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Using a single supercontinuum source for visible multispectral photoacoustic microscopy and 1300 nm optical coherence tomography

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Abstract: We present a bimodal system driven by a supercontinuum source to perform photoacoustic-based spectral selective absorption measurements from 500 nm to 800 nm and structural optical coherence tomography imaging at 1300 nm. An energy of 5 to 40 nJ is achieved on sample within a 50 nm bandwidth in the visible range in the photoacoustic channel. Also, a few mW power is also achieved on the sample in the optical coherence tomography channel.

OCIS codes: (110.4500) Optical coherence tomography; (110.4234) Multispectral and hyperspectral imaging; (110.5125) Photoacoustics; (190.4360) Nonlinear optics, devices; (360.6080) Sources

1. Introduction

Supercontinuum sources have recently shown a great potential in multispectral photoacoustic microscopy (MPAM) since it is the only source that allows a pulse repetition rate of tens of kHz with a broad spectrum typically from 500 nm to 2300 nm. This source can be applied to e.g. blood imaging and oxygen saturation measurements in the visible spectrum [1] as well as lipids imaging in the IR. It offers users the flexibility to be adapted to a large range of applications. However only a few supercontinuum sources deliver enough energy to allow *in-vivo* multispectral photoacoustic microscopy in the visible (500-600 nm), useful for oxygen saturation measurements.

Supercontinuum sources have already shown great promise in spectrometer-based optical coherence tomography (OCT) due to their broad spectrum of several hundreds of nm and tens of mW of average power with a central wavelength that can be chosen from the visible to the IR [2-4]. Thus an axial resolution better than 10 um is achieved and only limited by the spectrometer bandwidth.

Photoacoustic imaging and OCT are the fastest growing fields in biomedical imaging. PAM and OCT can be combined in a single set-up, however the majority of prior reports refer to each modality using its own light source [5,6]. Combining these two modalities is of a great interest since they give complementary information from a sample, i.e. absorption via PAM and scattering via OCT. In a previous report, we presented *in-vivo* images of a mouse ear with a single supercontinuum source from NKT Photonics A/S using 500-800 nm for PAM and 800-900 nm for OCT [1].

We present here a continuation of this work with a bimodal imaging system that combined MPAM and OCT using a similar supercontinuum source. The visible band (500-840 nm) is used for MPAM on three different absorbers and the IR band (1200-1400 nm) for OCT.

2. Experimental setup

A commercial supercontinuum source (Compact, NKT Photonics A/S) is used in combination with a dual band filter (VARIA, NKT Photonics A/S). A dichroic mirror allows the total bandwidth of the light source that extends from 500 nm to 2300 nm to be split into a visible band 500-840 nm and an IR band 840-2300 nm. The visible band is further filtered to allow tunability of the central wavelength and the bandwidth that can be narrowed down to 10 nm wide, Fig. 1 (a) represent the transmission of the VARIA [7]. In our configuration a longpass filter with a cut-off wavelength of 1150 nm (Thorlabs, FEL1150) is used to provide a spectrum centred at 1300 nm for OCT, Fig. 1 (b) is the spectrum on the line scan camera. The two bands which can be used separately are fibre-delivered to facilitate the implementation of the combined imaging system.

Fig. 1 (c) shows the schematic of the bimodal OCT-MPAM system. Both laser beams are combined together by a dichroic mirror, then reflected by two galvo-scanners that allow raster scanning of the sample in the lateral directions. A scanning objective lens (Thorlabs, LSM03) focuses the two beams on the sample. The sample is placed on a 3-

dimensional stage to facilitate its alignment. A customized unfocused needle ultrasonic transducer (University of Southern California, 30 MHz centre frequency, 60% bandwidth, 0.4 mm active element) is used to detect the photoacoustic signal coupled through water. Silver reflective collimators (Thorlabs, RC04APC-01 in the sample arms and RC08APC-01 in the reference arm) are used to ensure achromaticity. A 50/50 fibre coupler is used in the OCT channel (Thorlabs, TW1300R5A2). A homemade IR spectrometer that consists of a diffraction transmission grating (Wasatch Photonics, HP 1145 l/mm @ 1310 nm) and a line scan camera (Goodrich, SU1024-LDH1.7RT-0500/LC) is used. A polarisation controller is inserted in the reference arm only.

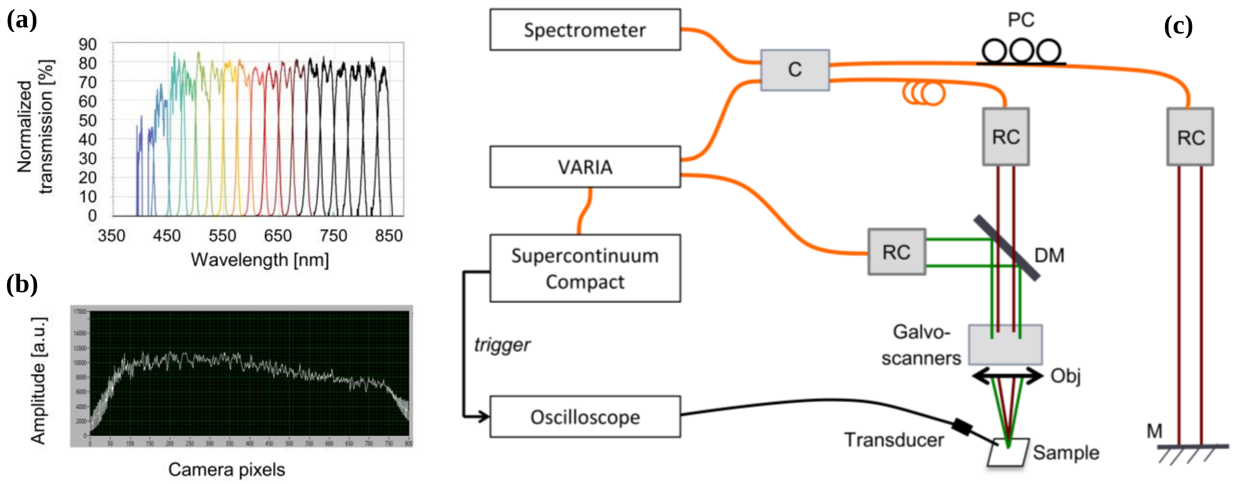

Fig. 1. (a) Visible spectrum offered by the VARIA, (b) IR spectrum on the spectrometer, and (c) Schematic of the OCT-MPAM system. C: 50/50 coupler, DM: dichroic mirror, M: mirror (OCT reference arm), Obj: objective lens, PC: polarisation controller, RC: reflective collimators.

3. Optical coherence tomography at 1300 nm.

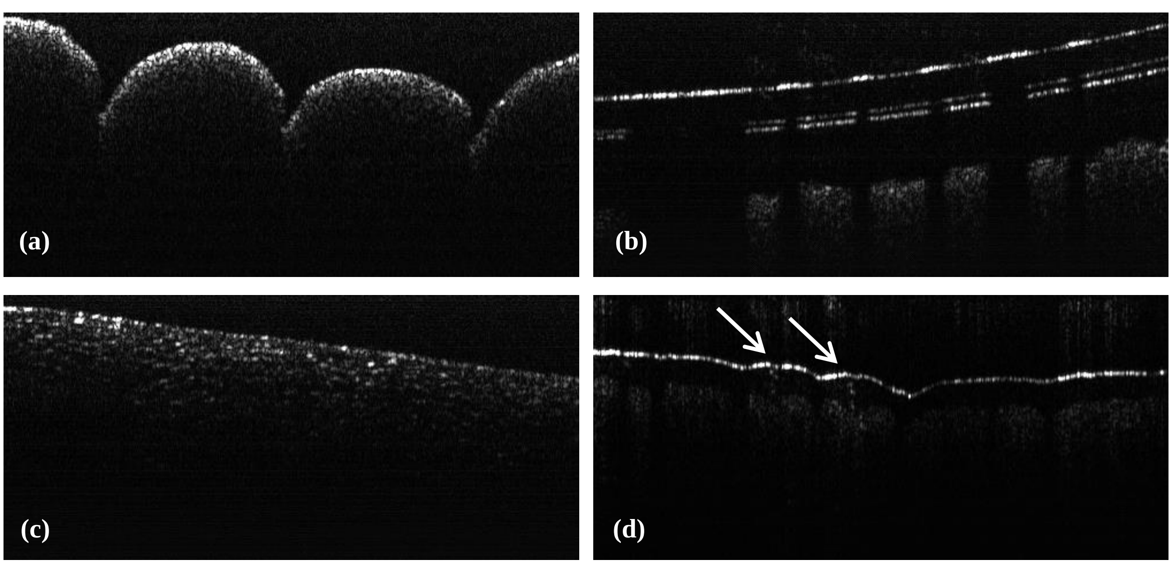

Fig. 2. OCT B-scans of (a) blu tack, (b) IR card with a black grid printed on a plastic film on the top, (c) cucumber, and (d) human finger skin with sweat glands indicated by the arrows.

The bandwidth is ~ 110 nm, giving a theoretical axial resolution in air of 6.8 um. A lateral resolution of ~ 8.7 um is achieved. The total power on sample is 1.6 mW, the integration time of the camera is set to 1.77 µs (35-36 pulses per A-scan). Fig. 2 shows B-scans of 4 samples, the lateral scanned area is of ~ 3.8 mm. The axial range of the B-scans shown in Fig. 2 is of 1.4 mm measured in air. The penetration depth is limited by the power on sample due to the low galvo-scanners transmission (40%), in the future this will be improved and a careful optimization of the spectrometer will allow better quality OCT. The OCT acquisition is running via a Labview program based on the complex master slave method developed by the University of Kent [8].

4. Multispectral photoacoustic microscopy in the visible

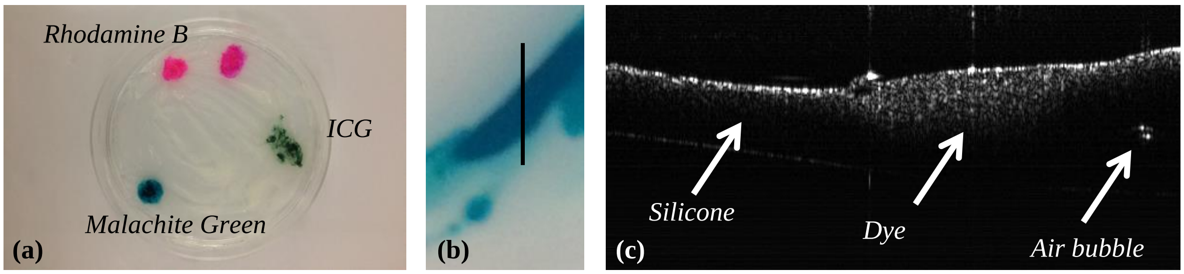

Fig. 3. (a) Rhodamine B, Malachite Green, and Indocyanine Green (ICG) silicon samples, (b) region scanned for the OCT, and (c) the corresponding OCT B-scan.

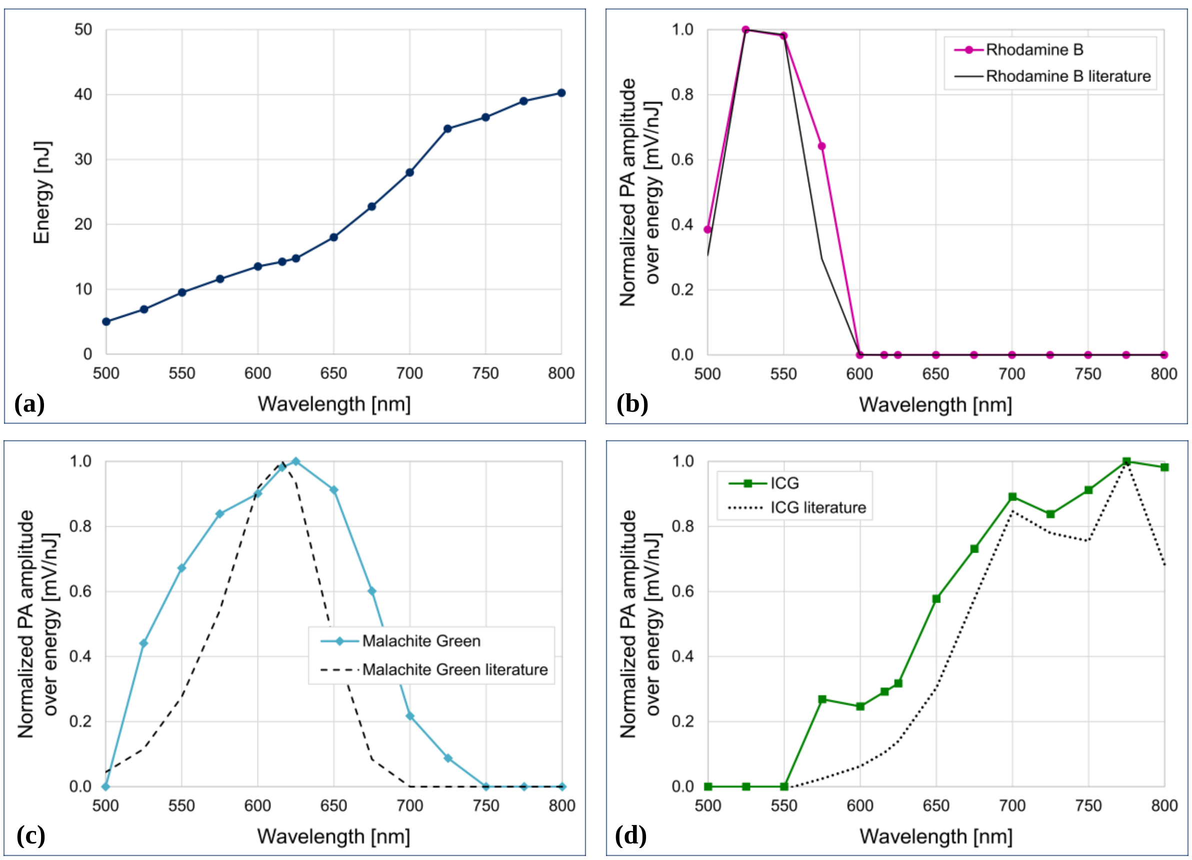

Fig. 4 (a) Energy measured on sample for the 3 different bandwidths, (b) Rhodamine B MPAM, (c) Malachite Green MPAM, and (d) ICG MPAM.

In order to demonstrate the principle of multispectral photoacoustic microscopy we performed photoacoustic measurements in steps of 25 nm with a bandwidth (BW) of 50 nm from 500 nm to 800 nm. Three different absorbers have been used, all mixed with silicone to create the phantoms: Rhodamine B (Flinn Scientific, absorption peak at 542.8 nm), Malachite Green (Sigma-Aldrich, absorption peak at 616 nm), and Indocyanine Green (ICG) (Akorn, absorption peak at ~ 695 and/or 780 nm depending on the concentration). Fig. 3 (a) shows the samples prepared for the multispectral measurements, (b) is the sample (Malachite Green) used for the OCT image in (c). Fig. 4 (a) is the energy measured on the sample for each wavelength. We performed multispectral photoacoustic microscopy for each of the absorbers, the results are presented in respectively Fig. 4 (b), (c) and (d) at maximum power. The photoacoustic signal amplitude is normalized by the energy on sample and the maximum absorption. We assumed that the response of the ultrasonic transducer is linear with power (preliminary work shows that this is valid for energies of more than 1 nJ). The black curves correspond to the literature absorption spectra that have been integrated over a bandwidth of 50 nm for Rhodamine B and 25 nm for Malachite Green and ICG [9].

The absorption peaks measured in the photoacoustic channel are generally broader than in the literature; we believe that this is due to the fact that the absorbers are mixed with silicone, which may have influenced the absorption differently than when diluted with water. Further measurements of the absorption by transmission will be able to confirm or not this hypothesis. These measurements are a proof of principle for multispectral photoacoustic operation using a supercontinuum source over the 500 nm to 800 nm range.

5. Conclusion

A dual-modal imaging system is demonstrated that combines structural information via optical coherence tomography at 1300 nm with spectral absorption of the sample via photoacoustics measurements. One can distinguish between different absorbers by using the spectral selection capabilities of the multispectral photoacoustic channel, enabled by the broadband excitation of the supercontinuum extending from 500 nm to 800 nm.

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7. References

[1] Shu, X., Bondu, M., Dong, D., Podoleanu, A., Leick, L. and Zhang, H. F., "Single all-fiber-based nanosecond-pulsed supercontinuum source for multispectral photoacoustic microscopy and optical coherence tomography," Opt. Lett. 41(12), 2743-2746 (2016).

[2] Bizheva, K., Tan, B., MacLelan, B., Kralj, O., Hajialamdari, M., Hileeto, D. and Sorbara, L., "Sub-micrometer axial resolution OCT for in-vivo imaging of the cellular structure of healthy and keratoconic human corneas", Biomed. Opt. Express, 8(2), 800-812 (2017).

[3] Lichtenegger, A., Harper, D. J., Augustin, M., Eugui, P., Fialová, S., Woehrer, A. Hitzenberger, C. K., and Baumann, B., "Visible light spectral domain optical coherence microscopy system for ex vivo brain imaging," in Optics in the Life Sciences Congress , OSA Technical Digest (online) (Optical Society of America, 2017), paper JTu4A.16.

[4] Yi, J., Wei, Q., Liu, W., Backman, V. and Zhang, H. F., "Visible-light optical coherence tomography for retinal oximetry", Opt. Lett. 38(11), 1796-1798 (2013).

[5] Song, W., Wei, Q., Liu, W., Liu, T., Yi, J., Sheibani, N., Fawzi, A. A., Linsenmeier, R. A., Jiao, S. and Zhang, H. F.,"A combined method to quantify the retinal metabolic rate of oxygen using photoacoustic ophthalmoscopy and optical coherence tomography", Sci. Rep. 4, article number: 6525 (2014).

[6] Zhang, E. Z., Povazay, B., Laufer, J., Alex, A., Hofer, B., Pedley, B., Glittenberg, C., Treeby, B., Cox, B., Beard, P. and Drexler, W., "Multimodal photoacoustic and optical coherence tomography scanner using an all optical detection scheme for 3D morphological skin imaging", Biomed. Opt. Express 2(8), 2202-2215 (2011).

[7] http://www.nktphotonics.com/lasers-fibers/product/superk-varia-tunable-single-line-filter/

[8] Bradu, A., Rivet, S. and Podoleanu, A., "Master/slave interferometry – ideal tool for coherence revival swept source optical coherence tomography", Biomed. Opt. Express 7(7), 2453-2468 (2016).

[9] http://omlc.org/spectra/