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Hybrid Optical-Ultrasonic Technique for Biomedical Diagnostics

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Abstract

We report the development of a diagnostic system combining time-resolved fluorescence spectroscopy and ultrasound backscatter microscopy and its application in diagnosis of tumors and atherosclerotic disease. This system allows for concurrent evaluation of distinct compositional, functional, and micro-anatomical features of normal and diseased tissues.

I. Introduction

Advances in disease diagnosis and disease response to therapy depend on the availability of tools to evaluate molecular and morphological changes in either animal models of human diseases or in clinics. Optical spectroscopy and imaging [1–4] and ultrasound [5–7] are actively investigated as research and diagnostic techniques for both biomedical research and clinical applications. Each of these techniques alone, however, cannot provide a comprehensive inside into the complexity of changes or processes which typically occur concurrently in biological systems. This includes morphological, biochemical, metabolic or physiological transformations which take place, for example, during progression of tumor or remodeling of an atherosclerotic plaque. Thus, multi-modal approaches that enable the simultaneous investigation of such changes *in vivo* are of importance. Yet, ultrasonic evaluation can compensate for the limited penetration depth of optical techniques. In this context, our objective is to research the development of tissue diagnostic systems that combines two complementary techniques fluorescence spectroscopy (in particular time-resolved or lifetime techniques) and ultrasonic backscatter microscopy (UBM) thus biochemical and morphological changes in tissues can be investigated simultaneously.

Fluorescence spectroscopy and imaging can non-invasively assess the biochemical changes associated with the development/progression of critical diseases such as cancer, and atherosclerosis. Both wide-field fluorescence imaging and steady-state point spectroscopy have been extensively investigated as methods for disease tissue characterization [1–4,8–10]. While these methods have shown promise to provide contrast between normal and pathologic conditions in tissue, some studies suggest that they may have limited specificity [10] – as such methods primarily rely on absolute fluorescence intensity measurements obtained from a relatively large tissue volume. The specificity of fluorescence measurement can be improved through either high-resolution spectroscopy/imaging [11] or time-resolved (lifetime) fluorescence [3,4,10] measurements.

Ultrasound backscatter microscopy (UBM) allows for non-invasive measurements of internal structure of living systems on a microscopic scale. UBM is based on the availability

of high frequency ultrasound transducers (20 to 55 MHz up to 100 MHz) that has resulted in the extension of pulse-echo (backscatter) methods, originally used in medical imaging, into the domain of microscopy [7,12]. UBM has been widely applied for the imaging of small animals, particularly in the longitudinal studies of pathogenesis or embryonic development of mice [5,12]. UBM Doppler ultrasound, used in the measurement of blood flow rates, can provide additional hemodynamical information as well from targeted organs. [6].

We report here the development of a hybrid diagnostic system combining time-resolved fluorescence spectroscopy (TRFS) and UBM. The latter technique provides structure information of tissue and enables localization of the region of diagnostic interest, while the former evaluates the chemical composition of tissue within this region. To validate the concept of the dual-modal system we integrated into a compact experimental apparatus a TRFS device developed by our research group [13] with an ultrasonic system [14] and test this hybrid apparatus in human atherosclerotic plaques and on an hamster oral carcinoma model.

II. Experimental design

The conceptual schematic diagram of the hybrid prototype is given in Fig. 1. The combined system consisted of four primary modules including the TRFS and UBM subsystem, the hybrid probe and the control unit.

Briefly, the TRFS component is based on a modular design consisting of: a) a nitrogen laser as an excitation source (337.1 nm, 700 ns pulse width, 100 mJ and 50 HZ maximum output and repetition rate respectively) equipped with a dye module (400–900 nm). b) A f/4 dual mode imaging spectrograph (f/3.8, 600 g/mm grating), c) a digital oscilloscope (2.5 GHz, sampling rate: 40 Gsamples/s), a computer workstation, and peripheral electronics. The emitted fluorescence is captured and directed, via the collection channel of the probe, into the entrance slit of the spectrometer and detected by a multichannel plate photo-multiplier tube (MCP-PMT: rise time 180 ps, spectral response 160–850 nm, BW: CW to 2.0 GHz). The temporal resolution of this system is ~200 ps. The scanning monochromator allows for TR-LIFS measurements at discrete steps across the emission spectrum - an important feature when the TR-LIFS is used to characterize a new biological system (e.g. tissue type) and to determine which spectral range(s) can be used to provide useful contrast.

The UBM system consist of a high voltage pulser generating one cycle of a rectangular wave (200 V peak-to-peak) with an adjustable period was used to drive the transducer. The receiving circuit was constructed with a commercial expander and limiter to protect receiving circuitry from high voltages required for transmission when using the same transducer for transmitting and receiving. The received RF echoes were amplified with a 30 dB low noise amplifiers and filtered with proper bandpass filters. A 12-bit AD converter with the sampling rate as high as 400M samples/s was employed, which maintained the SNR of the receiving RF signals and provided good resolution and dynamic range of 60 dB. A custom-built positioning assembly incorporating a miniature precision linear motorized 1 μ m resolution stage allowed for scanning and image formation. After digitization, signal processing algorithms were applied to detect the echo envelope and logarithmically compress the RF data and display the B-mode images. Various high frequency transducers with different specifications (40 MHz to 73 MHz) could be used in this system. The needle transducer (45 MHz, 0.4 mm aperture size, Resource Center for Medical Ultrasonic Transducer Technology, USC, Los Angeles, CA) used had a -6 dB two-way fractional bandwidth of 50%. The axial and lateral resolution were estimated from the image acquired by scanning across a 13 μ m tungsten wire (sub-resolution point scatterer), which was 38 μ m and 200 μ m, respectively.

A compact combined probe (Fig. 2) was constructed by assembling either parallel or concentrically the high frequency ultrasonic transducer and the optical fiber. The flexible design of the hybrid probe demonstrated that an ample range of transducers (with different frequencies, aperture sizes, focusing, and materials) and optical fibers (varied core sizes) could be chosen for specific purposes.

A bench top computer, acting as the control unit, was used to integrate the interfaces of the TR-LIFS and UBM subcomponents. Instrument control and user interface software in LabVIEW integrating both the UBM and TR-LIFS were developed for system parameter adjustments and various data acquisition tasks. The control procedure for the dual-modality system was accomplished through the software by scanning the sample with the ultrasound transducer to form and display a B-mode image, locating the region of interest, marking this point digitally on the UBM images for subsequent correlation between the optical and the ultrasound data, and then guiding the optical fiber to the proper location to perform TR-LIFS measurements. Similar procedures were repeated until all the regions of interest were examined by TR-LIFS along this ultrasound scanning line.

III. Hybrid trfs-ubm applications

A. Atherosclerotic Cardiovascular Disease

Current techniques used for diagnosing atherosclerotic plaques including angiography, intravascular ultrasound (IVUS), optical coherent tomography (OCT), thermography, intravascular magnetic resonance imaging (MRI), and optical spectroscopy, have their intrinsic advantages and are sensitive to either plaque structure or composition. However, none of them alone can provide complete information on various markers involved in plaque vulnerability and rupture which may encompass both structure and composition (e.g. a thin fibrous cap, a large lipid-rich pool, increased macrophage activity, and outward remodeling) [15]. New diagnostic techniques to localize, to identify anatomic features and to characterize the composition of high-risk plaques are needed in order to reduce incidence of acute cardiovascular syndromes and sudden death. The hybrid system described here can provide such an opportunity.

For example, optical fluorescence spectroscopy techniques detect elastin, collagen, lipids and other sources of label-free composition that allow for differentiation between normal and diseased arterial walls [3]. Yet, early studies have shown that fluorescence techniques including time-resolved techniques enable detection of plaques with a thin fibrous cap and discrimination of lipid-rich lesions, and allow for detection of fibrotic caps with macrophage and inflammatory cell infiltration [16]. Poor penetration depth, however, which is on the order of hundreds of microns, limits the application of optical fluorescence spectroscopy. Ultrasonic imaging can overcome such limitation. It can provide important structural information and improve the overall diagnostic ability of the optical system. Fig. 3 demonstrates the application of the hybrid fluorescence and ultrasonic system to the characterization of a human aortic plaque.

The preliminary data demonstrated a number of noteworthy findings. Notably, a) *ex-vivo*, spatially-correlated ultrasonic and TR-LIFS studies were feasible with the available hybrid probes, b) the close similarity between the tissue morphology shown by ultrasound and the corresponding histopathology demonstrated that ultrasound could be used as a guidance tool to indicate possible unstable plaque areas for subsequent fluorescence studies, and c) information (such as a calcified region) specifically detected by ultrasound demonstrated that ultrasound could provide complementary diagnostic information to TRLS analysis. Ultrasound imaging may serve as both guiding tool for the use of TR-LIFS and complementary tool for diagnosis of features of plaque vulnerability.

A. Tumor diagnosis

The progression of tumors is associated with multiple transformations in tissues including chemical, metabolic, physiological and morphological. The hybrid technique described here has the potential to impact the tumor diagnostic as well as the monitoring of tumor response to therapies. To demonstrate the applicability of this technique to this field we conducted *in-vivo* studies in a well characterized hamster oral carcinoma model [17]. TRFS was used to resolve molecular composition. In this carcinoma model the fluorescence originates from distinct fluorophores (NADH, collagen/crosslinks and porphyrins) which have distinct emission spectra and lifetimes. The UMB was used to delineate the tumor volume and to resolve specific morphological features within the tumor volume. Fig. 4 depicts a representative evaluation of this tumor model using the hybrid system.

Overall, current results have demonstrated that biochemical composition of oral cancerous lesions including NADH, collagen and porphyrins can be derived from the TRFS data, and that these data can be further complemented and by the corresponding ultrasonic images.

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Fig. 1. Schematic diagram of the combined TRFS/UBM system. The combined system consisted of four modules (from left to right), which were the TR-LIFS subsystem, the computer (control unit), the hybrid ultrasonic and spectroscopic probe, and the UBM subsystem.

Fig. 2.

Schematic diagram of the hybrid TRLIFS/UBM probes encapsulating an ultrasound transducer and a single optical fiber with (a) the parallel and (b) concentric assembly. (a) For the parallel hybrid probe, a 1 mm diameter transducer (60 MHz, unfocused, piezoelectric material of Lithium Niobate single crystal) and 600 μm (NA 0.22) optical fiber were assembled in a 3.5 mm shaft tube. (b) The concentric design of the hybrid probe used a 3.75 mm ring transducer (40 MHz, focused, 1 mm hole, 6 mm focal depth, BW 63%, Lithium Niobate) with a single optical fiber placed in the center.

Fig. 3.

Dual-modal measurements of the ex-vivo human aorta atherosclerotic plaque samples showing the complimentary information provided by TR-LIFS and UBM. (a) Trichrome/ elastin stained section showed the 650 μm fibrous cap (L) and the normal intima layer with the thickness of 80 μm (R). (b) UBM image demonstrated the fibrous cap (L) adjacent to the muscular media, compared to the thin layer of normal intima (R). (c, d) Fluorescence measurements were taken at normal and plaque position marked on the histology and ultrasound image (arrows). Fluorescence at normal position (circle) showed broad average emission spectrum (c) and relatively short-lasting decay as reflected by the average lifetime (d). Fluorescence at plaque position (triangle) showed narrower spectra (c), relatively long-lasting emission as reflected by the lifetime at blueshifted emission wavelength and their wavelength dependence (d).

Fig. 4.

Correlation between UBM and TR-LFIS measurements. Data were taken from hamster oral carcinomas *in-vivo*. *Upper panel:* UBM image showed the tumor on the left with a hypoechoic region, compared to the normal region on the right. *Lower panels:* (Left) Fluorescence emission spectra were compared between the tumor and normal areas. Fluorescence from the tumor showed broad emission spectrum with the emission peak of 460 nm, suggesting the contribution of NADH highly expressed in the tumor cells, as well as a peak at 630 nm mainly due to porphyrin. The spectrum from normal region showed the fluorescence characteristics of collagen, with an emission peak of 390 nm. (Right) Comparison of the average lifetimes as a function of wavelength. At the blue-shifted wavelength the tumor fluorescence emission was characterized by a relatively shorter lifetime values when compared with the lifetime of normal tissue.