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Molecular Photoacoustic Imaging of Energy Metabolism

Rui Cao, MS, and Song Hu, PhD, Department of Biomedical Engineering, University of Virginia

Optical imaging plays an indispensable role in biomedicine. It can directly probe biomolecules through their unique spectra. Pure optical imaging technologies fall into two spatially distinct categories: high-resolution (down to tens of nanometers) optical microscopy at shallow depth (less than 1 millimeter) and deep-penetrating (up to 7 centimeters) optical tomography with macroscopic resolution (~1/3 of the imaging depth), leaving a huge gap in between (1). Ultrasound imaging, in contrast, provides seamless spatial scalability by varying the acoustic frequency, but it is unable to directly detect chemical markers of tissue function and metabolism (2).

Combining the optical contrast and ultrasonic scalability, photoacoustic imaging (PAI) is among the most rapidly growing biomedical imaging modalities in recent years (1, 3). In PAI, short-pulsed or intensity-modulated laser light is absorbed by endogenous or exogenous chromophores in biological tissues, inducing transient heating. The subtle temperature rise (at the level of milliKelvin) leads to thermoelastic expansion of the tissue and subsequent emission of ultrasonic waves, which can be captured by an acoustic detector(s) to map the distribution of the optical absorber *in vivo* [Figure 1].

The conversion of optical excitation to acoustic emission brings two unique advantages: specific imaging contrast of optical absorption and excellent depth-to-resolution ratio across the optical and acoustic dimensions. The absorption contrast complements that of fluorescence imaging, the most widely used optical modality for molecular imaging *in vivo*. It enables label-free PAI of multiple endogenous biomolecules—in particular, hemoglobin and lipid, which are intrinsically weakly fluorescent and difficult to tag with exogenous contrast agents. Moreover, PAI detects emitted acoustic waves rather than fluorescent light. Biological tissues scatter light much more efficiently than ultrasound. Effective acoustic focusing in the optical diffusive regime leads to the depth-to-resolution ratio of ~200 in PAI, far exceeding that in diffuse optical tomography (1).

Recent advances in instrumentation and image analysis have significantly expanded the scope of PAI (4). Here, we focus on PAI of oxygen utilization, glucose uptake and lipid accumulation *in vivo*; these hold important implications for understanding energy metabolism.

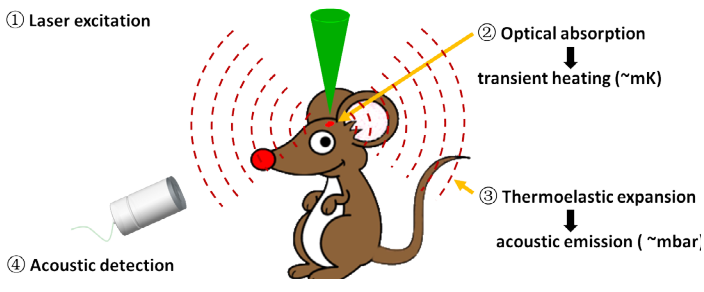

Figure 1. Principle of photoacoustic imaging. mK = milliKelvin.

PAI of Oxygen Utilization

As a major oxygen transporter in the blood circulation, hemoglobin plays a critical role in oxygen metabolism. Within the optical diffusion limit (~1 mm in biological tissues), the wavelength dependence of tissue attenuation of light is negligible. Thus, optical-resolution photoacoustic microscopy (OR-PAM, a high-resolution embodiment of PAI that operates within the optical diffusion limit) is able to differentiate oxy- and deoxy-hemoglobin based on their different optical absorption spectra, from which the oxygen saturation of hemoglobin (sO_2) can be calculated within individual microvessels (5, 6). In addition, statistical (7) and correlation (8) analysis of sequentially acquired OR-PAM signals allows quantifying the total concentration of hemoglobin (C_{Hb}) and blood flow

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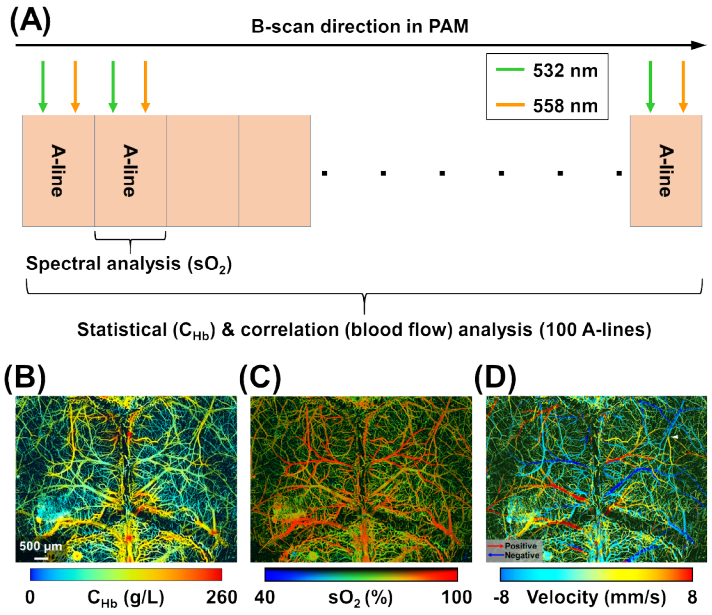

Figure 2. (A) Scanning mechanism of multiparametric OR-PAM for statistical, spectral and correlation analysis. (B) C_{Hb}, (C) sO_2, and (D) the speed and direction of blood flow in the mouse brain simultaneously imaged by OR-PAM. Reproduced from Reference 4 with permission.

in absolute values, respectively. Combining the hemodynamic parameters, OR-PAM has demonstrated label-free imaging of the metabolic rate of oxygen (MRO_2) in early-stage tumor xenografts (9) and electrically stimulated mouse brains (10).

Although encouraging, current oxygen-metabolic OR-PAM has a significant limitation. Unlike C_{Hb} and sO_2, the blood flow can be measured only at selected vessel locations. Thus, this technology can be applied only to quantify the total MRO_2 of selected tissue regions that have closed circulation, in which the total blood flow of all imaged feeding arteries must be identical to that of the draining veins (4).

To overcome this limitation, blood flow must be simultaneously imaged with C_{Hb} and sO_2 at the same spatial scale. To this end, we have developed multiparametric OR-PAM (11, 12). Statistical, spectral and correlation analysis of the same imaging dataset enables, for the first time, simultaneous quantification of C_{Hb}, sO_2 and the speed and direction of blood flow at the microscopic level *in vivo* [Figure 2]. Future development of algorithms to extend these measurements from the microvascular level to tissue level will ultimately enable us to derive microscopic MRO_2 using the Fick's law.

Carrying out these quantitative measurements in the optical diffusive regime has been an unmet challenge, mainly due to the incremental optical scattering and absorption of biological tissues and their wavelength dependences (13). Recently, exciting progress has been made. Tzoumas et al. have developed the eigenspectra PAI based on the novel finding that any light spectrum inside the tissue can be represented as a combination of four spectra—the mean incident light spectrum and three eigenspectra (14). With this advanced analysis, PAI is now able to quantify sO_2 at unprecedented depths (up to 1 centimeter). In

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In the Literature

Each month, the CMIIT Editorial Board selects the top molecular imaging research papers from all papers indexed by PubMed. Below are recent papers on molecular imaging research. The links below go to these references, including their abstracts and links to the full paper on PubMed.

Radiation Dosimetry of a Novel Adenosine A2A Receptor Radioligand [11C]Preladenant Based on PET/CT Imaging and Ex Vivo Biodistribution in Rats.
Zhou X, Elsinga PH, Khanapur S, Dierckx RA, de Vries EF, de Jong JR. PMID: 27539309

Near-Infrared Intraoperative Chemiluminescence Imaging.
Büchel GE, Carney B, Shaffer TM, Tang J, Austin C, Arora M, Zeglis BM, Grimm J, Eppinger J, Reiner T. PMID: 27471800

Fluorine-18 radiolabeling of a nitrophenyl sulfoxide and its evaluation in an SK-RC-52 model of tumor hypoxia.
Laurens E, Yeoh SD, Rigopoulos A, O'Keefe GJ, Tochon-Danguy HJ, Chong LW, White JM, Scott AM, Ackermann U. PMID: 27435268

Development of nanostars as a biocompatible tumor contrast agent: toward in vivo SERS imaging.
D'Hollander A, Mathieu E, Jans H, Vande Velde G, Stakenborg T, Van Dorpe P, Himmelreich U, Lagae L. PMID: 27536107

Positron emission tomography and nanotechnology: A dynamic duo for cancer theranostics.
Goel S, England CG, Chen F, Cai W. PMID: 27521055

Spatially selective depletion of tumor-associated regulatory T cells with near-infrared photoimmunotherapy.
Sato K, Sato N, Xu B, Nakamura Y, Nagaya T, Choyke PL, Hasegawa Y, Kobayashi H. PMID: 27535621

18-kDa translocator protein ligand 18F-FEMPA: Biodistribution and uptake into atherosclerotic plaques in mice.
Hellberg S1, Silvola JM1, Kiugel M1, Liljenbäck H, Savisto N, Li XG, Thiele A, Lehmann L, Heinrich T, Vollmer S, Hakovirta H, Laine VJ, Ylä-Herttuala S, Knuuti J, Roivainen A, Saraste A. PMID: 27225517

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Preclinical Imaging

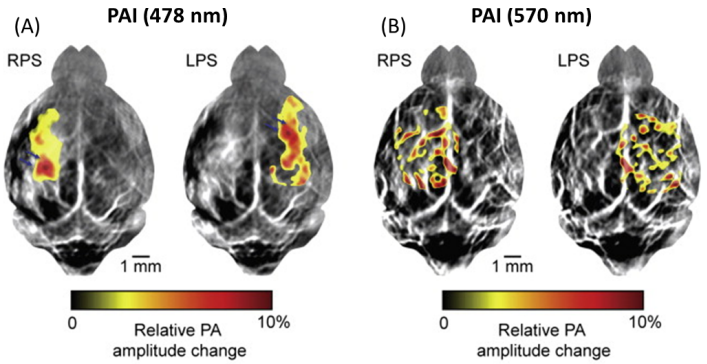

Figure 3. PAI of the cortical glucose and hemodynamic responses to right forepaw stimulation (RPS) and left forepaw stimulation (LPS). Relative changes in the photoacoustic (PA) amplitude at (A) 478 nm (2-NBDG absorption dominant) and (B) 570 nm (hemoglobin absorption dominant). Reproduced from reference 17 with permission.

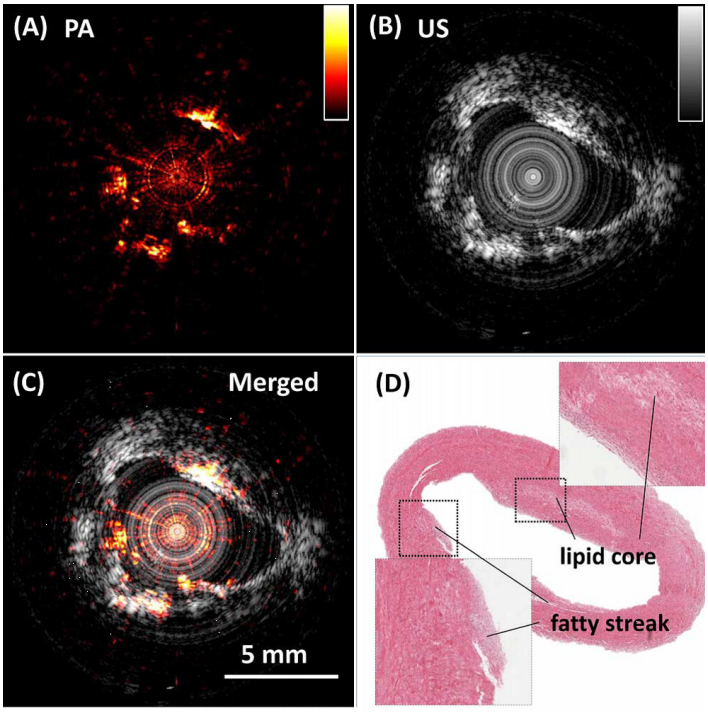

Figure 4. (A) IVPA, (B) IVUS and (C) merged IVPA/IVUS images of atherosclerotic artery. (D) Histology of the arterial cross-section of the imaged area. Reproduced from reference 23 with permission. Credit: Pu Wang, Teng Ma, Mikhail N. Slipchenko, Shanshan Liang, Jie Hui et al.; Scientific Reports, Nature Publishing Group.

parallel, Wang et al. have developed the ultrasonically encoded PAI for blood flow measurement in deep tissue (15). The photoacoustic effect is known to be temperature dependent (13). By thermally tagging the flowing blood with focused ultrasound, PAI is able to trace the blood flow deep inside the tissue through the heat propagation. Integration of these emerging techniques holds great potential for enabling oxygen-metabolic PAI at depth.

PAI of Glucose Uptake

As a raw material for both cellular respiration and fermentation, glucose plays a critical role in energy metabolism. With the aid of radioactive deoxyglucose analogs (e.g., ^{18}F-FDG), positron emission tomography has been widely used to image glucose uptake in humans. Recent advances in fluorescent deoxyglucose analogs (e.g., 2-NBDG) have refined the spatiotemporal resolution of glucose imaging and enabled new applications in preclinical settings (16). However, high-resolution imaging of glucose uptake beyond the optical diffusion limit remains a challenge.

PAI holds great potential for filling this technology gap. Due to the imperfect quantum yield of fluorescent deoxyglucose analogs, part of the photon energy absorbed by these probes undergoes nonradiative relaxation and leads to acoustic emission, which can be exploited for PAI. Taking advantage of the different optical absorption spectra of the deoxyglucose analog and blood hemoglobin, glucose uptake and hemodynamics can be simultaneously imaged by PAI in vivo. For instance, the absorption of 2-NBDG peaks at 478 nm, where hemoglobin has a relatively low absorption. On the contrary, at 570 nm, the absorption of hemoglobin outweighs that of 2-NBDG. With proper levels of light excitation, the signal contributions from 2-NBDG and hemoglobin are negligible at 570 nm and 478 nm, respectively (17). Utilizing this strategy, dual-wavelength PAI has demonstrated simultaneous imaging of glucose uptake and hemodynamic response of the intact mouse brain to the forepaw stimulation [Figure 3]. The high spatial resolution of PAI reveals a more homogenous and confined glucose response with a clear core (blue arrows in Fig. 3A), compared with the hemodynamic response. Future integration of the oxygen- and glucose-metabolic PAI will ultimately enable in vivo high-resolution imaging of the pathological metabolic reprogramming (i.e., the shift between oxidative metabolism and glycolysis) in neurodegeneration (18) and cancer (19).

It is worth noting that IRDye800-2DG, a near-infrared deoxyglucose analog, has also been applied for PAI of glucose uptake in vivo (20). The absorption peak of IRDye800-2DG sits in the optical window of tissue, thereby allowing extended imaging depth. However, the larger molecular weight of IRDye800-2DG (1,330 vs. 342 for 2-NBDG) makes it more difficult to cross the blood-brain barrier.

PAI of Lipid Accumulation

Lipids are not just the primary component of the cell membrane; they are an important energy source in metabolism. Imbalances in lipoprotein synthesis, processing and clearance can lead to accumulation of atherogenic lipid in the vessel wall, becoming a major risk factor of myocardial infarction (21).

Current technical mainstays—including intravascular ultrasound (IVUS) and optical coherence tomography—have enabled in vivo high-resolution imaging of atherosclerotic plaque morphology at different depths. However, it remains a challenge to determine the chemical composition of plaque in vivo. Intravascular PAI (IVPA) is capable of differentiating different types of lipids based on their absorption spectra in the near-infrared region, so it is ideally suited to address this challenge (22). Moreover, integration of IVUS and IVPA enables simultaneous morphological and chemical characterization of the plaque lesion [Figure 4] (23).

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Although encouraging, most existing IVPA systems are limited to ex vivo applications (24). Pushing this technology to in vivo settings requires further developments in high-repetition multiwavelength laser sources and high-sensitivity IVUS-compatible IVPA probes.

Conclusion

Photoacoustic imaging is uniquely capable of high-resolution imaging of multiple major metabolic substrates—including oxygen, glucose and lipids—beyond the optical diffusion limit. PAI opens a new window for studies of energy metabolism in preclinical setting. Of particular interest is metabolic reprogramming, which is closely associated with the progression of tumorigenesis and neurodegeneration.

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CMIIT News

Congratulations to all the CMIIT Award winners, who were recognized at the 2016 SNMMI Annual Meeting in San Diego!

CMIIT Laboratory Professionals Award Recipients:

Joseph L. Latoche, BS, University of Pittsburgh Cancer Institute
"Pre-clinical PET/CT Imaging at the University of Pittsburgh Cancer Institute (UPCI)"

Leonard H. Manson, BS, ED, University of Missouri Research Reactor Center
"Medical Isotope Research Production Development Program at the University of Missouri Research Reactor Center (MURR)"

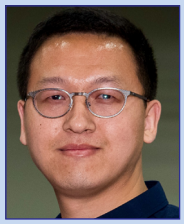
Lisa M. Muench, BS, Brookhaven National Laboratory
"Developing New Radiotracers and Assuring High Standards for Chemical and Radiochemical Purity for Preclinical and Clinical Studies"

The CMIIT Laboratory Professionals Award recognizes innovative/novel and high-impact tools, techniques, and practices of molecular imaging laboratory professionals, with the goal of promoting innovative efforts and exemplary accomplishments by individuals in the lab. The CMIIT Laboratory Professionals Award winners each received free registration to the SNMMI Annual Meeting and a $1,000 monetary award. This award was made possible through a grant from the Education and Research Foundation for Nuclear Medicine and Molecular Imaging.

CMIIT 2016 Young Investigator Award Recipients:

1st Place ($1,000)—Matthias Eiber, MD, PhD, Technical University Munich, Munich, Germany
"Systemic Radioligand Therapy with ^{177}Lu-PSMA I&T in Patients with Metastatic Castration-Resistant Prostate Cancer"

2nd Place ($500) —Eric Price, PhD, University of Saskatchewan
"PET Imaging to Determine HGF Levels in Tumors for Enhanced Patient Selection"



3rd Place ($250) —Hao Chen, PhD, Stanford University
"Self-Assembled NIR-II Dye Conjugated Small Peptides for Bone Imaging"

The Center's Young Investigators Awards recognize the best abstracts presented during the CMIIT Young Investigators Session at the 2016 SNMMI Annual Meeting. Young Investigators are defined as individuals within five years of completing a residency training program or a PhD program.

22. Jansen K, Wu M, van der Steen AFW, van Soest G (2014) Photoacoustic imaging of human coronary atherosclerosis in two spectral bands. *Photoacoustics* 2(1):12–20.
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mi In the News

MI Gateway presents a sampling of research and news of interest to the community of molecular imaging scientists. More **molecular imaging news** is available daily at **www.snmmi.org/cmiit**.

Researchers develop noninvasive PET technique to diagnose and monitor depression

DOTmed

Scientists in Japan have developed a noninvasive PET technique to image regions of the brain that are known to be particularly affected by depression. Clinicians will now be able to obtain objective evidence of depression and determine the effectiveness of treatment.

New PET scan tracer allows first imaging of the epigenetics of the human brain

Science Daily

A novel PET radiotracer, Martinostat, is able for the first time to reveal epigenetic activity—the process that determines whether or not genes are expressed—within the human brain.

PET imaging features linked to EGFR mutations in NSCLC

Healio

PET imaging features strongly correlated with EGFR mutations in non–small cell lung cancer, according to study results presented at the American Association of Physicists in Medicine Annual Meeting. The findings suggest radiomic features could help predict EGFR mutations and may lead to the development of a noninvasive imaging biomarker.

PET/CT calcium scores reveal chemo's damage to the heart

Aunt Minnie

By comparing baseline and follow-up coronary calcium scores from PET/CT scans of lymphoma patients after chemotherapy, researchers say they can better follow the cancer treatment's debilitating impact on the heart, and perhaps get a better handle on possible cardiotoxicity.

Old versus new neuroendocrine tumor imaging agents

Medscape

A systematic review and meta-analysis compared 68Ga-DOTATATE PET with 111In-DTPA-pentetreotide (octreotide) scintigraphy in patients with pulmonary and gastroenteropancreatic neuroendocrine tumors. The authors determined that 68Ga-DOTATATE provides superior image quality, lower radiation dosimetry, and more streamlined imaging acquisition and improved patient convenience. It should, therefore, be used in preference over 111In-DTPA-octreotide where available.

PET imaging may measure risk for suicidal ideation, attempts, death

Healio

Greater raphe nuclei serotonin1A binding potential, as indicated in PET imaging, predicted higher suicidal ideation and greater risk for death by suicide, according to recent findings.

AR protein in prostate cancer detected with new imaging technique

Prostate Cancer News Today

Researchers developed new imaging compounds that bind to a particular region common to the androgen receptor and its variant forms. Using SPECT/CT, the compounds were able to specifically detect prostate cancer cells expressing the androgen receptor in a mouse model of prostate cancer.

PET with tau agent sheds light on Alzheimer's disease

Aunt Minnie

PET imaging of tau proteins with a novel radiotracer based on fluorine-18 (F-18) is showing great promise in differentiating between Alzheimer's patients and cognitively normal individuals and identifying how the disease may develop, according to a study published online July 25 in JAMA Neurology.

New technique opens window into how brain cells communicate

AP – Yahoo

Using PET scans, Yale University researchers have developed a way to picture synapses in living brains.

New quantum dots excite in vivo imaging advancements

Photonics

With extended wavelength ranges and an absence of heavy metals, new quantum dots are poised to take optical in vivo imaging to the next level by enhancing a surgeon's ability to remove tumors.

PET/MRI technique shows promise for prostate cancer

Aunt Minnie

Researchers from the University of Michigan found that adding 18F-choline PET to mpMRI for targeted transrectal prostate biopsies better identified significant prostate cancer than MRI-guided or standard, nontargeted biopsies.

PET imaging for lung cancer helps monitor treatment response

Immuno-Oncology News

Research shows that PET might allow clinicians to better monitor atezolizumab treatments in non-small cell lung cancer (NSCLC) patients. The findings came from a study that focused on immunotherapy but also supports the potential for PET as a means to assess various treatment effects and prognosis.

Calendar of Events

Nuclear Medicine & Molecular Imaging Week—Committed to Quality. Dedicated to Patients.
October 2-8, 2016
http://www.snmmi.org/nmw

2016 Southeastern Chapter SNMMI Annual Meeting
October 7-9, 2016 • Chattanooga, Tennessee
http://www.secsnm.org

EANM'16—29th Annual Congress of the European Association of Nuclear Medicine
October 15-19, 2016 • Barcelona, Spain
www.eanm.org

International Conference on Medical Imaging & Diagnosis
October 20-21, 2016 • Chicago, Illinois
http://www.omicsonline.org

2016 Western Region SNM Annual Meeting
October 20-23, 2016 • Anaheim, California
http://www.wrsnm.org

2016 IEEE Nuclear Science Symposium and Medical Imaging Conference
October 29-November 6, 2016 • Strasbourg, France
http://ieee-npss.org

2016 Northeast Regional Meeting
November 4-6, 2016 • Stamford, Connecticut
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4th Theranostics World Congress
November 7-9, 2016 • Melbourne, Australia
http://www.unicornfoundation.org.au

13th Global Summit on Cancer Therapy
November 17-19, 2016 • Dubai, UAE
http://www.omicsgroup.com

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1850 Samuel Morse Drive
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