High-resolution Biophotonic Tomography

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URL:  http://oilab.tamu.edu
# Credits to Lab Members

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- A. Garcia-Uribe
- X. Jin
- R. Kothapalli
- C. Kim
- G. Ku
- L. Li
- M. Li
- K. Maslov
- J. Oh
- S. Sakadzic
- M. Sivaramakrishnan
- E. Smith
- K. Song
- M. Todorovic
- X. Xie
- M. Xu
- X. Xu
- H. Zhang
- R. Zemp

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- S. Jiao, PhD
- J. Li, PhD
- G. Marquez, PhD
- M. Mehrubeoglu, PhD
- H. Sun, PhD
- Y. Pang, MS
- X. Wang, PhD
- Y. Wang, MS
- Y. Xu, PhD
- G. Yao, PhD
- W. Yu, MS
- X. Zhao, MS

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Credits to Collaborators

- **Texas A&M University (Animal study):**
  - G. Stoica, DVM

- **UT MD Anderson Cancer Center (Clinical study & molecular contrast agents):**
  - M. Duvic, MD
  - B. Fornage, MD
  - K. Hunt, MD
  - C. Li, PhD
  - V. Prieto, MD

- **UT Dallas (Data classification):**
  - N. Kehtarnavaz, PhD

- **Nanospectra (Nanoshells):**
  - P. O’Neal, PhD
  - J. Schwartz, PhD
Introduction

Motivation and challenges

Example 1: Optical contrast in skin cancer detection

Example 2: Optical contrast in *Mueller* OCT

Ultrasound-modulated optical tomography (UOT)

Laser-induced photo-acoustic tomography (PAT)

RF-induced thermo-acoustic tomography (TAT)

Summary
Motivation for Optical Imaging

- Safety — Non-ionizing radiation: photon energy is $\sim$2 eV.
- Physics — Related to the molecular conformation of tissue.
- Optics — High intrinsic contrast:
  - Optical absorption: Angiogenesis, hyper-metabolism, apoptosis, necrosis, and exogenous contrast agents.
  - Optical scattering: Size of cell nuclei.
  - Optical polarization: Collagen, muscle fibers.
- Physiology — Functional imaging of physiological parameters:
  - Oxygen saturation of hemoglobin
  - Total hemoglobin concentration (related to blood volume)
  - Enlargement of cell nuclei
  - Orientation of collagen
  - Denaturation of collagen
  - Blood flow (Doppler)
- Physiology — Molecular imaging (exogenous contrast agents).
- .....

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Animated Propagation of Light in Biological Tissue

- 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: Photo-acoustic tomography

Simulation software available from http://oilab.tamu.edu
Outline

- Introduction
  - Motivation and challenges
  - Example 1: Optical contrast in skin cancer detection
  - Example 2: Optical contrast in Mueller OCT
- Ultrasound-modulated optical tomography (UOT)
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- Summary
Separation of Benign and Malignant Lesions in Group 1

Training

Prospective Blind Testing

Benign (24)  Cancerous (13)
AK: Actinic Keratosis (6)  SCC: Squamous Cell Carcinoma (8)
SK: Seborheic Keratosis (17)  BCC: Basal Cell Carcinoma (5)
W: Warts (1)


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Separation of Benign and Malignant Lesions in Group 2

**Training**

<table>
<thead>
<tr>
<th></th>
<th>Combined Image Feature CIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td><img src="#" alt="Graph" /></td>
</tr>
<tr>
<td>Dysplasia</td>
<td><img src="#" alt="Graph" /></td>
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</table>

**Prospective Blind Testing**

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<tr>
<td>Dysplasia</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Relative case number</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>0, 10, 20, 30, 40</td>
</tr>
<tr>
<td>DN</td>
<td>0, 10, 15, 20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>(29)</td>
</tr>
<tr>
<td>Pre-cancerous</td>
<td>(36)</td>
</tr>
</tbody>
</table>

|       | CN: Common Nevi | DN: Dysplastic Nevi |


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### Origins of Optical Signatures

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cancerous</td>
<td>Benign</td>
</tr>
<tr>
<td>Oxygen saturation of</td>
<td>0.46±0.02</td>
<td>0.49±0.02</td>
</tr>
<tr>
<td>hemoglobin (SO$_2$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total hemoglobin C$_{Hb}$</td>
<td>0.14±0.08</td>
<td>0.31±0.21</td>
</tr>
<tr>
<td>Size of cell nuclei (μm)</td>
<td>20.4±7.5</td>
<td>6.3±5.5</td>
</tr>
</tbody>
</table>

**Applied Optics 43, 2643 (2004).**
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Mueller Images of Porcine Tendon (0.5 mm × 1 mm)

- 10 micron resolution
- ~1 mm imaging depth
- Birefringence: \((4.2 \pm 0.3) \times 10^{-3}\) (e.g., density of collagen)
- Orientation: accurate to <5° (e.g., direction of collagen)
- Diattenuation: 0.26/mm (e.g., property of collagen)


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(a) Intensity
(b) Retardation (integrated)
(c) Retardation (differentiated)
(d) Histology (HE)

E – epidermis;
P – papillae;
B – burn

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## Motivation for Combining Light with Ultrasound

<table>
<thead>
<tr>
<th>Properties</th>
<th>Diffuse optical tomography</th>
<th>Ultrasonic imaging</th>
<th>Ultrasound-modulated optical tomography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contrast</td>
<td>Excellent (functional)</td>
<td>Poor in early cancers</td>
<td>Excellent (= DOT)</td>
</tr>
<tr>
<td>Resolution</td>
<td>Poor (~5-10 mm)</td>
<td>Excellent &amp; scalable</td>
<td>Excellent (= US)</td>
</tr>
<tr>
<td>Imaging depth</td>
<td>Good (~5 cm)</td>
<td>Good &amp; scalable</td>
<td>Good</td>
</tr>
<tr>
<td>Speckle artifacts</td>
<td>None</td>
<td>Strong</td>
<td>None</td>
</tr>
<tr>
<td>Scattering coefficient</td>
<td>Strong (~100 /cm)</td>
<td>Weak (~0.3 /cm)</td>
<td></td>
</tr>
</tbody>
</table>
Power Spectrum of Ultrasound-modulated Light

Modulation depth:

\[ M = \frac{I_1}{I_0} \]

References:

Physical Review E 72, 036620 (2005)

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Ultrasound-modulated Optical Tomography

- Laser
- Function Generator
- Power Amplifier
- Ultrasonic Transducer
- Sample
- CCD Camera
- Computer

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Image of 4.5-cm Thick Biological Tissue


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Ultrasound-modulated Optical Computed Tomography: Experimental Configuration

(Reflection detection) (Transmission detection)

CCD Biological sample CCD

Laser

Ultrasonic beam

Object

Ultrasonic transducer

Rotation

Linear scan

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Ultrasound-modulated Optical Computed Tomography: Tomogram vs. Photograph


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Frequency Encoding along Ultrasonic Axis (z)

Analogous to MRI

Snapshot of frequency

Ultrasonic Transducer

Laser

Ultrasonic Absorber

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Image of Biological Tissue Acquired with Frequency Sweep

Ultrasonic axis $z$

Lateral dimension $x$

Axial Resolution by Pulsed Ultrasound


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Principle of Photo-acoustic Tomography

(1) Laser pulse (<ANSI limit: e.g., 20 mJ/cm²)

(2) Local heating (~ mK)

(3) Ultrasonic emission

(4) Ultrasonic detection
Experimental Setup of Photo-acoustic Tomography

- Nd:YAG
- Dye-laser
- Step motor
- Concave lens
- Ground glass
- Water tank
- Transducer
- Oscilloscope
- Computer
- Amplifier
- Trigger

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Photo-acoustic Reconstruction: Inverse-source Problem

Forward problem :

\[ p(\mathbf{r}, t) = C \int \int \int \frac{d\mathbf{r}'}{|\mathbf{r}' - \mathbf{r}|} A(\mathbf{r}') \frac{\partial I(t')}{\partial t'} \bigg|_{t' = t - \frac{|\mathbf{r} - \mathbf{r}'|}{c}} \]

For an impulse source: \( I(t) = I_0 \delta(t) \)

Inverse solution when detection radius \( r_0 >> \) wavelength \( \lambda_a \) :

\[ A(\mathbf{r}) = C' \int \int d\Omega_0 \frac{1}{t} \frac{\partial p(\mathbf{r}_0, t)}{\partial t} \bigg|_{t = \frac{|\mathbf{r}_0 - \mathbf{r}|}{c}} \]


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Non-invasive Transcranial Photo-acoustic Image of a Rat Brain with a Lesion

Open-skull photograph

L: Lesion
LH: Left hemisphere
MF: Median fissure
RH: Right hemisphere
V: Blood vessel


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Functional Imaging of Whisker Stimulation In Vivo

PAT image (left stimulation)

PAT image (right stimulation)


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Non-invasive Transcranial Photo-acoustic Image of a Mouse Brain: 3D Imaging

PAT image (8 mm deep)

Histology

CB: Cerebellum
HC: Hippocampus
VL: Ventriculi lateralis
VQ: Ventriculi quarti


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Monitoring of Dynamic Optical Absorption of Nanoshells

Photo-acoustic Angiography of Rat Brains In Vivo

(A) Without ICG-PEG

(B) With ICG-PEG

(C) B – A

(D) Open-skull photo

• Speckle free
• High resolution: 60 μm
• High sensitivity: ~fmol

Spectroscopic Photo-acoustic Tomography: Molecular and Tumor Hypoxia Imaging

- PAT
- Molecular contrast
- Oxygen saturation

Open-skull photo

- Macro-fluorescence
- Thionine stained slice (Depth: ~2 mm from scalp)

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Spatial Resolution versus Bandwidth in Photo-acoustic Tomography

(a) (b) (c) (d)

Center freq.: 3.5 MHz 10 MHz 20 MHz Open-skull photo

Resolution: 210 µm 60 µm 30 µm

Deeply Penetrating Photoacoustic Tomography
with NIR Excitation & ICG Contrast


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Dark-field Confocal Photoacoustic Microscopy: Schematic and Photograph


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Dark-field Confocal Photoacoustic Microscopy: Resolution and Depth

B-scan of a black double-stranded cotton thread embedded in rat

- Center frequency: 50 MHz
- Lateral resolution: ~45 microns
- Axial resolution: ~15 microns
- Imaging depth: ~3 mm


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Dark-field Confocal Photoacoustic Microscopy: Multi-wavelength Functional Imaging

578.5 nm

584.7 nm

590.8 nm

596.6 nm

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Dark-field Confocal Photo-acoustic Microscopy: Structural and Functional Images

Structural image at 584-nm wavelength

Vessel-by-vessel oxygen saturation

Histology

Artery

Vein

Composite fluorescence image


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Dark-field Confocal Photo-acoustic Microscopy: Hemodynamics

Structural image at 584 nm

Normoxia to hypoxia

Normoxia to hyperoxia

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Dark-field Confocal Photo-acoustic Microscopy: Melanoma Imaging

Composite maximum intensity projection (MIP) image projected along z axis obtained with 584-nm and 764-nm wavelengths. MIP images projected along x, y axes obtained with 764-nm wavelength.


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1. Vessels at different depths are distinguished.
2. Vessels with different spatial orientations are visualized.
3. Relationship between the melanoma and vessels is revealed.

**Nature Biotech.** 24, 848 (2006).
In vivo Image of Tumor with Nanoshells as Contrast Agents

Original
(Contrast enhanced by nanoshells)

Focusing enhanced
(SAFT+CF)

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Dark-field Confocal Photo-acoustic Microscopy: Human Imaging


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# Modern High-resolution Optical Microscopy

<table>
<thead>
<tr>
<th>Modality</th>
<th>Year</th>
<th>Depth</th>
<th>Depth / Resolution</th>
<th>Contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confocal microscopy</td>
<td>1970s</td>
<td>~0.5 mm</td>
<td>&gt; 100</td>
<td>Scattering, fluorescence</td>
</tr>
<tr>
<td>Two-photon microscopy</td>
<td>1990s</td>
<td>~0.5 mm</td>
<td>&gt; 100</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>Optical coherence tomography</td>
<td>1990s</td>
<td>~1 mm</td>
<td>&gt; 100</td>
<td>Scattering, polarization</td>
</tr>
<tr>
<td>Confocal photoacoustic microscopy</td>
<td>2005*</td>
<td>~3 mm, scalable</td>
<td>&gt;100</td>
<td>Absorption</td>
</tr>
</tbody>
</table>


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Experimental System for Thermo-acoustic Tomography

Waveguide
Microwave generator
Amplifiers and scope
Water tank
Ultrasonic transducers
Stepper motor
Thermo-acoustic Image of a Mastectomy Specimen

- 11 cm diam. x 9 cm thick
- ~5:1 contrast
- Invasive lobular carcinoma

Tech. in Cancer Res. & Treatment 4, 559 (2005).

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Summary

- Physically combining ultrasonic and electromagnetic waves (light & RF) provides
  - improved spatial resolution compared to optical or RF imaging
  - new contrast mechanisms compared to ultrasound imaging.
- Spatial resolution is determined by the ultrasonic parameters.
- Spatial resolution is scalable with the ultrasonic parameters.
- Contrast is provided by the electromagnetic properties.
- Deep (~cm) tissue imaging can be achieved.
- Speckle artifacts do not exist.
- Functional imaging can be accomplished with endogenous contrast.
- Molecular imaging can be accomplished with exogenous contrast agents.
- Non-ionizing radiation is used.
- Costs are comparable to those of ultrasound systems.
Funding Sources

ACTIVE
- NIH
  - R01 CA106728
  - R01 NS46214 (BRP)
  - R33 CA094267
  - R01 CA092415
  - R01 EB000712
- NIST
- Whitaker Foundation

RECENTLY COMPLETED
- NIH
  - CA83760
  - CA71980
  - CA68562
  - EB000319
- NSF
- US Army
- Whitaker Foundation

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Welcome to the Optical Imaging Laboratory, a research laboratory dedicated to the developments of novel non-ionizing tomography and spectroscopy for the early detection of various cancers.