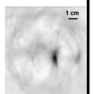
PHOTOACOUSTIC TOMOGRAPHY:
High-resolution Imaging of Optical Contrast In Vivo at New Depths



Lihong V. Wang, PhD
Gene K. Beare Distinguished Professor
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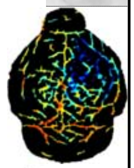
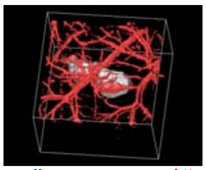
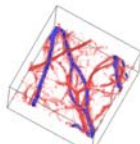
Washington University, St. Louis







Email: Photoacoustics@gmail.com

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- X. Cai
- A. Danielli
- C. Favazza
- A. Garcia-Uribe
- Z. Guo
- S. Hu
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- C. H. Kim
- G. Ku
- C. H. Li
- L. Li
- H. L. Liu
- K. Maslov
- M. Pramanik
- B. Rao
- L. Song
- V. Tsytsarev
- Y. Wang
- X. Xu
- J. M. Yang
- J. J. Yao

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- H. Fang, PhD
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- S. Jiao, PhD
- X. Jin, PhD
- J. Li, PhD
- M. Li, PhD
- Y. Li, PhD
- G. Marquez, PhD
- M. Mehrubeoglu, PhD
- J. Oh, PhD
- Y. Pang, MS
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- M. Sivaramakrishnan, MS
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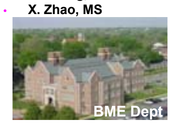
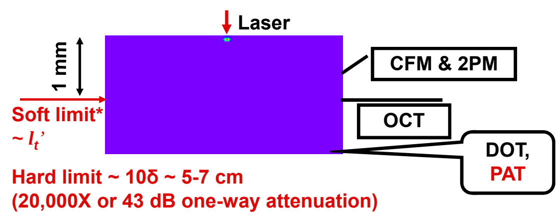
- Y. Xu, PhD
- X. Yang, PhD
- G. Yao, PhD
- W. Yu, MS
- R. Zemp, PhD
- H. Zhang, PhD
- X. Zhao, MS



Motivation for Optical Imaging

- Safety — Non-ionizing radiation: photon energy is ~2 eV
- Physics — Related to molecular conformation of tissue
- Optics — High intrinsic contrast:
  - Absorption: oxy- & deoxy-hemoglobin, melanin, water
  - Scattering: cell nuclei and mitochondria
  - Polarization: Collagen and muscle fibers
- Physiology — Functional imaging (endogenous contrast):
  - Concentration of hemoglobin (angiogenesis)
  - Oxygen saturation of hemoglobin (hyper-metabolism)
  - Blood flow (Doppler)
  - Enlargement of cell nuclei
  - Orientation and denaturation of collagen
- Physiology — Molecular imaging (exogenous contrast):
  - Integrin, VEGF (vascular endothelial growth factor).
  - Reporter genes
- ….

Major Challenge in Optical Tomography: High Resolution Beyond the Quasiballistic (~1-mm Depth) Regime



CFM: Confocal microscopy
2PM: Two-photon microscopy
OCT: Optical coherence tomography
DOT: Diffuse optical tomography
PAT: Photoacoustic tomography
l_t': Optical transport mean free path ~ 1 mm (mean free path ~ 0.1 mm)
δ: Effective penetration depth

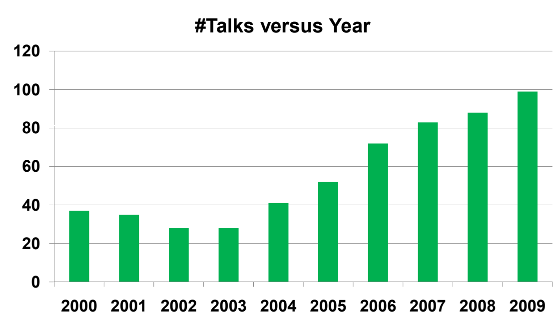
* Applied Optics 38, 4951 (1999). Simulation software MCML available on the web

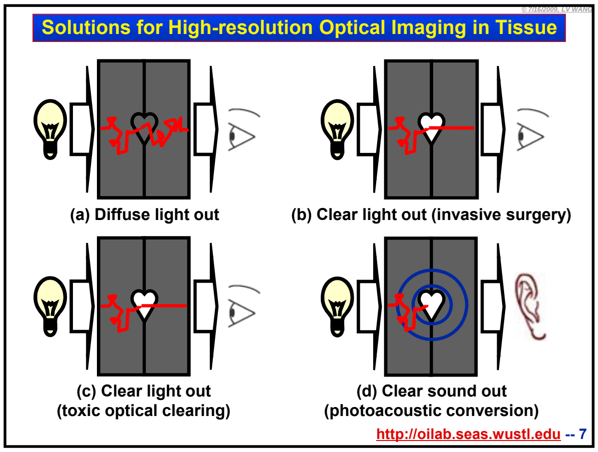
Optical Diffraction versus Diffusion

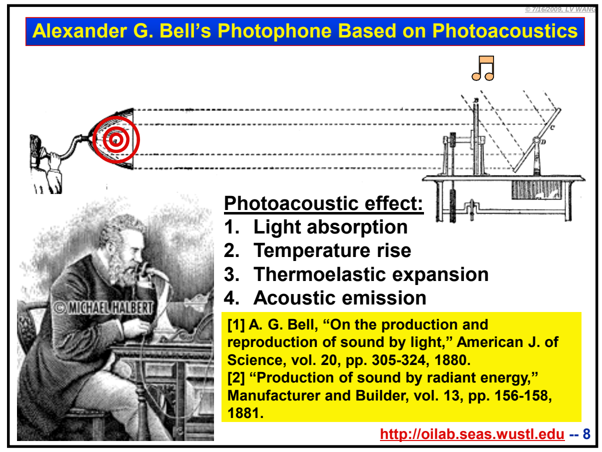
- Diffraction limits the spatial resolution of ballistic imaging
- Breaking the diffraction limit leads to super-resolution imaging

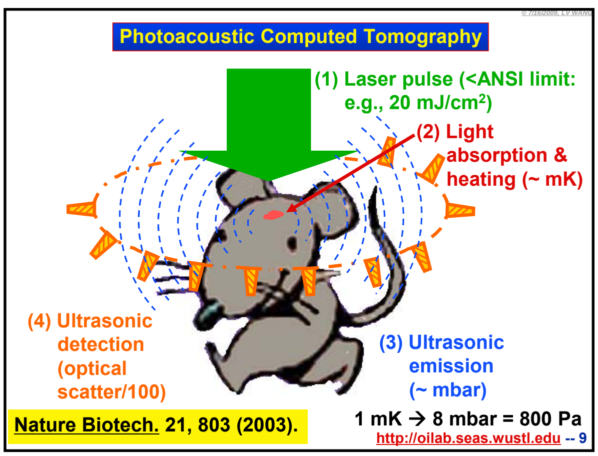
- Diffusion limits the penetration of ballistic imaging
- Breaking the diffusion limit leads to super-depth imaging

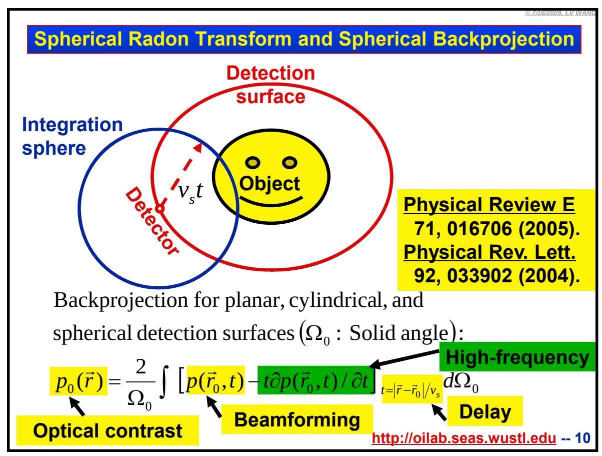
Growth of Photoacoustic Tomography: Data from Conference on Photons plus Ultrasound Chaired by Oraevsky and Wang




Solutions for High-resolution Optical Imaging in Tissue
(a) Diffuse light out
(b) Clear light out (invasive surgery)
(c) Clear light out
(toxic optical clearing)
(d) Clear sound out
(photoacoustic conversion)
http://oilab.seas.wustl.edu -- 7


Alexander G. Bell's Photophone Based on Photoacoustics
Photoacoustic effect:
1. Light absorption
2. Temperature rise
3. Thermoelastic expansion
4. Acoustic emission
© MICHAEL HALBERT
[1] A. G. Bell, "On the production and reproduction of sound by light," American J. of Science, vol. 20, pp. 305-324, 1880.
[2] "Production of sound by radiant energy," Manufacturer and Builder, vol. 13, pp. 156-158, 1881.
http://oilab.seas.wustl.edu -- 8


Photoacoustic Computed Tomography
(1) Laser pulse (<ANSI limit: e.g., 20 mJ/cm^2)
(2) Light absorption & heating (~ mK)
(3) Ultrasonic emission (~ mbar)
(4) Ultrasonic detection (optical scatter/100)
Nature Biotech. 21, 803 (2003).
1 mK → 8 mbar = 800 Pa
http://oilab.seas.wustl.edu -- 9


Spherical Radon Transform and Spherical Backprojection
Detection surface
Integration sphere
Detector
$v_s t$
Object
Physical Review E 71, 016706 (2005).
Physical Rev. Lett. 92, 033902 (2004).
Backprojection for planar, cylindrical, and spherical detection surfaces (Ω_0 : Solid angle):
$p_0(\vec{r}) = \frac{2}{\Omega_0} \int \left[p(\vec{r}_0, t) - t \partial p(\vec{r}_0, t) / \partial t \right]_{t=|\vec{r}-\vec{r}_0|/v_s} d\Omega_0$
High-frequency
Optical contrast
Beamforming
Delay
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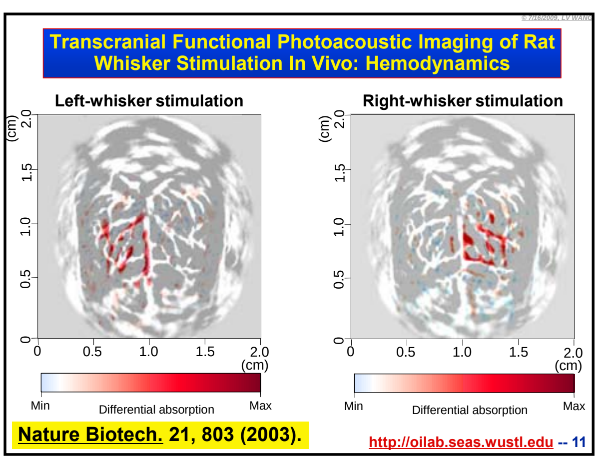
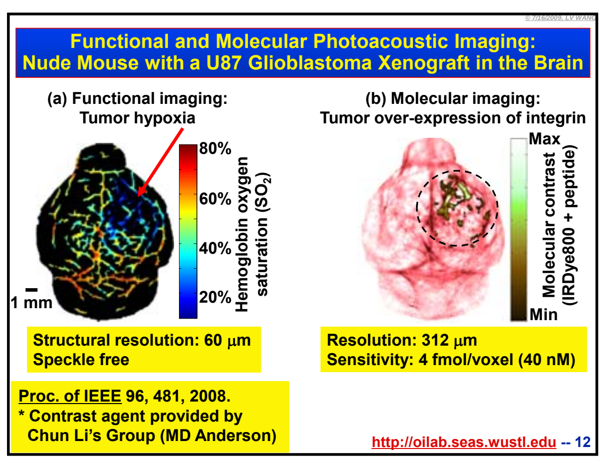

Transcranial Functional Photoacoustic Imaging of Rat Whisker Stimulation In Vivo: Hemodynamics
Left-whisker stimulation
Right-whisker stimulation
(cm)
Min
Differential absorption
Max
Nature Biotech. 21, 803 (2003).
http://oilab.seas.wustl.edu -- 11


Functional and Molecular Photoacoustic Imaging: Nude Mouse with a U87 Glioblastoma Xenograft in the Brain
(a) Functional imaging: Tumor hypoxia
(b) Molecular imaging: Tumor over-expression of integrin
80%
60%
40%
20%
Hemoglobin oxygen saturation (SO$_2$)
1 mm
Max
Min
Molecular contrast (IRDye800 + peptide)
Structural resolution: 60 μm
Speckle free
Resolution: 312 μm
Sensitivity: 4 fmol/voxel (40 nM)
Proc. of IEEE 96, 481, 2008.
* Contrast agent provided by Chun Li's Group (MD Anderson)
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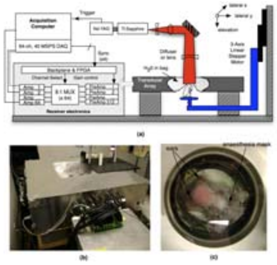
5-MHz Ring Ultrasonic Array

- Transducers:
  - Elements: 512-elements.
  - Center frequency: 5 MHz.
  - Bandwidth: 80% of center freq.
- Geometry:
  - Shape: Ring with 50 mm diam.
  - Pitch: 0.308 mm. Kerf: 0.1 mm.
  - Elevation height: 10 mm.
  - Elevation focal length: 19 mm.
- Data acquisition:
  - Channels: 64 channels A/D (8:1 MUX)
  - Sampling rate: 40 MHz.
  - Sampling dynamic range: 12 bits.
  - Frame rate: 0.9 Hz with a 7-Hz laser pulse repetition.
- Resolution:
  - In plane: ~200 μm.
  - Elevation: ~1.9 mm.



J. Biomed. Optics 13, 024007, 2008.
Optics Express, submitted, 2009.
* Detection system by Q. Zhu's Group (UConn).

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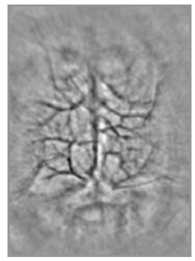
Noninvasive Photoacoustic Image of a Mouse Brain: 360° View, 0.9 Hz Frame Rate and Gel Coupling

Photoacoustic Image
Field of view: 10 mm x 14 mm



Photograph

C. Li et al., unpublished.

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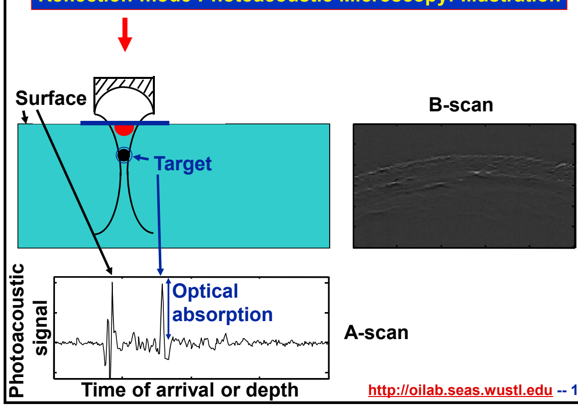
Reflection-mode Photoacoustic Microscopy: Illustration



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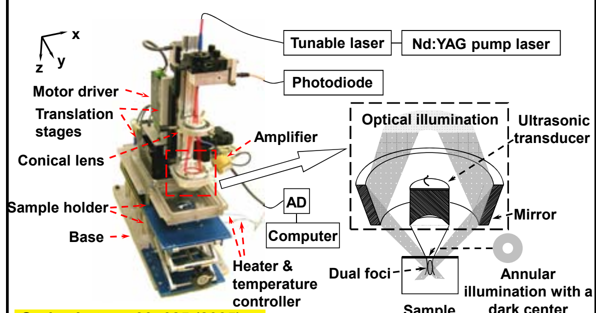
Reflection-mode Dark-field Confocal Photoacoustic Microscopy: System

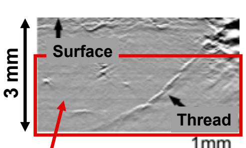


Optics Letters 30, 625 (2005).
Nature Biotech. 24, 848 (2006).
Nature Protocols 2, 797 (2007).

http://oilab.seas.wustl.edu -- 16

Photoacoustic Imaging Depth and Resolution: 50 MHz System (High Ultrasonic Frequency)

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



Super-depths for high-resolution optical imaging (beyond the quasiballistic regime)

- Imaging depth: ~3 mm
- Axial resolution: ~15 microns
- Depth/resolution: ~200 pixels

- Lateral resolution: ~45 microns
- Acquisition time: 2 μs/A-scan
- Signal averaging: None

Optics Letters 30, 625 (2005).

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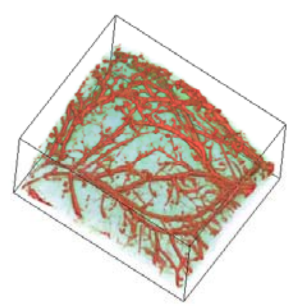
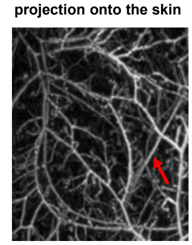
Volumetric Imaging of Rat Microvasculature In Vivo



Volume: 10 mm x 8 mm x 3 mm

Maximum amplitude projection onto the skin



1 mm

Optics Express 14, 9317 (2006).

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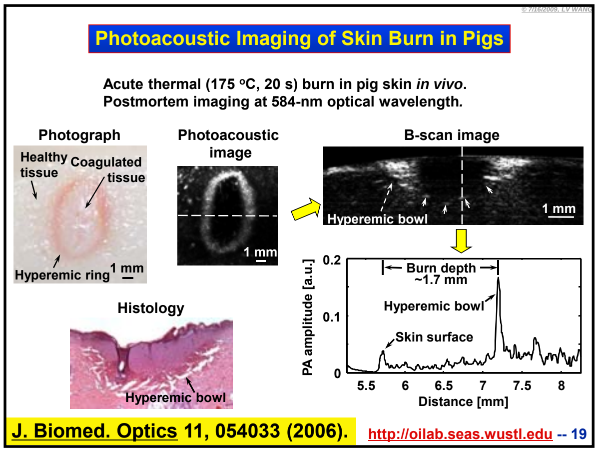
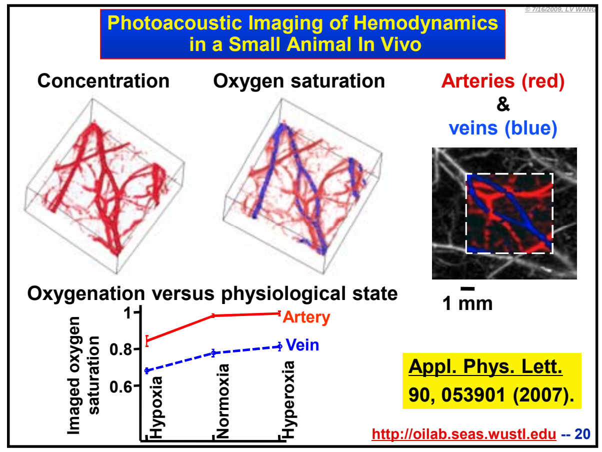
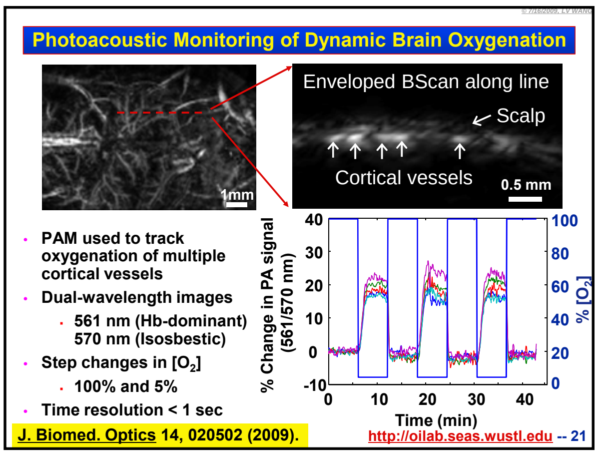
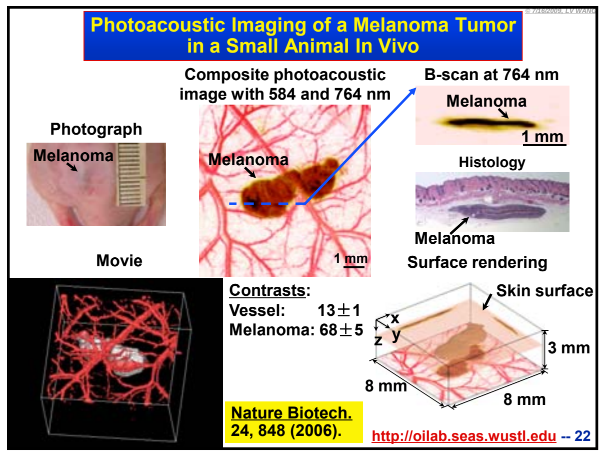
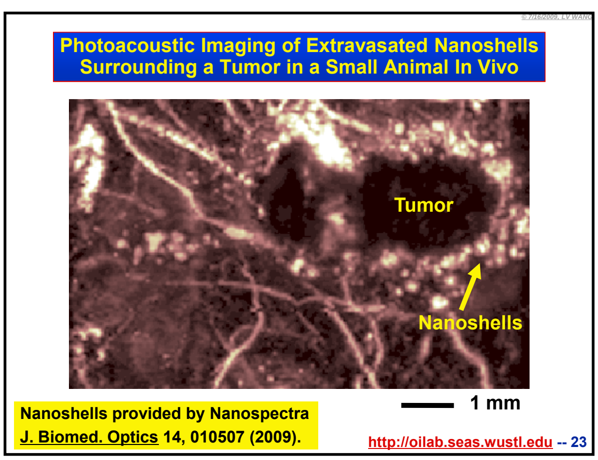
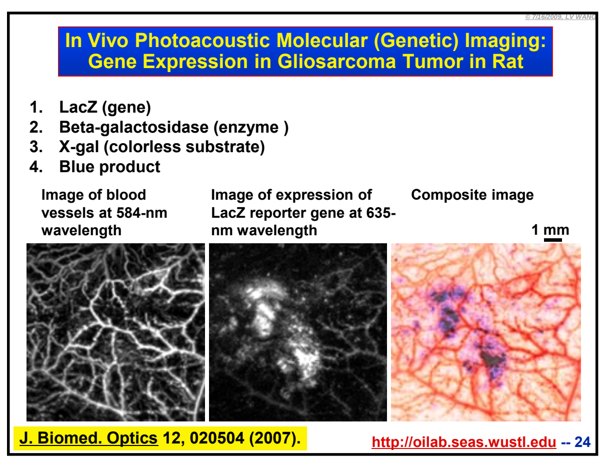

Photoacoustic Imaging of Skin Burn in Pigs
Acute thermal (175 °C, 20 s) burn in pig skin in vivo.
Postmortem imaging at 584-nm optical wavelength.
Photograph
Healthy tissue
Coagulated tissue
Hyperemic ring
1 mm
Photoacoustic image
1 mm
B-scan image
Hyperemic bowl
1 mm
Burn depth ~1.7 mm
Hyperemic bowl
Skin surface
PA amplitude [a.u.]
Distance [mm]
Histology
Hyperemic bowl
J. Biomed. Optics 11, 054033 (2006).
http://oilab.seas.wustl.edu -- 19


Photoacoustic Imaging of Hemodynamics in a Small Animal In Vivo
Concentration
Oxygen saturation
Arteries (red) & veins (blue)
1 mm
Oxygenation versus physiological state
Imaged oxygen saturation
Artery
Vein
Hypoxia
Normoxia
Hyperoxia
Appl. Phys. Lett. 90, 053901 (2007).
http://oilab.seas.wustl.edu -- 20


Photoacoustic Monitoring of Dynamic Brain Oxygenation
1mm
Enveloped BScan along line
Scalp
Cortical vessels
0.5 mm
PAM used to track oxygenation of multiple cortical vessels
Dual-wavelength images
561 nm (Hb-dominant)
570 nm (Isosbestic)
Step changes in [O2]
100% and 5%
Time resolution < 1 sec
% Change in PA signal (561/570 nm)
Time (min)
% [O2]
J. Biomed. Optics 14, 020502 (2009).
http://oilab.seas.wustl.edu -- 21

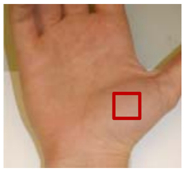

Photoacoustic Imaging of a Melanoma Tumor in a Small Animal In Vivo
Photograph
Melanoma
Composite photoacoustic image with 584 and 764 nm
Melanoma
1 mm
B-scan at 764 nm
Melanoma
1 mm
Histology
Melanoma
Movie
Surface rendering
Contrasts:
Vessel: 13±1
Melanoma: 68±5
Skin surface
3 mm
8 mm
8 mm
Nature Biotech. 24, 848 (2006).
http://oilab.seas.wustl.edu -- 22


Photoacoustic Imaging of Extravasated Nanoshells Surrounding a Tumor in a Small Animal In Vivo
Tumor
Nanoshells
1 mm
Nanoshells provided by Nanospectra
J. Biomed. Optics 14, 010507 (2009).
http://oilab.seas.wustl.edu -- 23

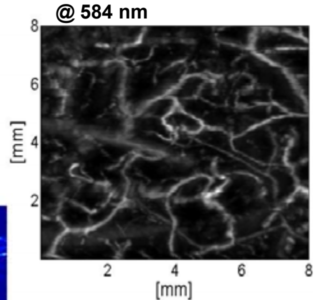

In Vivo Photoacoustic Molecular (Genetic) Imaging: Gene Expression in Gliosarcoma Tumor in Rat
1. LacZ (gene)
2. Beta-galactosidase (enzyme)
3. X-gal (colorless substrate)
4. Blue product
Image of blood vessels at 584-nm wavelength
Image of expression of LacZ reporter gene at 635-nm wavelength
Composite image
1 mm
J. Biomed. Optics 12, 020504 (2007).
http://oilab.seas.wustl.edu -- 24

Photoacoustic Imaging of Human Palm

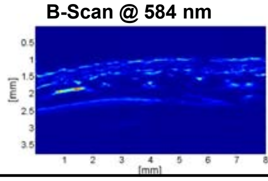
Photo



Max amplitude projection @ 584 nm



B-Scan @ 584 nm



C. Favazza, unpublished.

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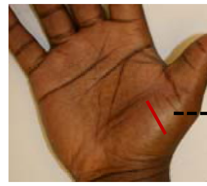
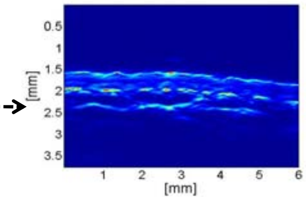
Photoacoustic Imaging of Human Palm: African American

Photo



B-Scan @ 584 nm



C. Favazza, unpublished.

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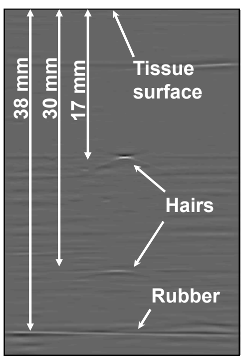
Deeply Penetrating (30 mm) 5-MHz Photoacoustic Imaging System



- Transducer frequency: 5 MHz
- Optical wavelength: 804 nm
- Exposure: <31 mJ/cm^2 (ANSI)
- Signal averaging: 30 times
- SAFT (synthetic-aperture focusing technique)
- Axial resolution: 144 μm
- Transverse resolution: 560 μm
- SNR: 24 dB at 30 mm depth

J. Biomed. Opt. 12, 060503 (2007).

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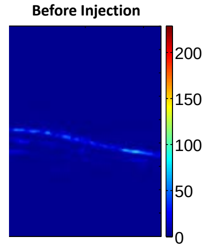
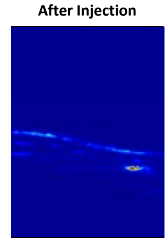
In Vivo Photoacoustic Imaging of Sentinel Lymph Node in Rat with Methylene Blue (L15-7 Transducer)

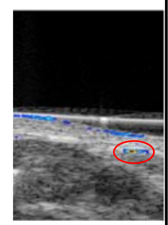
Photoacoustic: Before Injection



Photoacoustic: After Injection



Photoacoustic & Ultrasound



Field of view: 12 mm x 18 mm

In collaboration with Philips, unpublished.
Credit: C. Kim, T. Erpelding, L. Jankovic

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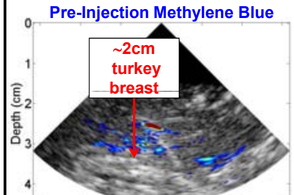
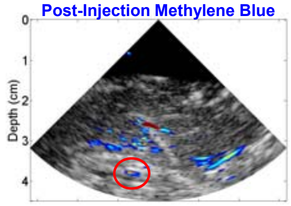
Photoacoustic Imaging of Sentinel Lymph Node in Rat at 2 cm Depth (S5-1 transducer, no averaging)

Pre-Injection Methylene Blue



Post-Injection Methylene Blue



Average depth of human axillary lymph nodes = 1.2 cm

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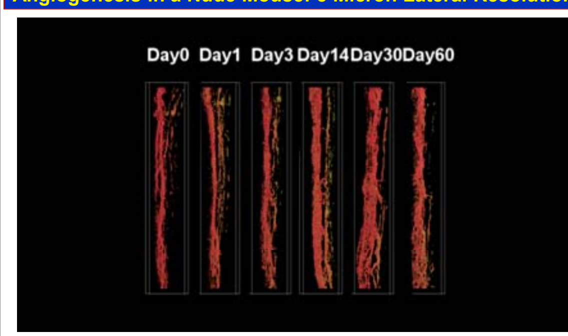
Optical Resolution Photoacoustic Microscopy of Angiogenesis in a Nude Mouse: 5 Micron Lateral Resolution



Each image:
4.00x1.00x0.45 mm^3

S. Hu et al., unpublished (Collaboration: Arbeit).

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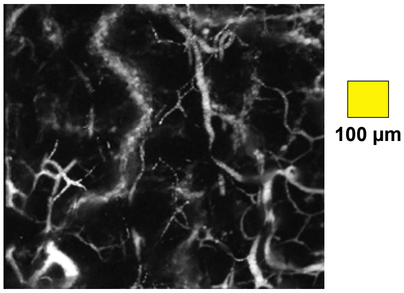
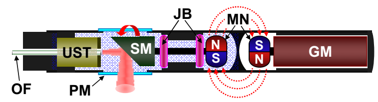
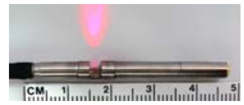
Optical Resolution Photoacoustic Microscopy of Single Red Blood Cells In Situ: 2 Micron Lateral Resolution

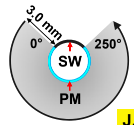


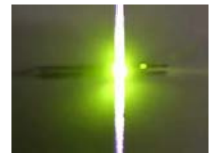
G. Ku et al., unpublished.

Photoacoustic Endoscopy



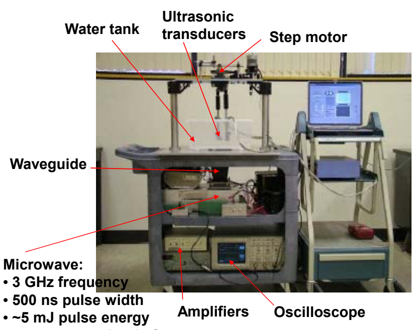




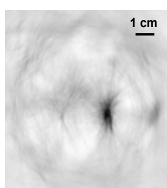


J. Yang et al., Optics Letters, 34, 1591(2009).

Microwave-induced Thermoacoustic Tomography (TAT): Experimental System and Image of Mastectomy Specimen



Microwave:
- 3 GHz frequency
- 500 ns pulse width
- ~5 mJ pulse energy
- 9 cm penetration in fat



- Invasive lobular carcinoma
- 11 cm diameter X 9 cm thick
- 5:1 contrast

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

Summary

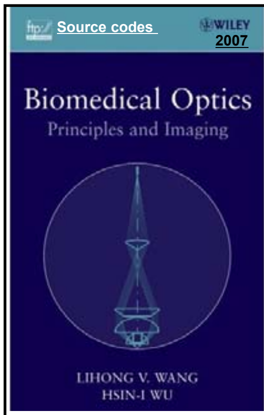
- Photoacoustic tomography achieved by optical excitation and ultrasonic detection: Physically integrated single modality.
- Depths beyond optical transport mean free path imaged
  - Absorption of both unscattered and scattered photons used
  - "Soft limit" (~1 mm in skin) broken (i.e., superdepths achieved)
  - "Hard limit" (~5 cm) reached
- Spatial resolution at superdepths determined ultrasonically
  - Number of resolvable pixels enhanced: Depth/resolution > 200
  - Depth and resolution scaled: Scalable multiscale imaging
  - 15 micron axial resolution at 3 mm depth shown
  - 150 micron axial resolution at 30 mm depth shown
- Spatial resolution within "soft limit" determined both optically and ultrasonically
  - 2-5 micron transverse resolution demonstrated
  - Single capillaries (smallest blood vessels) resolved
  - Single red blood cells imaged

Summary (Continued)

- Contrast provided by optical absorption properties
  - Ultrasonic or fluorescent contrasts complemented
  - Sensitivity to specific absorption (mJ/cc) shown to be 100%
  - High specificity attributed to high intrinsic contrast: Blood:bg>10:1
  - Multiple chromophores detected spectrally: Oxy- and deoxy-Hb
  - Functional imaging allowed by endogenous chromophores: sO_2
  - Molecular biomarker imaging enabled by targeted contrast agents: Organic IRDye800 bound to integrin
  - Molecular gene-expression imaging derived from reporter genes: Blue product from lacZ gene
- Frame rate limited theoretically by range/sound speed: 0.1 ms → 15 cm
  - 50 frames/second (Hz) achieved thus far
  - Limited practically by the number of ultrasonic transducers
  - Limited practically by the pulse repetition rate of the laser

Summary (Continued)

- Thermoacoustic tomography achieved by microwave/RF excitation and ultrasonic detection.
  - Optical "hard limit" broken
  - Water or ion contrasts imaged
  - Deep-penetrating nonionizing molecular imaging possible
- Speckle in optical coherence tomography or ultrasonography avoided
- Non-ionizing radiation used
- Costs estimated comparable to those of ultrasound imaging systems



Chapters

1. Introduction to biomedical optics
2. Single scattering: Rayleigh theory and Mie theory
3. Monte Carlo modeling of photon transport
4. Convolution for broad-beam responses
5. Radiative transfer equation and diffusion theory
6. Hybrid model of Monte Carlo method and diffusion theory
7. Sensing of optical properties and spectroscopy
8. Ballistic imaging and microscopy
9. Optical coherence tomography
10. Mueller optical coherence tomography
11. Diffuse optical tomography
12. Photoacoustic tomography
13. Ultrasound-modulated optical tomography

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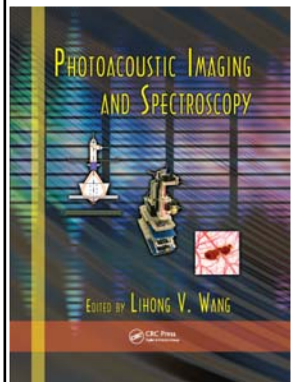

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