10.1109/ULTSYM.2015.0398

Low power continuous wave photoacoustic microscope for bioimaging applications

K. Sathiyamoorthy and Michael C. Kolios
Department of Physics, Ryerson University
350 Victoria Street, Toronto, Ontario, M5B 2K3 Canada
mkolios@ryerson.ca

Abstract— **A photoacoustic (PA) system based on low power CW laser and microphone was developed. The PA detection was done by a non-contact method using ambient air as an acoustic coupling medium. A resonant chamber coupled to a microphone was used to detect the PA signal. The system uses a He-Ne laser as source of excitation, modulated by an optical chopper. A resolution of 228 lines/mm for light focused by a 40x optical objective was achieved. Methemoglobin, a constituent of interest in blood pattern analysis by forensics, is used as a test sample. High SNR images methemoglobin we produced. The proposed technique is expected to find potential applications in forensics and biomedical analysis.**

Keywords— photoacoustic; microscopy; continuous wave; microphone; methemoglobin

I. INTRODUCTION

The photoacoustic effect was first reported by Alexander Graham Bell in 1880, though the widespread use of this technique for spectroscopic purposes started only almost after a century. From then, there has been a steady progress in the development of PA spectrometers in view of their potential application in biological applications and environmental studies [1]-[6]. A major improvement in the sensitivity of photoacoustic method has been brought about by the availability of laser sources, highly sensitive transducers and other efficient acoustic detection systems [6]-[9]. The invention of ultrafast high power laser has further expanded the utilization of the PA effect to many fields that includes NDT and biomedical imaging [10]-[19]. This hybrid method finds potential applications in biomedical imaging as it provides enhanced resolution due to contributions from both high optical contrast and high scalable ultrasound resolution [16]-[19].

Photoacoustic (PA) spectroscopy is a very powerful analytical tool for examining the optical absorption properties of solids as it measures directly the energy absorbed by the material exposed to light. Conventional optical absorption/transmission spectroscopy requires a homogenous and partially transparent sample and further, it cannot be used with highly scattering samples, opaque materials (diffuse optical reflectance can be used, but it requires special sample surface preparation), powders etc.

PA spectroscopy uses low power light such as incandescent lamp or low power continuous wave (CW) laser as the excitation source and uses less expensive acoustic detection systems like a microphone. The measurement is based on a non-contact technique with ambient air as the coupling medium.

The main object of this study is to employ this photoacoustic spectroscopic principle for photoacoustic microscopy imaging as it can be integrated to existing conventional optical microscope systems in a cost effective manner.

II. MATERIALS AND METHODS

A. Experimental set up

The main component of the PA microscope is the photoacoustic cell. The figure 1 represents the schematic diagram of a PA cell.

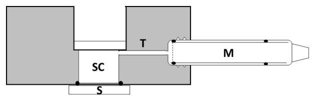

Fig. 1. A Photoacoustic cell where SC is the sample chamber, S is the sample, T is the tunnel and M is the microphone.

The PA cell is made of a perspex material comprising of two chambers: one for the sample (S) and one that leads to the detection microphone (M). Both sample (SC) and microphone (M) chambers are cylindrical in shape. The sample chamber has a radius of 3 mm and height of 3mm whereas the microphone chamber has a radius of 6.35 mm and a height of 10 mm. The microphone is enclosed in the microphone chamber with a gap 1 mm between its diaphragm and the front wall of the microphone chamber. The two chambers are connected by a tunnel (T) of diameter 1 mm and length 14 mm. The microphone is isolated from main sample chamber by the tunnel to prevent from light interference as the direct incidence of light on microphone diaphragm would result in an unwanted additional PA signal. Moreover the tunnel acts as a resonator air column whose resonance frequency depends on the length of the air column. The length of the tunnel can be detuned to support the frequency of the transducer/microphone. A Helium neon laser (633 nm) of beam diameter 2 mm and power of 3 mW is used for the present studies. The principle of PA is when the sample is illuminated with light at its absorption peak, it undergoes optical transition to higher states followed by spontaneous decay to ground state either radiative (eg: fluorescence), non-radiative excitation (heat) or both. The

978-1-4799-8182-3/15/$31.00 ©2015 IEEE

periodic heating through non-radiative de-excitation of sample would result in thermal expansion and contraction of the sample resulting in photoacoustic waves. The sample is periodically heated by means of intensity modulated CW light achieved using a SR540 optical chopper (Stanford Research Systems, Sunnyvale, California). In these experiments, the laser beam is modulated at frequency of 150 Hz. The generated PA signal is measured using a microphone (Brüel & Kjær Sound & Vibration Measurement, Pointe-Claire, Quebec, Canada) and lock-in amplifier (SR850) system. The PA interrogation is done with the help of custom optical microscope. The configuration of the optical system follows the commercially available standard conventional optical microscope system (figure 2). The optical system uses 10 X and 40 X microscope objectives.

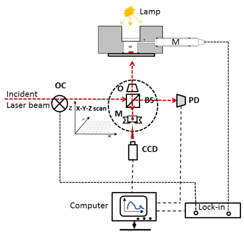

Fig. 2. Optical microscope setup where OC is the chopper, BS is the beam splitter, O is the objective, M is the mirror, PD is the photodiode and CCD is the camera.

The PA raster scan on sample is done using XY mechanical translation stage from Zaber technologies, Vancouver, Canada. The XY stage holds mirror, beam splitter and objective of the optical microscope. The light from the He-Ne source is directed to the 50:50 % beam splitter (BS). The beam is split into two beams, one as a reference beam and the other beam for the optical excitation of the sample. The light which is passed through the beam splitter is used as the reference beam whereas the light which is reflected by the splitter is passed through the objective (O) for optical excitation of the sample. The incandescent lamp is used for wide field optical imaging which is positioned on the top of the PA chamber. The light which illuminates the sample would passes through objective, beam splitter and finally mirror before it is captured by the CCD camera. The reference beam which is monitored by the photodiode (PD) is used to normalize the recorded PA spectrum to avoid photoacoustic signal variation due to light intensity fluctuation.

B. *USAF1951 resolution test target*

A USAF1951 test target from Thorlabs, Newton, New Jersey is used to identify the resolution of the proposed system. The target contains sets of 3 chrome lines patterned of resolution starting from 4 lines/mm to 228 lines/mm on soda lime glass. It's a positive chrome pattern obtained by photolithography.

C. *Blood*

Methemoglobin obtained from Sigma Aldrich is used for the studies. The hemoglobin sample is prepared in liquid form by dissolving 0.05 gm of methemoglobin in 10 ml PBS.

III. Results and Discussion

The developed system was calibrated using USAF 1951 chart from Thorlabs. The figure 3 represents the PA image of group 3 element 2 of USAF 1951 chart.

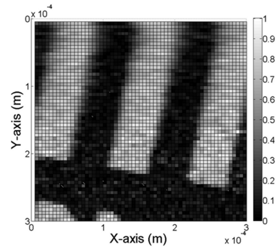

Fig. 3. PA image of group-3 element 2 of USAF 1951 chart.

The system exhibited a lateral resolution of 228 lp/mm as it can able to resolve group 7 element 6 of USAF chart. The performance of the PA system was investigated by imaging methemoglobin aggregates on glass substrate. Methemoglobin has unique visible spectra which has its characteristic optical absorption peak around 630 nm. The figure 4 represents the PA image of methemoglobin aggregate.

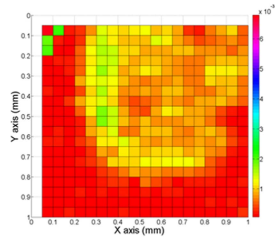

Fig. 4. PA image of methemoglobin aggregates.

Bloodstain pattern analysis (BPA) is one of several techniques used in the field of forensic science [20]-[22]. Blood stains contain increased levels of methemoglobin. The spectrophotometer is generally used for BPA analysis, but this device has limitations as it cannot be used for non-homogeneous and opaque sample. The developed PA microscope can find potential application in the forensic and biomedical sciences.

IV. Conclusion

We have developed a PA microscopy imaging system based on low power CW laser and microphone. The measurement is a non-contact method which uses ambient air as acoustic coupling. The system exhibits the resolution of about 228 lines/mm. The application of system was examined by studying the methemoglobin aggregate pattern on glass substrate. Methemoglobin is the important constituent analyzed in blood stains of deceased person by forensic department. The developed PA microscope can find potential applications in the forensic and biomedical sciences. The developed system is cost effective as it utilizes a less expensive low power laser source and microphone, and hence it can find wide clinical applications. Since the system is very compact as there is no usage of bulky ultrafast laser system and expensive sophisticated transducer as in most commercial PA systems, the proposed system can be readily integrated with commercial available optical microscopes, enabling multimodal imaging.

ACKNOWLEDGMENT

This work was funded by Natural Sciences and Engineering Research Council of Canada (216986-2012) and Ryerson University. Funding to purchase the equipment was provided by the Canada Foundation for Innovation, the Ontario Ministry of Research and Innovation, and Ryerson University.

REFERENCES

[1] Allan Rosencwaig, Photoacoustics and photoacoustic spectroscopy, 1st edn., vol. 57. John Wiley & Sons Inc, 1980, 309 pages.

[2] Allan Rosencwaig, "Photoacoustic spectroscopy," Ann. Rev. Biophys. Bioeng., vol. 9, pp. 31-54, June 1980.

[3] Allan Rosencwaig, "Photoacoustic spectroscopy of solids," Optics Communications, vol. 7, no. 4, pp. 305-308, Apr. 1973.

[4] Jan Paul Favier, Jos Buijs, Andras Miklos, Andras Lorincz, and Dane Bicanic, "Photoacoustic characterization of different food samples," Z Lebensm Unters Forsch, vol. 199, pp. 59-63, Dec. 1994.

[5] Richard Martel, Christophe N. N'Soukpoe-Kossi, Paul Paquin, and Roger M. Leblanc, "Photoacoustic Analysis of Some Milk Products in Ultraviolet and Visible Light," Journal of Dairy Science, vol. 70, no. 9, pp. 1822-1827, Sept. 1987.

[6] L.B. Kreuzer, N.D. Kenyon, and C.K. Patel, "Air Pollution: Sensitive Detection of Ten Pollutant Gases by Carbon Monoxide and Carbon Dioxide Lasers," Science, vol. 177, no. 4046, pp. 347-349, July 1972.

[7] Edwin L. Kerr and John G. Atwood, "The laser illuminated absorptivity spectrophone: a method for measurement of weak absorptivity in gases at laser wavelengths," Applied Optics, vol. 7, no. 5, pp. 915-922, May 1968.

[8] L.B. Kreuzer, "Ultralow Gas Concentration Infrared Absorption Spectroscopy," J.Appl.Phys., vol. 42, no. 7, pp. 2934-2943, Dec. 1971.

[9] Frank Muller, Alexander Popp, Frank Kuhnemann, and Stephan Schiller, "Transportable, highly sensitive photoacoustic spectrometer based on a continuous-wave dual cavity optical parametric oscillator," Optics Express, Vol. 11, No. 22, 2820-2825, (2003).

[10] Pavel A. Fomitchov, Alexei K. Kromine, and Sridhar Krishnaswamy, Photoacoustic probes for nondestructive testing and biomedical applications, Applied Optics 41(22), 4451-4459, Aug. 2002.

[11] Ivan Pelivanov, Takashi Bumac, Jinjun Xia, Chen-Wei Wei, and Matthew O'Donnell, "NDT of fiber-reinforced composites with a new fiber-optic pump-probe laser ultrasound system," Photoacoustics vol. 2, no. 2, pp. 63-74, June 2014.

[12] Yuhua Cheng, Yiming Deng, Jing Cao, Xin Xiong, Libing Bai, and Zhaojun Li, "Multi-Wave and Hybrid Imaging Techniques: A New Direction for Nondestructive Testing and Structural Health Monitoring," Sensors, vol. 13, no. 22, pp. 16146-16190, Nov. 2013.

[13] C. B. Hirschmann, J. Lehtinen, J. Uotila, S. Ojala, and R. L. Keiski, "Sub-ppb detection of formaldehyde with cantilever enhanced photoacoustic spectroscopy using quantum cascade laser source," Appl. Phys. B, vol. 111, pp. 603-610, Mar. 2013.

[14] J.A. Johnson, K.J. Manke, D.G. Veyssett, A.A. Maznev, K.J. Ramos, D.E. Hooks, and K.A. Nelson, "Photoacoustic determination of the speed of sound in single crystal cyclotrimethylene trinitramine (RDX) at acoustic frequencies from 0.5 to 15 GHz," J. Appl. Phys., vol. 110, 113513, Dec 2011.

[15] T. Pezeril, F. Leon, D. Chateigner, S. Kooi, and K.A. Nelson, "Picosecond photoexcitation of acoustic waves in locally canted gold films," Appl. Phys. Lett., vol. 92, 061908, Feb. 2008.

[16] Amos Danielli, Konstantin Maslov, Alejandro Garcia-Uribe, Amy M. Winkler, Chiye Li, Lidai Wang, Yun Chen, Gerald W. Dorn II, and Lihong V. Wang, "Label-free photoacoustic nanoscopy," Journal of Biomedical Optics, vol. 19, no. 8, 086006, Aug. 2014.

[17] Minghua Xu, and Lihong V. Wang, "Photoacoustic imaging in biomedicine," Review of Scientific Instruments, vol. 77, 041101, Apr. 2006.

[18] Ben Cox, Jan G. Laufer, Simon R. Arridge, and Paul C. Beard, "Quantitative spectroscopic photoacoustic imaging: a review," J Biomed Opt., vol. 17, no. 6, 061202, Jun. 2012.

[19] Paul Beard, "Biomedical photoacoustic imaging," Interface Focus, vol. 1, pp. 602-631, June 2011.

[20] Minori Nishiguchi, Hideyuki Nushida, Noriyuki Okudaira, and Hajime Nishio, "An Autopsy Case of Fatal Methemoglobinemia due to Ingestion of SodiumNitrite," J Forensic Res., vol. 6, no. 1, 262, Dec. 2015.

[21] A. Cruz-Landeira, M. J. Bal, O. Quintela, and M. López-Rivadulla, "Determination of methemoglobin and total hemoglobin in toxicological studies by derivative spectrophotometry," J Anal Toxicol., vol. 26, no. 2, pp. 67-72, Mar. 2002.

[22] N. Watanabe, K. Terazawa, and M. Sakaihara, "Blood storage for forensic hemoglobin analysis using CO-oximeter," Hokkaido Igaku Zasshi., vol. 78, no. 4, pp. 289-295, July 2003.