Dr. Lihong Wang has disclosed the following financial relationships. Any real or apparent conflicts of interest related to the content of this presentation have been resolved.

- **Consultant/Share**
  - Microphotoacoustics, Inc.

**Outline**

- Motivations and challenges
- Photoacoustic tomography
  - Photoacoustic computed tomography
  - Photoacoustic microscopy
- Time-reversal wavefront engineering
- Compressed ultrafast photography
Motivations for Imaging with Light

- Light-matter interaction uniquely positioned at the molecular level

Electromagnetic spectrum

<table>
<thead>
<tr>
<th>Wavelength:</th>
<th>1 mm</th>
<th>1 µm</th>
<th>1 nm</th>
<th>1 pm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiowave/microwave</td>
<td>Optical</td>
<td>X-ray</td>
<td>Gamma</td>
<td></td>
</tr>
<tr>
<td>Non-ionizing (safe)</td>
<td>Isotizing (DNA damage)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Motivations for Imaging with Light

- Light-matter interaction uniquely positioned at the molecular level
- Fundamental role of molecules in biology and medicine
- In vivo functional imaging analogous to functional MRI
- In vivo metabolic imaging analogous to PET
- In vivo molecular imaging of gene expressions or disease markers
- In vivo label-free histologic imaging of cancer without excision

Oxy- & deoxy-hemoglobins
Brain activation
Glucose uptake
Melanoma hallmark
Photoacoustic microscopy of cell nuclei


Challenges in Optical Penetration

<table>
<thead>
<tr>
<th>Depth</th>
<th>Photon propagation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mm</td>
<td>Classical planar optical microscopy</td>
</tr>
<tr>
<td>10 µm</td>
<td>Confocal or two-photon microscopy</td>
</tr>
<tr>
<td>100 µm</td>
<td>Optical coherence tomography</td>
</tr>
<tr>
<td>1 mm</td>
<td>Diffusion limit: 10/1 scattering coefficient</td>
</tr>
<tr>
<td>1 cm</td>
<td>Photoacoustic tomography</td>
</tr>
<tr>
<td>10 cm</td>
<td>Dissipation limit: 10/1 attenuation coefficient</td>
</tr>
<tr>
<td>1 m</td>
<td>wavefront engineering with internal guide stars</td>
</tr>
<tr>
<td>10 m</td>
<td>Absorption limit: 10/1 absorption coefficient</td>
</tr>
</tbody>
</table>

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Photoacoustic Computed Tomography:
Deep Penetration with Optical Contrast and Ultrasonic Resolution

(1) ns laser pulse (within safety limit)
(2) Absorption of photons
(3) Rapid heating (~ mK)
(4) Ultrasonic emission:
1 mK → 8 mbar (800 Pa), detectable
(5) Ultrasonic detection of unscattered phonons
(acoustic scattering ~ optical scattering/1000)


First Functional (Also First In Vivo) Photoacoustic Tomography
in Small Animals with Intact Scalp and Skull

Left-whisker stimulation

Right-whisker stimulation

Contralateral hemodynamic response

5 mm

Growth of Photoacoustic Tomography

Largest conference since 2010 in 20,000-attendee Photonics West

Number of papers

Omniscale In Vivo Photoacoustic (PA) Tomography with Consistent Contrast

• Omniscale biological research from organelles to small-animal organisms
• Translation of microscopic lab discoveries to macroscopic clinical practice


Single Impulse Panoramic Photoacoustic Computed Tomography

Lei Li, Liren Zhu, Cheng Ma, Konstantin Maslov, LV Wang, unpublished
60 Hz Frame-Rate Photoacoustic CT of Mice In Vivo: Liver/Portal Vein Region

Lei Li, Liren Zhu, Cheng Ma, Konstantin Maslov, LV Wang, unpublished

Photoacoustic Computed Tomography of the Whole Brain of a Mouse In Vivo

Pengfei Zhang, ... LV Wang, unpublished

Reversibly Switchable Photoacoustic Genetic Imaging of Mouse Brain Tumor


OFF = BG
ON = OFF = signal
Overlay
In Vivo Human Breast Panoramic Photoacoustic Computed Tomography

Photoacoustic Computed Tomography of Human Breast In Vivo: Volunteer #1

Omniscale In Vivo Photoacoustic (PA) Tomography with Consistent Contrast

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**In Vivo Photoacoustic Microscopy of the Human Skin**

*Photo of fingers with B-scan PAM @ 584 nm*

- Epidermal-dermal junction
- Stratum corneum
- Dermis
- Subpapillary plexus

**Omniscale In Vivo Photoacoustic (PA) Tomography with Consistent Contrast**

![Graph illustrating depth-resolution ratio = 200](image)

- Lateral resolution
- Axial resolution
- Low-freq PA tomography
- PA macroscopy
- Acoustic-resolution PAM
- Optical-resolution PAM
- Submicron PAM
- Sub-wavelength PAM
- PA nanoscopy

**In Vivo Photoacoustic Microscopy of Cerebral Hemodynamic Response to Electric Hindpaw Stimulation**

- 3D imaging rate: 1 Hz
- Oxygen saturation
- Photoacoustic signal change

**Collaboration:** LA Cornelius

**References:**
- OILAB.SEAS.WUSTL.EDU
- J Bliemol Optics 16, 016015, 2011
- LV Wang, J Biomed Optics 16, 016015, 2011
- C Favazza, O Jassim, LA Cornelius, LV Wang, J Bliemol Optics 16, 016015, 2011
- LV Wang, S Hu, Science 335, 1458, 2012
- LV Wang, Nature Photon 3, 503, 2009
- JJ Yao, LD Wang, JM Yang, XJ Maslov, TTW Wong, Lei Li, CH Huang, J Zou@TAMU, LV Wang, Nature Methods 12, 407, 2015; Featured by Science, doi:10.1126/science.aab0393
**In Vivo Single-Cell Photoacoustic Flowoxigraphy: Flow and Oxygenation Imaging**

In Vivo Single-Cell Photoacoustic Flowoxigraphy: Flow and Oxygenation Imaging

1 Hz B-scan

20 Hz B-scan

Single red blood cells (200 Hz B-scan, 20 3D images/s)

Oxygen saturation of hemoglobin (sO₂)

Normalized photoacoustic amplitude

Human cuticle capillary


**In Vivo Photoacoustic Microscopy and Short-Pulsed Laser Therapy of Single Circulating Tumor Cells**

In Vivo Photoacoustic Microscopy and Short-Pulsed Laser Therapy of Single Circulating Tumor Cells

Goals:

1. Remove primary melanoma
2. Clear circulating tumor cells
3. Uncage antigens alive
4. Elicit immunoresponse
5. Destroy metastases

Yun He, LD Wang, J Shi, J Zou @ TAMU, ... LV Wang, unpublished

**Label-Free Photoacoustic Histology by Imaging DNA & RNA in Cell Nuclei**

Label-Free Photoacoustic Histology by Imaging DNA & RNA in Cell Nuclei

Photoacoustic microscopy without staining

Histology with hematoxylin and eosin staining

D Yao, R Chen, K Maslov, Q Zhou, LV Wang, J Biomed Optics 17, 056004, 2012; Collaboration: Q Zhou @ USC
Label-Free Photoacoustic (PA) Nanoscopy of a Mitochondrion with Sub-Organellar Resolution: Beat Optical Diffraction Nonlinearly


PA microscopy (Resolution: 234 nm)
PA nanoscopy (Resolution: 90 nm)
Comparison Conventional PAM
Electron microscopy

Omniscale In Vivo Photoacoustic (PA) Tomography with Consistent Contrast


Low-freq PA tomography
PA macroscopy
Acoustic-resolution PAM
Optical-resolution PAM
Submicron PAM
Sub-wavelength PAM
PA nanoscopy

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Time-Reversed Ultrasound-Encoded (TRUE) Optical Focusing


Speckle Pattern Concentration by Photoacoustic Wavefront Shaping (PAWS)


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Watch a Flying Laser Pulse with Single-Shot Compressed Ultrafast Photography at 100 Billion Frames/Second

Video slowdown: 10 billion x


Watch a Flying Superluminal Mach Cone with Single-Shot Compressed Ultrafast Photography at 100 Billion Frames/Second

[L. Liang, C. Ma, L. Zhu, L.V. Wang, unpublished]

Single-Shot Compressed Ultrafast Photography: 100 Billion Frames per Second

Boost from 100 Billion to 10 Trillion Frames per Second

100 GHz 2.3 THz

[L Gao, J Liang], C Li, LV Wang, Nature 516, 74, 2014
J Liang, ..., LV Wang, unpublished

Simulated Ultrafast Imaging of Action Potential Propagation: Fastest 2-KHz Commercial Camera versus the Proposed 1 MHz CUP Camera

Liren Zhu, LV Wang, unpublished

Financial Interest Disclosure and Funding Sources

FINANCIAL INTEREST
- Microphotoacoustics, Inc.

ACTIVE GRANTS
- NIH DP1 EB016986: NIH Director’s Pioneer Award
  Program Directors: Richard Conroy/Ravi Basavappa
- NIH R01 CA186567: NIH Director’s Transformative Research Award
  Program Directors: Bob Nordstrom/Ravi Basavappa
- NIH R01 EB016963: Ring PACT
  Program Director: Richard Conroy
- NIH U01 NS090579: BRAIN Initiative
  Program Director: Ned Talley
- March of Dimes: Prematurity Birth
  Program Director: Joe Leigh Simpson
Further Information

- YouTube videos on “Photoacoustic tomography”
- Web at HTTP://OILAB.SEAS.WUSTL.EDU
- Books

Email: Photoacoustics@gmail.com

Relocation to Caltech

http://www.mede.caltech.edu/people

Hiring:
- Postdocs
- Students
- Technicians

Noise Equivalent Concentration or Number

Molar extinction coefficient (cm⁻¹/M)
Noise equivalent molar concentration (M)

Hb
Melanin
Microbubble
ICG
IRDye800
MB
mCherry
EGFP
RFP
iRFP
EB
GNC
GNR
GNB
SWNT

Imaging depth (mm)

NEC of hemoglobin (M)
NEN of hemoglobin (mol/voxel)

SW-PAM
OR-PAM
AR-PAM
PAMac
Optical diffusion limit

Endogenous Absorbers

Scalability of Resolution and Penetration

Acoustic spatial resolution $\propto \frac{1}{\text{Acoustic bandwidth}}$

Acoustic penetration limit $\propto \frac{1}{\text{Acoustic bandwidth}}$

Penetration limit
Spatial resolution $=$ Constant

Photoacoustic Conversion Efficiency and SNR

Thermal expansion coefficient $\approx 8$ mbars/mK

Compressibility

- Noise equivalent pressure $\sim$ sub mbar
- SNR at photoacoustic source $\sim 10^5$
- Attenuation over a $10^3$ voxel range $\sim 10^3$
- SNR at tissue surface $\sim 10^2$
Photoacoustic and Fluorescence Detection of Calcium-Sensitive Protein GCaMP5G in the Fruit Fly Brain In Vivo

RY Zhang, B Rao, HY Rong, B Raman@WUSTL, LV Wang, unpublished

Photoacoustic Microscopy of Calcium-Sensitive Protein GCaMP5G in the Fruit Fly Brain In Vivo

RY Zhang, B Rao, HY Rong, B Raman@WUSTL, LV Wang, unpublished

Photoacoustic Computed Tomography of the Whole Brain of a Rat In Vivo

Li Lin, Lei Li, Liren Zhu, Cheng Ma, Konstantin Maslov, LV Wang, unpublished
Photoacoustic Imaging of Hemoglobin and Glucose Metabolism in Mouse Brain in Vivo with Electric Forepaw Stimulation

In Vivo

Analogous to fMRI

$[\text{Hemoglobin}] \at 570 \text{ nm}$ (isosbestic wavelength)

Right paw stimulation

Left paw stimulation

Analogous to PET

$[2-\text{NBDG}] \at 478 \text{ nm}$ (Glucose analog)

Right paw stimulation

Left paw stimulation

Glucose analog: $2-\text{NBDG} = 3\text{-deoxy-3-[(7\text{-nitrobenz-2-oxa-1,3-diazol-4-yl)}amino]-D-glucopyranose}$

Photoacoustic image

Noninvasive In Vivo Photoacoustic Tomography of Resting-State Functional Connectivity in Mouse Brain

Olfactory bulb

Paxinos atlas

Photoacoustic image

[JM Nasiriavanaki, J Xia, H Wan, A Bauer, J Culver@WUSTL, LV Wang, PNAS 111, 21, 2014]