ISSN: 2347-3487

# Photoacoustic imaging: hybrid technology for small animals holding potentials translatable for clinical applications

Berritto Daniela, Iacobellis Francesca, Belfiore Maria Paola, La Porta Michele, Grassi Roberto.

*Radiology Department, Second University of Naples, P.zza L. Miraglia 2, 80132-Napoli, Italy*

berritto.daniela@gmail.com

*Radiology Department, Second University of Naples, P.zza L. Miraglia 2, 80132-Napoli, Italy*

francesca.iacobellis@libero.it

*Radiology Department, Second University of Naples, P.zza L. Miraglia 2, 80132-Napoli, Italy*

mariapaola.belfiore@virgilio.it

*Radiology Department, University of Foggia-Foggia, Italy*

m.laporta.neuro@gmail.com

*Radiology Department, Second University of Naples, P.zza L. Miraglia 2, 80132-Napoli, Italy*

roberto.grassi@unina2.it

## ABSTRACT

Photoacoustic imaging is an emerging modality that exploits the photoacoustic effect to combine the high contrast of optical imaging with the spatial resolution and penetration depth of ultrasound. A key feature of PA imaging methods is that they exploit optical contrast but employ US detection principles. The PA effect offers a way to take advantage of the ability of light to penetrate into the body and let us defeat light diffusion by using US waves to “see” the penetrating light. The main advantage of this hybrid approach is that the optical properties of biological tissue, including high contrast and spectral specificity, are encoded in an ultrasound signal. Resolutions of better than 1 mm can be obtained at depths measured in centimeters (up to 7) and not in millimeters, depending on the laser wavelength and transducer frequency used, opening up entirely new regimens of “optical imaging.” From a clinical standpoint, PA imaging is complementary in nature and synergetic with US and a combined US and PA imaging system can be easily implemented due to the presence of a shared detector and associated electronics. Furthermore, such a system will be readily accepted by clinicians familiar with US imaging.

## Indexing terms/Keywords

Photoacoustic imaging; Medical and biological imaging; functional imaging; animal model.

## Academic Discipline And Sub-Disciplines

Diagnostic imagind

## SUBJECT CLASSIFICATION

Photoacustic imaging

## TYPE (METHOD/APPROACH)

Quasi-Experimental

ISSN: 2347-3487

## BRIEF ARTICLE

Photoacoustic (PA) imaging represents exciting and rapidly growing areas of research that holds great promise for addressing important biomedical applications and has been specifically designed for non-invasive real-time in vivo research in small animals.

This method, hybrid in nature, combines optical contrast and ultrasound (US) resolution and penetration in such a way that the result is a modality with characteristics superior to each of the component imaging techniques, while circumventing their limitations.

In the medical and preclinical imaging world there are several technologies that are capable of imaging at various scales from the anatomical to the functional to the molecular [1-7].

For example, Positron Emission Tomography (PET) and single photon emission computed tomography (SPECT) are capable of imaging at the functional and molecular scale relying on the use of nuclear radioisotopes for gamma-ray detection but have poor spatial resolution; Magnetic Resonance Imaging (MRI) is capable of providing anatomical, functional and molecular imaging but requires high costs and long acquisition times

PET-Computed Tomography (PET-CT) spans the spectrum of imaging scale, but has to use ionizing radiation.

Pure optical imaging has received great attention in biomedicine because of its rich contrast and nonionizing radiation. However, due to strong light scattering, pure optical imaging modalities suffer from either shallow penetration depth (maximum penetration depth ~1 mm) or poor spatial resolution.

Pure US imaging methods can image deep structures at high spatial resolution but the image contrast based on differences in mechanical properties of tissue is typically much weaker than optical-based contrast for soft tissue.

A combination of optical and ultrasound also covers the spectrum with the added advantages of having real-time, instantaneous image acquisition in vivo, with high resolution and throughput.

It takes advantage of the PA effect, whereby an acoustic wave is generated by an object which is illuminated by pulsed electromagnetic radiation. A major advantage of this technique is that optical absorption is sensitive to biological processes so that PA may be used for functional imaging [8-10].

A key feature of PA imaging methods is that they exploit optical contrast but employ US detection principles.

The PA effect offers a way to take advantage of the ability of light to penetrate into the body and let us defeat light diffusion by using US waves to “see” the penetrating light [11-13].

The PA technology employs integrated fiber-optic transducers to deliver nanosecond laser pulses into deep anatomical targets. Tissues differentially and specifically absorb the light causing transient thermoelastic expansions which generate acoustic pressure waves with a frequency determined by the pulse repetition rate of the laser.

Transmitted US pulses are detected by a piezoelectric transducer generating high-resolution images of microscopic anatomical structures.

The resolution of the system will be determined by the resolution of the US detector, not by the diffusion of the light.

Since acoustic waves are scattered far less than photons in tissue, photoacoustic signals can be detected at far greater depths than traditional optical imaging techniques, with depths of up to 7 cm reported in living subjects and not in millimeters, depending on the laser wavelength and transducer frequency used, opening up entirely new regimens of “optical imaging.” [14-16].

PA imaging utilizes endogenous [17, 18] as well as exogenous [19-21] light absorbers as entities providing the optical contrast in biological tissues.

The primary endogenous optical absorber in tissue in the near infrared spectrum is hemoglobin (Hb). The absorption coefficient of hemoglobin is several orders of magnitude greater than the absorption of surrounding tissues [22].

Hemoglobin in red blood cells absorbs light and thus imaging of vasculature and calculation of oxygen saturation and total hemoglobin can be performed independent of blood flow, while the system’s Doppler imaging capabilities could give blood flow information. This can all be performed non-invasively in real-time.

The differing optical absorption spectra of oxygenated ($HbO_2$) and deoxygenated hemoglobin (Hb) make PA an ideal modality for in vivo imaging of vascular oxygen saturation ($sO_2$), early tumor detection and angiogenesis.

Oxygen saturation measurements have important therapeutic applications in widespread clinical use. In humans, low $sO_2$ levels in tissues could indicate a hypoxic environment, permitting tumor proliferation, inhibiting tissue perfusion or even indicating the presence of necrotizing fasciitis [23, 24].

Furthermore, $sO_2$ measurements can provide information on anemia, fetal development, and ischemia/stroke.

PA imaging offers new methods for quantification via oxygen saturation and new visualization possibilities through the endogenous heme signal.

ISSN: 2347-3487

In vivo PA imaging of blood vasculature [25] has been used to monitor tumor angiogenesis, vasa vasorum in atherosclerotic plaques, blood oxygenation [26] functional brain mapping [27] and also skin melanomas [28].

The ability to image total Hb in addition to sO2 in an ischemic or reperfused tissue could prove to be a valuable research tool in the study of ischemia and potential interventions and treatments.

Ischemia - or the lack of blood supply to a tissue - and subsequent reperfusion induces physiological and biochemical changes in the affected tissue and is an important area of study since the damage that occurs as a result is clinically important in diabetes and stroke.

At wavelengths above 1100 nm, optical absorption of other tissue components such as lipid may dominate, which is helpful in imaging other tissue types [29, 30].

Furthermore, multi-wavelength, or spectroscopic, PA imaging can be used to reconstruct the local optical absorption spectrum in the imaged regions of interest [29].

To further improve PA contrast, various exogenous contrast agents have been introduced to target specific regions or pathologies. The properties of these contrast agents will affect the biodistribution and efficacy of nanoparticle contrast agents in vivo. Each parameter should be designed for optimal uptake, distribution and retention of the contrast agent in the targeted tissue or organ to be imaged.

Exogenous contrast agents can produce photoacoustic contrast with several fold higher magnitude than intrinsic endogenous contrast alone.

The goal of contrast agents as molecular biology tools is to identify biomolecular markers of disease using in vivo imaging technologies.

The ability to distinguish between normal and diseased tissue in vivo in addition to obtaining molecular information about the tissue of interest has important applications in cancer research in particular.

In conclusion the PA imaging technology combines the sensitivity and specificity of optical

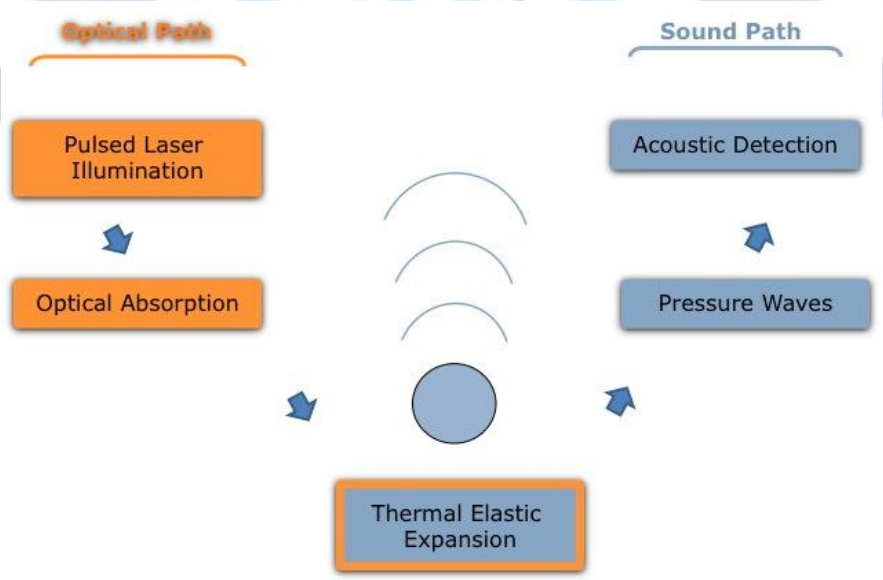
imaging with the high-resolution of US offering unique advantages over existing imaging modalities. This combination is based on the complementary nature of these imaging modalities, where the PA image can be co-registered on a B-Mode US image to provide anatomical information about the PA signal.

The ability to image differences in optical absorption in tissue makes it an effective strategy for differentiating between healthy and diseased tissue. Furthermore, real-time PA imaging can be performed in vivo.

Although the technology is still in its infancy and is limited by its ability to scan bone and air, much work has been done in the pre-clinical area, and PA imaging is fast approaching the clinical setting. The imaging field is broad with many exciting applications for detecting and diagnosing diseased tissue or processes.

From a clinical standpoint, PA imaging is complementary in nature and synergetic with US and a combined US and PA imaging system can be easily implemented due to the presence of a shared detector and associated electronics. Furthermore, such a system will be readily accepted by clinicians familiar with US imaging.

## FIGURES/CAPTIONS



ISSN: 2347-3487

**Figure 1: PA imaging consists of pulsed laser illumination with optical absorption by specific tissue chromophores such as haemoglobin, melanin, water or lipids followed by rapid conversion to heat; that produces thermal expansion and a pressure waves generation which can be acoustically detected.**

**The detected acoustic signal is used to generate an image. PA image can be regarded as an ultrasound image in which the contrast depends not on the mechanical and elastic properties of the tissue, but its optical absorption.**

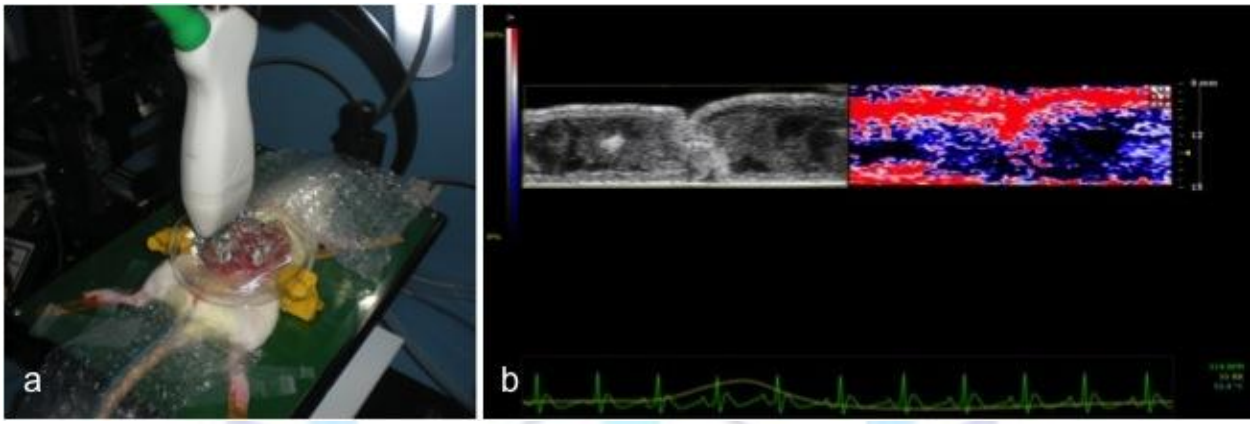



**Figure 2. In a) the animal (Sprague-Dawley rat) on the handling and positioning system with exposed bowel which is arranged on a dedicated support and covered with US-gel (black arrowhead). The Nd:YAG laser, Second Harmonic Generator (SHG), and the Optical Parametric Oscillator (OPO) together (white arrow), provide a tunable light source from 680-970 nm at 20 Hz enabling real-time imaging of a variety of endogenous and exogenous signals. In b) the bowel segment analyzed by PA probe providing for b-Mode image on the right and its physiological wall signal on the left, which appear red.**

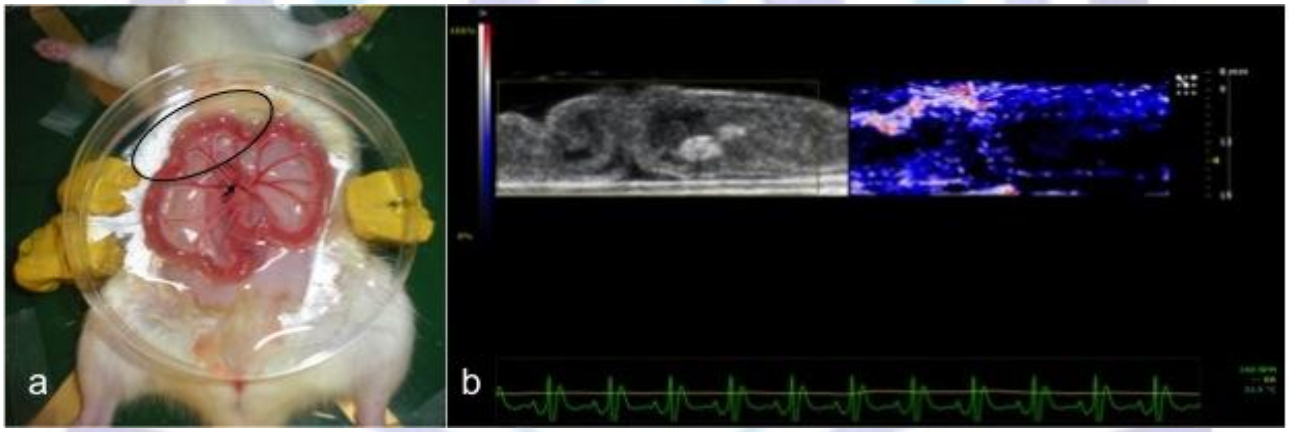



**Figure 3. In a) acute ischemia of the bowel segment in the circle was induced using suture thread as a tourniquet (arrow). In b) note that the oxygen saturation in the ischemic (right) part of the bowel drops significantly after the tourniquet is tightened appearing with blue signal.**

## REFERENCES

[1] Grassi R1, Cavaliere C, Cozzolino S, Mansi L, Cirillo S, Tedeschi G, Franchi R, Russo P, Cornacchia S, Rotondo A. Small animal imaging facility: new perspectives for the radiologist. Radiol Med. 2009 Feb;114(1):152-67. doi: 10.1007/s11547-008-0352-8

[2] Berritto D, Iacobellis F, Belfiore MP, Rossi C, Saba L, Grassi R. Early MRI findings of small bowel obstruction: an experimental study in rats. World J Gastroenterol. 2013 Oct 28;19(40):6825-33. doi: 10.3748/wjg.v19.i40.6825.

[3] Saba L1, Berritto D, Iacobellis F, Scaglione M, Castaldo S, Cozzolino S, Mazzei MA, Di Mizio V, Grassi R. Acute arterial mesenteric ischemia and reperfusion: macroscopic and MRI findings, preliminary report. J Biol Regul Homeost Agents. 2013 Jul-Sep;27(3):771-9.

[4] Berritto D, Iacobellis F, Somma F, Corona M, Faggian A, Iacomino A, Feragalli B, Saba L, La Porta M, Grassi R. 7T mMR in the assessment of acute arterial mesenteric ischemia in a rat model. Cardiovasc Ultrasound. 2011 Dec 8;9:39. doi: 10.1186/1476-7120-9-39.

[5] Belfiore MP, Berritto D, Iacobellis F, Rossi C, Nigro G, Rotundo IL, Cozzolino S, Cappabianca S, Rotondo A, Grassi R. A longitudinal study on BIO14.6 hamsters with dilated cardiomyopathy: micro-echocardiographic evaluation. World J Gastroenterol. 2012 Apr 7;18(13):1496-501. doi: 10.3748/wjg.v18.i13.1496.

[6] Iacobellis F, Berritto D, Somma F, Cavaliere C, Corona M, Cozzolino S, Fulciniti F, Cappabianca S, Rotondo A, Grassi R. Magnetic resonance imaging: a new tool for diagnosis of acute ischemic colitis? Radiol Med. 2011 Sep;116(6):829-41. doi: 10.1007/s11547-011-0676-7. Epub 2011 Apr 19.

[7] Berritto D1, Somma F, Landi N, Cavaliere C, Corona M, Russo S, Fulciniti F, Cappabianca S, Rotondo A, Grassi R. Seven-Tesla micro-MRI in early detection of acute arterial ischaemia: evolution of findings in an in vivo rat model. Radiol Med. 2011 Sep;116(6):829-41. doi: 10.1007/s11547-011-0676-7.

[8] Li C, Wang LV. Phys Med Biol. 2009;7:54(19):R59-97. doi: 10.1088/0031-9155/54/19/R01

[9] Liu, JH, Wei CW, Sheu YL, et al. Design, Fabrication and Testing of a Dual Band Photoacoustic Transducer Ultrason Imaging 2008;30:217-227 doi:10.1177/016173460803000403

[10] Anastasio MA, Beard PC Photoacoustic imaging and sensing J Biomed Opt 2012;17:6 doi:10.1117/1.JBO.17.6.061201

[11] Mezrich R Photoacoustic Imaging—Can It Let Us See Color and Function Deep Inside the Body? Radiology 2010;256:1 doi: 10.1148/radiol.100470.

[12] L. V. Wang "Multiscale photoacoustic microscopy and computed tomography," Nat. Photonics 2009;3(9):503–509. doi: 10.1038/nphoton.2009.157

[13] C. Kim, C. Favazza, and L. V. Wang, "In vivo photoacoustic tomography of chemicals: high-resolution functional and molecular optical imaging at new depths," Chem. Rev. 2010;110(5),2756–2782. doi: 10.1021/cr900266s

[14] Wang LV, Hu S Photoacoustic tomography: in vivo imaging from organelles to organs. Science 2012;335:1458-1462 doi: 10.1126/science.1216210

[15] L. V. Wang, H. F. Zhang, and K. Maslov, Biomedical Optics, Technical Digest (CD) Optical Society of America, 2006, Paper No. WD1 doi:10.1117/1.2355667

[16] Mezrich R Photoacoustic Imaging—Can It Let Us See Color and Function Deep Inside the Body? Radiology 2010;256:1 doi: 10.1148/radiol.100470

[17] Wang X, Pang Y, Ku G, et al. Noninvasive laser-induced photoacoustic tomography for structural and functional in vivo imaging of the brain Nat Biotechnol. 2003;21:803-806 doi:10.1038/nbt839

[18] Ermilov SA, Khamapirad T, Conjusteau A, et al. Laser optoacoustic imaging system for detection of breast cancer J Biomed Opt. 2009;14:024007 doi: 10.1117/1.3086616

[19] De la Zerda A, Zavaleta C, Keren S, et al. Carbon nanotubes as photoacoustic molecular imaging agents in living mice Nat Nanotechnol. 2008;3:557-562 doi:10.1038/nnano.2008.231

[20] Lu W, Huang Q, Ku G, Wen X, et al. Photoacoustic imaging of living mouse brain vasculature using hollow gold nanospheres Biomaterials 2010;31:2617-26 doi:10.1016/j.biomaterials.2009.12.007

[21] Pan D, Pramanik M, Senpan A, et al. Molecular photoacoustic tomography with colloidal nanobeacons Angew Chem Int Ed Engl. 2009;48:4170-3 doi: 10.1002/anie.200805947

[22] Prahl, SA. Optical properties spectra compiled by Scott Prahl. 2001. [cited July 1, 2010]; Available from: http://omlc.ogi.edu/spectra/

[23] Lima A, Van Bommel J, Jansen TC et al. Low tissue oxygen saturation at the end of early goal-detected therapy is associated with worse outcome in critically ill patients Critical Care 2009;13:S13 doi: 10.1186/cc8011

[24] Wang TL, Hung CR Role of tissue oxygen saturation monitoring in diagnosing necrotizing fasciitis of the lower limbs Ann Emerg Med 2004;44:222-8 doi:10.1016/j.annemergmed.2004.03.022

[25] Hu S, Wang LV. Photoacoustic imaging and characterization of the microvasculature. J Biomed Opt 2010;15(1):011101–15 doi: 10.1117/1.3281673

[26] Laufer JG, Elwell CE, Delpy DT, et al. Spatially resolved blood oxygenation measurements using time-resolved photoacoustic spectroscopy. Adv Exp Med Biol 2006;578:155–60

[27] Stein EW, Maslov K, Wang LV. Noninvasive, in vivo imaging of blood-oxygenation dynamics within the mouse brain using photoacoustic microscopy. J Biomed Opt 2009 Mar-Apr;14(2):020502. doi: 10.1117/1.3095799

[28] Oh JT, Li ML, Zhang HF, et al. Three-dimensional imaging of skin melanoma in vivo by dual- wavelength photoacoustic microscopy. J Biomed Opt 2006 May-Jun;11(3):34032 doi:10.1117/1.2210907

[29] Sethuraman S, Amirian JH, Litovsky SH, et al. Spectroscopic intravascular photoacoustic imaging to differentiate atherosclerotic plaques. Opt Express 2008;16(5):3362–7. http://dx.doi.org/10.1364/OE.16.003362

[30] Wang B, Su JL, Amirian J, et al. Detection of lipid in atherosclerotic vessels using ultrasound- guided spectroscopic intravascular photoacoustic imaging. Opt Express 2010;18(5):4889– 97 doi: 10.1364/OE.18.004889.