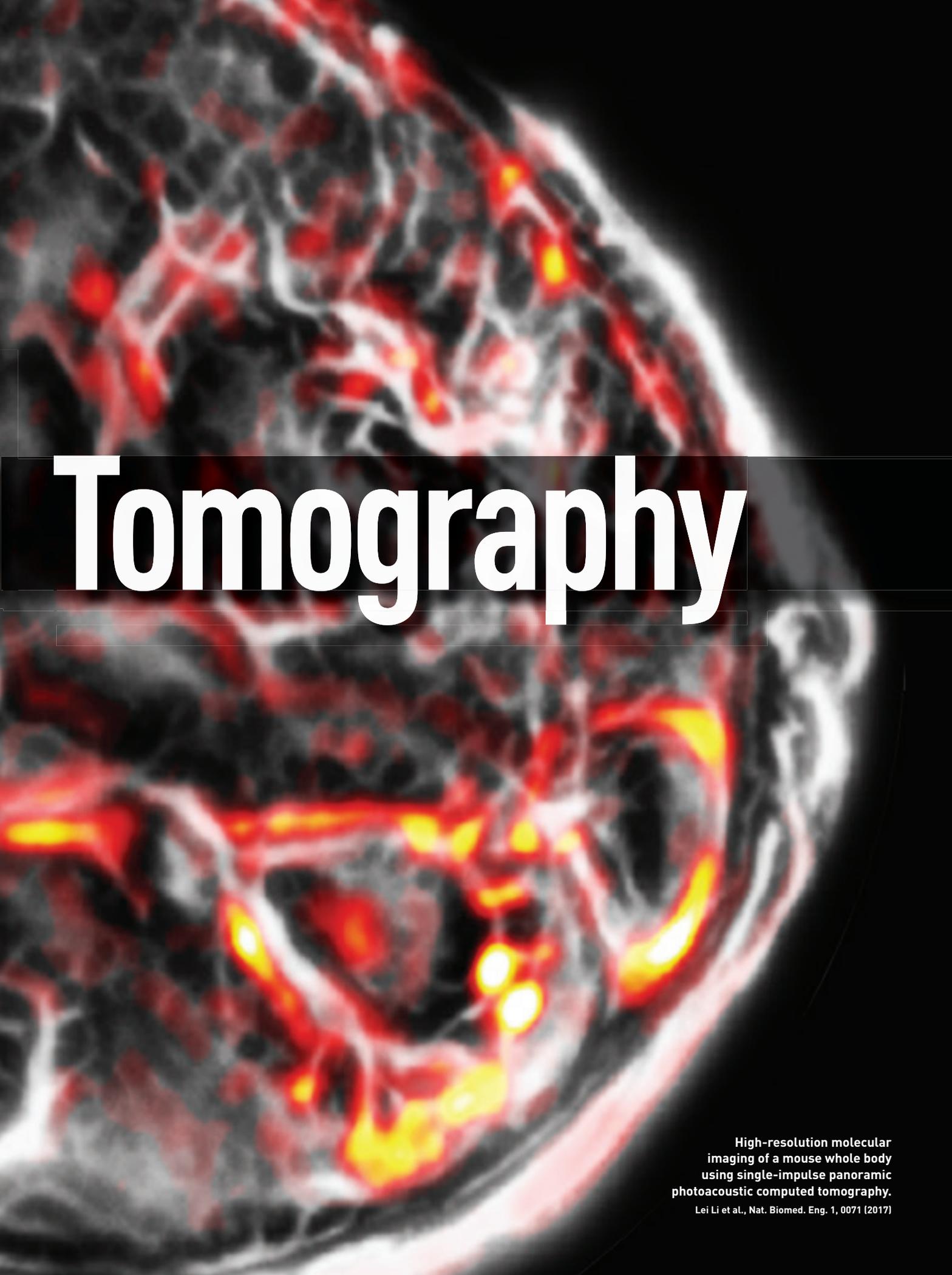


# Multiscale Photoacoustic

Lei Li, Li Lin and Lihong V. Wang

Photoacoustic tomography can bridge microscopic insights and macroscopic observations, providing imaging over a range of spatial scales—from organelles and cells to tissues, small animals and human organs.



# Tomography

High-resolution molecular  
imaging of a mouse whole body  
using single-impulse panoramic  
photoacoustic computed tomography.

Lei Li et al., Nat. Biomed. Eng. 1, 0071 (2017)

**B**iomedical imaging has revolutionized modern medical care and life science. Peering inside complex biological systems, imaging technologies have recently achieved finer spatiotemporal resolution, richer contrast, deeper penetration, and greater detection sensitivity and specificity. The result has been an ever-more-comprehensive view of biological structures and functions.

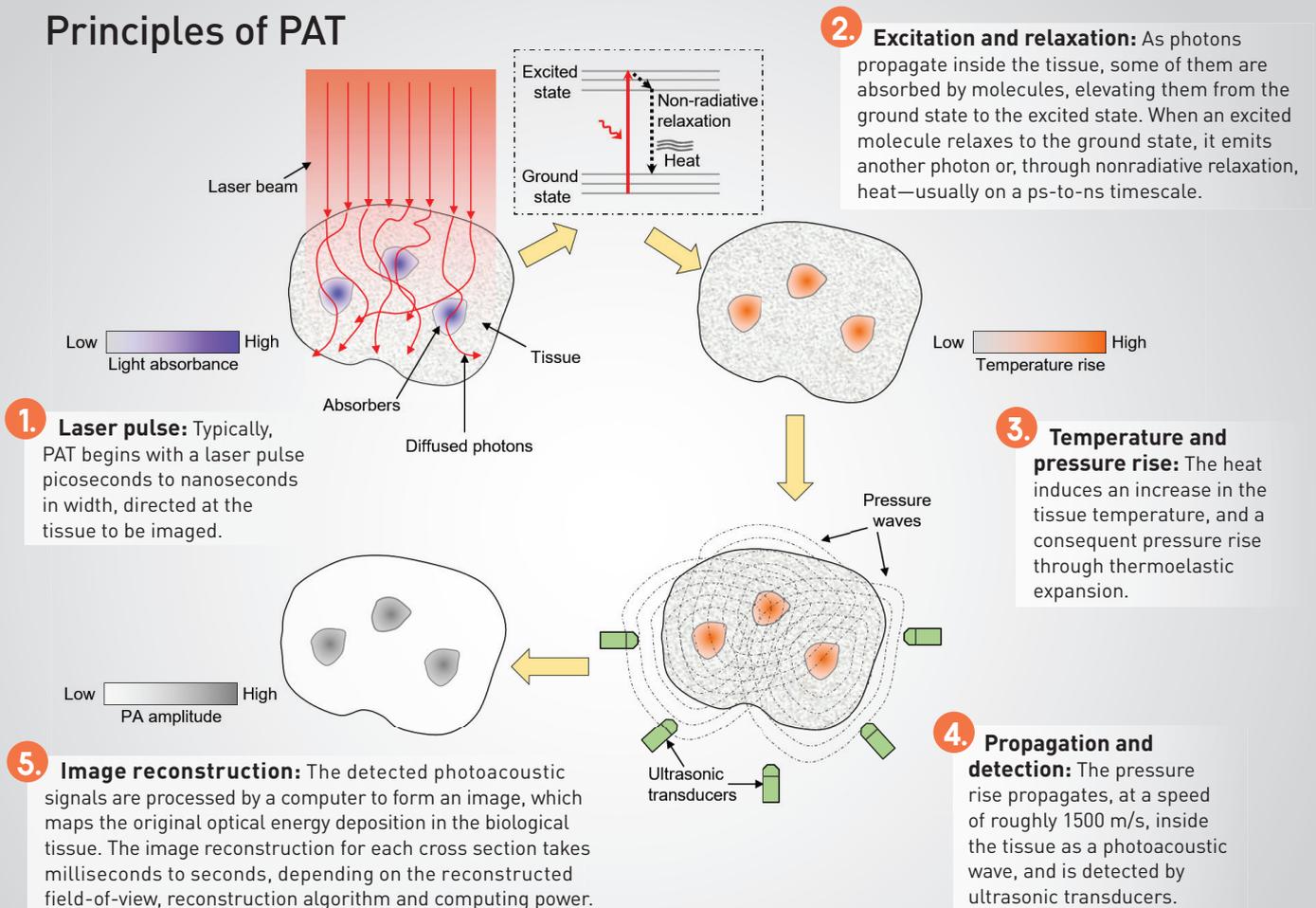
One persistent challenge, however, lies in understanding biological problems across scales. Optical imaging approaches, especially optical microscopy, can visualize biological phenomena with subcellular and suborganelle resolutions, at superficial depths of approximately 1 to 2 mm. Non-optical approaches—including magnetic resonance imaging (MRI), X-ray computed tomography (X-ray CT), positron emission tomography (PET) or single-photon emission computed tomography (SPECT),

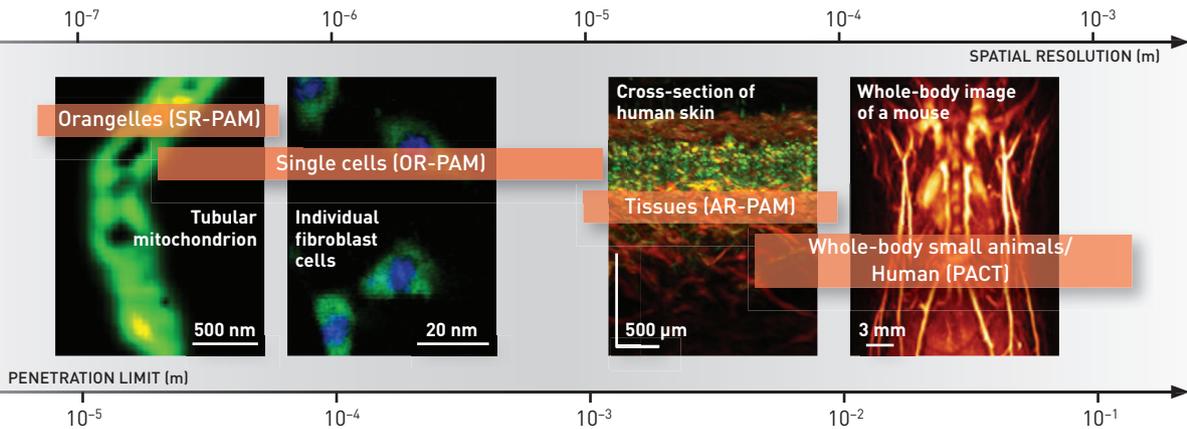
and ultrasound tomography (UST)—can achieve excellent penetration for clinical diagnosis. But these different imaging modalities employ different imaging-contrast mechanisms, and each works only within a certain spatial scale.

Photoacoustic tomography (PAT), also referred to as optoacoustic or thermoacoustic tomography, offers a prominent exception to this scale constraint. PAT can image at scales ranging from the organelles of cells, such as nuclei and mitochondria, to entire small-animal bodies to human organs, using a consistent contrast mechanism—optical absorption. Thus, PAT connects microscopic and macroscopic imaging, and can efficiently bring together anatomical, functional, metabolic, histological and molecular information about biological tissue.

PAT technology has advanced rapidly in recent decades. Here, we look at PAT's principles, explore some cutting-edge technical developments and applications,

## Principles of PAT





## Photoacoustic tomography at multiple scales

Spatial resolutions of different PAT techniques scale with their penetration depths.

A. Danielli et al., *J. Biomed. Opt.* **19**, 086006 (2014); C. Zhang et al., *J. Biomed. Opt.* **18**, 020504 (2013); J. Aguirre et al., *Nat. Biomed. Eng.* **1**, 0068 (2017); X.L. Deán-Ben et al., *Light Sci. Appl.* **6**, e16247 (2017).

and discuss the challenges for next-generation PAT development—along with the great potential of PAT in biomedical studies and clinical translation.

### Leveraging light and sound

PAT is a hybrid imaging technique that combines two forms of energy—optical and acoustic energy—via the photoacoustic effect, a natural phenomenon that converts absorbed photons into acoustic waves. When tissues are hit by a short laser pulse, some of the molecules are elevated to an excited state, and a portion of the absorbed energy can be re-released as heat, on a ps-to-ns timescale, through non-radiative relaxation. The heat gives rise to a pressure increase that propagates, at a speed of around 1500 m/s, inside the tissue as a photoacoustic wave, which is detected within microseconds to milliseconds by a single-element ultrasound transducer or transducer array. Finally, computer processing converts the detected photoacoustic signals into an image.

In PAT, the amplitude of the photoacoustic wave is proportional to the optical energy deposition, which is the product of the optical absorption and the optical fluence. Thus, a given fractional change in optical absorption maps to an identical fractional change in the photoacoustic signal amplitude, providing PAT with 100-percent sensitivity to small variations in

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absorption. In principle, any molecule has its own absorption spectral signature, and usually has a fluorescence quantum yield of less than 100 percent, which provides contrast for PAT. In recent decades, PAT has demonstrated spectroscopic imaging of many endogenous biological molecules, such as oxy- and deoxy-hemoglobin, melanin, water, lipids, DNA, RNA and cytochromes.

By combining aspects of optical and ultrasound imaging, PAT inherits the advantages of both:

**Optical sensitivity.** PAT is sensitive to tissue's optical absorption. By preferentially exciting different molecules with carefully selected optical wavelengths, PAT reveals abundant contrasts based on a tissue's chemical compositions. Absorption by endogenous molecules, such

as hemoglobin, cytochrome and DNA/RNA, makes PAT useful in anatomical, functional, metabolic and histologic imaging. And, by exploiting exogenous contrast agents such as organic dyes, proteins and nanoparticles, PAT can perform molecular and cellular imaging.

**Acoustic penetration.** PAT directly detects acoustic waves induced by the excitation photons, regardless of whether they are ballistic photons or scattered/diffused photons. Thus PAT can achieve far greater penetration

than optical microscopy. More important, scattering of acoustic waves in biological tissue is about three orders of magnitude weaker than optical scattering on a per-unit-path-length basis, so PAT can provide orders-of-magnitude higher spatial resolution at tissue depths greater than 2 mm than can pure optical imaging technology.

## Multiscale PAT

PAT has two major incarnations, depending on how the image is formed. In one incarnation, photoacoustic microscopy (PAM), a single focused transducer is mechanically scanned across the sample to form an image. The other incarnation, photoacoustic computed tomography (PACT), employs wide-field light illumination and detects the resulting acoustic waves at multiple spatial locations, with the image reconstructed using inverse modeling. In PACT, the acoustic detection can assume a linear, circular, planar, or spherical or partly spherical geometry.

PAM can be further categorized into optical-resolution PAM (OR-PAM), in which light is focused much more tightly than the acoustic focus, and acoustic-resolution PAM (AR-PAM), in which the diffused optical beam is wider than the acoustic focus. In both cases, the axial resolution is determined acoustically. Super-resolution PAM (SR-PAM) uses nonlinear mechanisms to break the optical diffraction limit.

In most PAT implementations, the center frequency and the bandwidth of the acoustic detection determine the spatial resolution. Those parameters are

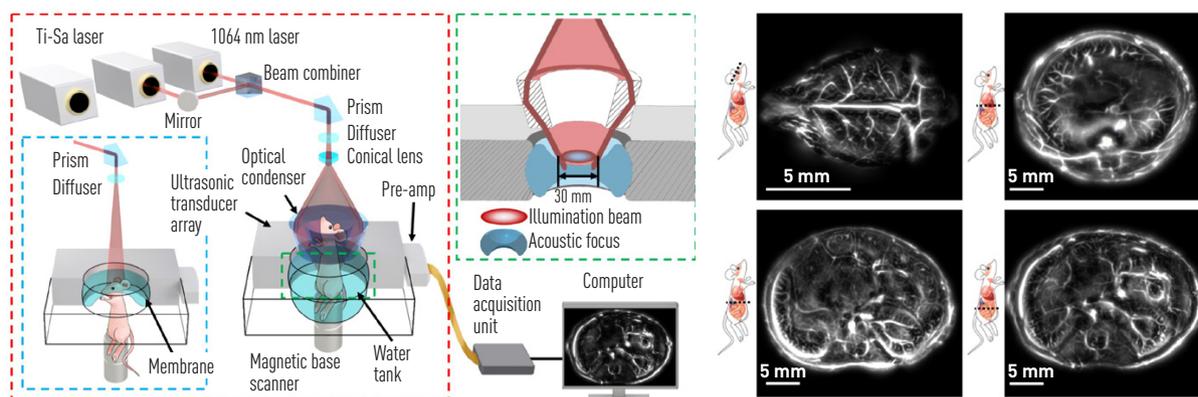
selected based on the desired penetration and, hence, the expected bandwidth of the photoacoustic signals. The higher the central frequency and the broader the bandwidth, the better the spatial resolution but the shallower the penetration; thus, PAT's spatial resolution scales with the desired penetration depth.

Recent advances have broadened the multiscale imaging capability of PAT. SR-PAM has imaged the detailed structures of single mitochondria, the energy factories of cells. Label-free OR-PAM has spectrally resolved the cytoplasm and nuclei of fibroblast cells, the main cells making up connective tissue in animals. AR-PAM has provided mesoscopic images of detailed vascular structures of human skin, from the epidermis to the dermis. And PACT has volumetrically imaged the whole trunk of a live mouse. Very recently, PACT has imaged a healthy human breast *in vivo*, with detailed vasculature resolved up to a depth of 4 cm. In the sections that follow, we look at some of the preclinical and clinical applications of PAT, ranging from whole-body imaging of laboratory animals to imaging of tissues and tumors in human patients.

## Small-animal whole-body imaging

Small-animal whole-body imaging provides physiological, pathological and phenotypic insights into biological processes, making it indispensable to pre-clinical research. With high spatiotemporal resolution and functional contrast, small-animal whole-body imaging can visualize biological dynamics *in vivo*, advancing both biology and translational medicine.

## Whole-body small-animal imaging



Left: PACT system for imaging of the brain (blue dashed box) and trunk of mice; green dashed box shows confocal alignment of optical delivery and acoustic detection. Right: Cross-sectional views showing brain cortex vasculature, liver, upper abdominal cavity, and lower abdomen. L. Li et al., Nat. Biomed. Eng. 1, 0071 (2017)

Revealing how the human brain works is a grand challenge, the pursuit of which can illuminate profound mysteries in neuroscience.

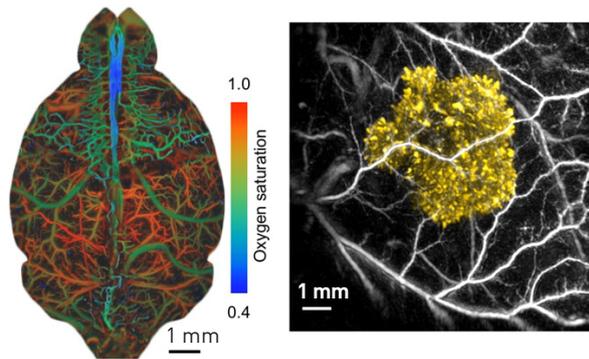
A recent, significant advance in PACT technology, single-impulse panoramic photoacoustic computed tomography (SIP-PACT), has allowed the capture of anatomical, functional and molecular small-animal whole-body images with unprecedented quality and speed. The SIP-PACT experiment used 512-element, full-ring acoustic detection, with simultaneous one-to-one mapped pre-amplification and digitization for maximum imaging signal-to-noise ratio (SNR) and speed.

In the setup, a single laser beam, with a pulse width of several ns—sufficiently short to be treated as an impulse—was formed in a full-ring shape to excite photoacoustic waves, which were detected within 50  $\mu$ s for 2-D imaging of a cross-section of the mouse body (immersed in water for better coupling with the acoustic waves). The 2-D panoramic acoustic detection scheme provided 125- $\mu$ m, isotropic in-plane resolution within the whole cross-section of the mouse trunk, as well as full-view fidelity. A half-time dual-speed-of-sound universal back-projection algorithm compensated for the first-order acoustic inhomogeneity between the mouse tissue and the surrounding water, allowing a more detailed picture of features inside the body.

More recently, a PACT system based on a half-spherical transducer array has allowed dynamic imaging of small-animal organs—especially cardiac imaging, in which the technique’s high imaging speed can reveal heartbeat cycles in detail.

### Imaging the brain and cancer

Revealing how the human brain works is a grand challenge, the pursuit of which can illuminate profound mysteries in neuroscience and also provide a key to the treatment of neurological diseases. A single-wavelength pulse-width-based OR-PAM developed for high-resolution fast brain functional imaging has recently achieved a 1-D rate of 100 kHz for imaging blood oxygenation, with capillary-level, resolution of the mouse brain cortex *in vivo*. Further, capitalizing on the endogenous hemoglobin contrasts of cytochrome proteins and lipids, PACT has



### PACT for brain and cancer imaging

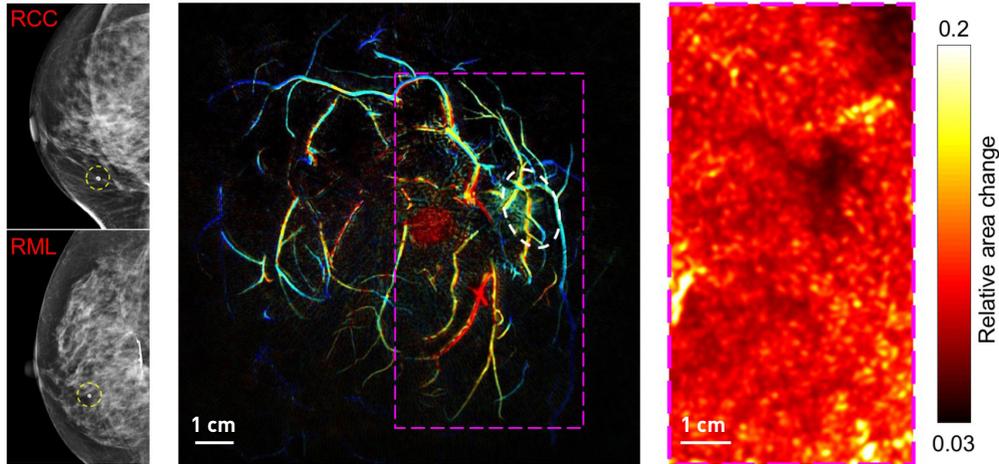
Left: Label-free OR-PAM maps the oxygen saturation of hemoglobin in a mouse brain cortex, clearly differentiating the arteries, shown in red, and the veins, shown in blue/green. Right: PACT image of a tyrosinase-expressing K562 tumor (yellow), with surrounding blood vessels shown in gray.

Y. Yao et al., Nat. Meth. 12, 407 [2015]; A.P. Jathoul et al., Nat. Photon. 9, 239 [2015]

achieved label-free imaging of structures in whole mouse brains *ex vivo*.

Neural imaging of the entire brain is a challenging task—and one for which PAT, used in conjunction with novel, voltage-sensing photoacoustic proteins or dyes, holds great potential. Recently, taking advantage of its deep penetration and high sensitivity, PACT has also been able to image through a rat’s whole brain, revealing detailed vasculature and functional connectivity throughout the brain in a coronal plane. And PAT promises a new horizon for neural imaging in the deep brain of action-potential pulses—a key electro-chemical phenomenon at the cell membrane in the transmission of neural signals.

PAT’s molecular and cellular imaging capabilities make it a powerful tool for cancer research and drug development. Used in conjunction with recently developed genetically encoded photoswitchable proteins, PAT has imaged deep seated tumors with high sensitivity and specificity. And even without exogenous contrast agents such as proteins, dyes and nanoparticles, PAT has proved an effective tool for label-free imaging of melanoma, using melanin as



## PAT in breast cancer imaging

Standard X-ray mammograms of an affected breast (left; RCC, right cranial-caudal; RML, right mediolateral), compared with depth-encoded angiogram of the same breast acquired by SBH-PACT (middle). The white circle identifies the location of the tumor. A map of the relative area change of tissue during breathing (right), in the region outlined by the magenta dashed box in the angiographic image, demarcates the hard tumor as a zone of little relative areal change (dark spot).

the endogenous contrast agent. (Melanin has orders of magnitude higher optical absorption in the red and NIR spectral ranges than hemoglobin.) PACT has successfully imaged transgenic cancer cells expressing high-level melanin with a tyrosinase-based genetic reporter, and has traced melanoma cells migrating in the bloodstream.

### Human breast cancer imaging

Breast cancer is the second leading cause of cancer-related deaths in the United States. A recent advance in breast cancer imaging technology, single-breath-hold PACT (SBH-PACT), can obtain a 2-D cross-sectional breast image with a single laser pulse, or acquire a 3-D image of the entire breast by fast scanning within a single breath hold (approximately 15 s).

Capitalizing on its panoramic acoustic detection, optimized illumination, and signal amplification, SBH-PACT has clearly revealed detailed angiographic, or blood-vessel, structures in the human breast, using the hemoglobin in blood as the contrast agent. The technique's high imaging speed also allowed elastographic imaging for tumor detection, by assessing deformations caused by breathing.

PACT also has the potential to complement and improve on conventional X-ray mammography. Compared with X-ray mammography, SBH-PACT uses safer, non-ionizing radiation, and has shown early promise for sensitivity in radiographically dense breasts

that provide relatively poor results in conventional mammograms. Further, in the near-infrared region, the average breast's optical attenuation coefficient is less than twice that for mammographic X-rays, indicating that PACT can still provide sufficient penetration. Meanwhile, the optical contrast of soft tissue is much higher than the X-ray contrast.

For breast imaging, PACT can exploit these advantages to the fullest, offering high sensitivity and high spatiotemporal resolution with sufficiently deep, non-ionizing penetration. PACT thus, in the near term, could well serve as an adjunct tool to X-ray mammography, reducing the false-positive rate significantly. And SBH-PACT can potentially monitor—again in a non-invasive way—the response of breast tumors to chemotherapy as treatment proceeds.

More than 60 percent of breast cancer patients are treated with breast-conserving surgery to completely remove all of the cancer. However, no intraoperative tools are currently available for microscopically demarcating the tumor's extent in lumpectomy specimens. As a result, in 20 to 60 percent of those patients, second surgeries are required to ensure that tumors have been completely removed. Another variant of PAT, UV-based PAM (UV-PAM), could address this critical need by assessing tumor margins in real time during surgery. Studies have shown a high correlation of the UV-PAM images with conventional histologic images, and that UV-PAM imaging can allow rapid

# PAT offers a comprehensive tool for biomedical imaging, complementing other techniques—and forming a bridge to multiscale biomedical imaging.

diagnosis based on nuclear size and packing density. And UV-PAM can be performed—and its results acted upon—during an operation, because, unlike histological methods, it does not require tissue processing or staining.

## Looking ahead

As the examples above suggest, rapid expansion of PAT applications in both basic, preclinical biomedical research and clinical translation should provide strong momentum for the technique's continued development. PAT does face a number of engineering challenges for next-gen implementations, but none are beyond reach:

- ▶ For imaging neuronal activities in the deep mouse brain, novel voltage- or calcium-sensitive indicators of action potentials with high optical absorption in the red or NIR spectral ranges need to be found or engineered. Nonfluorescent NIR bacteriophytochromes could be candidates to report action potentials for photoacoustic imaging.
- ▶ The biggest barrier to translating PACT to human brain imaging is the skull, which strongly attenuates and distorts photoacoustic waves. One potential solution is to combine PACT with X-ray CT or MRI, which provides measurements for accurate skull modeling. With that model in hand, the PACT reconstruction can be acoustically corrected for skull distortions.
- ▶ Although NIR photons are least attenuated in biological tissues, photon dissipation limits the ultimate optical penetration in live tissue to around 10 cm, which hinders imaging human internal organs using PACT. One solution may lie in using microwaves rather than light as the source of non-ionizing radiation. Microwaves heat tissues by producing molecular rotations and torsions, and biological tissues are more transparent to microwaves than to light. Thermoacoustic computed tomography (TACT) using microwave pulses instead of optical

pulses could extend the penetration depth to about 30 cm, enabling imaging of human internal organs or even whole-body imaging of infants.

As these challenges are met, PAT should find expanding applications in biomedicine. It offers a comprehensive tool for biomedical imaging, complementing other techniques in its contrast mechanism, spatiotemporal resolution and penetration—and forming a bridge to multiscale biomedical imaging. [OPN](#)

*Lei Li and Li Lin contributed equally to this feature.*

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