



HAL
open science

Development of an enhanced photoacoustic microscopy

Khaled Metwally, Damien Gasteau, Serge Mensah, Anabela da Silva

► **To cite this version:**

Khaled Metwally, Damien Gasteau, Serge Mensah, Anabela da Silva. Development of an enhanced photoacoustic microscopy. European Conferences On Biomedical Optics (ECBO), Jun 2017, Munich, Germany. hal-03515443

HAL Id: hal-03515443

<https://hal.science/hal-03515443>

Submitted on 6 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Development of an enhanced photoacoustic microscopy

Khaled Metwally¹, Damien Gasteau², Serge Mensah¹, Anabela Da Silva²

¹ Aix Marseille Univ, CNRS, Centrale Marseille, LMA, Marseille, France

² Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, Marseille, France

anabela.dasilva@fresnel.fr

Abstract: In this work, the development of a photoacoustic microscope is carried out, the resolution of which can be selected between either optical resolution (OR-PAM) or acoustic resolution (AR-PAM) according to the scanning technique. An efficient optoacoustic combiner material is proposed.

OCIS codes: 170.5120,170.3880.

1. Introduction

Photoacoustic imaging gains a lot of attention due to its synergistic combination of high optical contrast with the high observation depth of acoustic probing [1–3]. Photoacoustic (PA) effect mechanism is basically explained as follows: when light energy is absorbed by tissue; the temperature rises locally and a pressure wave is generated due to the thermoelastic effect.

From instrumentation point of view, excitation and reception could be separately developed as in the case of PA tomography. However, they should share the same pathway in PA microscopy. In this aim, optoacoustic combiners are used to integrate both optical and acoustic pathways in order to be shared by both excitation and detection [4]. Yao summarizes the types of optoacoustic combiners into three types: i) coaxially aligned, ii) redirected beam for both optical or acoustic pathways by using either an optically transparent but acoustically reflective layer or acoustically transparent but optically reflective layer and iii) finally, reflective dark-field optical objective. The pathways of the optical illumination and the reflected acoustic wave are usually combined through a glass combiner (prism), with eventually a coating or a material highly reflecting the acoustic wave. The final resolution depends on the scanning approach. First, a mechanical scanning is applied to the acoustic detector (most of the time, focused transducer), and the resolution is guided by the size of the focal volume, this microscopic protocol is known as acoustic resolution microscopy (AR-PAM). The other mode is known as optical resolution microscopy (OR-PAM), where the laser spot is scanning a region contained in the acoustic focal volume.

In the present work, these two modes are combined in order to have access to one or the other, and an optimal optoacoustic combiner material, called OptiCellTM (Biocrystal, Ohio, USA), is proposed.

2. PA microscope instrumental development

A schematized description of the system is presented on figure 1. At first, the mechanical scanning mode scans line by line a two dimensional pattern; i.e. acoustic image resolution is a function of the acoustic spot size. The second mode scans optically the zone inside the acoustic spot leading to OR images as shown in figure 1.

Two motorized linear stages (M-403.8PD and M-403.4PD, pitch step size 100 μm from Physik Instruments) were fixed perpendicular to each other in order to control the movement in x and z-axis respectively. Motors were directly connected to their controllers (Mercury C863 DC motor controller, PI) which allow networking between multi-axis and controlled from one computer via a single USB interface. The y-axis was separately scanned using a 25 mm motorized translation stage (PT1/M-Z8, Thorlabs) which is controlled via K-cube DC servo motor controller.

Laser fibered solution was derived from the optical table after several manipulation of the beam, which provide a pulsed laser of 3 ns pulse duration, 532 nm wavelength and 10 Hz repetition rate. Dual-axis scanning galvanometric system with metallic coated mirrors was used to control the laser beam onto the defined locations via turning scanning mirrors. The mirrors' motors were controlled via electronic circuits connected to National instruments multifunction data acquisition system (analog input/outputs, digital I/O).

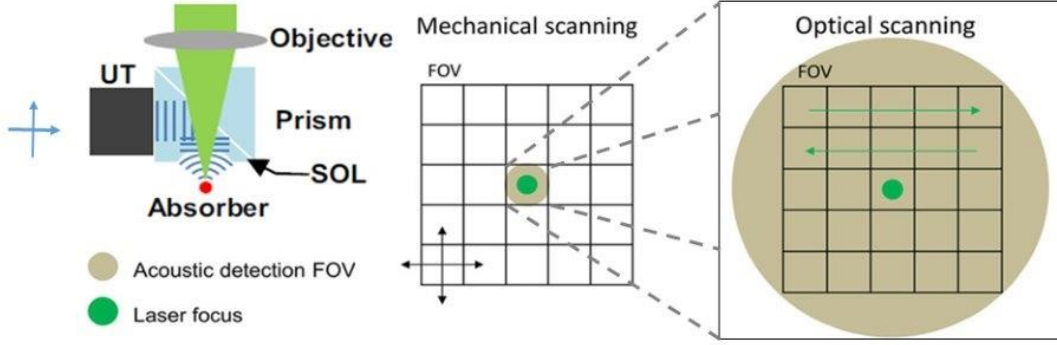


Fig. 1. Photoacoustic microscope: Left, Sketch of the PAM head scanner [4]; Right, Scanning modes for both resolutions AR-PAM and OR-PAM [3]

The acoustic pathway starts from the generation of PA signal in tissue passing through the coupling gel and media towards the acoustic reflector inside the optoacoustic combiner which redirect the signal towards the ultrasound transducer. The collected signal is then preamplified (preamplifier Olympus, France, gain 40 dB, cutting frequency 10 MHz) and driven into an oscilloscope (Tektronix, USA, 4 channels, bandpass width 200 MHz) for signal analyses. Finally, a Labview computer interface program was developed to manage and control motors and signal acquisition.

3. Optoacoustic combiner

As previously explained in the introduction the optoacoustic combiner merge the pathways. This combiner is sought-after dual goal: to minimally modify the optical path and to show the highest acoustic reflectance. In this aim an experiment was conducted to determine the reflectivity of different materials of the reflective layer of the optoacoustic combiner as shown in figure 2.

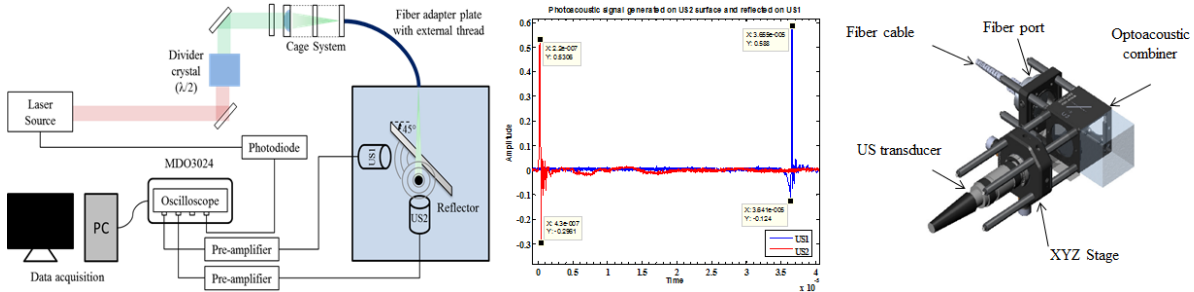


Fig. 2. Optoacoustic combiner: a) Schematic diagram for reflectivity experiment, b) PA signals measured by both ultrasonic transducers, c) Designed PA measuring head with the optoacoustic combiner.

Among tested materials, OptiCellTM [5] is a good optoacoustic combiner candidate. It is a sealed environment between two optically clear gas-permeable growth surfaces in a standard microliter plate-sized frame. Self-sealing ports provide easy aseptic access to the interior and contents using a syringe. The use of optiCell enables us to change its interior filling fluid (air, silicone oil, etc.). The acoustic reflection coefficient has been measured and the results are presented in Table 1.

The reflection coefficient is defined as the ratio between the reflected wave amplitude to the incident wave amplitude. It is given by:

$$R = \frac{Z_1 - Z_2}{Z_1 + Z_2}. \quad (1)$$

Table 1. Material choice for better reflectivity

	Density [kg/m^3]	Sound speed [m/s]	Acoustic impedance [$MRayls = (kg/(s.m^2)) \times 10^6$]	Reflection efficiency with water [%]
Opticell (air filled)	1.2	344	400×10^{-6}	99%
Opticell (Silicone oil filled)	818	960	0.74	30%
Glass	2402.4	3240	7.78	67%

The acoustic impedance Z is given by $Z = \rho c$, where c is the speed of sound within the material. Thus whenever a sound wave encounters a boundary between two different acoustic impedances, an echo is generated. The wave is also partially transmitted, with the amplitude (relative to the incident amplitude) given by $T = 1 - R$. Ultrasound transducer, (Panametrics C388, central frequency of 3.5 MHz, focal distance of 25.4 mm) was used. The signals are acquired using a digital oscilloscope (Tektronik MDO3024) and averaged over 8 measurements to reduce noise.

These measurements show that OptiCellTM, when empty (air filled), has a higher acoustic reflectivity (99%) compared to glass while preserving a high optical transmittance which leads to a higher signal to noise ratio.

4. Conclusion

The development of a photoacoustic microscope is carried out in both simulation and experiments. The primary tests for optoacoustic combiner were done to determine the efficiency of acoustic reflecting layers. OptiCellTM has been selected as an optimal optoacoustic combiner. The improvement in quantifying the inclusion properties will be investigated.

5. Acknowledgment

The authors wish to acknowledge financial support from ITMO Cancer AVIESAN (Alliance Nationale pour les Sciences de la Vie et de la Santé, National Alliance for Life Sciences & Health) within the framework of the Cancer Plan (GRAVITY project).

References

1. E. M. Strohm,, E. S. Berndl, M. C. Kolios. "High frequency label-free photoacoustic microscopy of single cells." *Photoacoustics*, **1(3)**, 49-53 (2013).
2. L Li, C Yeh, S Hu, L Wang, BT Soetikno, R Chen, Q Zhou, KK Shung, KI Maslov, LV. Wang. "Fully motorized optical-resolution photoacoustic microscopy." *Optics letters*,**39(7)**, 2117-2120 (2014).
3. S. Y. Lee, Y. H. Lai, K. C. Huang, Y. H. Cheng, T. F. Tseng, C. K. Sun, In vivo sub-femtoliter resolution photoacoustic microscopy with higher frame rates. *Scientific reports*, **5**, (2015).
4. J. Yao, L. V. Wang, Sensitivity of photoacoustic microscopy. *Photoacoustics*, **2(2)**, 87-101, (2014).
5. T. Tsurumoto, T. Matsumoto, A. Yonekura, H. Shindo, Nanobacteria-like particles in human arthritic synovial fluids. *Journal of proteome research*, **5(5)**, 1276-1278, (2006).