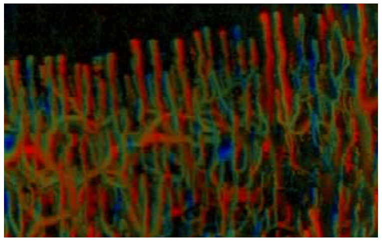
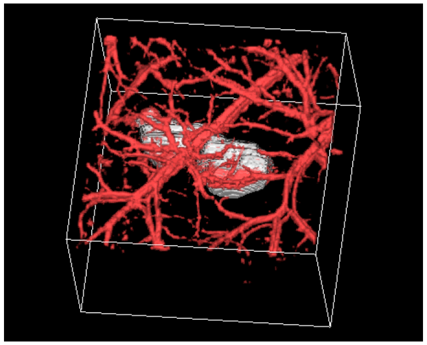
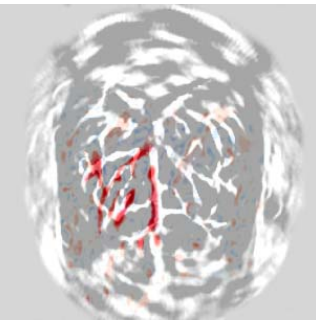
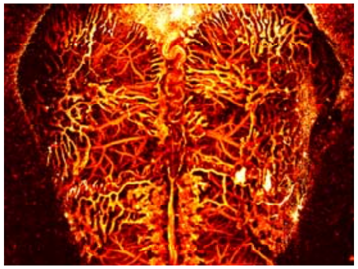
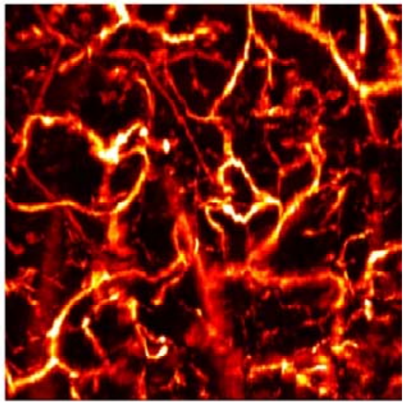
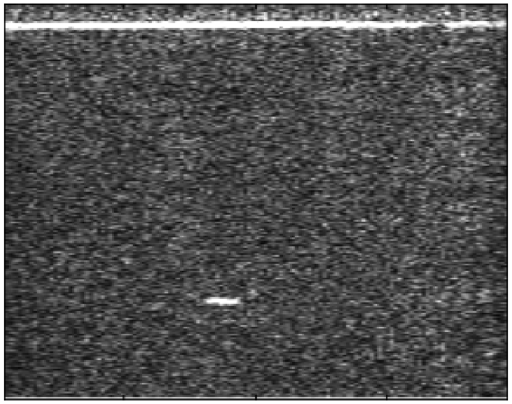
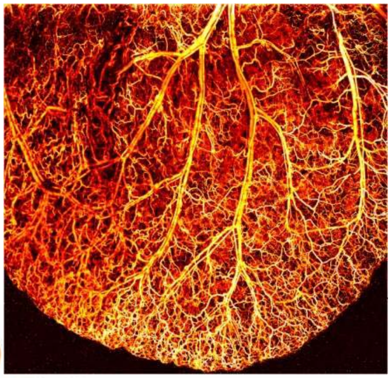
PHOTOACOUSTIC TOMOGRAPHY:
Ultrasonically Breaking through the Optical Diffusion Limit

Lihong V. Wang
Gene K. Beare Distinguished Professor
Optical Imaging Laboratory
Departments of Biomedical Engineering,
Radiology, and
Electrical & Systems Engineering
Washington University, St. Louis

Email: Photoacoustics@gmail.com

Financial Interest Disclosure and Funding Sources

FINANCIAL INTEREST

- Microphotoacoustics, Inc.
- Endra, Inc.

ACTIVE GRANTS

- NIH/NCI
  - U54 CA136398/NTR Ctr: SLN PA
  - R01 CA134539: Chemo TAT/PAT
  - R01 CA157277: PA endoscopy
- NIH/NIBIB
  - R01 EB000712: PAM
  - R01 EB008085: DOT/PAT
  - R01 EB010049: Brain TAT

COMPLETED GRANTS

- NIH
  - R21 CA83760: SLT
  - R01 CA71980: UOT
  - R29 CA68562/FIRST: UOT
  - R21 EB000319: MOCT
  - R01 EB000712: PAM
  - R33 CA094267: UOT
  - R01 CA092415: OCT
  - R01 NS46214/BRP: PACT
  - R01 CA106728: OIR
- NSF
  - BES-9734491/CAREER: UOT
- DOD Breast Program
  - DAMD17-00-1-0455: TAT
- Whitaker Foundation
  - RG-96-0221: OIR

Outline

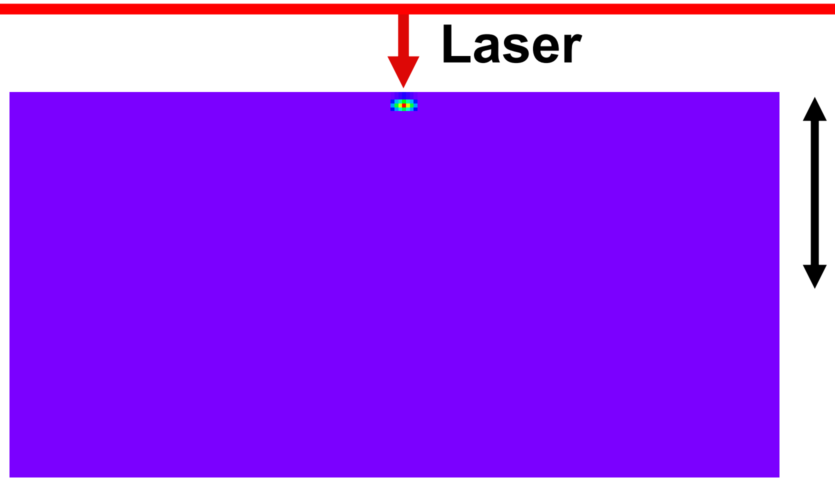
- Motivation and challenges
- Photoacoustic computed tomography
  - Circular geometry
  - Linear geometry
- Photoacoustic microscopy
  - Acoustic resolution
  - Optical resolution
- Discussion and summary

Motivation for In Vivo Optical Imaging (Partial List)

- Safety — Non-ionizing radiation: photon energy is ~2 eV
- Physics — Molecular structure and composition of tissue
- Optics — High intrinsic contrast. E.g., absorption probes
    - Oxy-hemoglobin & deoxy-hemoglobin
    - Melanin
    - Lipid
    - Water
    - DNA & RNA
- Physiology — Endogenous contrast for functional imaging
    - Concentration of hemoglobin (angiogenesis)
    - Oxygen saturation of hemoglobin (hyper-metabolism)
    - Cell nuclei
    - Blood flow (Doppler)
- Biology — Exogenous contrast for molecular imaging
    - Biomarkers (Integrin, VEGF, HER2, etc.)
    - Reporter genes

Fundamental Challenges in High-resolution Optical Imaging: Diffraction and Diffusion

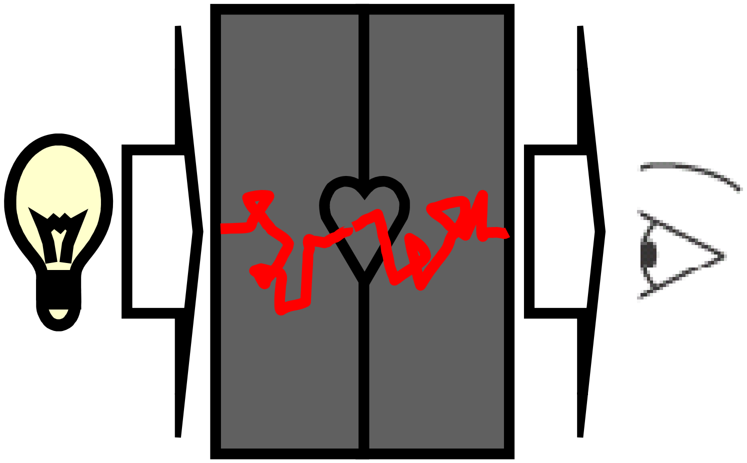
- Diffraction (wave phenomenon)
  - Limits the spatial resolution of ballistic imaging (planar, confocal, & two-photon microscopy, optical-coherence tomography).
  - Has been overcome for super-resolution imaging (PALM/STORM).
- Diffusion (scattering phenomenon)
  - Limits the penetration of ballistic imaging to ~1 mm in skin.
  - Has been overcome for super-depth imaging (PAT).



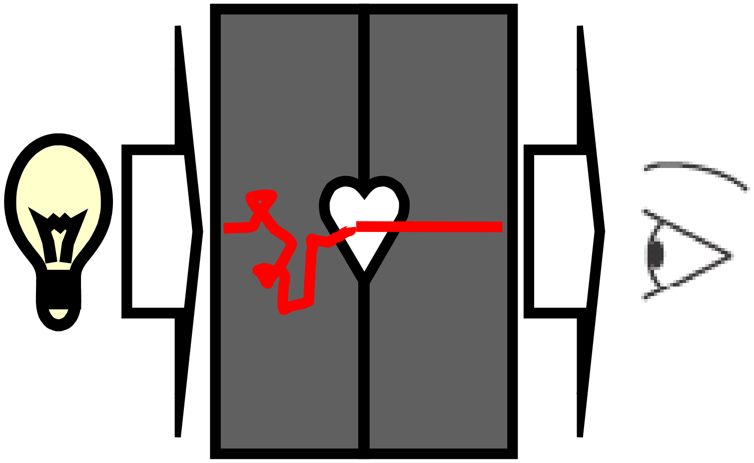
Diffusion limit ~ 1 mm
(Transport mean free path)

Ballistic decay constant ~ 0.1 mm
Diffuse decay constant ~ 10 mm

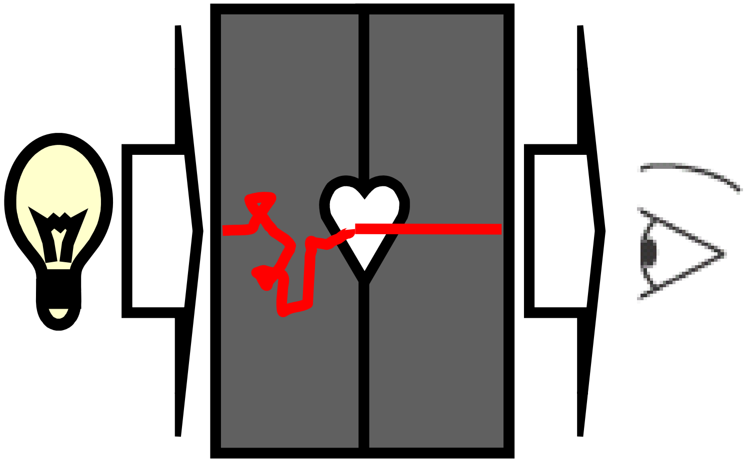
High-resolution Imaging by Clear Detection Despite Diffuse Illumination



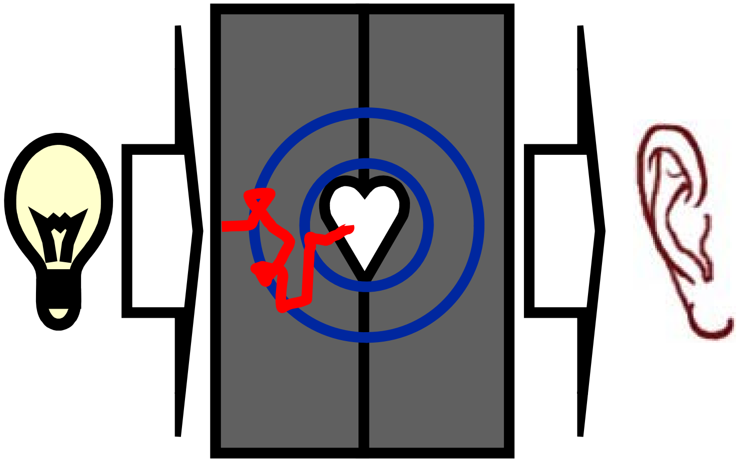
(a) Diffuse light out



(b) Clear light out (invasive surgery)

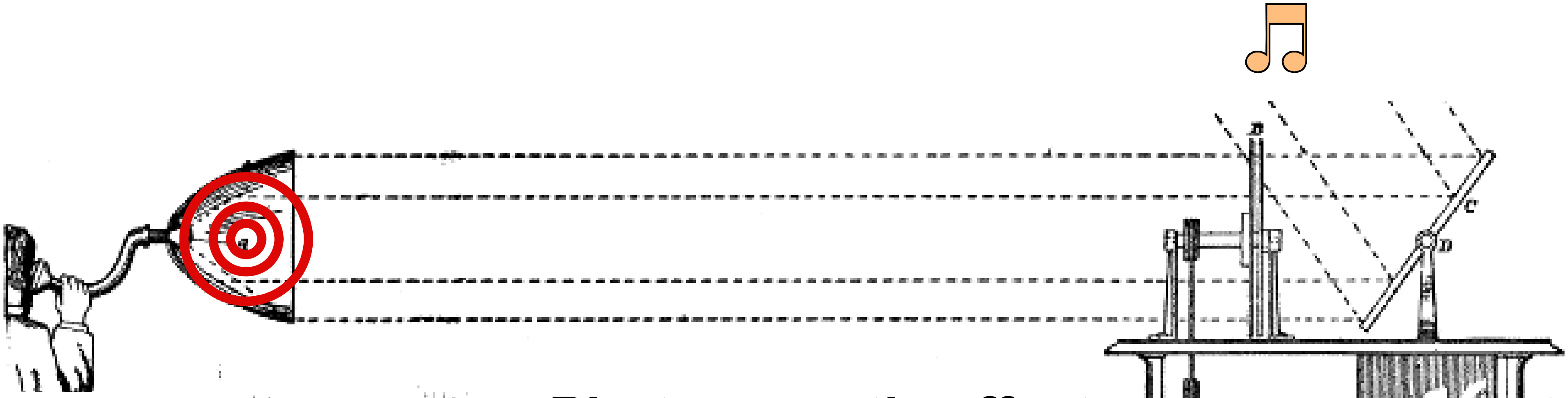


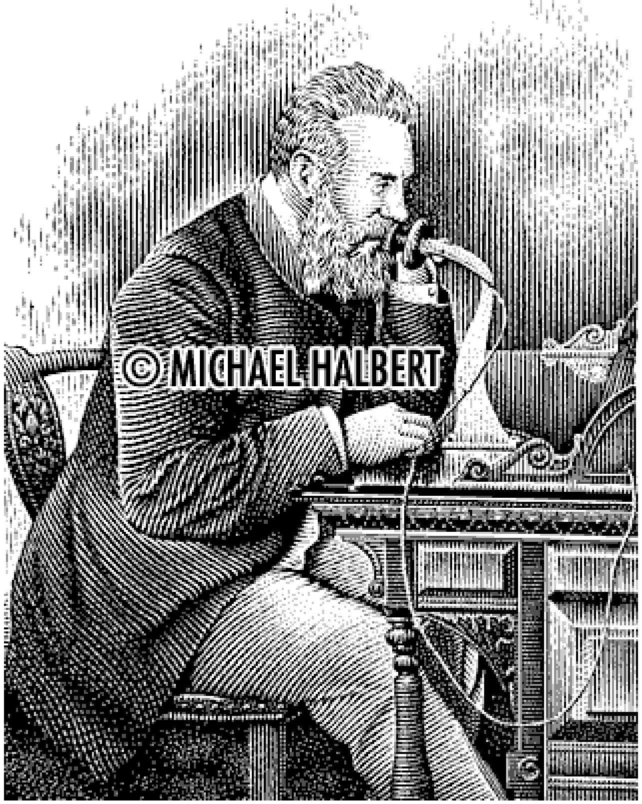
(c) Clear light out
(toxic optical clearing)



(d) Clear sound out
(photoacoustic conversion)

Alexander G. Bell's Photophone Based on Photoacoustics





Photoacoustic effect:

1. Light absorption
2. Temperature rise
3. Thermoelastic expansion
4. Acoustic emission

[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.

General Photoacoustic Wave Equation

$$(\nabla^2 - \frac{1}{v_s^2}\frac{\partial^2}{\partial t^2})p(\mathbf{r},t) = -\frac{\beta}{\kappa v_s^2}\frac{\partial^2 T\left(\vec{r},t\right)}{\partial t^2}$$

v_s : speed of sound

T : temperature rise

β : isobaric volume expansion coefficient

κ : compressibility

Short-pulsed Laser-induced Initial Photoacoustic Pressure

p_0 [Initial photoacoustic pressure]

$\propto$

T [Temperature rise]

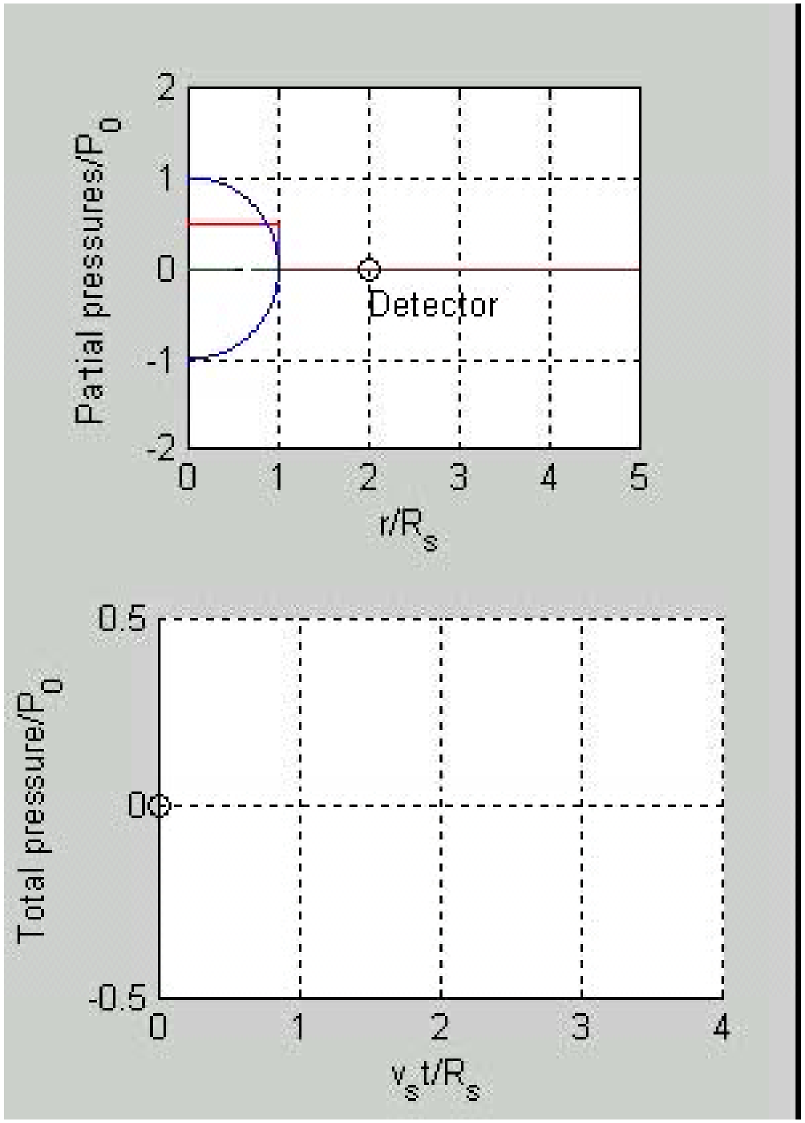
$\propto$

μ_a $\left[\text{Optical absorption coefficient in /m}\right]$

$\times$

F $\left[\text{Optical fluence}\left(\text{scalar flux}\right)\text{in J/m}^2\right]$

Photoacoustic Spherical Wave From a Sphere in an Optically Scattering Medium



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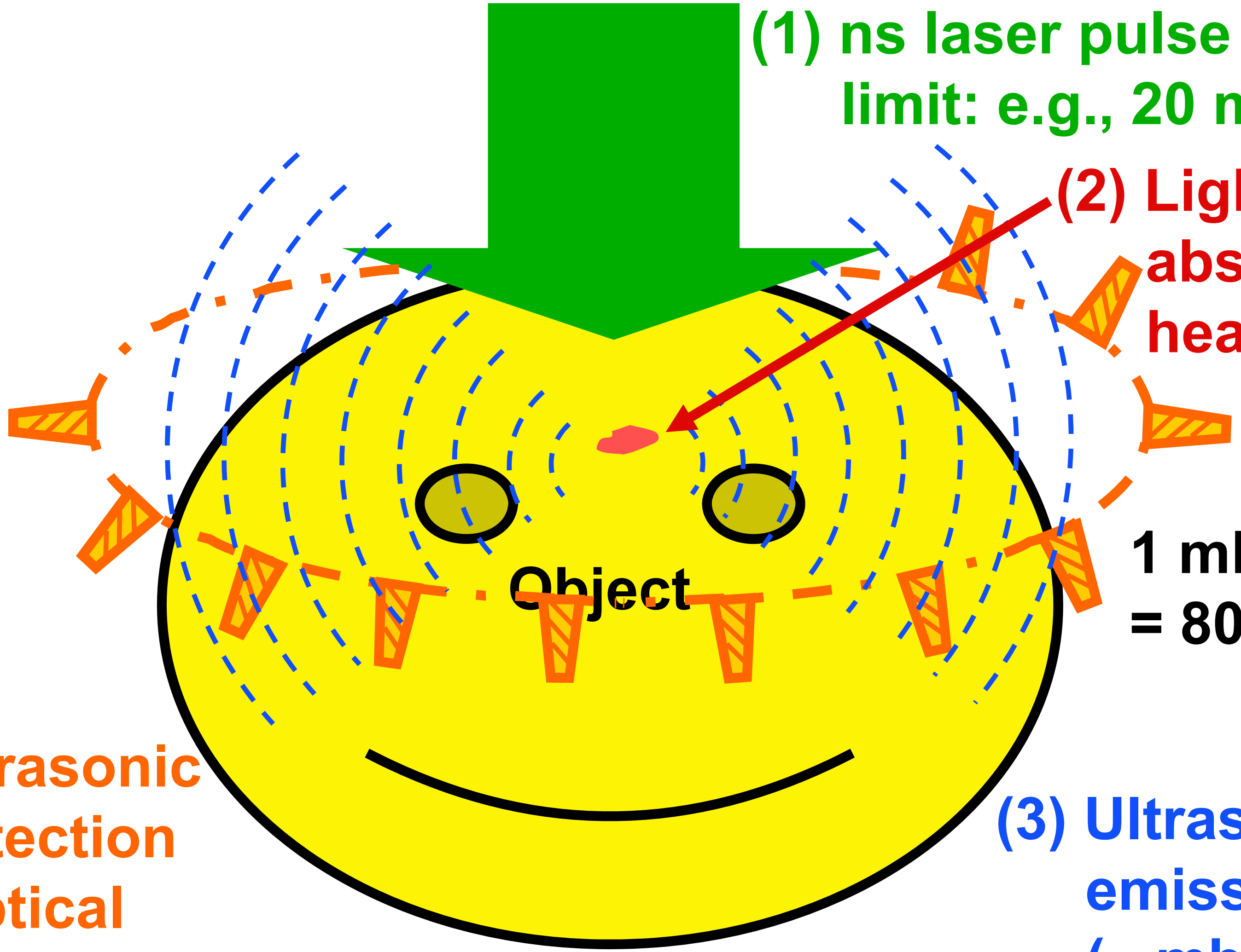
Photoacoustic Computed Tomography in Circular Geometry



(1) ns laser pulse (<ANSI limit: e.g., 20 mJ/cm^2)

(2) Light absorption & heating (~ mK)

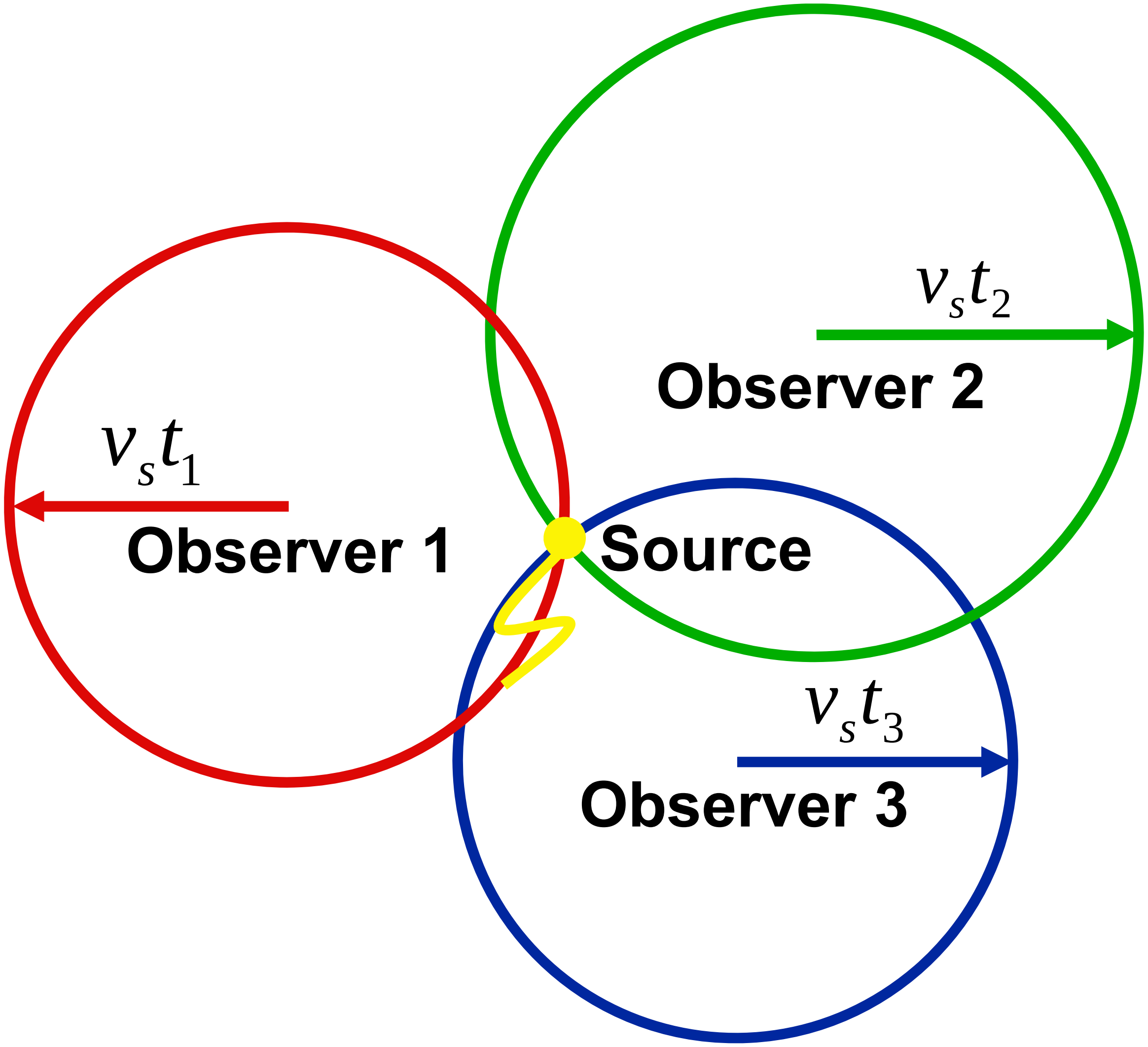
1 mK → 8 mbar = 800 Pa!

(3) Ultrasonic emission (~ mbar)

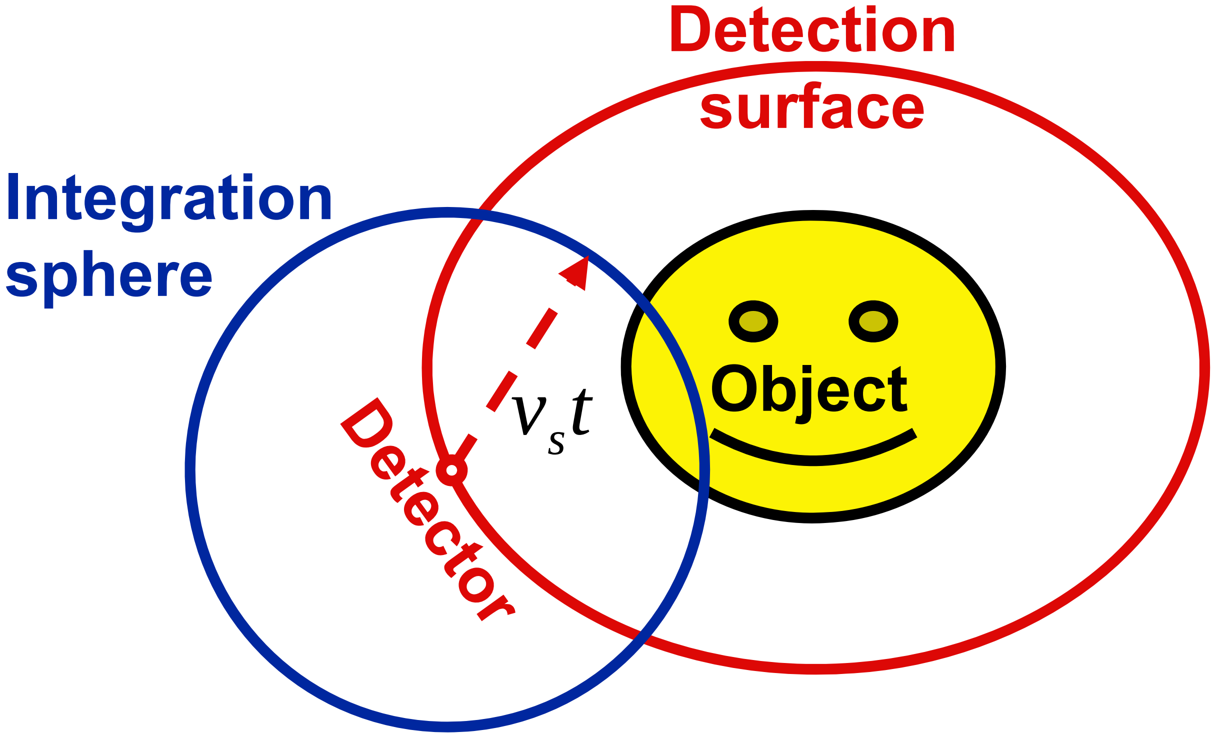
(4) Ultrasonic detection (optical scatter/1000)

<u>Nature Biotech.</u> 21, 803 (2003).

Imaging of a Single Sound Source by Triangulation



Spherical Radon Transform and Spherical Backprojection



Physical Review E 71, 016706 (2005).
Physical Rev. Lett. 92, 033902 (2004).

Universal backprojection for planar, cylindrical, and spherical detection surfaces $(\Omega_0 :\ \text{Solid angle})$:

$$p_0(\vec{r}) = \frac{2}{\Omega_0}\int \left[p(\vec{r}_0,t) - t\partial p(\vec{r}_0,t)/\partial t\right]_{t=|\vec{r}-\vec{r}_0|/v_s} d\Omega_0$$

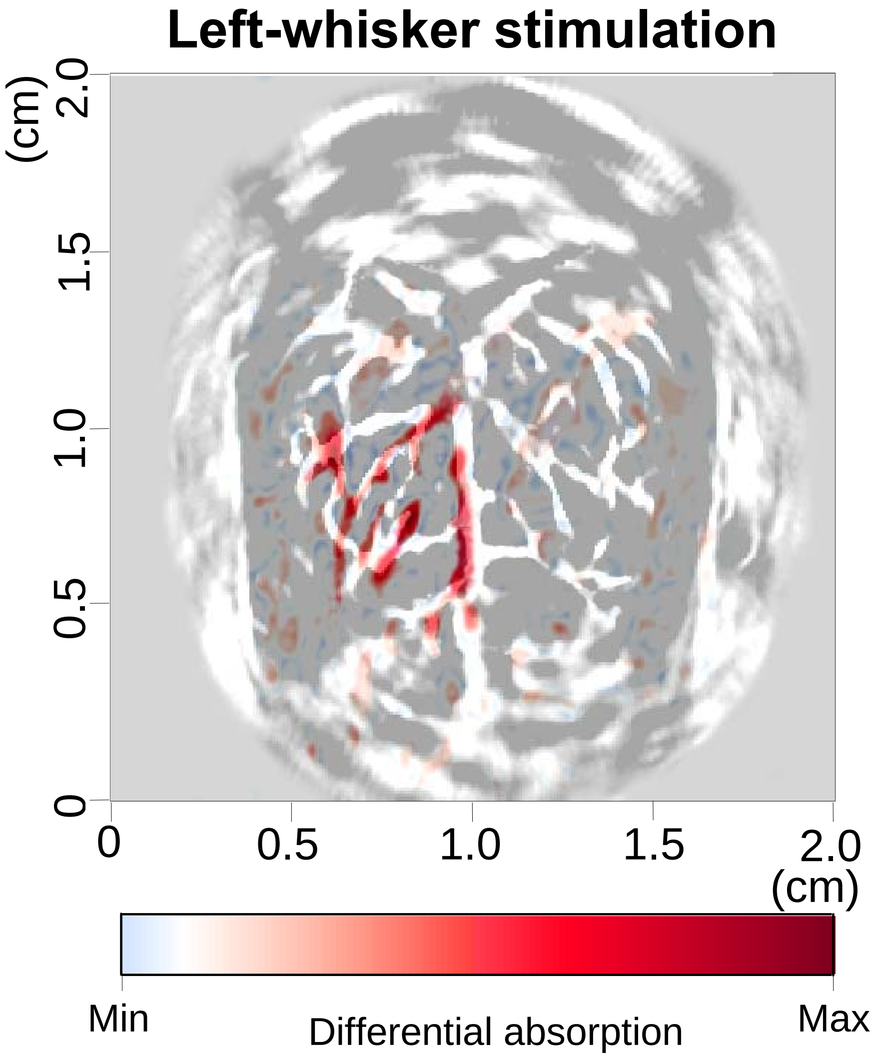
High-frequency

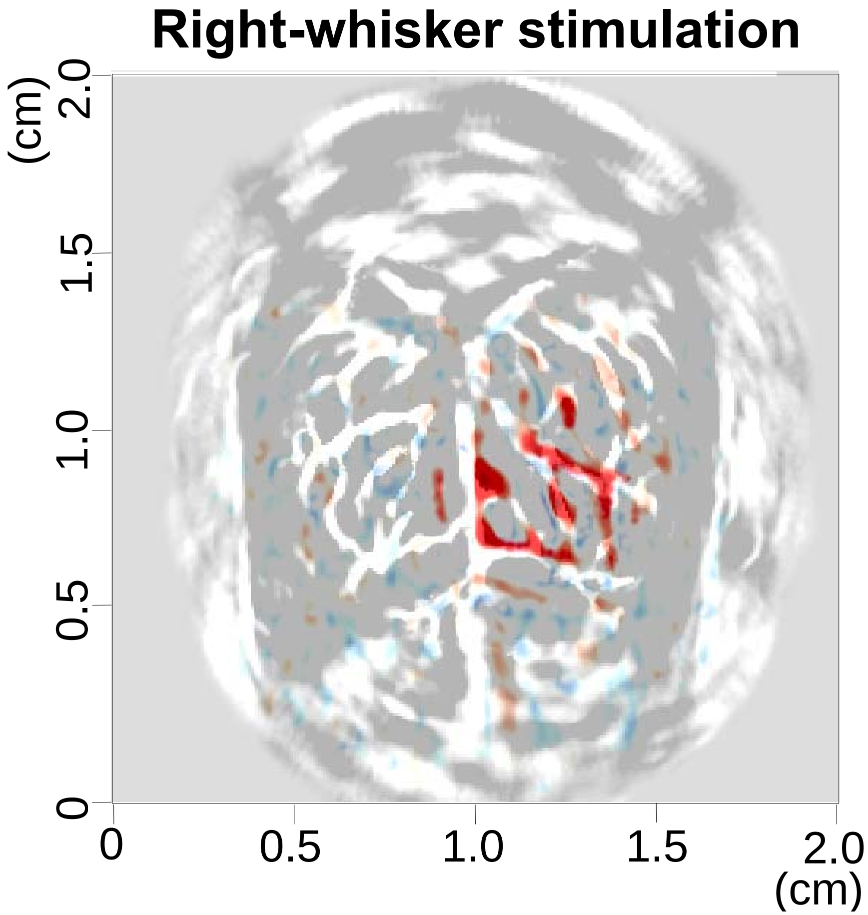
Optical contrast

Beamforming

Delay

Non-invasive Functional Photoacoustic Imaging of Rat Whisker Stimulation In Vivo: Hemodynamic Response

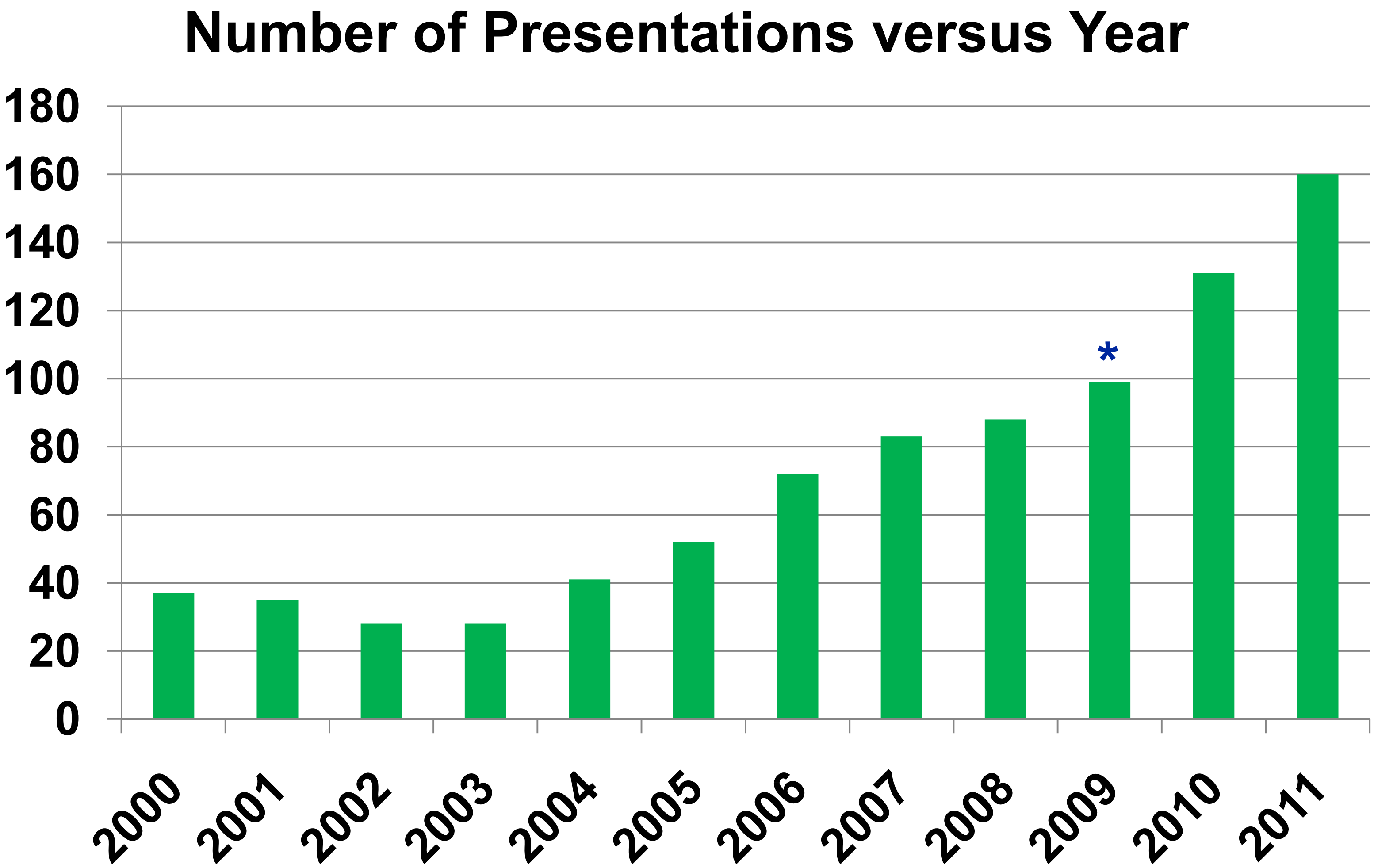

Left-whisker stimulation


Right-whisker stimulation

Through intact scalp and skull
In-plane resolution: 0.2 mm

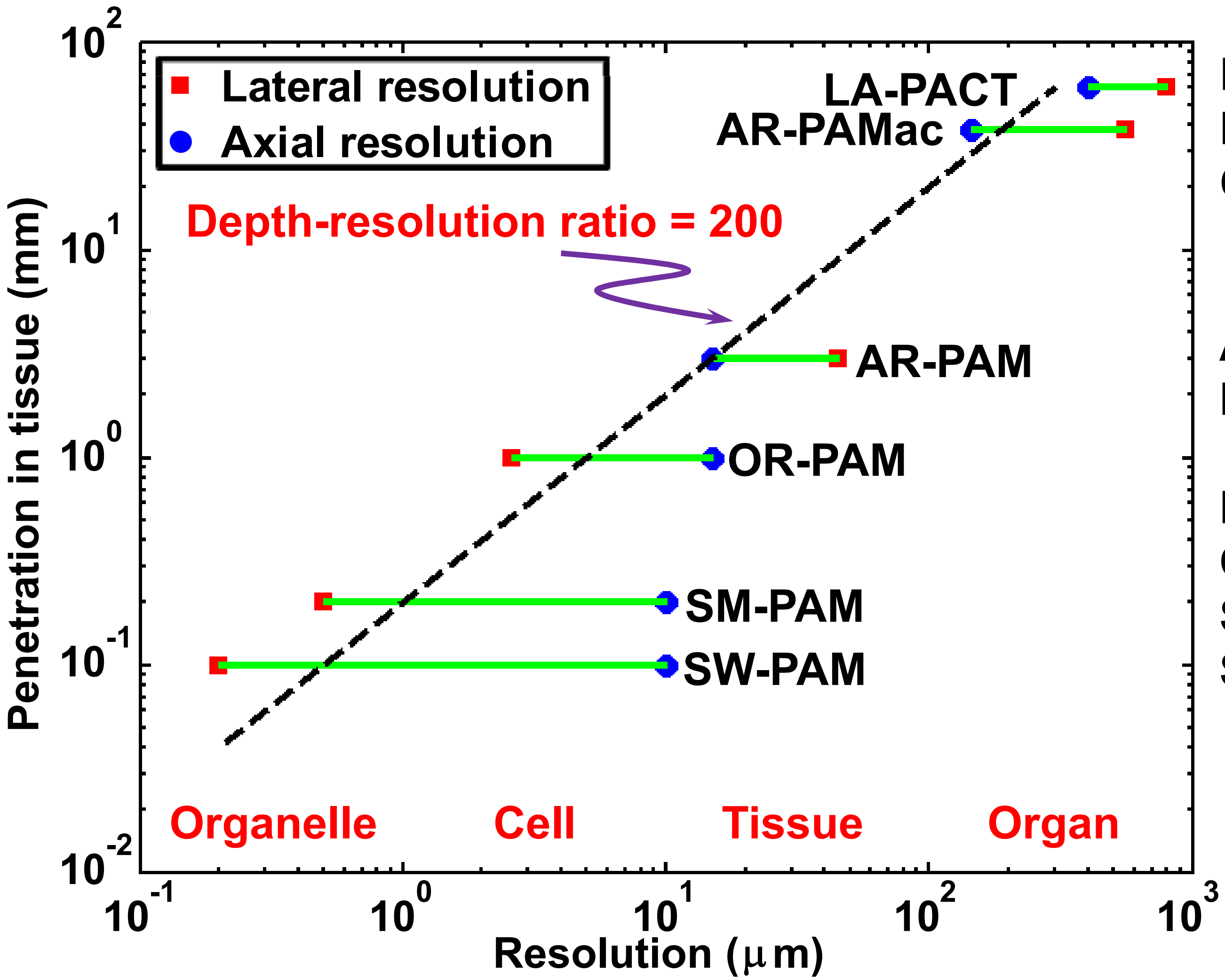
<u>Nature Biotech.</u> 21, 803 (2003).

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



* Largest in Photonics West

Multiscale Photoacoustic Tomography In Vivo with Consistent Contrast



LA: Linear array
PA: Photoacoustic
CT: Computed tomography

AR: Acoustic resolution
Mac: Macroscopy

PAM: PA microscopy
OR: Optical resolution
SM: Sub-micron
SW: Sub-wavelength

1. Enable systems biology research at multiple length scales
2. Accelerate translation of microscopic lab discoveries to macroscopic clinical practice

<u>Nature Photonics</u> 3, 503 (2009).

Scalability of Resolution and Penetration: Multiscale Imaging

$$\text{FWHM lateral resolution} \approx 0.71\frac{\lambda_0}{\text{NA}} = 0.71\frac{v_s}{f_0\text{NA}} \propto \frac{1}{f_0}$$

λ_0 : center acoustic wavelength; f_0 : center frequency.

NA : numerical aperture (# f/2); v_s : speed of sound.

$$\text{Axial resolution} \approx 0.88\frac{v_s}{\Delta f} = 0.88\frac{v_s}{\eta f_0} \propto \frac{1}{f_0}$$

Δf : one-way acoustic bandwidth; η : fractional acoustic bandwidth (~ constant).

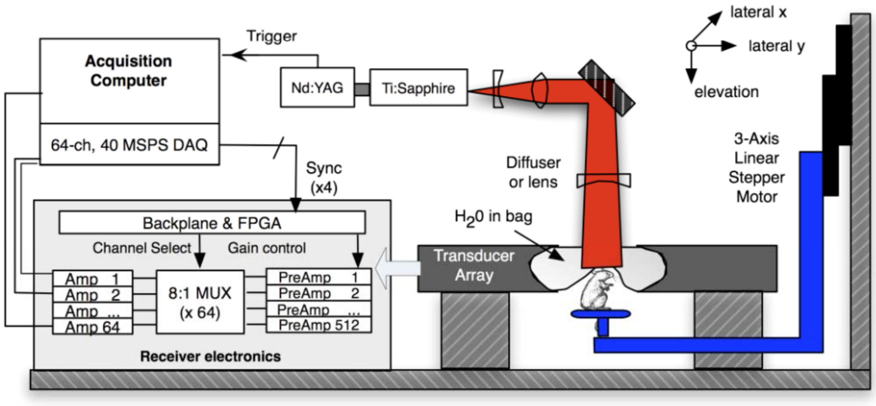
Ultrasonic attenuation = coefficient a × acoustic frequency f ; a ~ 0.5 dB/cm/MHz

$$\text{Penetration limit} \propto \frac{1}{f_0} \propto \text{Resolution.}$$

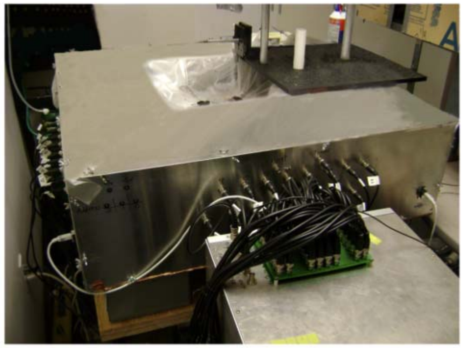
$$\frac{\text{Penetration limit}}{\text{Axial resolution}} \approx \text{Constant} \approx 200 \text{ (within optical penetration)!}$$

5-MHz Ring Ultrasonic Array

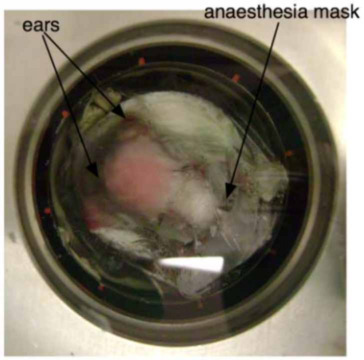
- Transducers:
  - Elements: 512-elements.
  - Center frequency: 5 MHz.
  - Bandwidth: 80% of center freq.
- Geometry:
  - Shape: Ring with 50 mm diam.
  - Pitch: 0.308 mm. Kerf: 0.1 mm.
  - Elevation height: 10 mm.
  - Elevation focal length: 19 mm.
- Data acquisition:
  - Channels: 64 channels A/D (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.



(a)



(b)

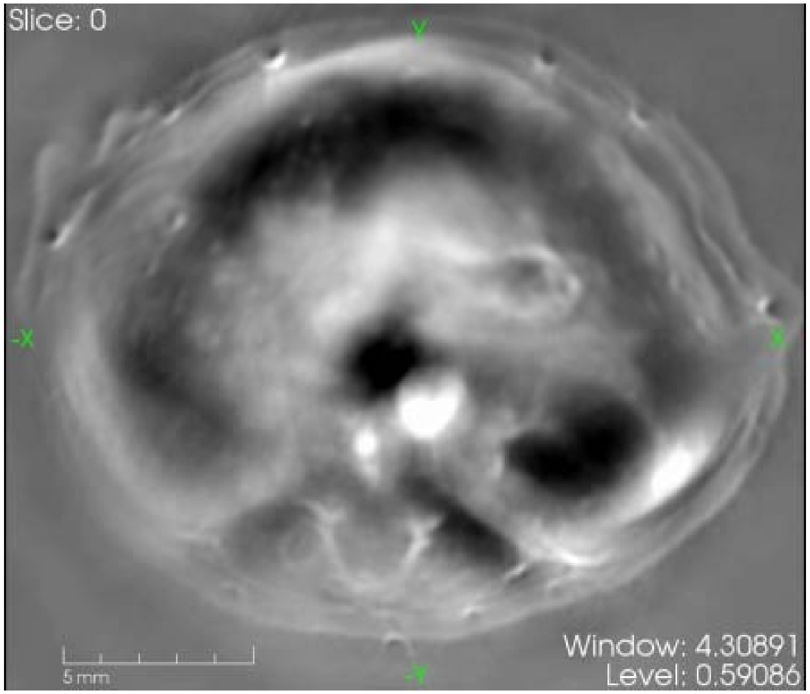


(c)

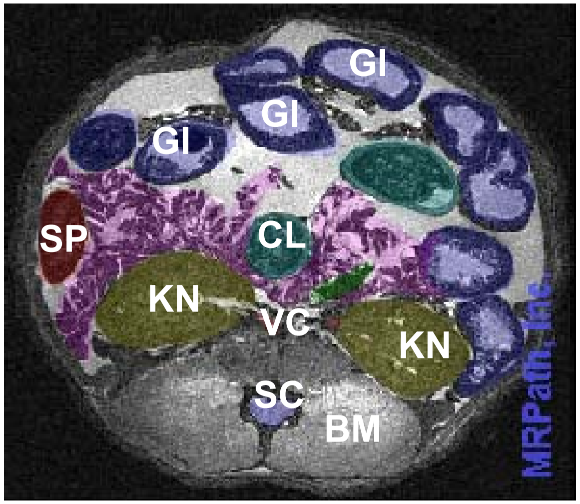
J. Biomed. Optics 13, 024007, 2008.
Optics Express, 17 (13), 10489, 2009.
Collaboration: Q. Zhu's Group @ UConn.

In Vivo Photoacoustic Computed Tomography of a Mouse

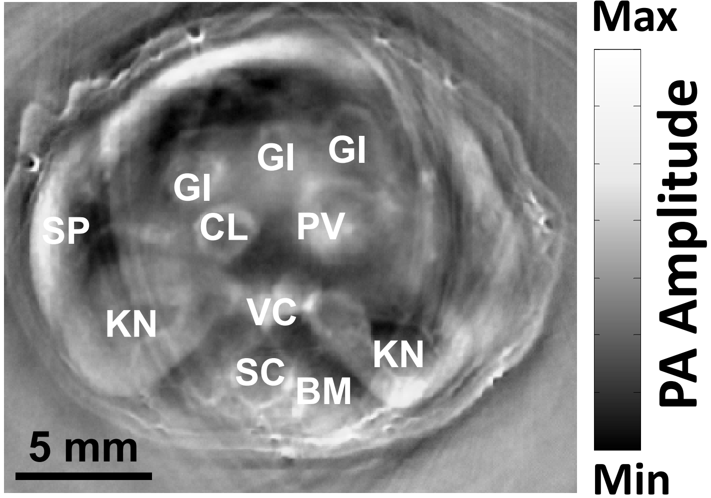
Photoacoustic images at varying depths
(0.1 mm per step scanning downward)



Visible mouse atlas (MRPath, Inc.)



Photoacoustic image



BM	Back bone muscle	PV	Portal vein
CL	Colon	SC	Spinal cord
GI	GI tract	SP	Spleen
KN	Kidney	VC	Vena cava

J Xia et al., unpublished.

Photoacoustic Tomography of Monkey Brain with Intact Cranium

Photoacoustic tomography image with cranium intact

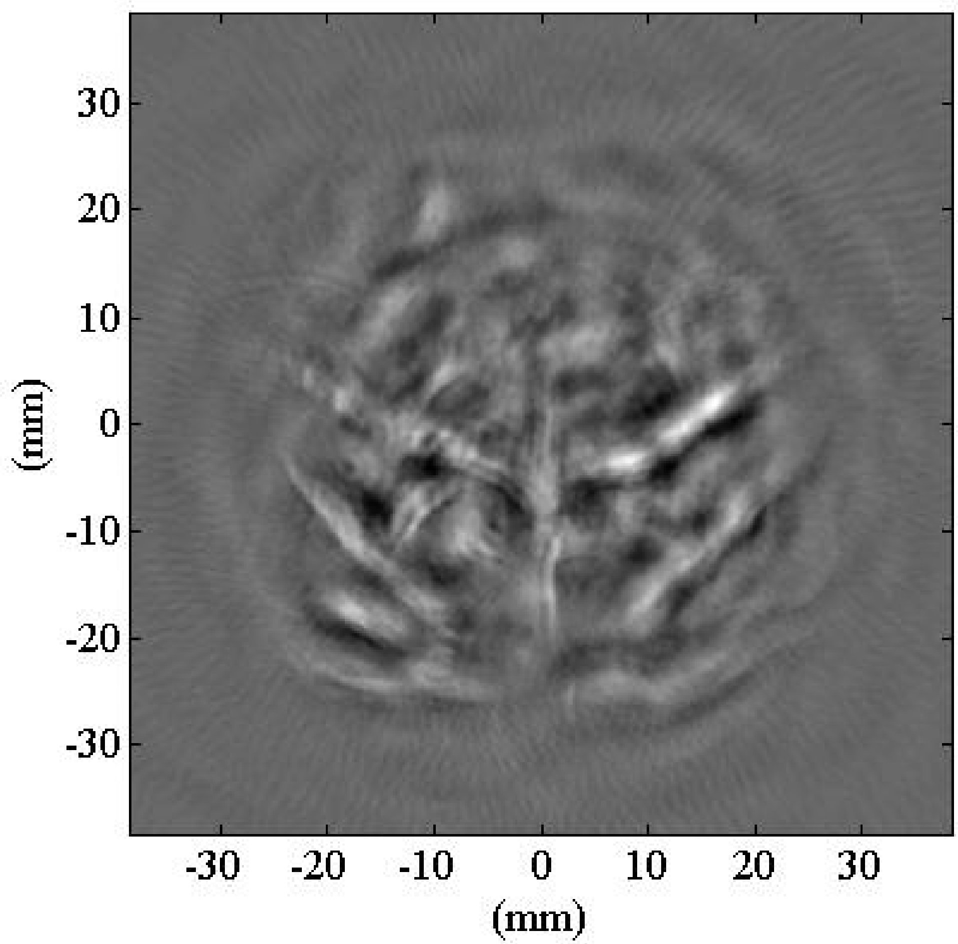
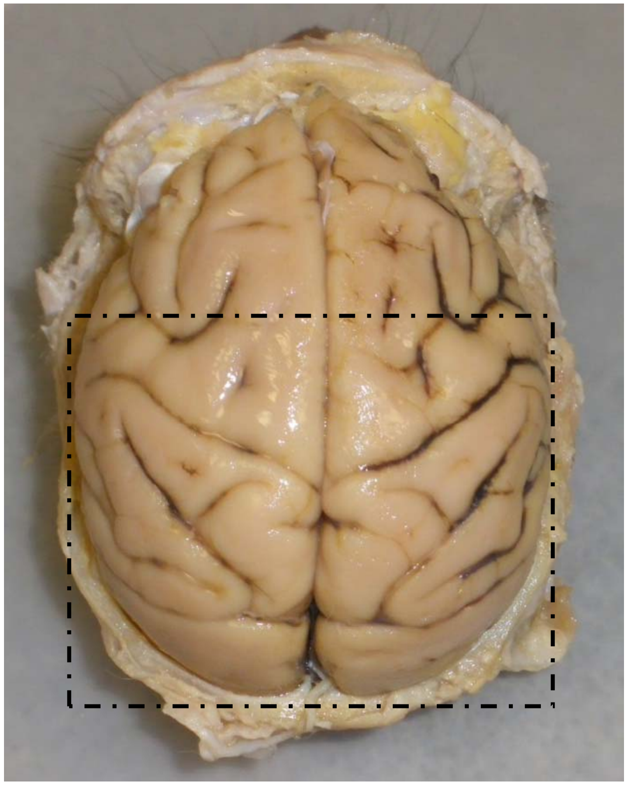


Photo with cranium removed



L. Nie, et al., unpublished.

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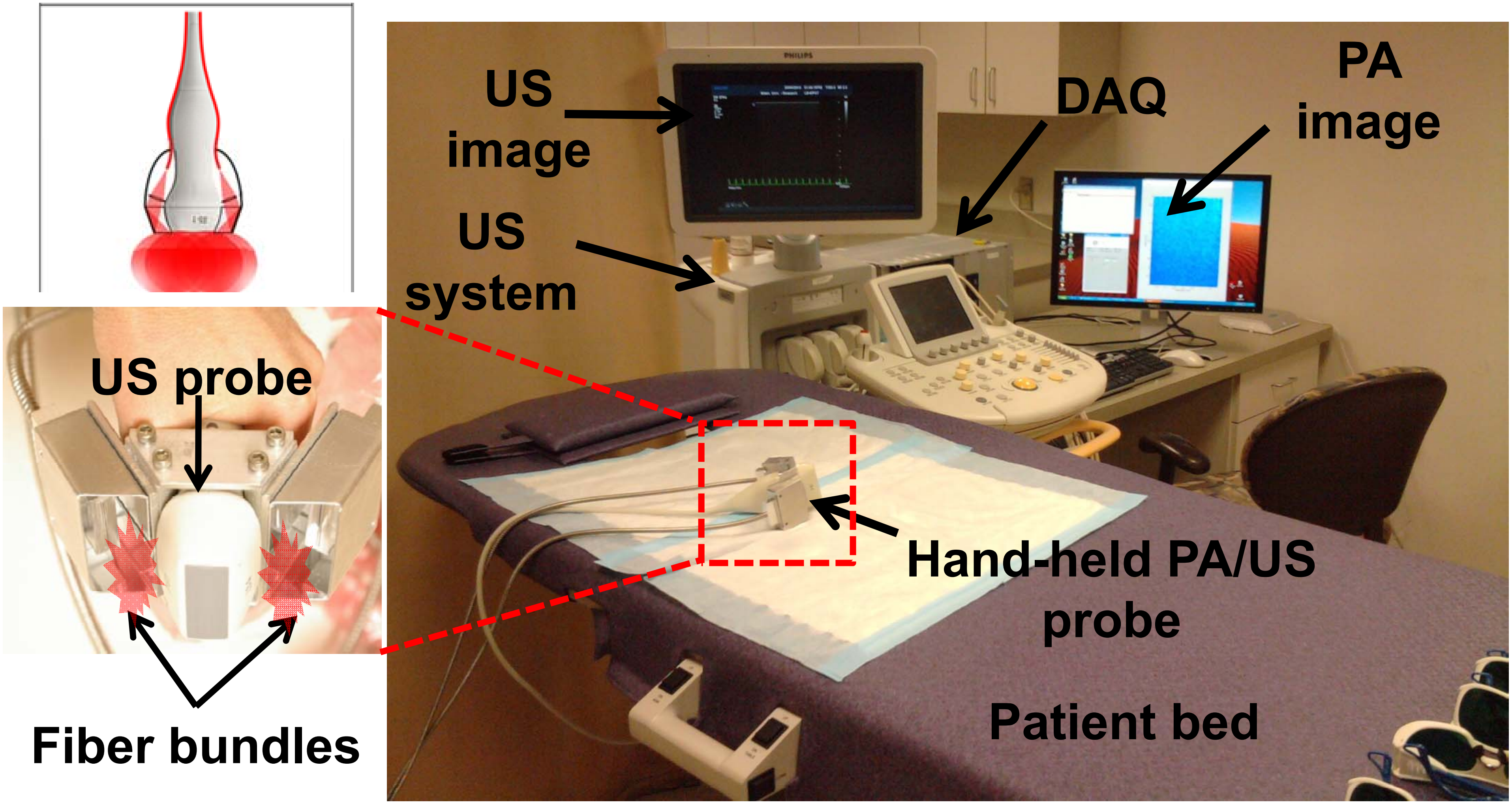
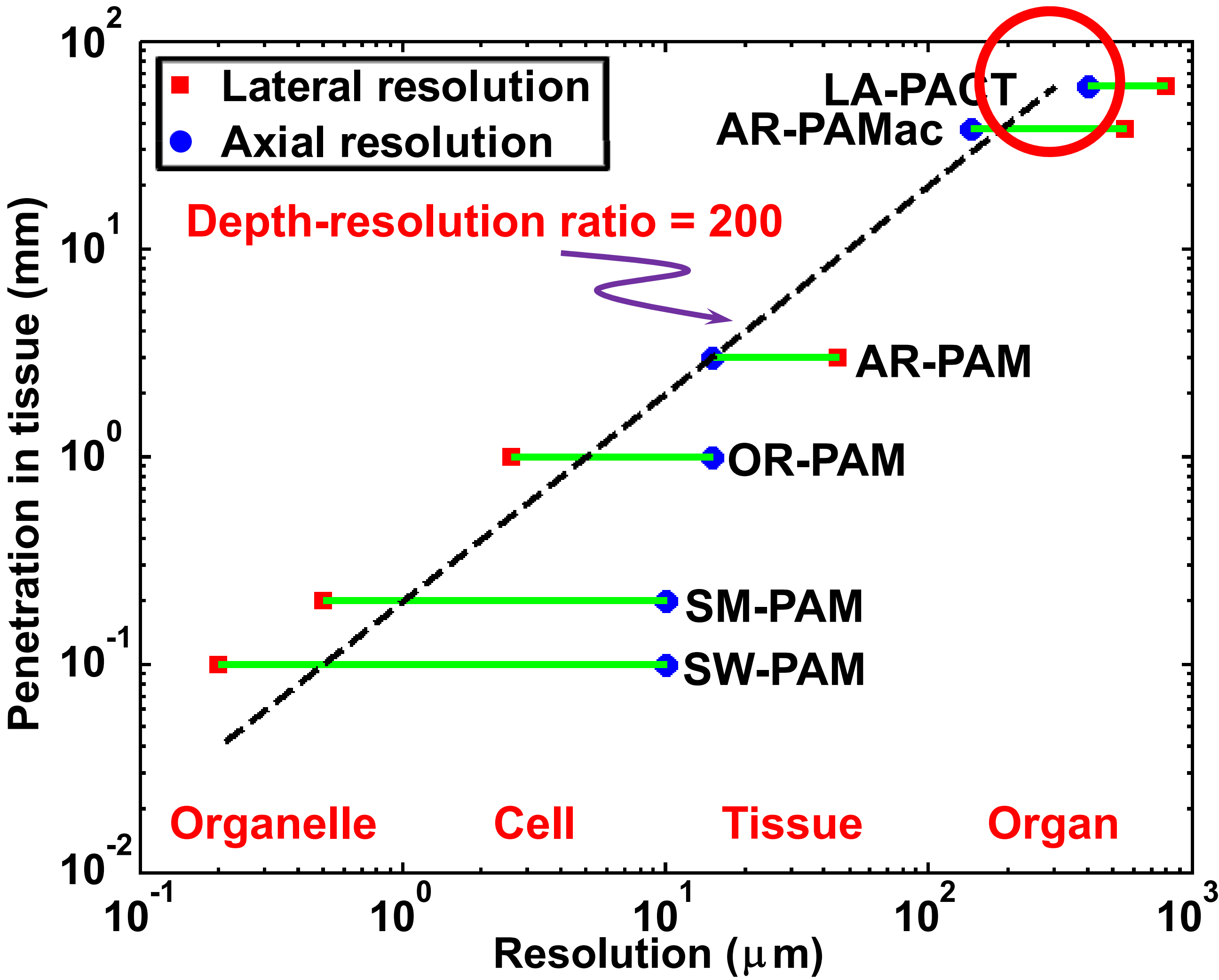
Hand-held Photoacoustic/Ultrasonic Imaging Probe using Modified Clinical Ultrasound Scanner



Biomed Optics Express 1, 278 (2010).

Collaboration: Philips Research

Multiscale Photoacoustic Tomography In Vivo with Consistent Contrast



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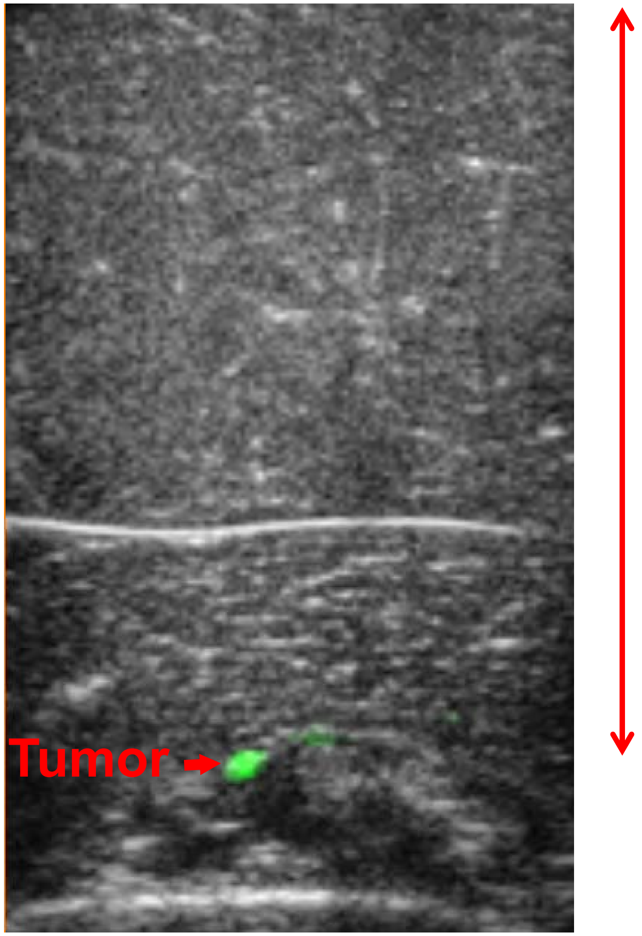
<u>Nature Photonics</u> 3, 503 (2009).

In Vivo Photoacoustic Molecular (Reporter Gene) Imaging: Gene Expression in Gliosarcoma Tumor in Mouse



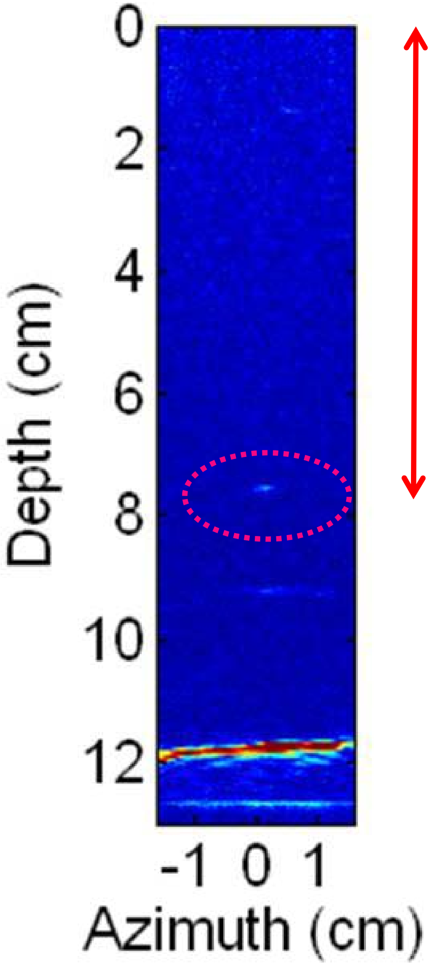
1. **LacZ (gene)**
2. **Beta-galactosidase (enzyme)**
3. **X-gal (colorless substrate)**
4. **Blue product**

- **Philips US array: L8-4**
- **2 mJ/cm^2 @ 650 nm**
- **1/10 of ANSI exposure limit**
- **100X averaging**
- **Contrast: ~2.5**

L. Li, et al., unpublished.

Collaboration: Philips Research

Deeply Penetrating Photoacoustic Imaging in Biological Tissue Enhanced with Methylene Blue



>7 cm

- **S5-1 transducer**
- **Beam size: 2.5 cm**
- **Fluence: 20 mJ/cm^2**
- **Methylene blue at 1%**
- **200X averaging**
- **1/e penetration depth: 0.7 cm**
- **Target depth: >7 cm**

H. Ke, T. Erpelding, et al., unpublished.
Collaboration: Philips Research

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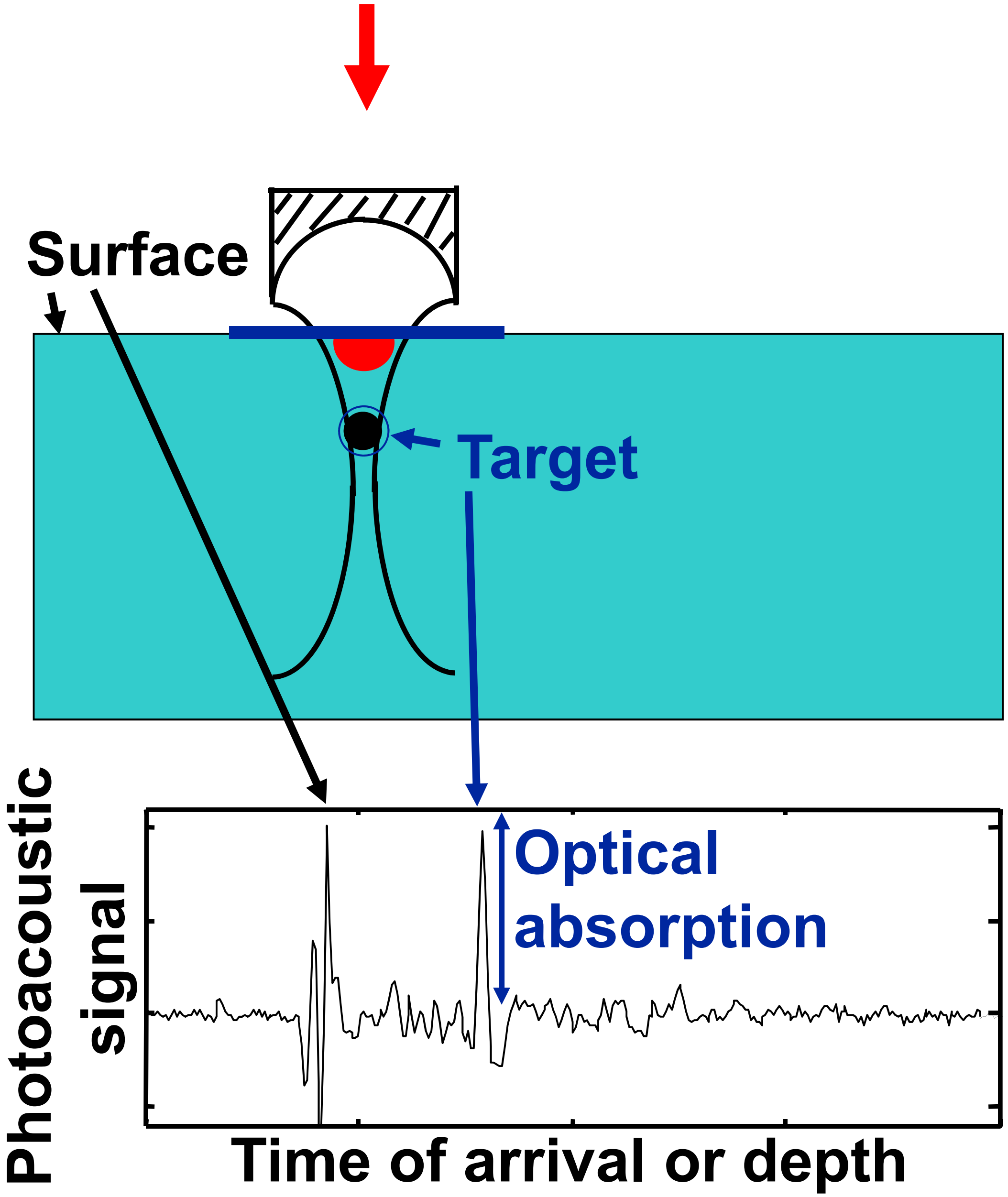
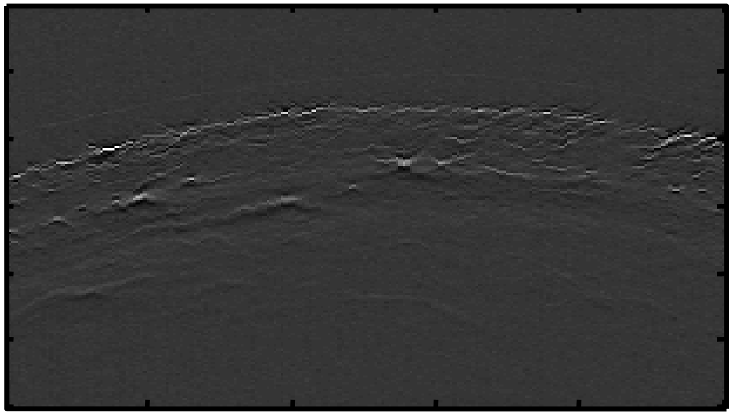
Reflection-mode Photoacoustic Microscopy: Illustration

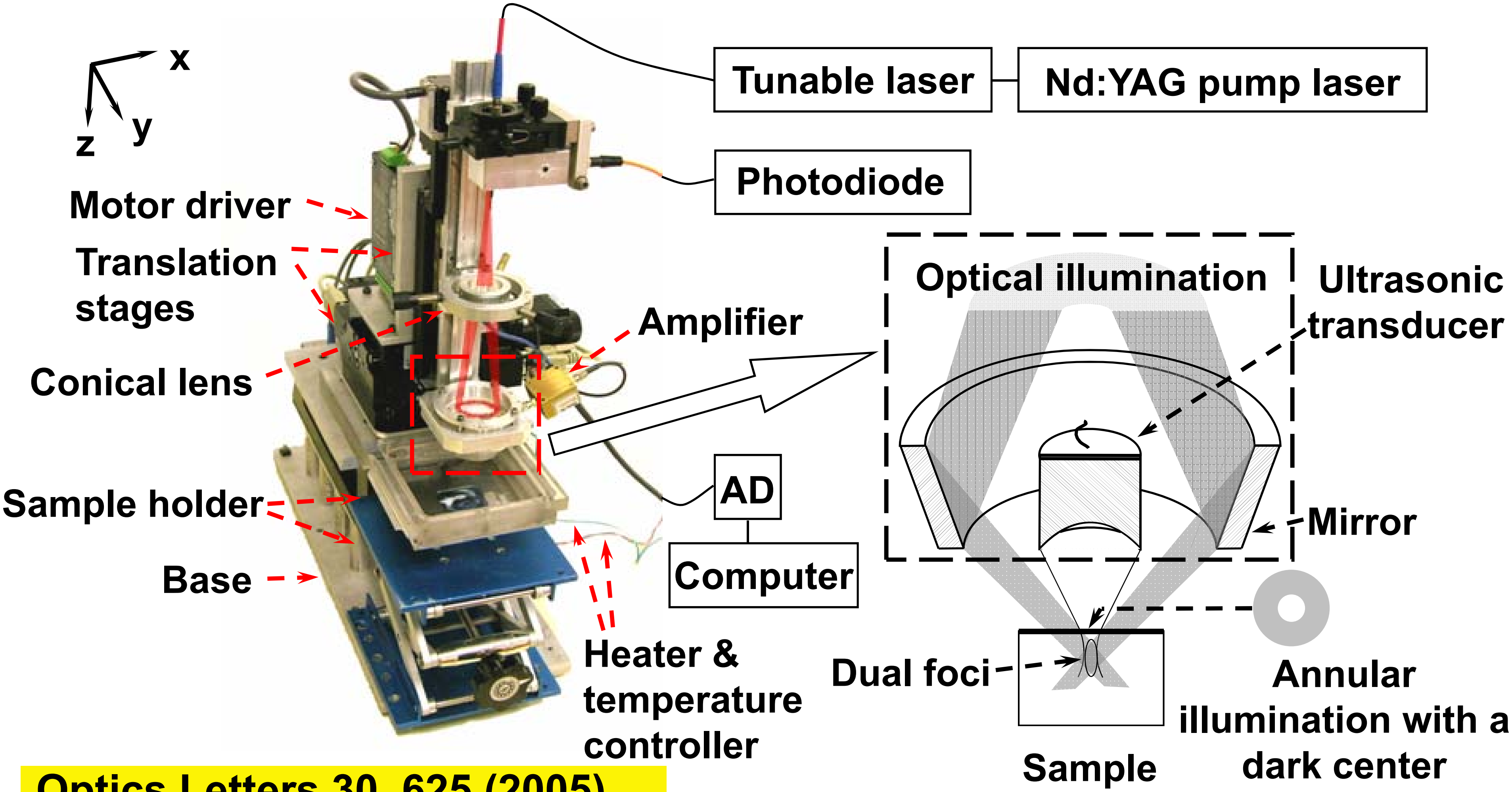


A-scan

B-scan



Dark-field Confocal Photoacoustic Microscopy: 3 mm Penetration at 50-MHz Ultrasonic Frequency

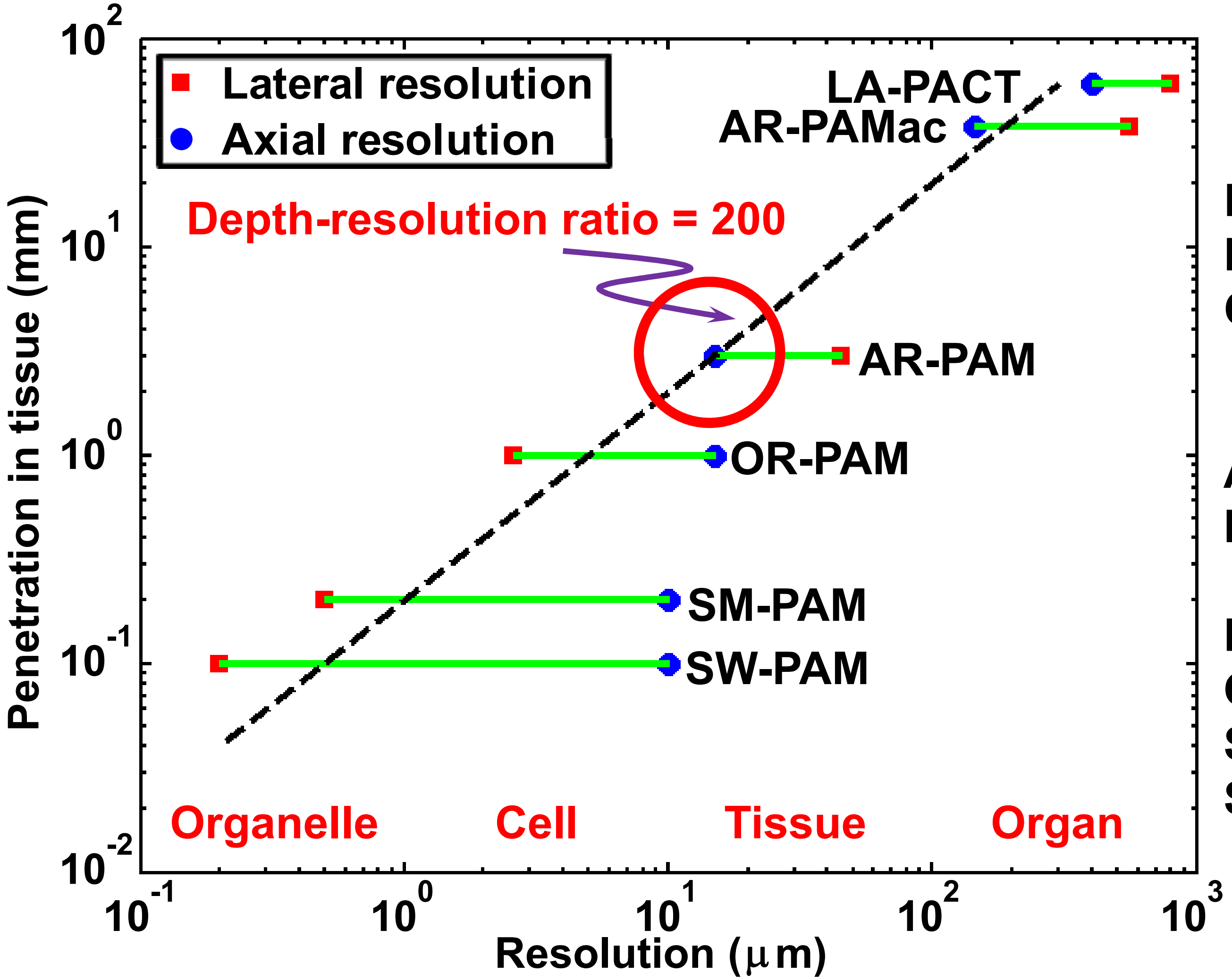


Optics Letters 30, 625 (2005).

Nature Biotech. 24, 848 (2006).

Nature Protocols 2, 797 (2007).

Multiscale Photoacoustic Tomography In Vivo with Consistent Contrast



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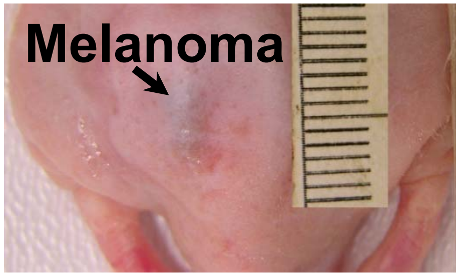
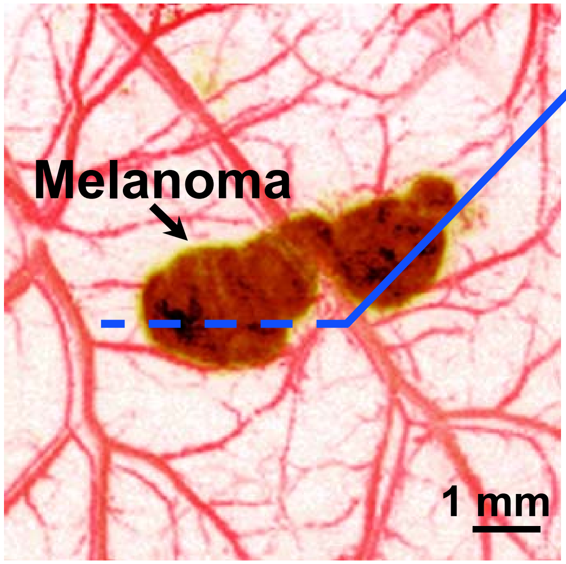
Nature Photonics 3, 503 (2009).

Photoacoustic Imaging of a Melanoma Tumor in a Small Animal In Vivo

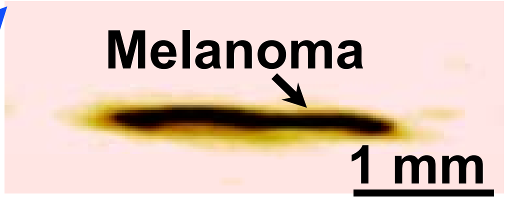
Photograph



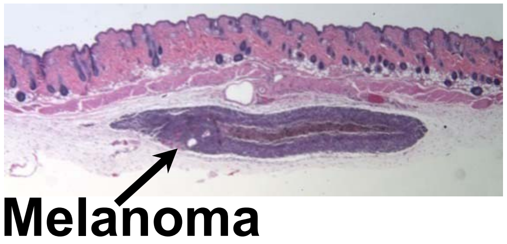
Composite photoacoustic image with 584 and 764 nm



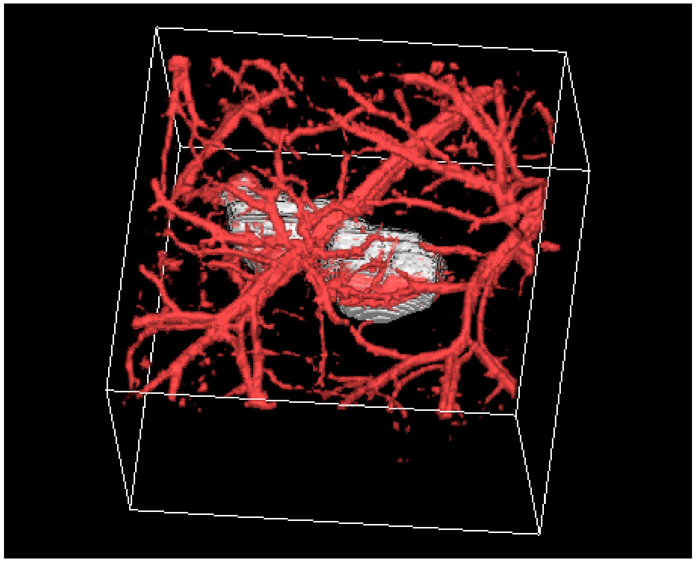
B-scan at 764 nm



Histology



3D Movie

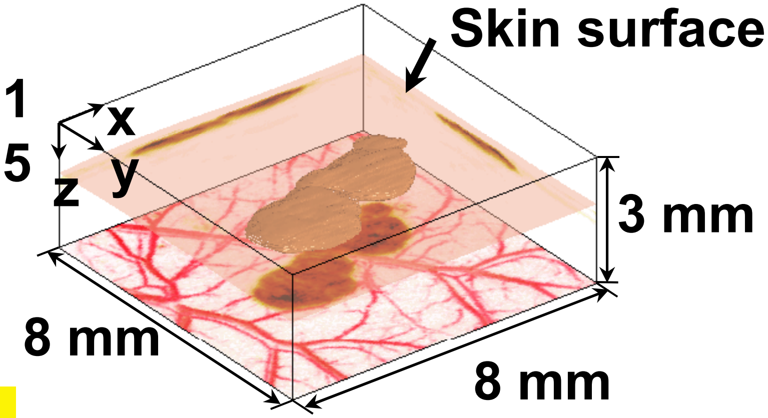


Contrasts:

Blood:	13±1
Melanoma:	68±5

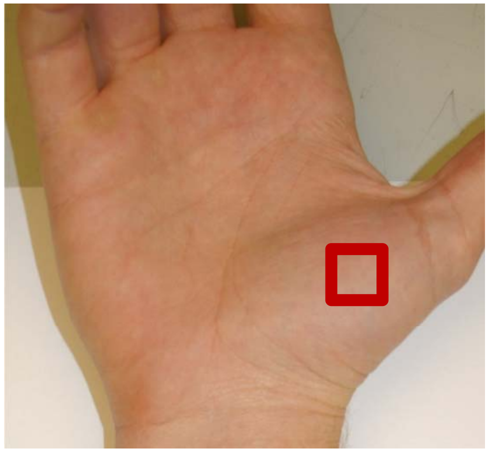
Nature Biotech. 24, 848 (2006).

Surface rendering

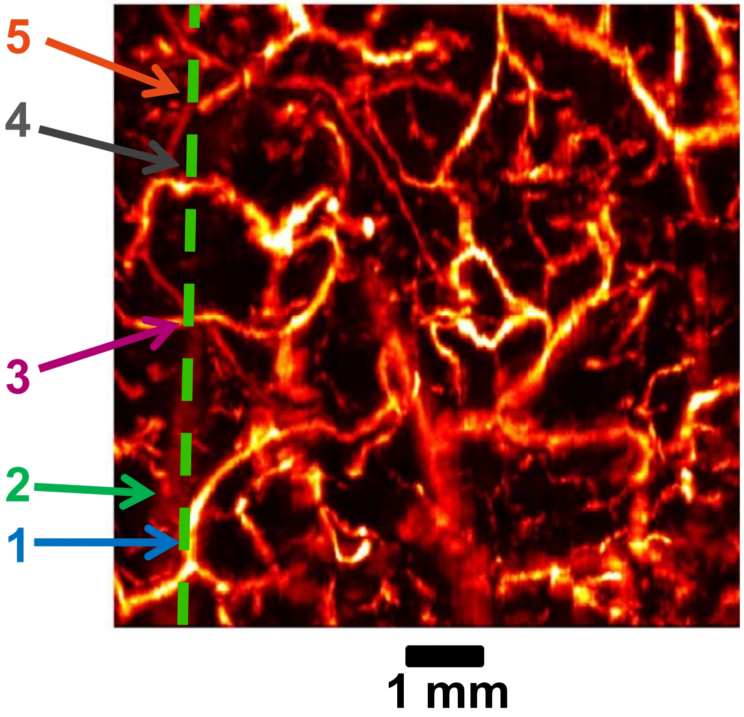


Photoacoustic Microscopy of Human Palm

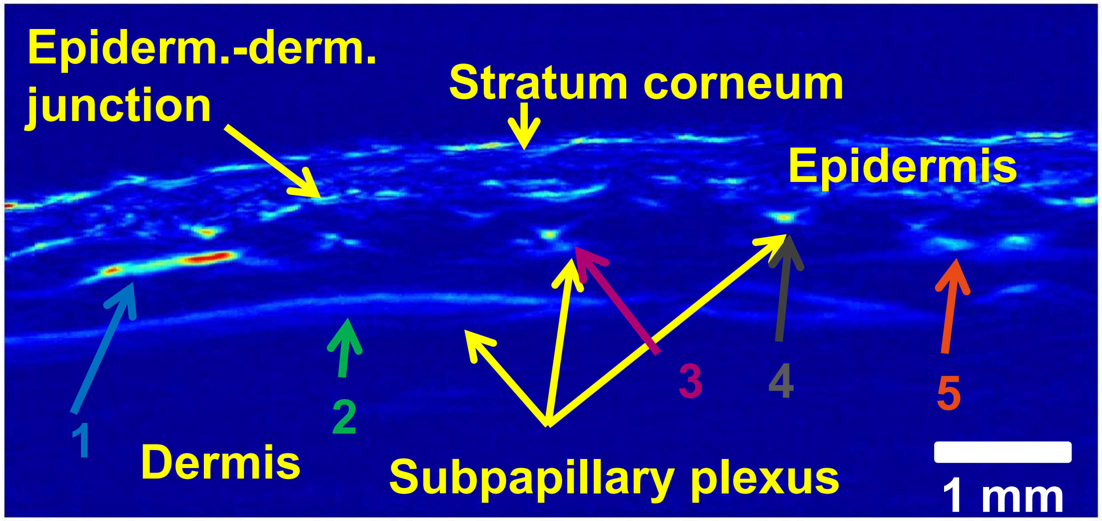
Photo



Max amplitude projection



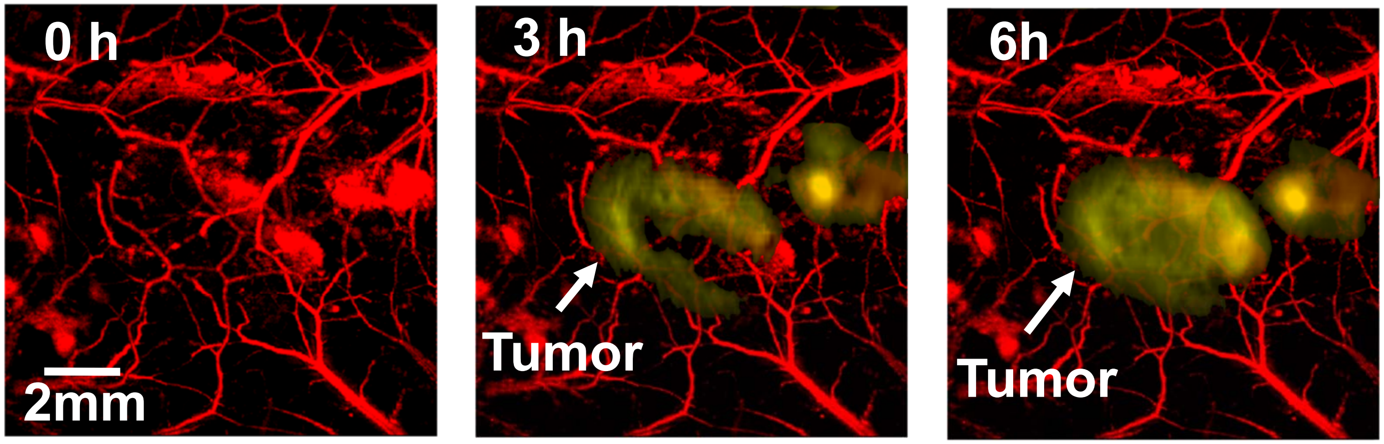
B-Scan @ 584 nm



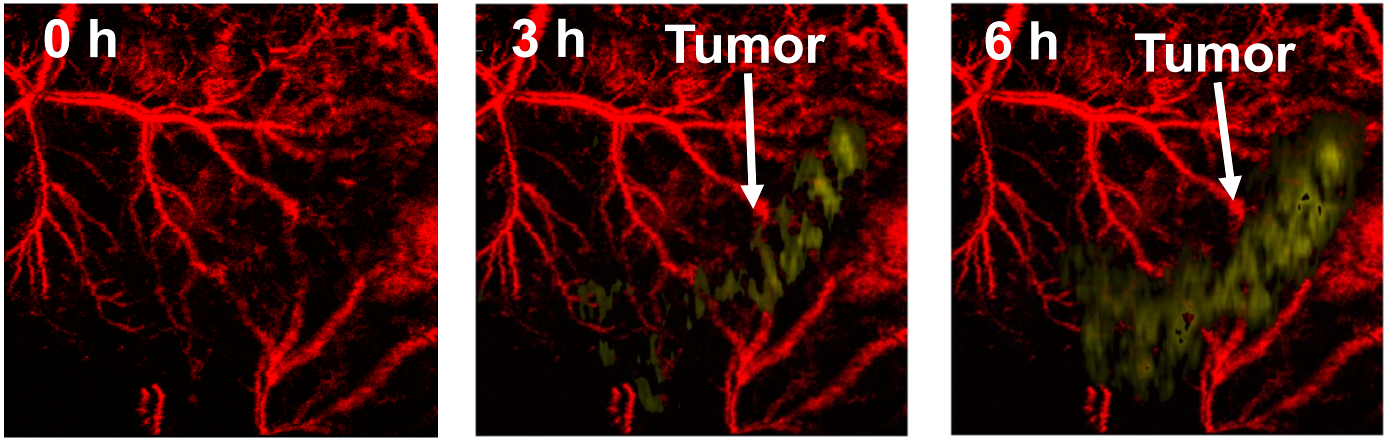
J Biomed Opt 16, 016015 (2011)
Collaboration: L. Cornelius

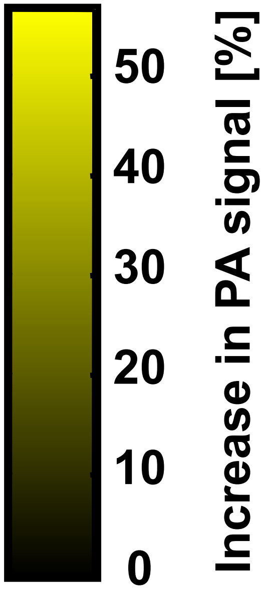
In Vivo Molecular Photoacoustic Imaging of B16 Melanoma in a Rat Using Targeted Gold Nanocages (AuNCs)

[Nle^4,D-Phe^7]-α-MSH-AuNCs → 38 ± 3 (%) at 6 hr (3X control)



PEG-AuNCs → 13 ± 1 (%) at 6 hr



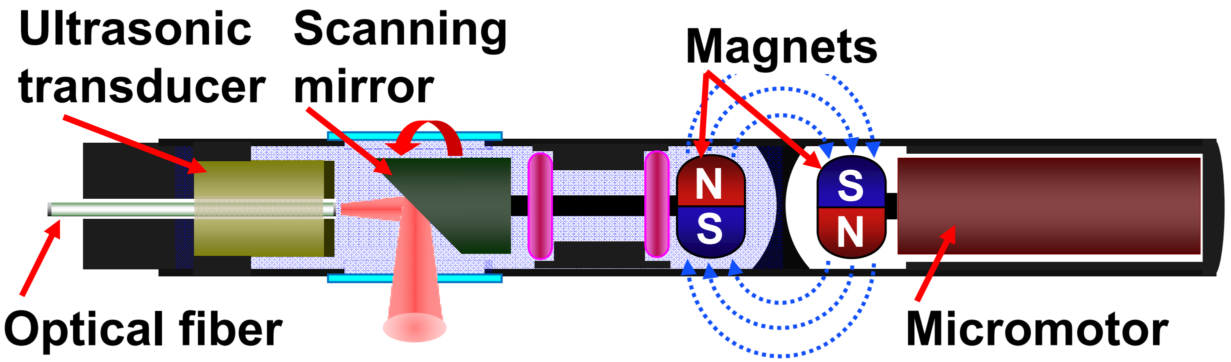


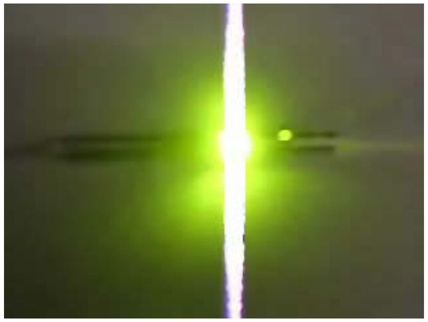
Sensitivity :
~ 5000 AuNCs / voxel

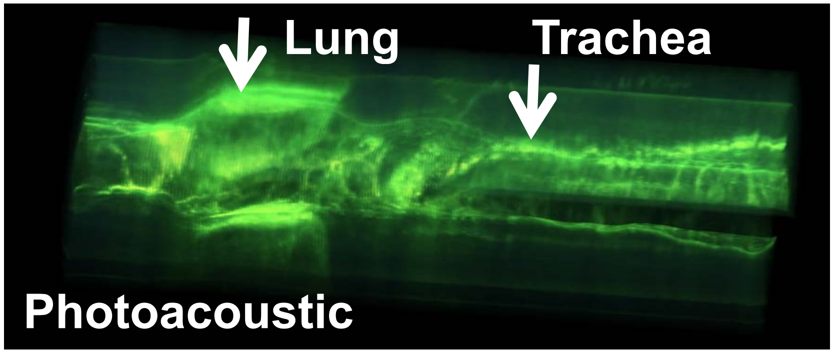
MSH: melanocyte-stimulating hormone

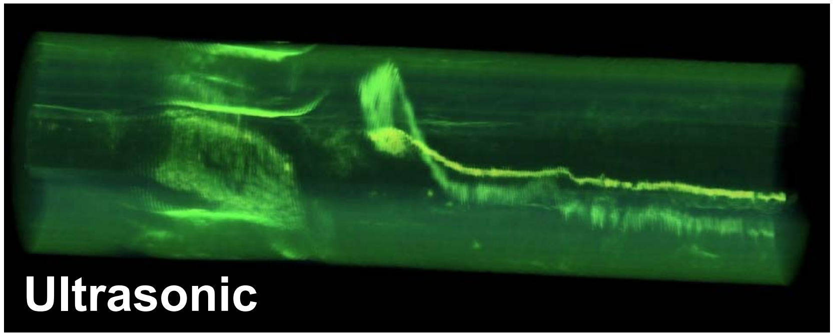
ACS Nano 4, 4559 (2010). Collaboration: Y. Xia
Controlled drug release: Nature Mat. 8, 935 (2009)

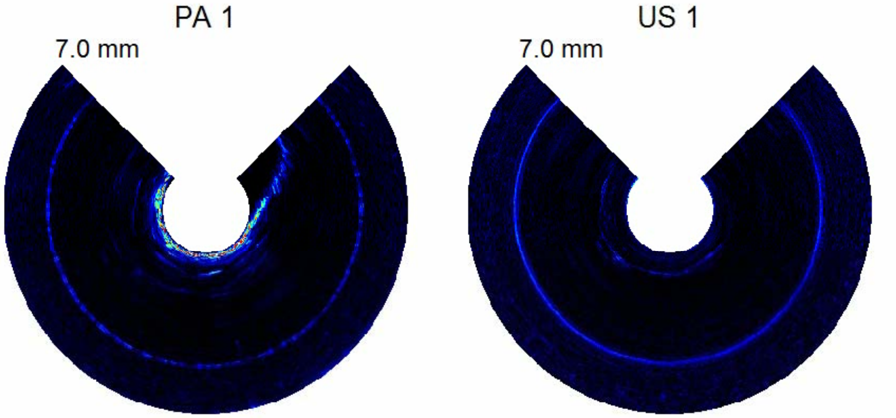
Photoacoustic Endoscopy of Rabbit Esophagus In Vivo











Axial resolution: 55 microns
Lateral resolution: 80 microns
Diameter of probe: 3.8 mm
Ultrasonic frequency: 36 MHz

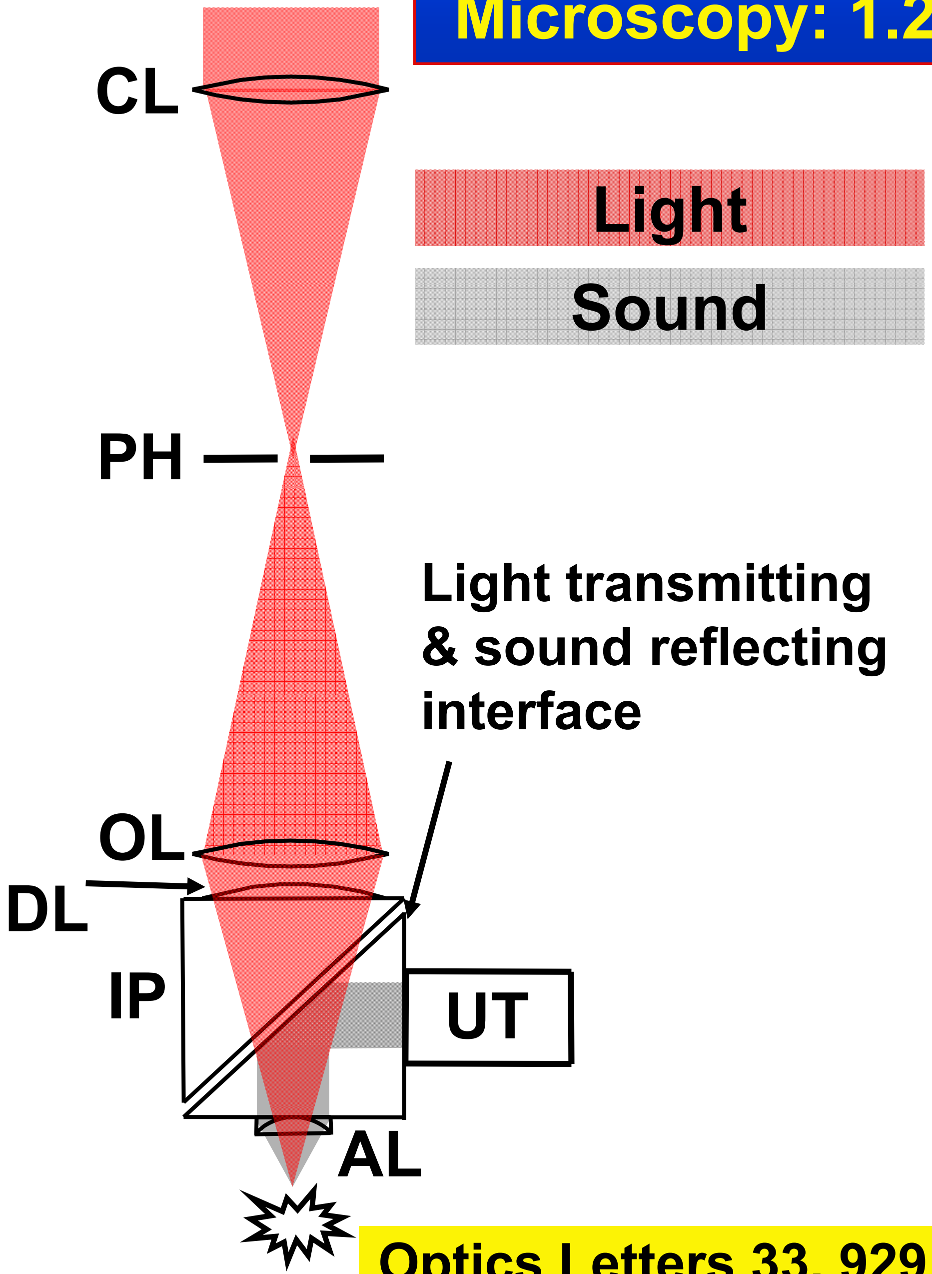
Yang et al., unpublished.
Optics Letters 34, 1591 (2009).

Collaboration: Zhou & Shung, USC

Outline

- Motivation and challenges
- Photoacoustic computed tomography
    - Circular geometry
    - Linear geometry
- Photoacoustic microscopy
    - Acoustic resolution
    - Optical resolution
- Discussion and summary

Optical Resolution Photoacoustic Microscopy: 1.2 mm Penetration



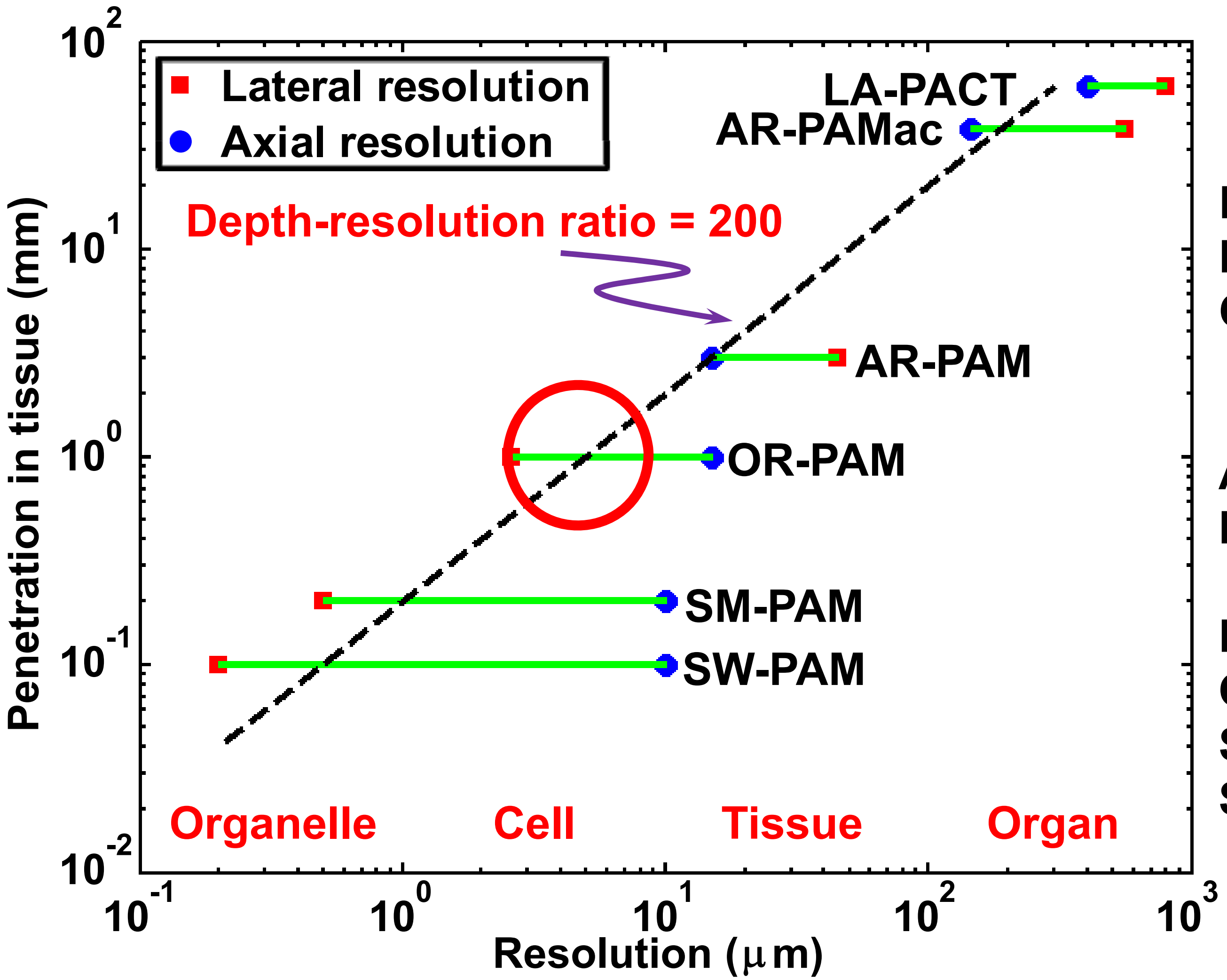
CL: Condenser lens
PH: Pinhole
OL: Objective lens
UT: Ultrasonic transducer
DL: De-aberrating lens
IP: Isosceles prism
AL: Acoustic lens

- Optic NA = 0.1
- Lateral resolution = 2.6 µm

- Acoustic NA = 0.45
- Center f ~ 85 MHz
- Axial resolution < 15 µm

Optics Letters 33, 929 (2008).

Multiscale Photoacoustic Tomography In Vivo with Consistent Contrast



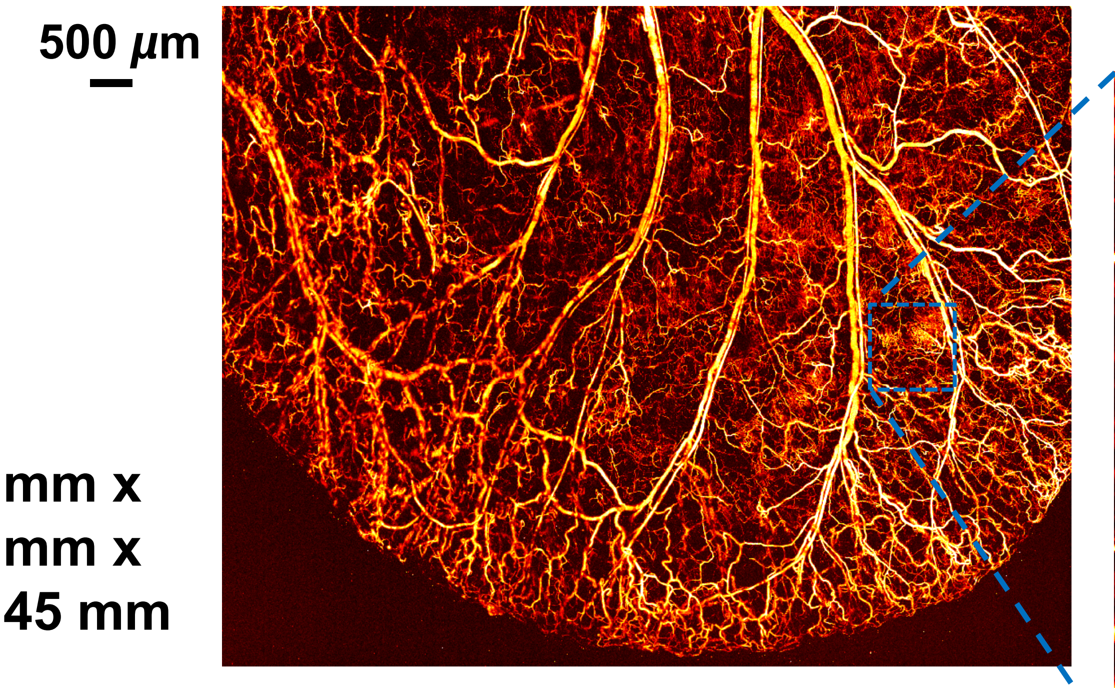
LA: Linear array
PA: Photoacoustic
CT: Computed tomography

AR: Acoustic resolution
Mac: Macroscopy

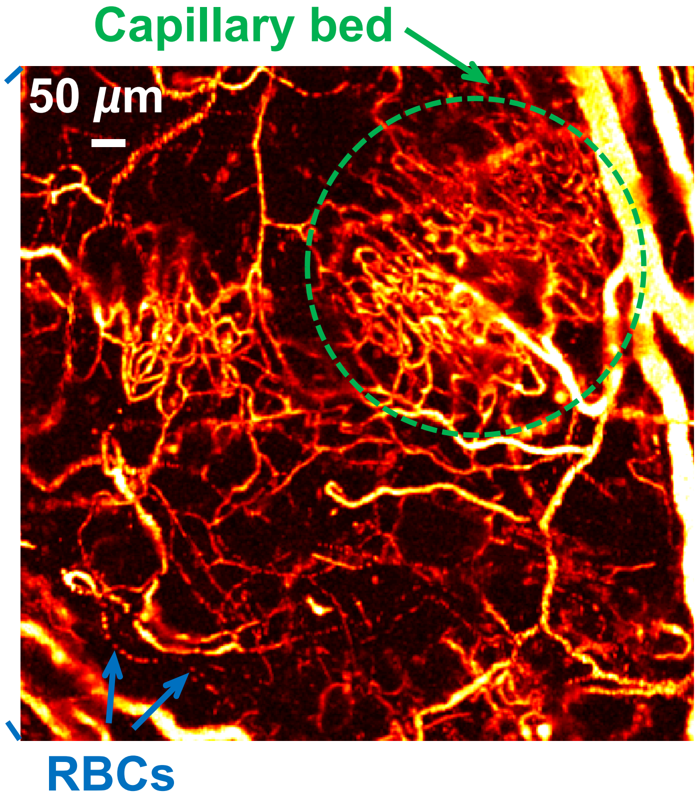
PAM: PA microscopy
OR: Optical resolution
SM: Sub-micron
SW: Sub-wavelength

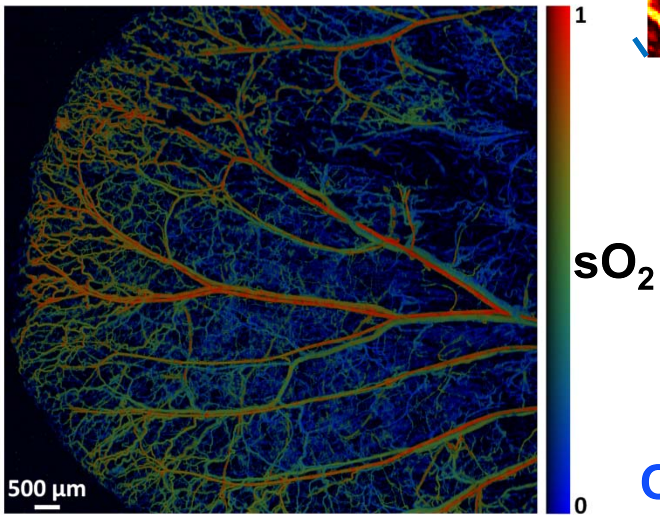
__Nature Photonics__ 3, 503 (2009).

In Vivo Optical-Resolution Photoacoustic Microscopy of Mouse Ear: 2.6 Micron Lateral Resolution



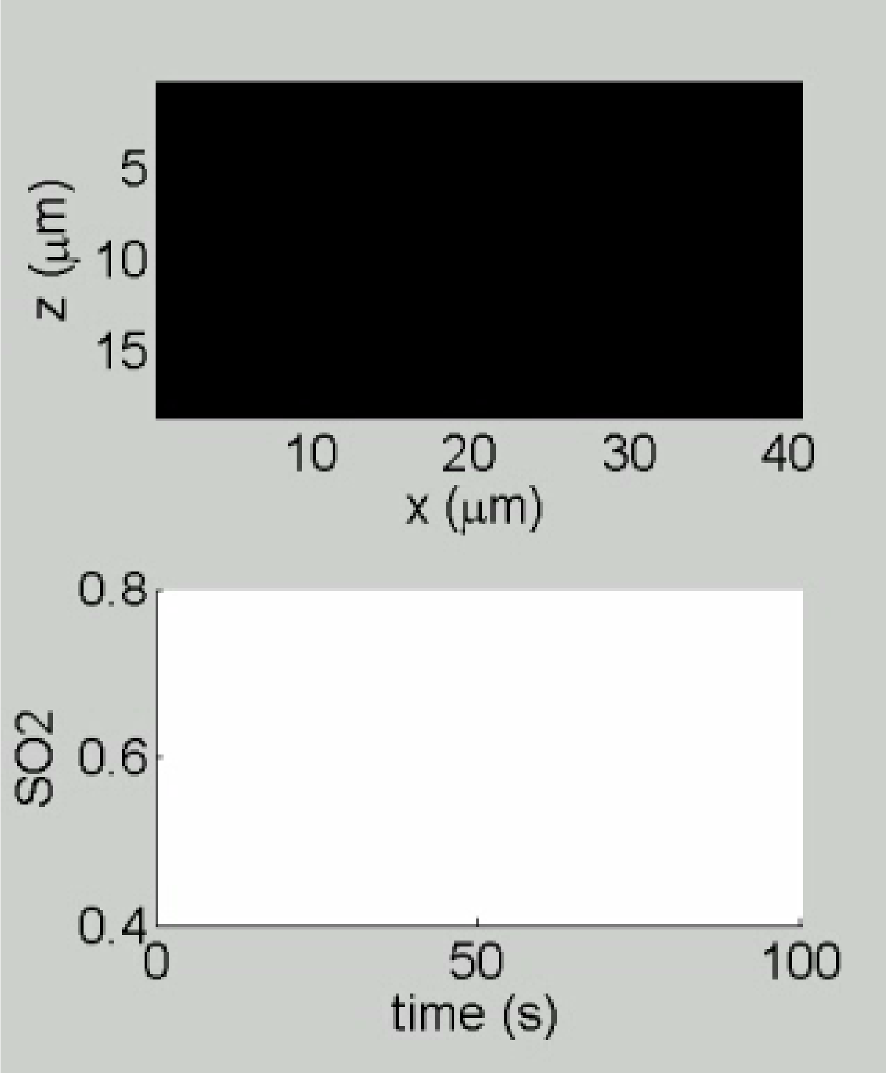
5 mm x
5 mm x
0.45 mm

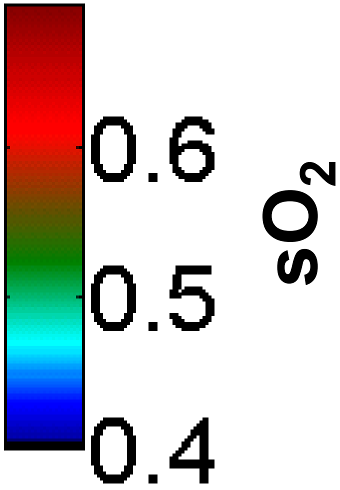




<u>Optics Lett</u> 36, 1134, 2011.

Oxygen Release by Single Red Blood Cells Imaged In Vivo



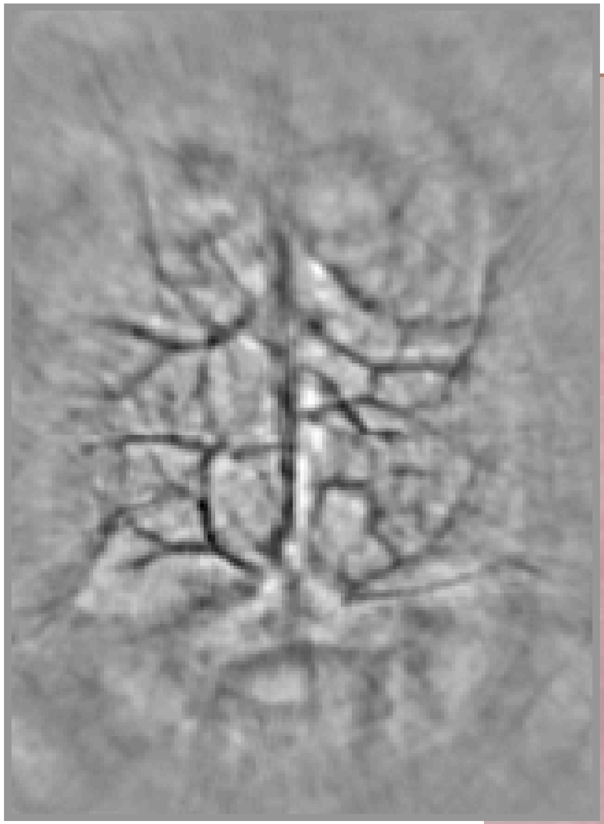


sO_2: oxygen saturation of hemoglobin

LD Wang & K Maslov et al., unpublished.

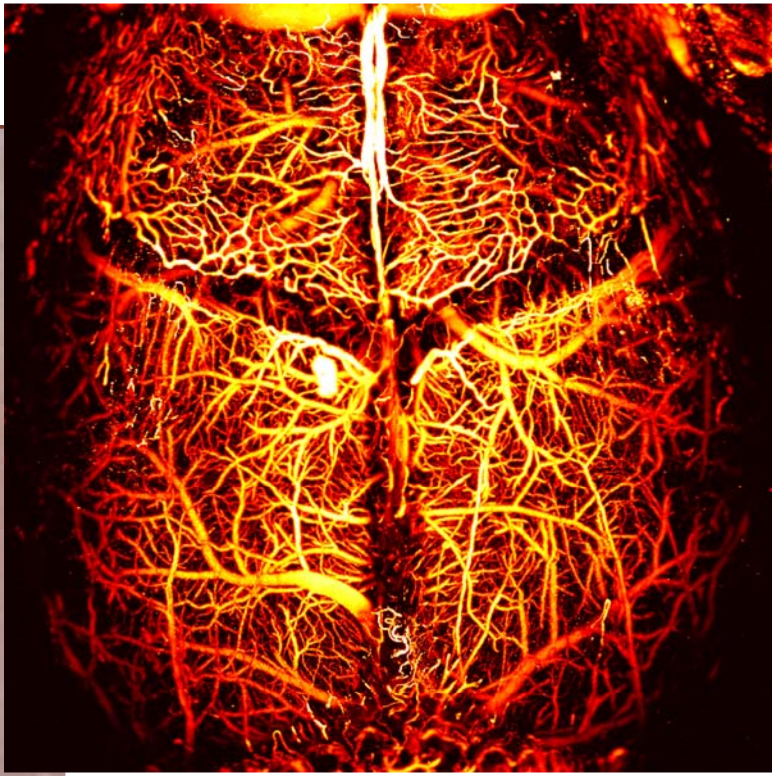
Transcranial Imaging in Living Mouse

Photoacoustic CT with intact scalp/skull



Invasive photography

Optical-resolution photoacoustic microscopy with intact skull

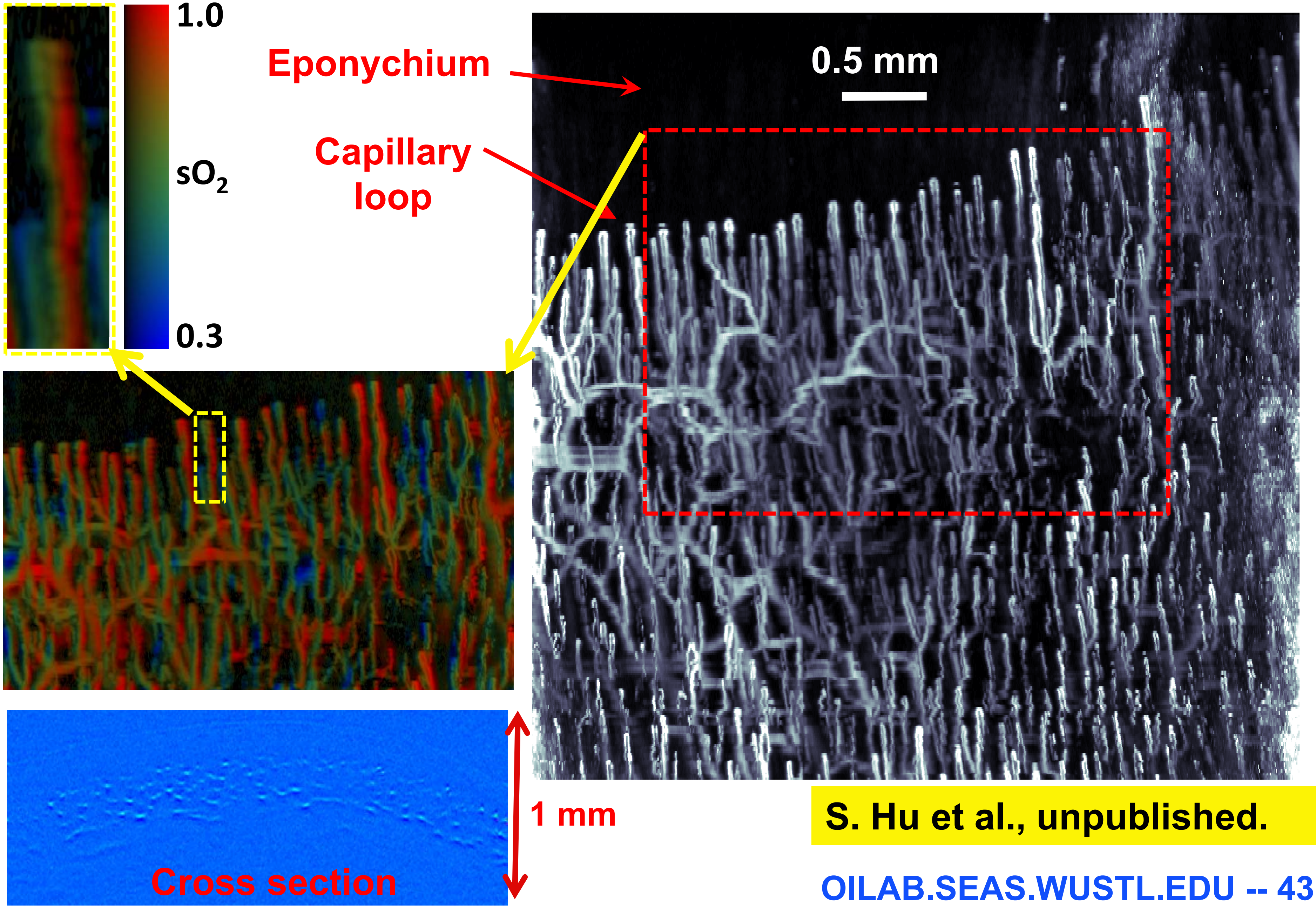


500 µm

Optics Lett 36, 1134, 2011.

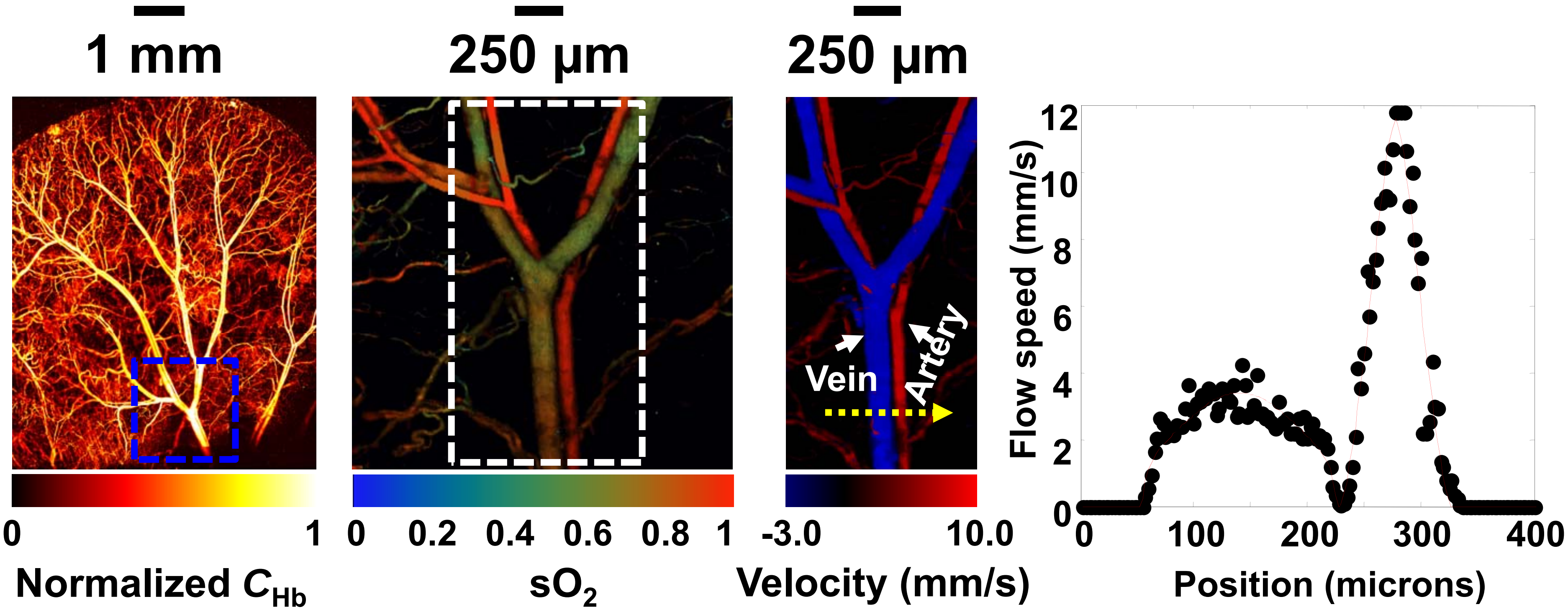
J Biomed Optics 15, 010509 (2010).

In Vivo Photoacoustic Microscopy of Human Finger Cuticle



S. Hu et al., unpublished.

Doppler Photoacoustic Microscopy of Blood Flow in Mouse



C_{Hb} : concentration of total hemoglobin

sO_2: oxygen saturation of hemoglobin

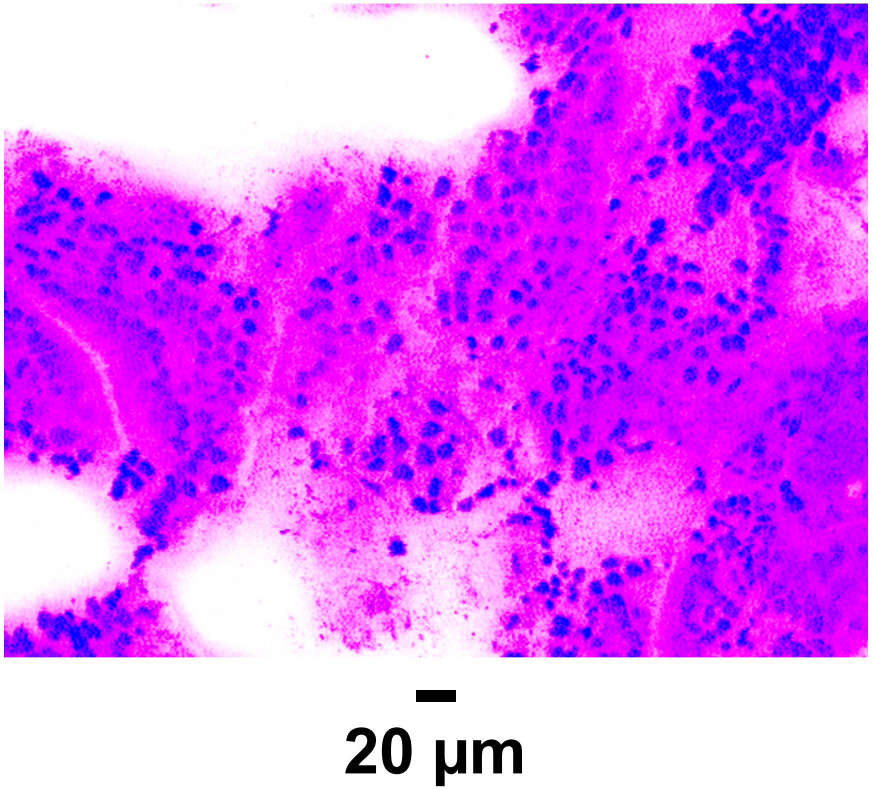
Metabolic rate of oxygen quantified

Measureable range:
0.1-12 mm/s

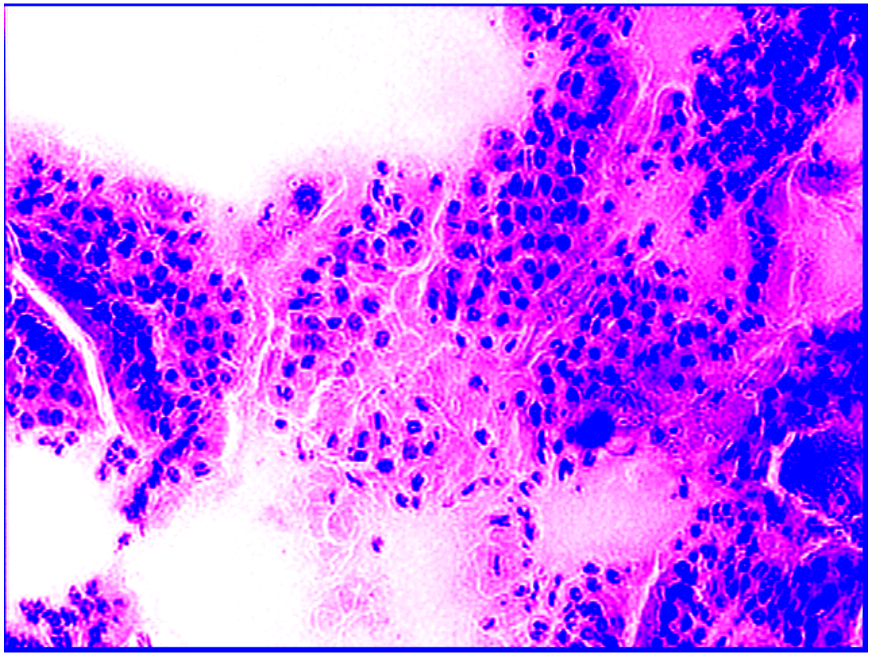
J. Yao et al., unpublished.
PRL 99, 184501, 2007.

Label-free In Vivo Histology by Photoacoustic Microscopy of DNA & RNA in Cell Nuclei

Photoacoustic microscopy without staining

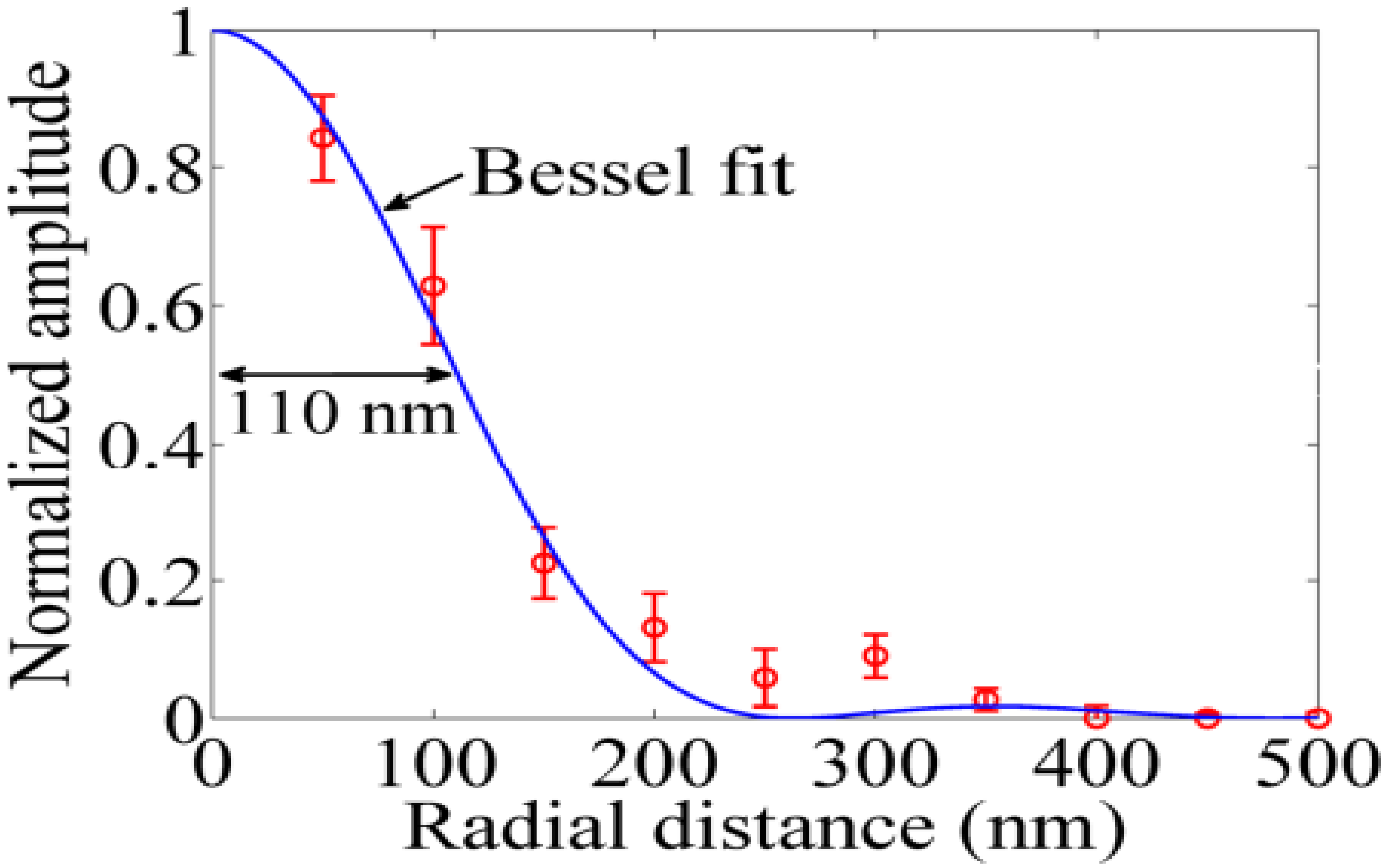


Histology with hematoxylin and eosin staining



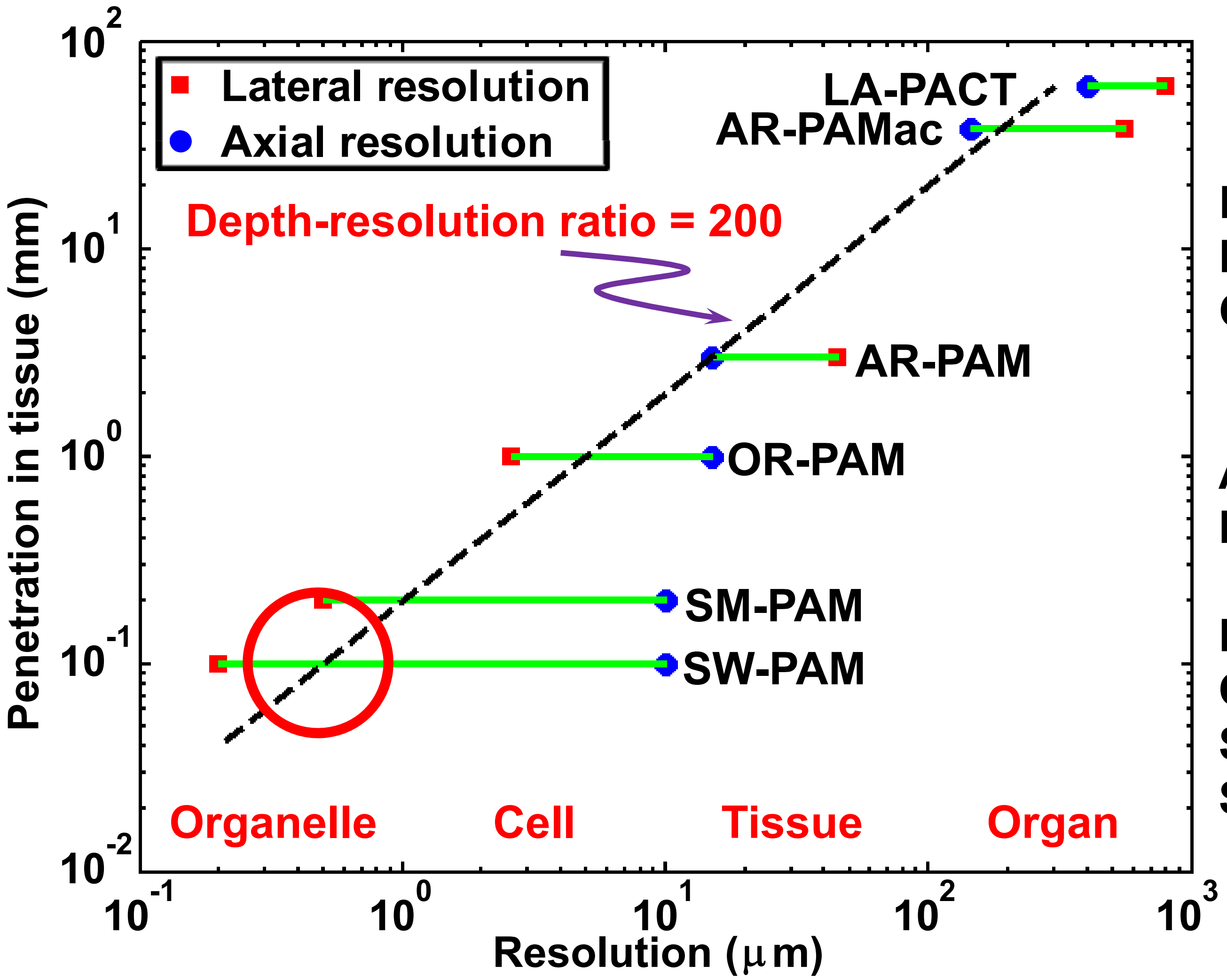
Optics Lett 35, 4139 (2010).

Photoacoustic Microscopy with 220-nm Resolution



Optics Lett 35, 3195 (2010)

Multiscale Photoacoustic Tomography In Vivo with Consistent Contrast



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Nature Photonics 3, 503 (2009).

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  - Acoustic resolution
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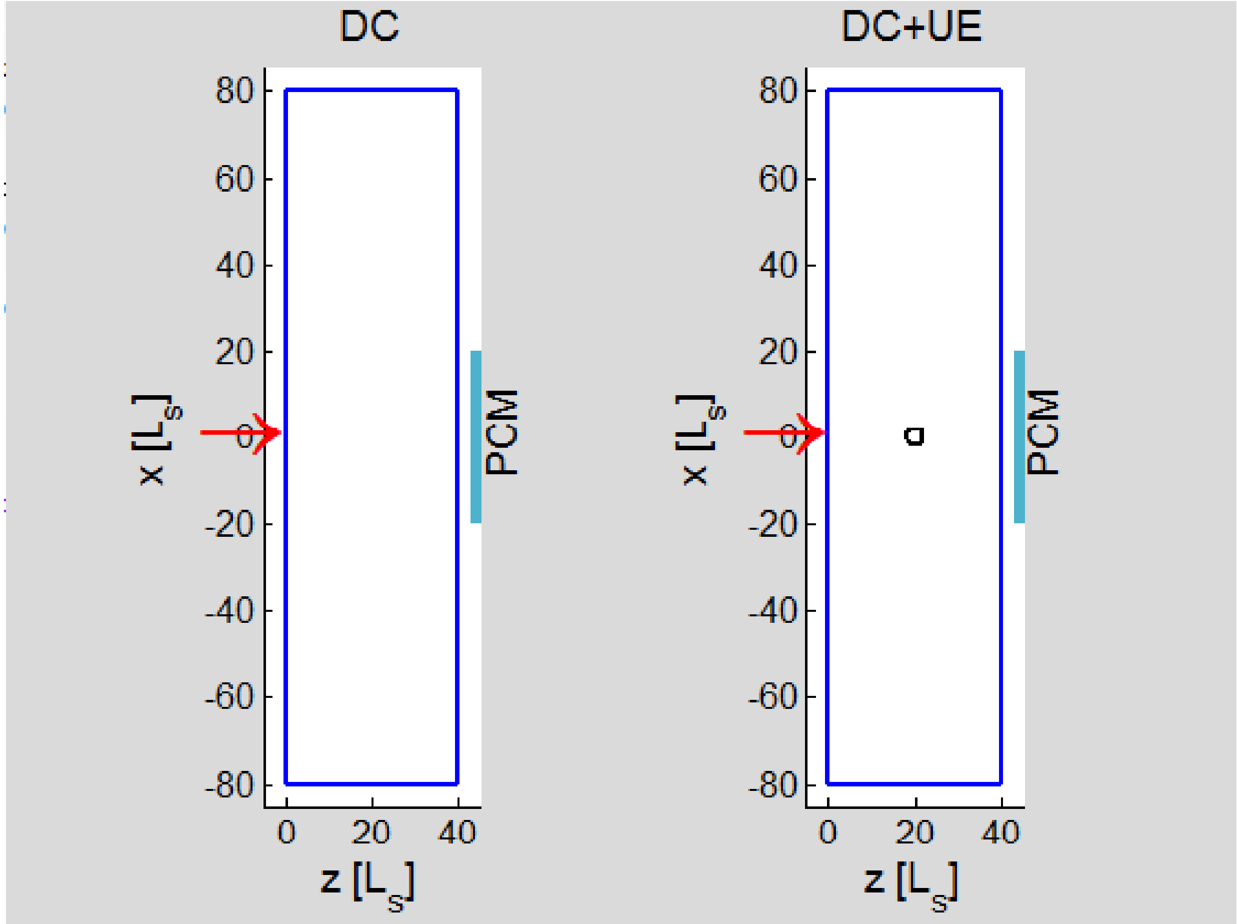
Time-reversed Ultrasound-encoded (TRUE) Optical Focusing



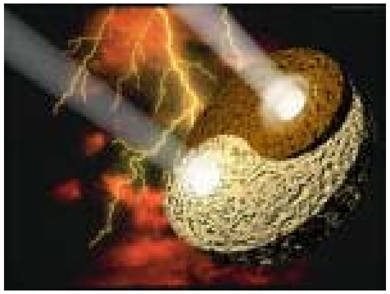
1. Virtual guide star
2. Arbitrary focal position
3. Label free
4. Applications in
   - Imaging (fluorescence)
   - Sensing (oxygenation)
   - Manipulation (opto-genetics)
   - Therapy (photodynamic therapy)

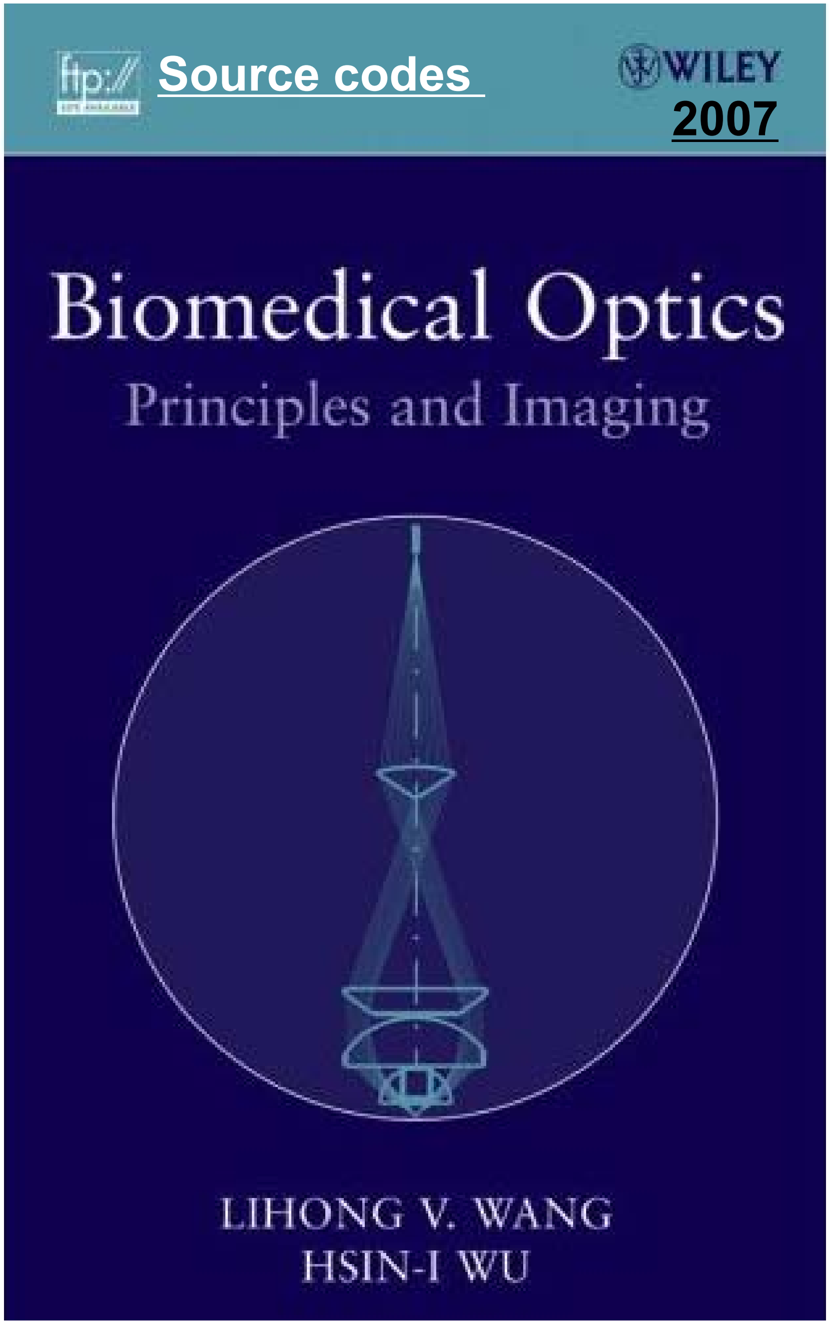
PCM: phase conjugate mirror
DC: un-modulated light
TR: time-reversed
UE: ultrasonic encoding

Nature Phot., 10.1038/NPHOTON.2010.306 (2011)

Summary of Photoacoustic Tomography

1. **Optical excitation and ultrasonic detection integrated**
2. **Diffusion limit (~1 mm) broken: Super-depths (up to 7 cm) reached**
3. **Single capillaries, cells, and organelles resolved in vivo (0.22 μm resolution)**
4. **Multiscale imaging achieved by scaling depth and resolution**
5. **Background-free detection built-in (no absorption, no signal)**
6. **Sensitivity to optical absorption maximized to 100%: highest among all**
7. **Either non-fluorescent or fluorescent pigments detected**
8. **Multiple chromophores resolved spectrally**
9. **Functional imaging derived from endogenous chromophores**
10. **Molecular imaging enabled by targeted contrast agents**
11. **Reporter genes imaged**
12. **Doppler imaging of flow demonstrated**
13. **Data acquired fast: 1 μs for 1.5 mm depth; 100 μs for 15 cm depth**
14. **Speckle artifacts avoided**
15. **Non-ionizing radiation used**
16. **Costs kept relatively low**
17. ***Gel or liquid applied for ultrasonic detection***
18. ***Ultrasound transmission attenuated through cavities and thick bones***



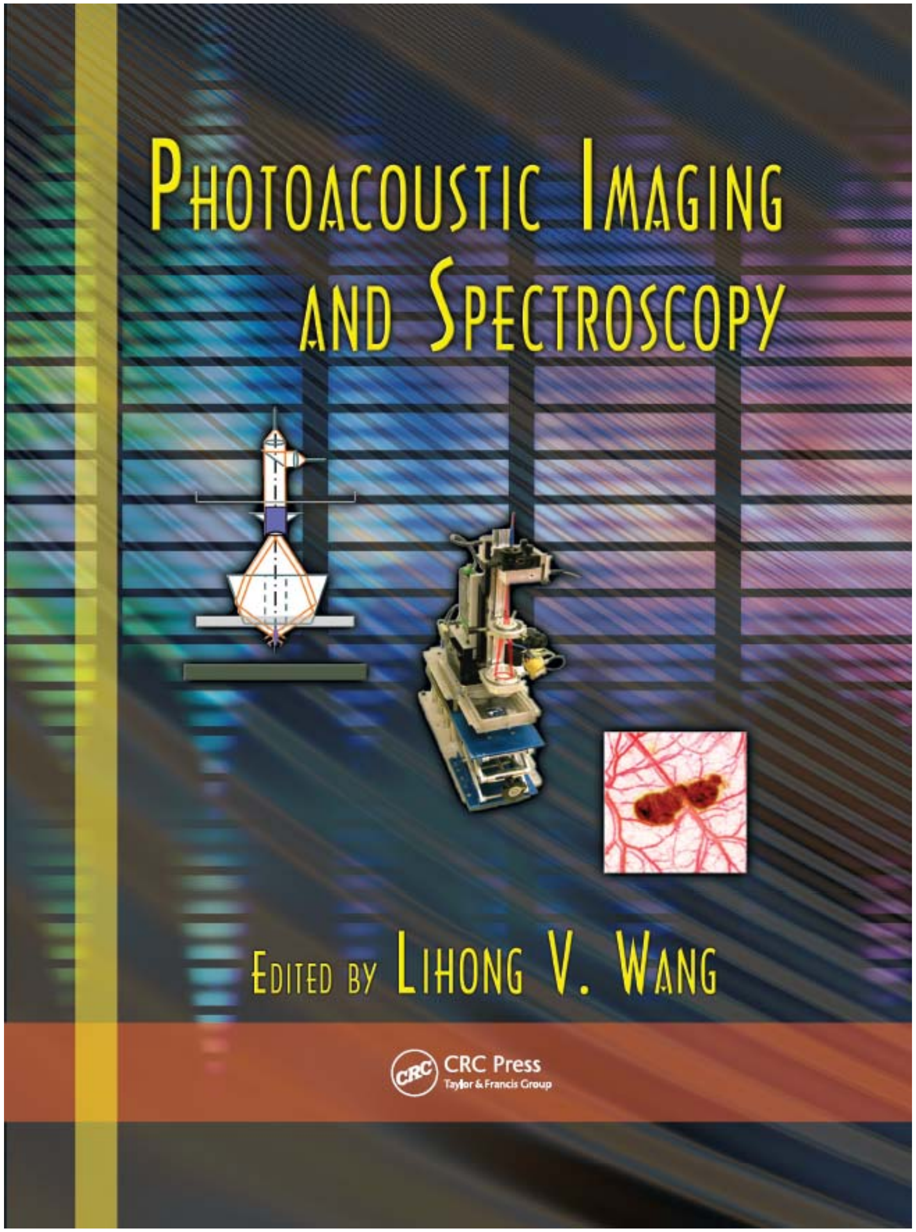


Chapters

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

Homework solutions provided for instructors

Joseph W. Goodman Book Writing Award



PHOTOACOUSTIC IMAGING AND SPECTROSCOPY

EDITED BY LIHONG V. WANG

CRC Press
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