Motivation for Optical Imaging

- Safety — Non-ionizing radiation: photon energy is \( \sim 2 \text{ eV} \).
- Physics — Related to the molecular conformation of tissue.
- Optics — High intrinsic contrast:
  - Optical absorption: oxyhemoglobin, deoxyhemoglobin, melanin, and exogenous contrast agents.
  - Optical scattering: Size of cell nuclei.
  - Optical polarization: Collagen and muscle fibers.
- Physiology — Functional imaging of physiological parameters:
  - Oxygen saturation of hemoglobin (related to hyper-metabolism)
  - Total hemoglobin concentration (related to angiogenesis)
  - Enlargement of cell nuclei
  - Orientation of collagen fibers
  - Denaturation of collagen fibers
  - Blood flow (Doppler)
- Physiology — Molecular imaging
  - Integrin, VEGF, etc.
  - Reporter genes

Why PA

The conversion from optical to ultrasonic energy immediately brings several advantages:

- PAT breaks through the optical diffusion limit by capitalizing on the low acoustic scattering in tissue—about three orders of magnitude less than optical scattering in tissue per unit path length.
- PAT enables multiscale high-resolution imaging of biological structures, ranging in size from organelles to organs, using the same contrast.
- By exciting different molecules at different optical wavelengths, PAT reveals rich optical contrasts according to chemical composition.
- PAT images optical absorption with 100% sensitivity, two orders of magnitude greater than those of confocal microscopy and optical coherence tomography.
Why PA cont’

- PAT provides inherently background-free detection because the photoacoustic amplitude is proportional to the optical absorption; nonabsorbing tissue components present no background.
- Unlike fluorescence imaging, PAT ensures no leakage of excitation photons into detectors.
- Unlike optical coherence tomography and ultrasonography, PAT is speckle-free.
- All molecules are optically absorbing at some wavelengths and can potentially be imaged by PAT, whereas far fewer molecules are fluorescent. Although both conventional ultrasound imaging and PAT are based on ultrasonic detection, the former measures only mechanical contrasts and the latter optical and thermoelastic contrasts.

WHAT IS PHOTOACOUSTIC?

- Conversion of photons to acoustic wave due to absorption and localized thermal excitation.
- Pulses of light is absorbed, energy will be radiated as heat.
- Heat causes detectable sound waves due to pressure variation.

Ultrasound Imaging

<table>
<thead>
<tr>
<th>Ultrasound Imaging</th>
<th>Low MHz</th>
<th>10 – 20 MHz</th>
<th>&gt; 20 MHz (UBM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penetration depth (mm)</td>
<td>100</td>
<td>10</td>
<td>0.01</td>
</tr>
<tr>
<td>Resolution (mm)</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

High Relative Resolution: Depth-to-Resolution Ratio

<table>
<thead>
<tr>
<th>Modality</th>
<th>Max depth</th>
<th>Axial resolution</th>
<th>Depth / Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confocal/two-photon microscopy</td>
<td>~0.2-0.5 mm</td>
<td>~1-2 microns</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Optical coherence tomography</td>
<td>~1 mm</td>
<td>~10 microns</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Magnetic resonance imaging / Ultrasonography</td>
<td>~100-200 mm</td>
<td>~1 mm</td>
<td>&gt;100</td>
</tr>
<tr>
<td>X-ray CT</td>
<td>~200 mm</td>
<td>~0.1 mm</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>
Photoacoustic

- Laser pulse (~5 ns)
- Heat absorption
- Temp. rise (~0.01 °C)
- Thermal expansion (strain ~ $10^{-5}$)
- Acoustic propagation
- Detection and Source reconstruction

Photoacoustic Forward Solution in an Infinite Medium: Plane Wave

Reconstruction-based Photoacoustic Tomography

1. Laser pulse (<ANSI limit: e.g., 20 mJ/cm²)
2. Local heating (~ mK)
3. Ultrasonic emission (~ mbar)
4. Ultrasonic detection (scattering/100)
Photoacoustic Signal Generation

- For effective PA signal generation, the laser pulse duration is normally within several nanoseconds.
- The PA pulse is less than both the thermal and stress confinement times.
- The thermal confinement indicates that thermal diffusion during laser illumination can be neglected:
  \[ \tau < \tau_{th} = \frac{d_c^2}{4D_T}, \]
  where \( \tau_{th} \) is the thermal confinement threshold, \( d_c \) the desired spatial resolution, and \( D_T \) the thermal diffusivity.

- The stress confinement means the volume expansion of the absorber during the illumination period can be neglected:
  \[ \tau < \tau_{st} = \frac{d_c}{v_s}, \]
  where \( v_s \) is the speed of sound.
Photoacoustic Signal Generation

- To generate acoustic waves, the thermal expansion needs to be time variant.
- This requirement can be achieved by using either a pulsed laser or a continuous-wave laser with intensity modulation at a constant or variable frequency.
- Following a short laser pulse excitation, the local fractional volume expansion $dV/V$ can be expressed as

\[
\frac{dV}{V} = -\kappa p(\vec{r}) + \beta T(\vec{r})
\]

Photoacoustic measurements

\[
\tau = \frac{1}{4k\mu_a^2}
\]

Femtosecond laser pulses are much shorter than the acoustic transit time $d/v$, with $d$ the optical penetration depth and $v$ the acoustic velocity. Therefore, it is reasonable to assume that the time component of the source function can be approximated with a delta distribution.

Photoacoustic Imaging

- Light pulse is absorbed in blood cell
- Adiabatic heating
- Pressure pulse emerging
- Detection at tissue surface
Photoacoustic measurements

\[ S(r,s) = I_0 \exp \left[ \frac{2r^2}{\omega^2} \right] \]

\[
\frac{1}{c} \frac{\partial \Psi(r,t)}{\partial t} - s \cdot \nabla \Psi(r,t) + (\mu_s(r) + \mu_a(r))\Psi(r,t) = \\
- \mu_s(r) \int_{4\pi} \Psi(r,t)p(s,s')d\Omega' + S(r,s')
\]

Photoacoustic measurements

\[ S(r,s',t) = \delta(r)\delta(s)\delta(t) \]

Temperature rise

\[ \Delta T = \frac{\mu_a \Psi}{\rho C_p} \]

Photoacoustic Imaging

Two erythrocytes

Diameter: \( \approx 10 \ \mu m \) (compared with a 12\( \mu m \) blue polystyrene sphere)

detection distance: \( \approx 1.7 \ mm \ ( = 1.15 \ \mu m \times 1500 \ m/s) \)

medium: water/PBS

Acoustic Signal

Two erythrocytes

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Acoustic Pressure

- Acoustic pressure increases linearly with optical input energy
- Thermal damage threshold: 25 $\mu$J delivered to a spot size of 25 $\mu$m
- Acoustic pressure at thermal damage threshold: 500 kPa at 10 mm

Photoacoustic Imaging

Double-Ring Detector
One-fiber illumination

Disk-shaped Detector
Ring illuminator
Photoacoustic Imaging

A human hair in chicken breast tissue.

Depth: ≈ 6 mm
(≈ 4 μs x 1500 m/s)
PA-imaging of Blood Vessels in Rabbit Ear:
Flushing with Saline.

Photoacoustic Imaging

Vascular tree from a branching epigastric artery of a rat.
Ex-vivo; medium: intralipid 1 % (≈ tissue).
Depth (Z-coord.) ≈ 5 mm: indicated in figure.
Laser power 532 nm, 2mJ/pulse through fiber ≈ 600 μm.
Depth resolution / lateral resolution: 10/100 μm respectively.

PA-imaging of Blood Vessels in Human Arm

Imaging of Human Palm In Vivo

Maximum amplitude projection onto the skin.
Optical absorption
Reconstruction-based Photoacoustic Tomography

Planar, cylindrical, and spherical detection surfaces:

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

\( d\Omega_0 \) : solid angle subtended by detection element

Transcranial Functional Photoacoustic Imaging of Rat Whisker Stimulation In Vivo: Hemodynamics

Photoacoustic Angiography of Rat Brains In Vivo

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Photoacoustic Inverse Solution in an Infinite Medium: Time-domain Reconstruction

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


Functional and Molecular Photoacoustic Imaging: Nude Mouse with a Glioblastoma in the Brain

(a) Functional imaging: Tumor hypoxia

(b) Molecular imaging: Tumor over-expression of integrin

Spectroscopic Photo-acoustic Tomography: Molecular and Tumor Hypoxia Imaging

Deeper Reflection-Mode Photoacoustic Imaging System: Schematic

- Laser source:
  - Wavelength: 804 nm
  - Pulse width: <15 ns
  - Repetition rate: 10 Hz

- Transducer:
  - Frequency: 5 MHz or 10 MHz
  - Focal length: 1 in.
  - Aperture: 0.75" diameter
  - f-number: 1.33

http://oilab.tminab.com
Deeply Penetrating Photoacoustic Tomography with NIR Excitation & ICG Contrast

Imaging Depth and Resolution
- Imaging depth: ~3 mm
- Axial resolution: ~15 microns
- Depth/resolution: ~200 pixels
- Lateral resolution: ~45 microns
- Acquisition time: 2 µs/A-scan
- No signal averaging

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

Volumetric Imaging of Rat Microvasculature In Vivo

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.

Maximum amplitude projection onto the skin

Histology

Distance [mm]

PA amplitude [a.u.]

Burn depth

~1.7 mm

Skin surface

Hyperemic bowl

0

0.1

0.2

5.5 6 6.5 7 7.5 8

Distance [mm]
Imaging of Hemoglobin Oxygen Saturation (SO₂) In Vivo

- Total hemoglobin
- SO₂ in segmented venules and arterioles
- Histology
- Arterial microsphere perfusion

Hemodynamics In Vivo (578, 584, 590, and 596 nm)

- Total hemoglobin
- Oxygen saturation
- Arteries and veins

- Change in oxygenation

Dark-field Confocal Photo-acoustic Microscopy: Hemodynamics

- Structural image at 584 nm
- Normoxia to hypoxia
- Normoxia to hyperoxia

Imaging of Melanoma In Vivo

- Composite photoacoustic image acquired at 584 and 764 nm
- Photograph
- Movie
- Histology
- Surface rendering

Contrasts:
- Vessel: 13
- Melanoma: 69
Maximum Imaging Depth in Chicken Breast Tissue

- 804 nm optical wavelength
- Exposure: <31 mJ/cm² (ANSI)
- 5 MHz transducer
- 30 times average
- SAFT
- Axial resolution: 144 µm
- Transverse resolution: 560 µm
- SNR:
  - 37 dB (17 mm)
  - 24 dB (30 mm)

Summary

- Physically combining ultrasonic and electromagnetic waves (light & RF) provides
  - improved spatial resolution compared with optical/RF imaging,
  - new contrast mechanisms compared with 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 with those of ultrasound systems.

ADVANTAGES

1. Ability to detect deeply situated tumor and its vasculature
2. Monitors angiogenesis
3. High resolution
4. Compatible to Ultra Sound
5. High Penetration depth

DISADVANTAGES

1. Limited Path length
2. Temperature Dependence
3. Weak absorption at short wavelengths

Limitations and Future Directions

- Limitation of light penetration: up to ~5 cm (~10 cm thickness)
- Limitation of ultrasound penetration through cavities and bones
- Commercialization of photoacoustic microscopic and macroscopic imaging technologies
- Adaptation of commercial ultrasound technologies
- Development of reporter genes that produce optically absorbing gene expression products for molecular (genetic) imaging
- Development of RF contrast agents for molecular imaging
<table>
<thead>
<tr>
<th>Imaging modality</th>
<th>Primary contrast</th>
<th>Imaging depth</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confocal microscopy</td>
<td>Fluorescence/scattering</td>
<td>~0.2 mm</td>
<td>~1-2 microns</td>
</tr>
<tr>
<td>Two-photon microscopy</td>
<td>Fluorescence</td>
<td>~0.5 mm</td>
<td>~1-2 microns</td>
</tr>
<tr>
<td>Optical coherence tomography</td>
<td>Optical scattering</td>
<td>~1-2 mm</td>
<td>~10 microns</td>
</tr>
<tr>
<td>Ultrasonography (5 MHz)</td>
<td>Ultrasonic scattering</td>
<td>~60 mm</td>
<td>~300 microns</td>
</tr>
<tr>
<td>Photoacoustic microscopy (50 MHz)</td>
<td>Optical absorption</td>
<td>~3 mm</td>
<td>~15 microns</td>
</tr>
<tr>
<td>Photoacoustic tomography (3.5 MHz)</td>
<td>Optical absorption</td>
<td>~50 mm</td>
<td>~700 microns</td>
</tr>
</tbody>
</table>

Super-resolution photoacoustic microscopy

![Image of super-resolution photoacoustic microscopy](image-url)