Video Article

Photoacoustic Tomography for Blood and Lipid Imaging

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Abstract

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Photoacoustic tomography (PAT) is an emerging biomedical imaging modality that utilizes light generated acoustic waves to obtain compositional information from tissue. PAT can be used to image blood and lipid components that can be useful for a wide variety of applications ranging from cardiovascular to tumor imaging. Currently used imaging techniques have their own inherent limitations that restrict their use with researchers and physicians. For instance, long acquisition times, high costs, use of harmful contrast, and minimal to high invasiveness are all factors that limit use of various modalities in the laboratory and clinic. Currently the only comparable imaging techniques to PAT are emerging optical modalities that also have their own disadvantages, such as limited depth of penetration and need for exogenous contrast. PAT, on the other hand, provides meaningful information in a rapid, noninvasive, label-free manner. If coupled with ultrasound, PAT can also be used to obtain structural, hemodynamic, and compositional information from tissue, thereby complementing currently used imaging techniques. The advantages of PAT illustrate its capabilities to make an impact in both the preclinical and clinical space.

Video Link

The video component of this article can be found at https://www.jove.com/video/10395/

Introduction

As previously mentioned, PAT is a hybrid modality that utilizes light-induced acoustic waves to obtain compositional information from tissue. Acoustic propagation is attributed to thermoelastic expansion where the tissue expands due to ambient temperature rise due to light absorption by specific chemical bonds. To elaborate, specific chemical bonds absorb light, causing the molecule to vibrate and convert some of this vibrational energy to heat. This production of heat causes local tissue expansion, which induces acoustic propagations that can be detected by an ultrasound transducer. To induce the photoacoustic effect, both the thermal and stress confinement conditions must be met to minimize heat dissipation and allow thermoelastically-induced pressure to build up within tissue. The resulting photoacoustic pressure wave can be characterized by equation (1), which states that the light-induced acoustic wave (P_o) is governed by the temperature-dependent Grueneisen parameter (Γ), absorption coefficient (μ_a), and local optical fluence (F).

$P_o = \Gamma \mu_a F$ Equation 1

As a result, each mK rise in temperature characteristically produces a 800 Pascal pressure wave that can be detected using an ultrasound transducer. This bond-selective absorption of light allows users to target various biological components by tuning the wavelength of light such as using 1100 nm light to target blood and 1210 nm light to target lipids. Additionally, since light is being used to induce acoustic wave propagation, this technique can be used to typically image deeper structures than other optical techniques without the need for contrast agents or invasive procedures. This specific method using long-wavelength light in the second near-infrared window light to induce acoustic waves provides numerous advantages to the user, allowing vibrational PAT (or VPAT) to be potentially used for a wide range of biomedical applications.

Protocol

The following procedure describes the methods needed to set up VPAT for blood and lipid imaging of the infrarenal aorta in apolipoprotein-E deficient ($apoE^{-/-}$) mice.

1. Laser-ultrasound coupling

1. Obtain a Nd:YAG pulsed optical parametric oscillator laser and a ultrasound system.
2. Acquire a pulse and function generator along with 5 BNC cables, T female to male to female, and straight female-to-female connectors.
3. Attach three BNC cables together using a T female to male to female and straight female-to-female connectors. These BNC cables should then be connected to the pulse generator "gate trig" port, function generator "output" port, and laser "control" port.
4. Connect a BNC cable into the pulse generator "port A" and the ultrasound "Trigger In", as well as another BNC cable into pulse generator "port B" and the laser "Port In".
5. Align the fiber optic cable with the laser and attach the fiber ends to the sides of the 40 MHz ultrasound transducer.
6. Set pulse generator to produce a square wave with frequency of 10 Hz, high level of 5 V, and offset of 2.5 V with a duty cycle of 50%.

7. Set pulse generator output A to 0.0012946 s and output B to 0.0015 s.

2. Animal Preparation and Image Acquisition

1. Anesthetize an apoE$^{-/-}$ mouse using 3% isoflurane in a knockdown chamber. Once the animal is anesthetized move the mouse to a nose cone to deliver 1 - 2% isoflurane.
2. Apply eye lubricate to the animals eyes to prevent corneal desiccation. Tape the mouse's paws to electrodes built into the heated stage to monitor animal's respiration and heart rate. Finally, insert rectal probe to monitor body temperature.
3. Remove hair from animal's abdomen by applying depilatory cream.
4. Place ultrasound transducer on the animal's abdomen and locate the infrarenal aorta. The left renal vein and the aortic trifurcation into the tail artery are two landmarks that will help the user locate this area.
5. Run the laser to output 1100 nm light to target blood followed by 1210 nm light to target lipid. Use appropriate laser safety goggles when laser is in use.

Representative Results

Here, VPAT methods were used to perform lipid and blood specific imaging *in vivo*. By coupling a laser and ultrasound system, light was delivered to tissue and the resulting acoustic waves were detected. Ultrasound imaging allowed us to obtain structural information of the infrarenal aorta (**Figure 1a**) that can be used to better interpret VPAT compositional information. Specifically, a 1100 nm light was used to image blood within the aorta (**Figure 1b**), and a 1210 nm light was used to image subcutaneous and periaortic fat accumulation (**Figure 1c**). From the ultrasound and VPAT images, one can see that the subcutaneous fat follows the geometry of the skin, the periaortic fat follows the contour of the aorta, and the blood signal originates from within the aorta. These results confirm that, indeed, VPAT can be used to image blood and lipid accumulation *in vivo*.

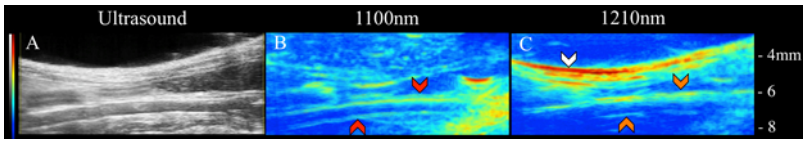

Figure 1: Ultrasound (left), blood VPAT (middle), and lipid VPAT (right) images of ApoE$^{-/-}$. The subcutaneous fat (white arrows), periaortic fat (orange arrows), and blood (red arrows) is clearly visible.

Discussion

VPAT is a rapid, noninvasive, label-free method to image blood and lipid accumulation *in vivo*. By delivering pulsed laser light to tissue, acoustic propagations were induced to obtain relative density and location of biological components. When coupled with ultrasound imaging, compositional, as well as structural and hemodynamic information from tissue can be resolved. Current limitation of this technique is a penetration depth of roughly 3mm for lipid-based imaging. While this is better than current optical techniques, improvements can be made to light delivery techniques to improve depth of penetration. One way to improve this is by developing a photoacoustic transducer that maximizes light delivery to region of interest while redirecting reflected light back into tissue. While VPAT is an imaging technique that is still in its infancy, it has received a great deal of interest in recent years making it likely that this technique will be used in more laboratories and clinics in the near future.

The described protocol can be used for a wide variety of applications in both the preclinical and clinical space. Three potential VPAT applications include utilizing the technique to 1) study lipid-based disease progression, 2) evaluate promising therapeutics, and 3) improve diagnosis of lipid-based diseases. The capability of tracking structural, hemodynamic, and compositional information makes VPAT an appealing technology to study how vascular lipid accumulates in small animals models (**Figure 1**). Moreover, since VPAT is a noninvasive method it can be applied to evaluate the effects of therapeutics in longitudinal studies. This would specifically lower cost of research by decreasing the number of animals needed for therapy validation. Finally, the ability of VPAT to provide compositional information makes it an attractive technique to image different types of plaques in patients that suffer from atherosclerotic-related diseases like carotid and peripheral artery disease. One of the current challenges in cardiovascular medicine is predicting which plaques are rupture-prone, and thus have potential to induce myocardial infarction and ischemic strokes. Therefore, VPAT may also play an important role in characterizing vulnerable versus stable plaques, due to its ability to differentiate biological components. Taken together, VPAT has potential to make a significant impact in both research and clinical practice of medicine.

Materials List

Name	Company	Catalog Number	Comments
VPAT Equipment			
Ultrasound System	VisualSonics	Vevo2100	
Nd:YAG OPO Laser	Ekspla	NT352C	
Sapphire Pulse Generator	Quantum Composers	9200	
Function Waveform Generator	Keysight Technologies	33220A	
BNC Cables	Thor Labs	2249-C-120	Outer diameter 0.2", length of BNC cable depends on user preference.
BNC T Female to Male to Female	Thor Labs	T3285	
BNC Straight Female to Female	Thor Labs	T3283	
Optical Goggles	LaserShields	#37 0914 UV400	Any goggle with OD 7+ will suffice.