Executive Committee

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Committee Member: Guido Perrone, Associate Professor, Politecnico di Torino

Committee Member: Felix Fanjul-Velez, Associate Professor, University of Cantabria

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Where to find information about the group

Therapeutic Laser Applications (BA)

Get Involved

Therapeutic Laser Applications

This group focuses on the use of lasers in surgery or in other treatments of disease. This includes the use of lasers as surgical tools for tissue cutting, welding, and coagulation, as well as the use of optics to initiate cell damaging photothermal reactions for the treatment of diseases such as cancer. In addition, optics, spectroscopy, and imaging provide unique tools that may allow real-time diagnostics of the efficacy of clinical procedures. For many of these applications, the development of optical tools for appropriate light delivery, especially for fiber-based or endoscopic delivery to tissues that are not directly accessible, is critical. In addition, this group emphasizes basic science studies of the mechanisms by which light can affect tissue in adverse or therapeutic ways.

Upcoming Technical Group Webinars

Photoacoustic Imaging of the Eye

Hosted By: Therapeutic Laser Applications Technical Group

24 October 2019, 10:00 AM - 11:00 AM

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- Interested in presenting your research? Have ideas for technical group events? Want to reach out to your fellow group members?
  - Contact us at elina.vitol@gmail.com or TGactivities@osa.org
Upcoming webinars

1 November 2019, 12 pm EST

**Recent advances in tissue biomechanics using Dynamic Optical Coherence Elastography**

Kirill Larin, PhD, University of Houston

21 January 2020, 11am EST*

**Thermomechanical effect of infrared laser for cartilage regeneration**

Yulia M. Alexandrovskaya, PhD

Institute of Photon Technologies, Federal Scientific Research Centre “Crystallography and Photonics” of the Russian Academy of Sciences (RAS)
Welcome to today’s webinar!

PHOTOACOUSTIC IMAGING OF THE EYE

Yannis M. Paulus, M.D., F.A.C.S.

Assistant Professor, University of Michigan
Department of Ophthalmology and Visual Sciences, and
Department of Biomedical Engineering

OCTOBER 24, 2019
Photoacoustic Imaging of the Eye

Yannis M. Paulus, M.D., F.A.C.S.
Assistant Professor
Department of Ophthalmology & Visual Sciences
Department of Biomedical Engineering
University of Michigan Kellogg Eye Center

OSA Therapeutic Laser Applications Technical Group
Webinar
October 24, 2019
Disclosures

- Inventor University of Michigan patents
  - Method and Apparatus for Removing Microvessels
  - RetinaScope Apparatus
  - Photomediated Ultrasound Therapy Method and Apparatus
  - Purely Organic Phosphorescent Nanoparticles for In Vivo Oxygen Sensing
  - Laser Ultrasound Body Sculpting
  - Multi-modal imaging for cell tracking
- Co-Founder companies PhotoSonoX LLC, OcuBell.
- CEO of PhotoSonoX LLC
- Consultant for Oraya Therapeutics, Quattro Consulting, Sonify Biosciences, Allergan Regional Advisory Board, Putnam Associated Consulting, Roda Consulting, ENDRA Life Sciences, MediBeacon Inc
- Will discuss several preclinical systems not approved by the FDA
Imaging is critical

- We can understand and diagnose what we can see
- Early disease detection
- Improved diagnosis
- Improved disease monitoring
- Better patient outcomes
- Precision medicine tailored to each patient’s molecular profile
- Improved understanding of pathophysiology
  - Change name: Central Serous Retinopathy to Central Serous Chorioretinopathy
  - Acute Posterior Multifocal Placoid Pigment Epitheliopathy (APMPPE) to Acute Multifocal Placoid Choroidopathy (AMP-C)\(^1,2\)
- **Retina is very unique.** The eye is optically transparent, so we can directly visualize neurons and microvasculature with high resolution optical imaging.

\(^1\)Zhang AY, Han IC, Goldberg MF. Renaming of Acute Posterior Multifocal Placoid Pigment Epitheliopathy (APMPPE) to Acute Multifocal Placoid Choroidopathy (AMP-C). JAMA Ophthalmol. 2017 Mar 1;135(3):185

Retinal Imaging today

- Fundus photography 1853
- Ultrasonography (A & B) 1956
- Fluorescein angiography 1961
- Indocyanine green angiography 1972
- Scanning laser ophthalmoscopy 1981
- Optical Coherence Tomography 1991
  - Time domain
  - Spectral domain
  - OCTA
  - Swept Source
  - Intra-operative
  - Hand-held/peds
  - Different wavelength
  - Doppler OCT
- Ultra-wide field imaging
- Fundus autofluorescence (FAF)
- Retinal oximetry
- Fluorescent lifetime imaging ophthalmoscopy (FLIO)
- Real-time image-guidance of laser photocoagulation
- Adaptive Optics
- Handheld/Smartphone-based fundus imaging
- Automated interpretation/ deep learning
- Photoacoustic Imaging
- Multimodal Imaging
- Molecular Imaging
Photoacoustic effect: conversion light to sound. 
Optoacoustic = thermoacoustic

**Advantages**
1. Deep Penetration
2. High Resolution
3. Speckle-Free

1880: Alexander G. Bell describes photoacoustic princ. 
Need t < 20 nsec. Most ~ 5 nsec
**Photoacoustic Ocular Imaging**

**Advantages:**
- Non-invasive, and high contrast
- **Structural:** 3D vessel structure
- **Functional:** hemoglobin concentration, oxygen saturation, blood flow
- **Molecular information** with contrast agents: integrin, growth factor
Photoacoustic Ocular Imaging

Endogenous absorbers:
- Hemoglobin
- Oxy and deoxy
- Melanin
- DNA/RNA
- Lipid
- H2O

Exogenous contrast:
- Methylene blue
- Indocyanine green
- Organic nanoparticles
- Gold nanorods
- Microbubbles
Photoacoustic Ocular Imaging

3 µm resolution:
✓ OCT image
✓ Blood distribution (angiogenesis)
✓ Oxygen saturation levels (ischemia)
✓ Tissue blood content
✓ ICG Photoacoustic Angiography
Molecular Imaging

Anatomic changes are the end result from complex molecular pathways

**Mechanism of Choroidal Neovascularization**

- **Capillary Nonperfusion**
- **HYPOXIA**
  - \(\uparrow\) Platelet aggregation
  - \(\uparrow\) Leukocyte adhesion
  - \(\uparrow\) ICAM, \(\downarrow\) Prostacyclin
  - HIF-1
  - \(\uparrow\) VEGF, \(\uparrow\) Flt-1
  - \(\uparrow\) IGF-1, \(\uparrow\) Ang2
- **Integrins**
  - \(\uparrow\) \(\alpha\upbeta3\)
  - \(\uparrow\) \(\alpha\upbeta5\)
- **Proteases**
  - \(\uparrow\) MMP-2, \(\uparrow\) MMP-9
  - \(\uparrow\) Urokinase
- **Lysis of ECM & BM**
- **Cell Migration**
- **Cell Proliferation**

**Inhibitors**
- PEDF
- TIMP
- Endostatin
- Angiostatin
- TGF \(\beta\)
Photoacoustic Imaging System

- Tunable, pulsed Nd:YAG laser
- Rep rate 10 Hz, pulse width 5 ns
- Fiber optic ring light
- Retinal laser density 0.5 mJ/cm², below ANSI limit
- US transducers 15 & 25 MHz acquire pulse-echo + PA signals
- Axial resolution: 83 & 50 μm
- Lateral resolution: 200 & 240 μm

ICG Photoacoustic Imaging

Tissue mimicking phantom filled with ICG
Results: Enucleated Pig Eye

Pig’s Eye

Deep penetration into choroid & sclera

Pig eye: 22 mm
Human eye: 24 mm

700nm (laser depth vs PA (melanin)), 5ns pulses, 0.5mJ/cm², 15MHz
Enucleated Pig Eye

Reconstructed 3D:
Amira, Visage Imaging
63 A-line scans
250 μm apart
8 averages
60 sec acquisition
Live Rat Eye
Live Rabbit Eye

Photograph rabbit Vertical Slice

Parasagittal Ultrasound

Parasagittal Photoacoustic

➢ Good signal within safe laser level

New Zealand rabbits, 6-8 weeks of age

740nm laser, 5ns pulses, 0.5mJ/cm², 25MHz focused transducer
Live Rabbit Eye

Coronal Horizontal Slice

Coronal Photoacoustic

➢ Visualize individual blood vessels

740nm laser, 5ns pulses, 0.5mJ/cm², 25MHz focused transducer
Live Rabbit Eye 3D Reconstruction

**3D Rendering**

- **Cornea**
- **Lens**
- **Blood Vessels**
- **Optic Nerve**

32 averages
Area: 12 mm x 8 mm
250 μm steps

740nm laser, 5ns pulses, 0.5mJ/cm², 25MHz focused transducer
Schematic multimodal photoacoustic microscopy (PAM), OCT, fluorescence microscopy
Tunable pulsed laser (Ekspla NT-242, Lithuania) with 1,000 Hz repetition rate
Ultrasound transducer center frequency: 35 MHz
Thorlabs Ganymede-II-High resolution system 36 kHz
A chrome grating was imaged at the focal plane of the scan lens for lateral resolution calibration. Initial Laser pulse energy: ~30 nJ (half ANSI safety limit)
PAM/OCT Retina

A: External photo.
B: Fundus photograph.
C: Photo retinal medullary ray vessels.
D: PAM of retinal (RV) and choroidal vessels (CVs).
E: 3D PAM.
F: 2D orthogonal slices of the PAM image.
G: OCT image showing RVs, CVs, NFL (nerve fiber layer), and retinal layers.

PAM/OCT Choroid

A: Fundus photograph.
B: PAM signal of the choroid
C: OCT image.

GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; OLM: outer limiting membrane; MZ: myoid zone; EZ: ellipsoid zone; OS: outer segment; IZ: interdigitation zone; BM, Bruch’s membrane.

PAM can be performed using a safe laser exposure dose (~80 nJ) below the ANSI safety limit (160 nJ) at 570 nm. Now down to 5% ANSI limit.
Normal New Zealand white rabbits

(a) Color fundus photo; (b) PAM (c) FM; (d) FA; (e) OCT; (f) 3D PAM
Normal Pigmented rabbits

(a) Color fundus photo; (b) PAM (c) FM; (d) FA; (e) OCT; (f) 3D PAM
Retinal Vein Occlusion Rabbit Model

Spectroscopic PAM analysis of the retina and choroid

A. Retinal vessels
B. Choroidal vessels

C and D. 3D reconstructions of A and B
Quantification of spectroscopic PAM
Multimodal imaging retinal blood vessels

(a) Color photo; (b) FA (c) PAM; (d) 3D PAM; (e) 3D OCT

Retinal vein occlusion

(a,c,e) Color photo;
(b,d) FA
(a1-4) OCT images;
(f) PAM;
(g) PAM amplitude
Retinal neovascularization in RVO

(a) Color photo;
(b,c) FA early and late;
(d) OCT;
(e) 3D OCT;
(f) PAM;
(g) 3D PAM;
(h) Label type vessels

Retinal neovascularization in RVO

(a) Color photo;
(b,c) FA early and late;
(d) OCT;
(e) 3D OCT;
(f) PAM;
(g) 3D PAM;
(h) Label type vessels
Retinal neovascularization over time

(a,b,c) Color photo; (d,e,f) FA; (g,h,i) OCT; (j,k,l) PAM
Retinal neovascularization in RVO

(a) Color photo; (b,c) FA; (d) OCT; (e) PAM; (f) 3D PAM
Quantify vessel diameter photo, OCT, and PAM
New Zealand white rabbit RNV

(a) Color fundus photo; (b) PAM (c) FM; (d) FA; (e) OCT; (f) 3D PAM
Pigmented rabbits RNV

(a) Color fundus photo; (b) PAM (c) FM; (d) FA; (e) OCT; (f) 3D PAM

New Zealand white rabbit neovascularization
PAM pigmented rabbit neovascularization
PAM normal vasculature compared to neovascularization

(a) Normal retinal vessels in New Zealand white rabbit;
(b) RNV induced by VEGF in NZ rabbit;
(c) composite pseudo color image of NZ rabbit showing the retinal vessels before and after VEGF injection;
(d) normal retinal vessels in pigmented rabbit;
(e) RNV induced by VEGF injection in a pigmented rabbit;
(f) composite pseudo color image of pigmented rabbit showing the retinal vessels before and after VEGF injection;
(g) quantification of retinal vessels and RNV in NZ and pigmented rabbits before and after VEGF injection;
(h) quantification of retinal vessels and RNV in NZ rabbits before and after VEGF injection using the vessel size;
(i) quantification of retinal vessels and RNV in pigmented rabbits before and after VEGF injection using the vessel size.
Gold-nanoparticle enhanced PAM imaging

Photoacoustic imaging with gold nanoparticles (AuNP) can significantly enhance signal of PAM and OCT.
PA signal with AuNP

(a) Photograph phantoms AuNP various concentrations
(b-k) PA images of phantoms wavelength from 500 to 570nm
(l) PA signal as function of AuNP concentration and wavelength
(m) photographs phantoms blood and blood + AuNP 1:1
(n) corresponding PA image
(o) PA blood 3-fold higher background; PA blood + AuNP 17.5 fold higher
PA imaging retinal vessels with PEG-AuNP 2 mg/mL

(a) Color fundus image of retina.
(b–i) PAM images before and after injection of PEG-AuNPs.
(j) PA signal amplitude increase 0.22 to 0.34
(k) Pre and (l) post injection 3D PAM
PA imaging retinal vessels with PEG-AuNP 5 mg/mL

(a) color fundus photo
(b) close-up photo
(c–q) MIP PA before and after PEG-AuNPs,
(r) PA signal amplitude over time
(s) pre and (t) post-injection
3D PAM
PA retinal vessels in pigmented rabbits with PEG-AuNP 5 mg/mL

(a) color fundus photo
(b) FA
(c–j) MIP PA before and after PEG-AuNPs, (k) pre and (l) post-injection 3D PAM
(m) Subtraction of post-pre
PAM imaging choroidal vessels with PEG-AuNP 2 mg/mL

(a) Color fundus photo
(b–k) PAM images before and after injection of PEG-AuNPs.
(l) PA signal amplitude increasing after AuNP
(m) Pre and (n) post injection 3D PAM
Real-time photoacoustic signal guided therapy

Schematic diagram of real-time PA signal guided PUT system
HIFU: High-intensity focused ultrasound  
Switch: Pulse/Receiver Switch  
DG: Delay generator  
FG: Function generator

Time sequencing of the system

Real-time photoacoustic signal guided therapy

Shrinkage

No change

Rupture

Before

After

Change of Photoacoustic (PA) signal intensity

Change of PA signal intensities over time
Conclusions

- Photoacoustic imaging is a promising modality to noninvasively image blood distribution with a high depth of penetration (retina and choroid)
- Photoacoustic microscopy can be achieved with a high resolution (2.6 µm lateral resolution)
- PAM can be utilized with a safe laser intensity below the ANSI safety limit
- PAM can visualize blood vessels in human-sized eyes (rabbit)
- PAM can visualize retinal vascular pathology such as retinal neovascularization (like in diabetes) and retinal vein occlusion
- Gold nanoparticles can serve as contrast agents to enhance PAM imaging
- Photoacoustic monitoring of retinal vasculature for automated dosimetry and reproducible burns “Smart laser”
Paulus Advanced Retinal Imaging and Laser Laboratory
Retina Division UM
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- University of Michigan Department of Ophthalmology and Visual Sciences
Thank you for your attention!

Discussion & Questions?