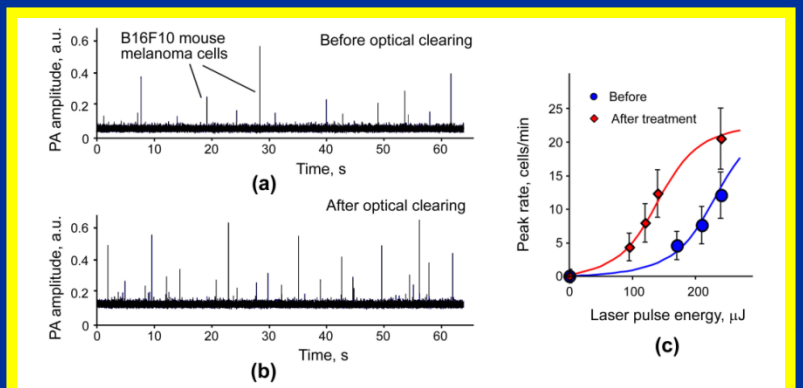
- Photoacoustic microscopy and flow cytometry

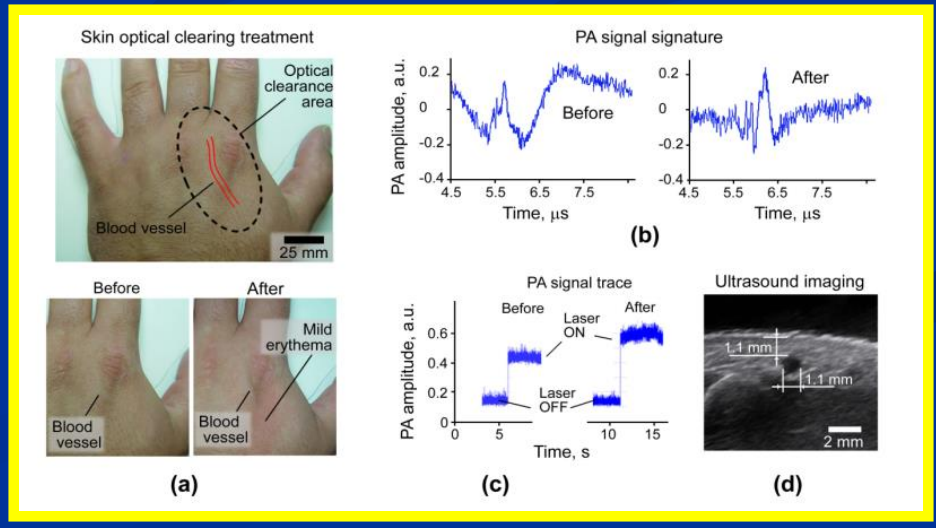
Photoacoustic microscopy and flow cytometry

Y.A. Menyaev, D.A. Nedosekin, M. Sarimollaoglu, M.A. Juratli, E.I. Galanzha, V.V. Tuchin, and V.P. Zharov, Skin optical clearing for in vivo photoacoustic flow cytometry, Biomed. Opt. Express 4 (**12**), 3030-3041 **(2013)**

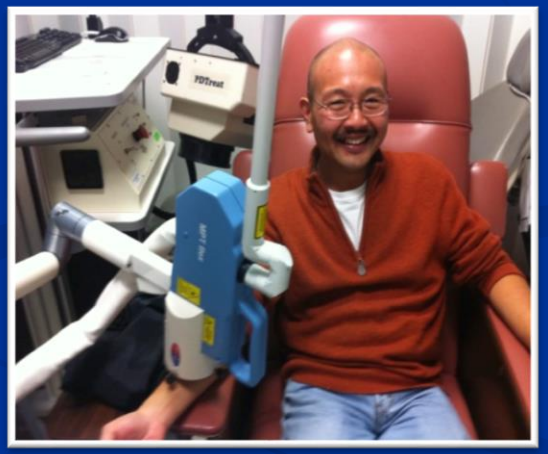






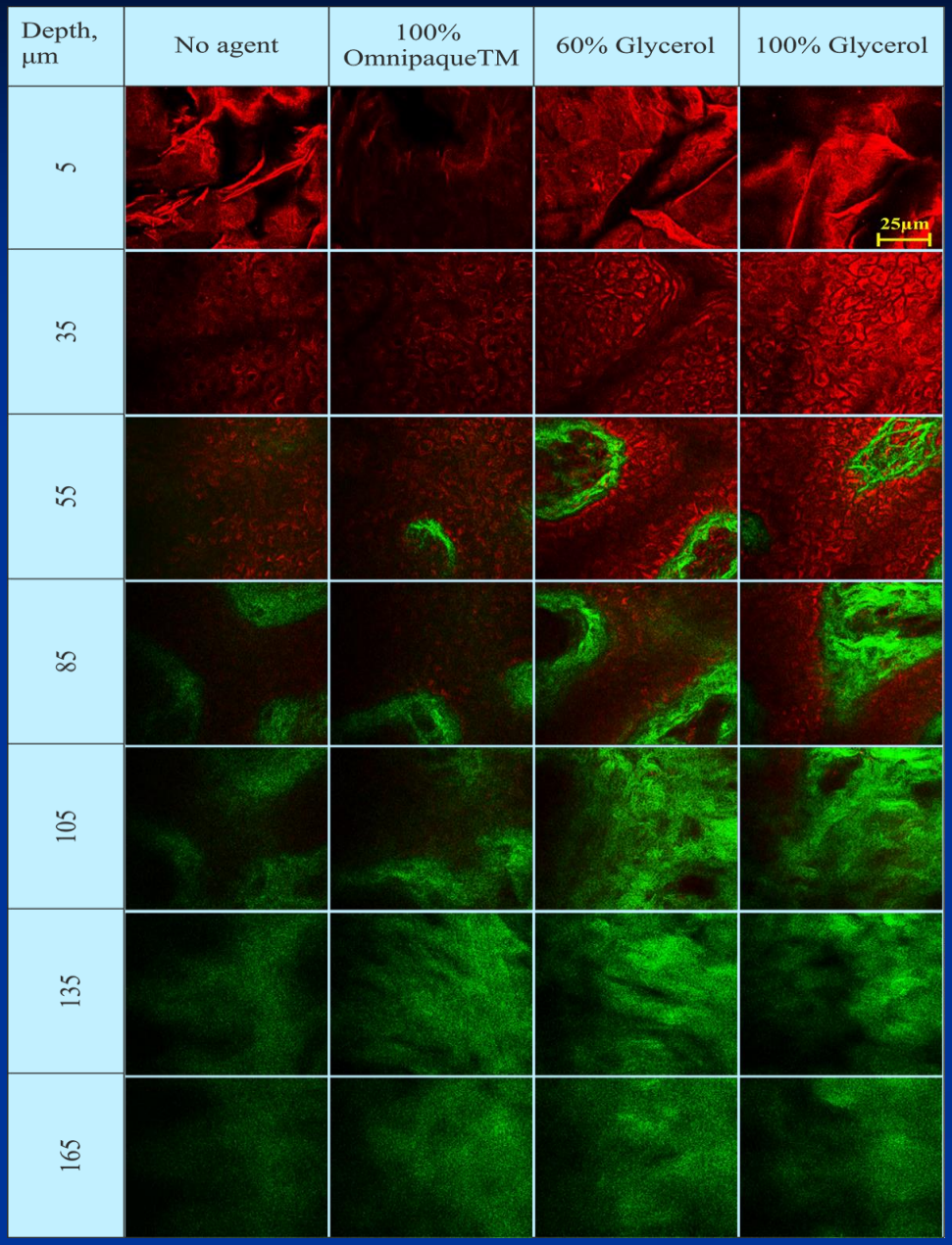


Nonlinear Microscopy



JenLab GmbH, Jena/Saarbrücken (http://www.jenlab.de/). Multi-photon microscope MPTflex-CARS

A. Sdobnov, M. E. Darvin, J. Lademann, V. Tuchin, Enhanced Two-photon Microscopy of Skin by Immersion Optical Clearing, *J. Biophotonics* (2017)

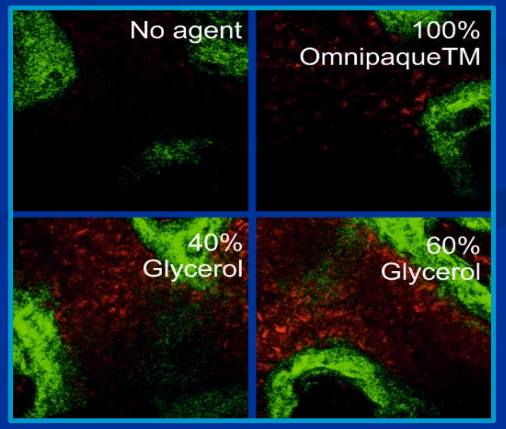


TPEAF and **SHG** images of skin layers obtained *ex vivo* on porcine ear skin samples for **Omnipaque™** and **glycerol** solutions

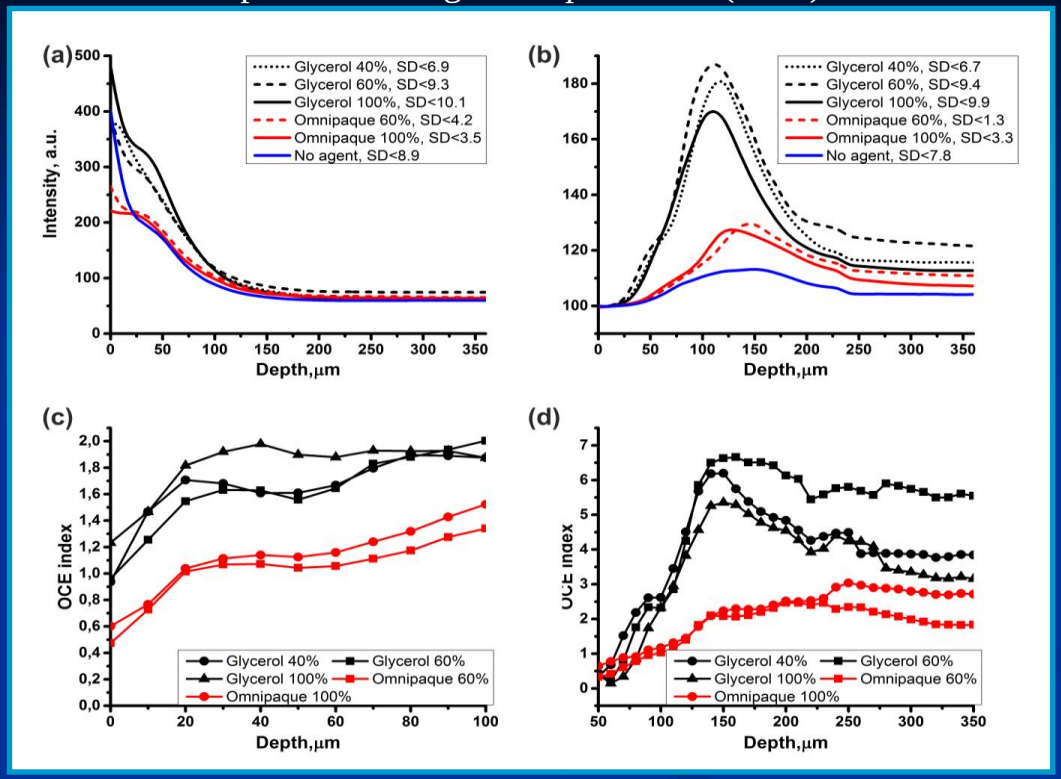
Red – **TPEAF** signal

Green - **SHG** signal

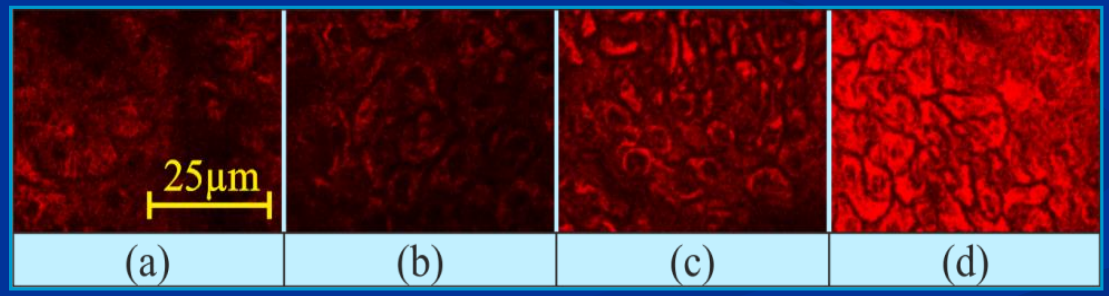
OCA	Glycerol			Omnipaque	
Concentration,%	40	60	100	60	100
Refractive index, n	1.384	1.413	1.474	1.392	1.432
Osmolarity, Osm/L	5.5	8.2	10.9	0.33	0.465
Viscosity, cp	3.7	10.8	1410	3.1	11.8



A. Sdobnov, M. E. Darvin, J. Lademann, V. Tuchin, Enhanced Two-photon Microscopy of Skin by Immersion Optical Clearing, *J. Biophotonics* (2017)



Averaged depth-dependent intensity profiles and OCE indices for **TPEAF** (a, c) and **SHG** (b, d) signals



Comparison of skin epidermal layer structure on **35 μm-depth**: without (a) and with [(b) – (d)] OCA treatment; **100%-Omnipaque™(300)** (b); **40% glycerol** (c); **100% glycerol** (d).

Confocal Raman spectroscopy of skin

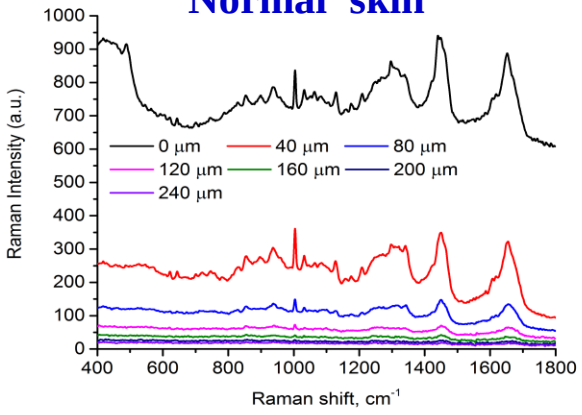
River Diagnostics, Model 3510 SCA; Rotterdam, The Netherlands

Fingerprint Raman spectra of porcine ear skin at OC

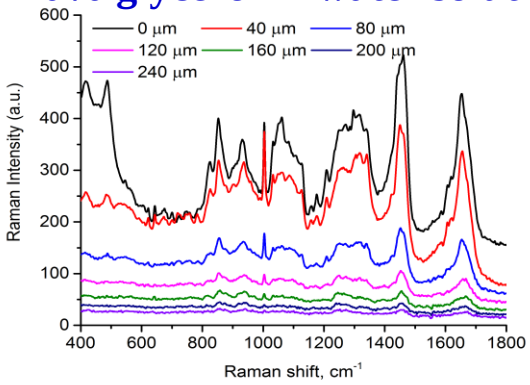
A. Yu. Sdobnov et. al. J. Physics D: Appl. Phys. (2017)

Confocal Raman Microscope (CRM) for *in vivo/ex vivo* skin measurements
River Diagnostics, Model 3510 SCA, Rotterdam, The Netherlands: 785 nm, oil objective ×50, laser power 20 mW, exposure 5 s, resolutions ≤ 5 μm and 2 cm^{-1}

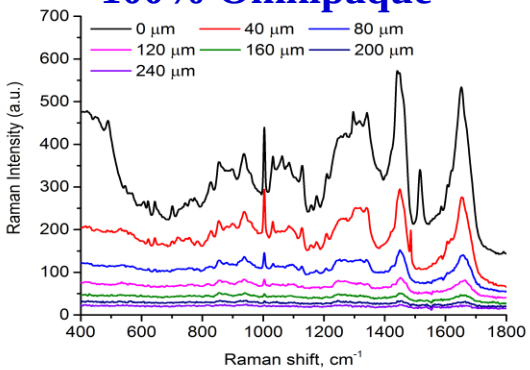
Normal skin



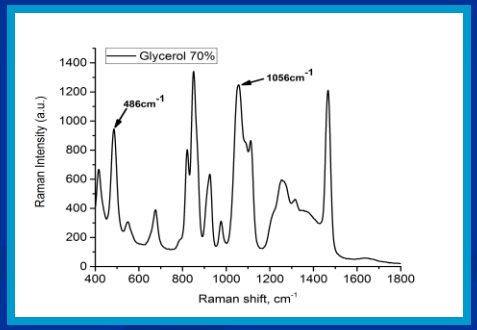
70% glycerol in water solution



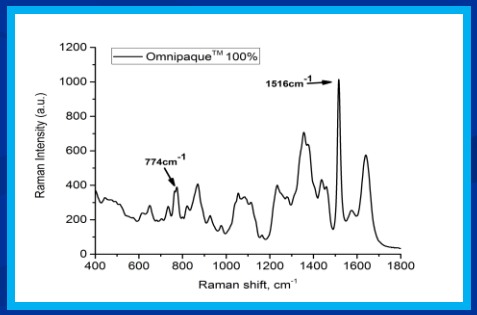
100% Omnipaque™



Glycerol in



Omnipaque™

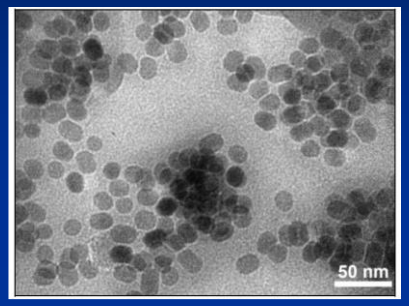


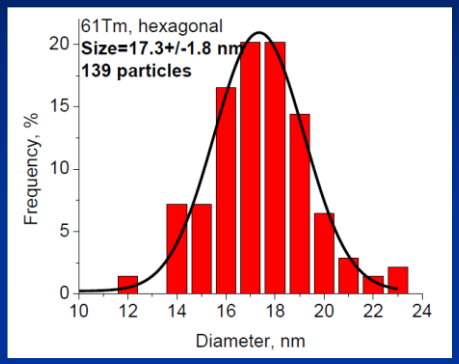
- Nanoparticle labeling

Upconversion nanoparticles (UCNP) for deep-tissue imaging

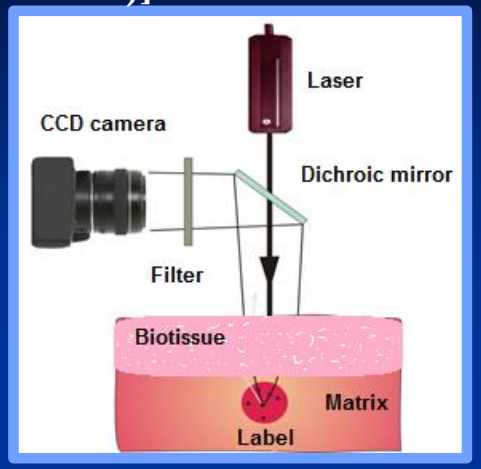
A.P. Popov, E.V. Khaydukov, A.V. Bykov, V.A. Semchishen, V.V. Tuchin, Enhancement of upconversion deep-tissue imaging using optical clearing, Proc. of SPIE-OSA **9540,** 95400B-5, 2015

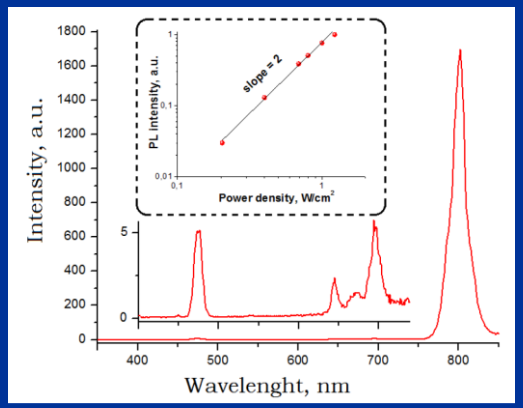
[NaYF4 matrix is doped with ions of ytterbium, erbium and thulium)]



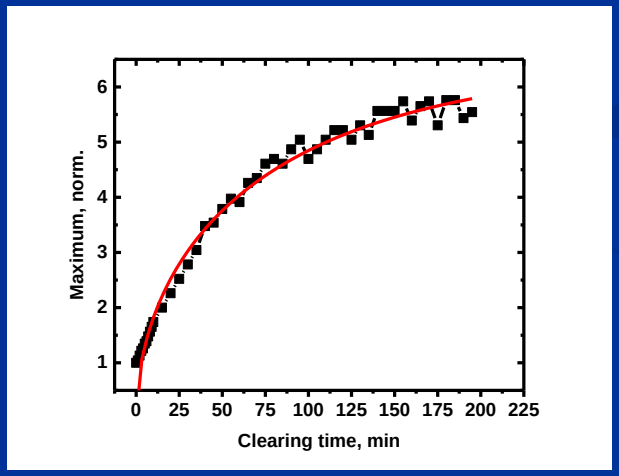


Size distribution

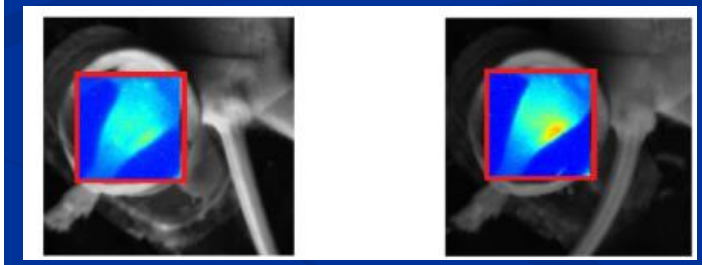




Spectra of luminescent radiation at pumping on 975 nm



Upconversion luminescence of a star-shaped label at **800 nm,** glycerol clearing of 6-mm-thick porcine muscle tissue

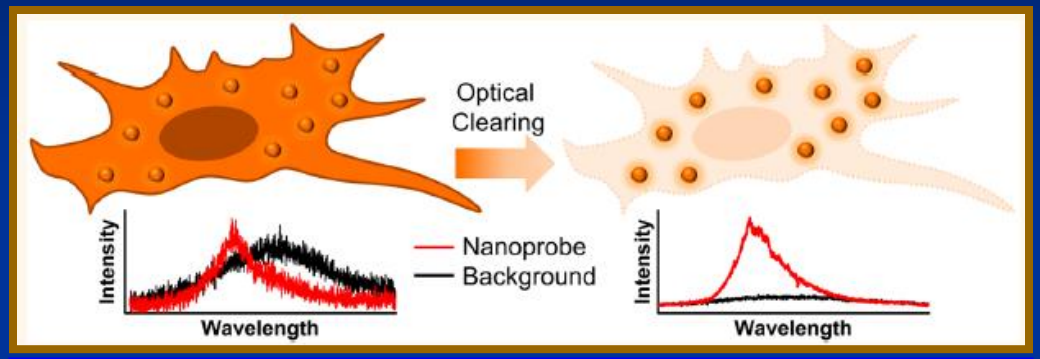


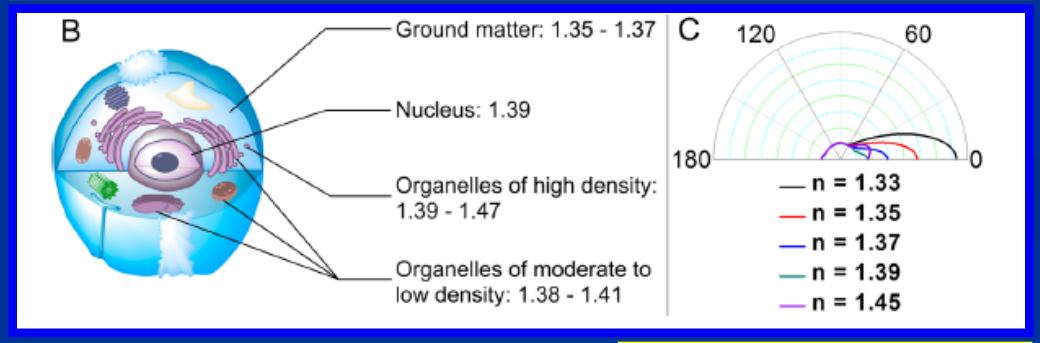
Before and after **255 min** of **glycerol** clearing mouse leg ***in vivo***

Optical Clearing for Ultrasensitive Imaging of Single Cells

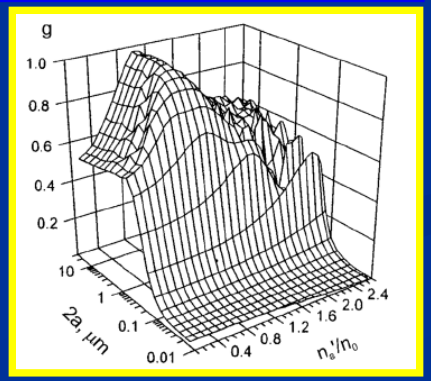
Kinnunen M., et al, Optical Clearing at Cellular Level. ***J. Biomed. Opt***. 2014, 19, 71409.

Yi Cui, et al., Optical Clearing Delivers Ultrasensitive Hyperspectral Dark-Field Imaging for Single-Cell Evaluation, ***ACS Nano*** (2016)



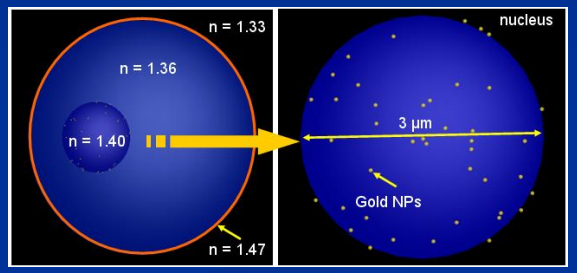


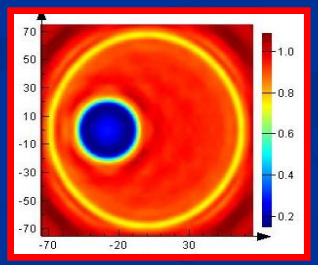
From: V. Tuchin, Tissue Optics, SPIE Press, 2015

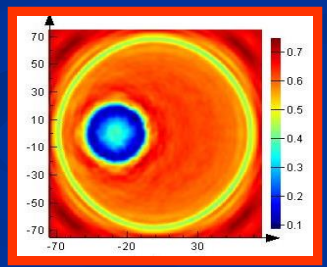


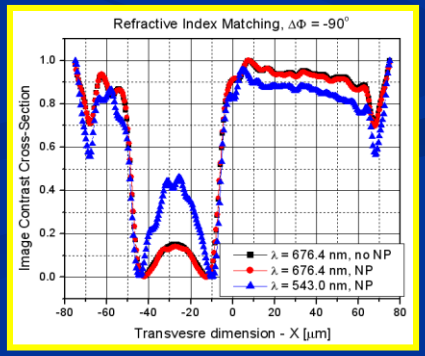
Tanev-Tuchin, 2006

Tanev, S., et al. ***J. Biomed. Opt***. 11(6), 064037-1-6 (2006)



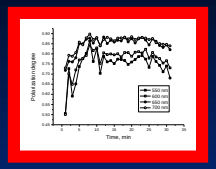




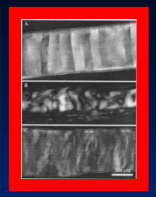


Summary: Optical clearing benefits

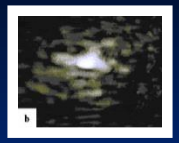
Polarization



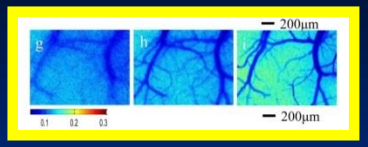
Birefringence

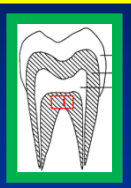


Speckles

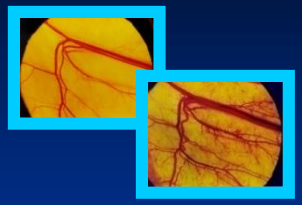


Skull, tooth, bone, cartilage

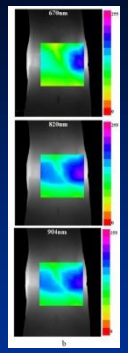




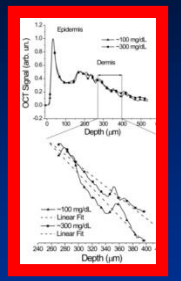
Blood vessel imaging



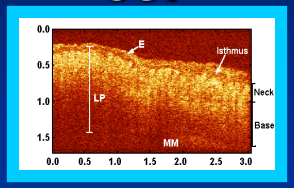
Rheumatoid arthritis



Glucose sensing

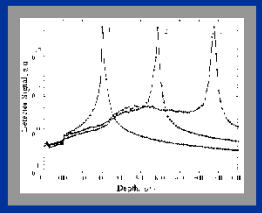


OCT

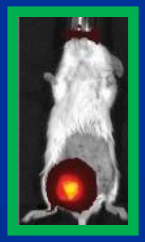


Weak fluorescence /luminescence

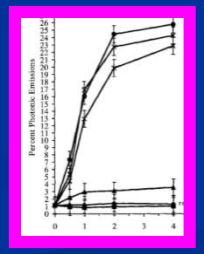
Confocal microscopy



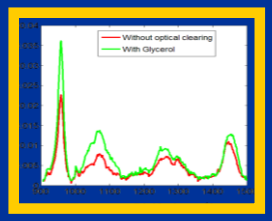
Tumor imaging

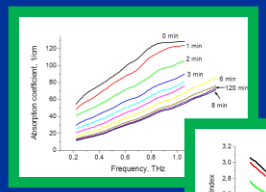


Salmonella detection

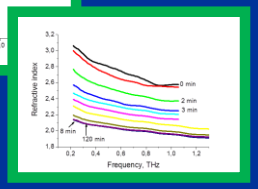


Raman spectroscopy

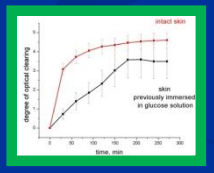




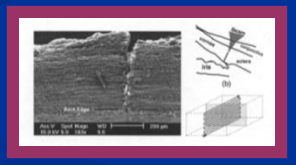
THz clearing



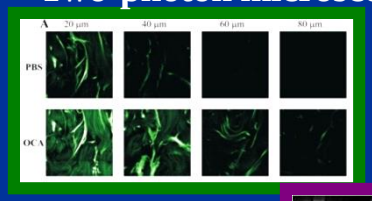
Glycated tissue differentiation



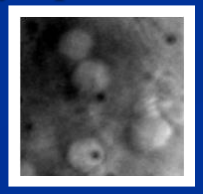
Precise laser surgery



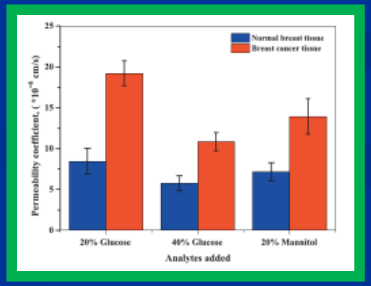
Two-photon microscopy



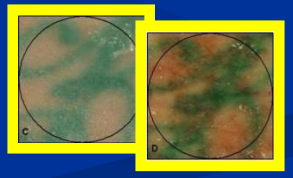
Cell imaging in lymph nodes



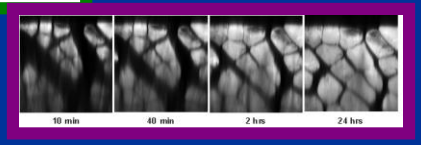
Cancerous tissue differentiation



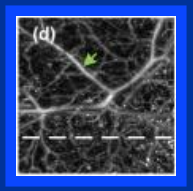
Laser tattoo removal



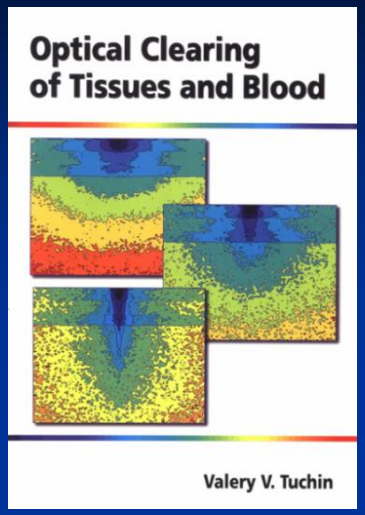
SHG imaging

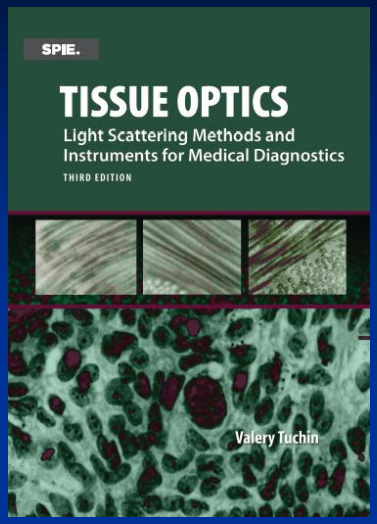


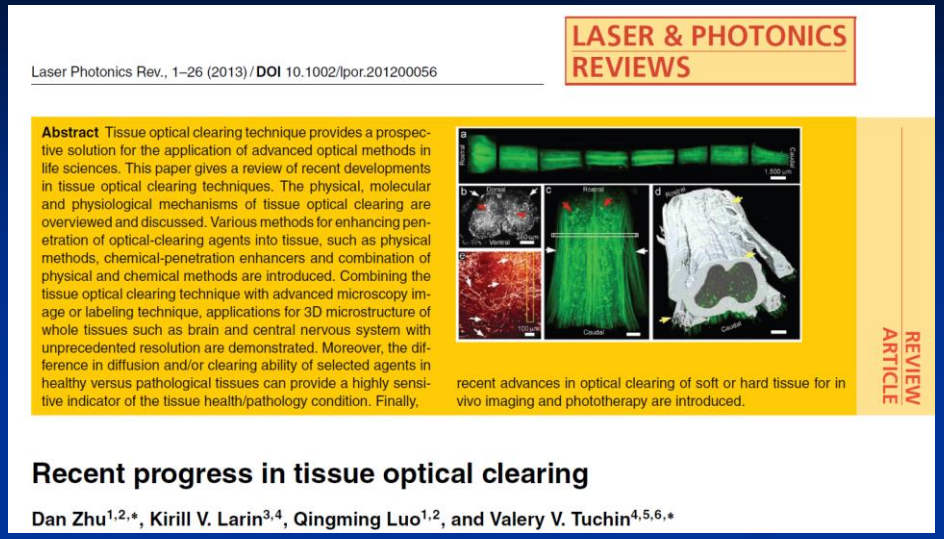
PA microscopy & flow cytometry



For reading







A new special section of the *Journal of Biomedical Optics*

Tissue and Blood Optical Clearing for Biomedical Applications

Guest Editors:

Dan Zhu

Huazhong University of Science and Technology

Bernard Choi

University of California, Irvine

Elina Genina

Valery V. Tuchin

Saratov National Research State University

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INTRODUCTION AND MOTIVATION
SFM'17, Saratov, September 25-29

Editorial Board

Welcome to JBPE

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 start-up 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

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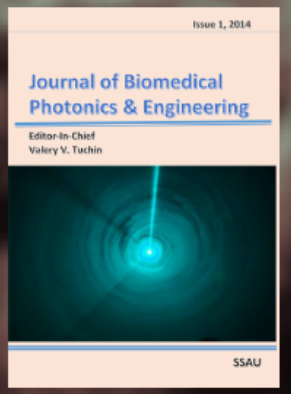

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