Three-Dimensional Clinical Coherent Chemically-Sensitive Imaging – 3D3CSI

1. Introduction

Coherent Anti-Stokes Raman scattering (CARS) and Optical Coherence Tomography (OCT) are well-established imaging modalities. CARS microscopy is a label-free, chemical-specific nonlinear microscopy technique for relatively small volumes (penetration depth ~0.3 mm), while OCT permits transverse scanning of larger volumes of scattering tissue up to 1.5 mm in depth. Independently, these non-invasive imaging systems have the ability to extract highly-contrasted molecular fingerprints of small specimen to monitor physiologic activity (CARS) or can quickly visualize larger three-dimensional structures with limited functional information (OCT). In this project we demonstrate a Fourier Transform (FT) CARS system with an ultrabroad bandwidth, high power Ti:Sapphire laser, which allows parallel acquisition of OCT data using a single light source capable of overlapping structural with chemical information enabling molecular sensitivity in OCT. Achieving noninvasive simultaneous chemical and structural changes in healthy as well as diseased tissue can help to diagnose diseases long before structural changes occur.

2. Method

An ultrabroad bandwidth Ti:Sapphire laser with more than 1.2 W output power and variable bandwidth between 30-300 nm at a repetition rate of 75 MHz centered at 780 nm to guarantee ultrashort pulses at the sample was realized. The bandwidth can be tweaked to ~4000 cm-1 to allow for CARS imaging from fingerprint to lipids and UHR OCT. Based on the ultrafast Ti:Sapphire laser the FTCARS system provides a simple scheme for obtaining high resolution CARS spectra with a single femtosecond light source. An approximately 8 mm wide excitation beam is focused with a high NA objective onto a sample to enable the collection of a forward (F)-CARS signal with a photomultiplier after re-collimation with a second objective. In FTCARS optical pulses have to be in the femtosecond range at the sample under investigation to evoke a nonlinear process (impulsive stimulated Raman scattering). Hence, the multimodal imaging system combining UHR OCT and CARS developed in this work necessarily has to use ultrashort laser pulses with pulse durations in the sub 15 fs to 10 fs region at the sample.

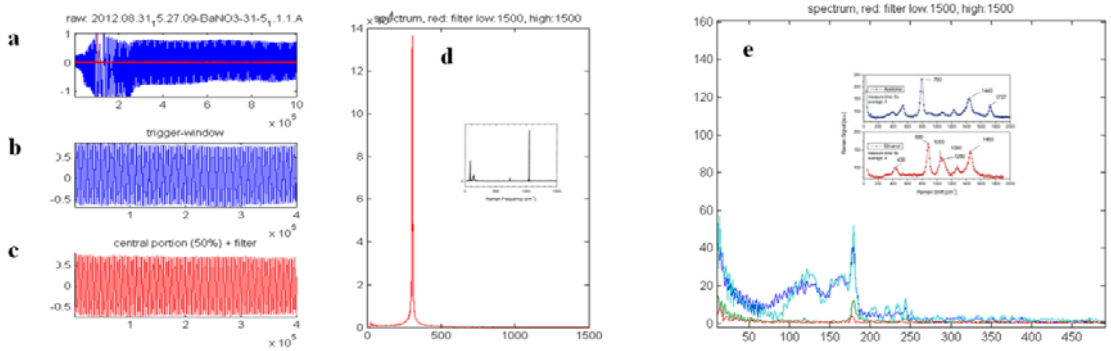

Fig. 1 CARS spectroscopy: (a) shows the time domain signal from the DAQ card for $Ba(NO_3)_2$, (b) the signal 3000 fs away from the zero delay and (c) the filtered signal Fourier transformed in (d). Inset in (d) shows the corresponding Raman spectrum. (e) reveals the CARS spectrum for Acetone with inset from corresponding Raman spectra from Acetone and Ethanol. All CARS spectra are not calibrated.

To yield simultaneously real time information about the intensity of the CARS signal a real time preview of CARS spectra obtained from the time-domain CARS signal by the FFT is implemented. Numerical FTCARS simulations are performed to evaluate the time domain FTCARS signal (cf Fig. 2c and 2g).

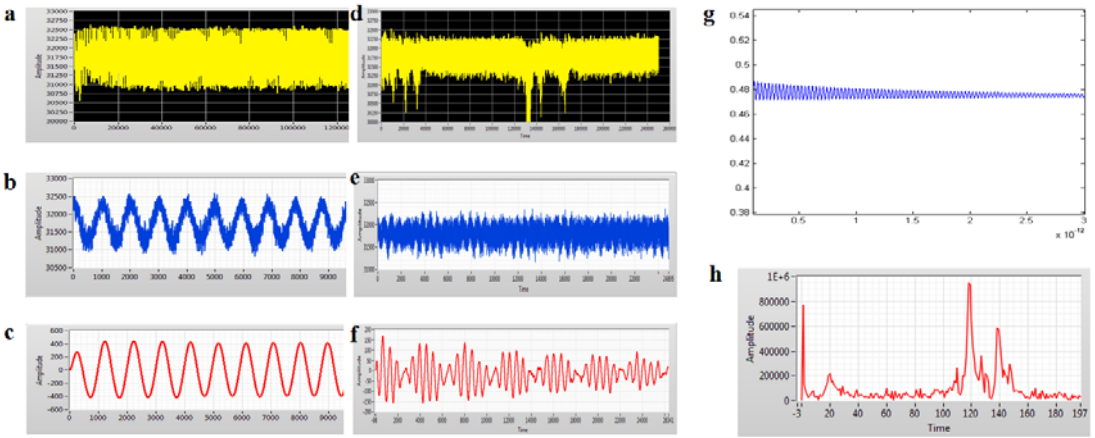

Fig. 2: Real time preview for FT CARS: CARS signal $Ba(NO_3)_2$ (a)/KGW (d). (b) Zoom in between 60000-80000 a.u. from (a) and between 40000-65000 from (d) in (e). (c) and (f) Denoised signals from (b) revealing the typical CARS signal of a single Raman line and (e) the beating signal of multiple Raman lines in KGW with corresponding FT in (h). (g) Numerical simulation of $Ba(NO_3)_2$ in good agreement with measured signal (c).

The hybrid ultrabroad bandwidth FTCARS/OCT set-up (cf. Fig. 3) requires careful management of material dispersion which was achieved with novel dispersive mirrors providing a pulse duration of < 10 fs at the sample measured with an interferometric autocorrelator at the focus of the microscope objective. The experiment uses a Newport objective with 20x magnification, 0.4 NA and 75 % throughput. A piezo linear stage is implemented to obtain FTCARS spectra. Razor Edge filters avoid leakage of the Ti:Sapphire laser. FTCARS imaging was performed with various samples ($Ba(NO_3)_2$, KGW, Acetone – cf. Fig. 1 and Fig. 2).

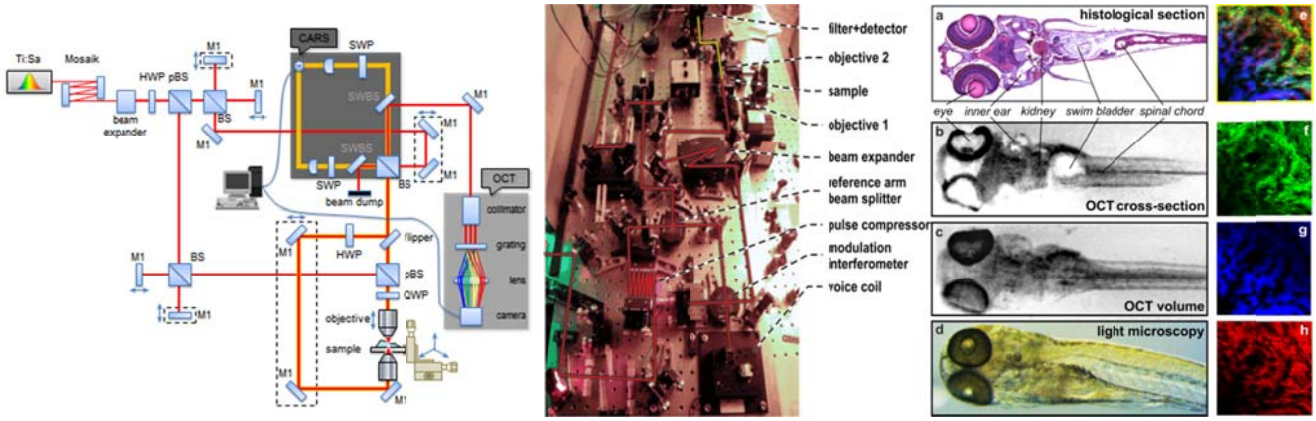

Fig. 3 FTCARS-OCT setup (left): The ultrafast Ti:Sapphire laser is the key element of the hybrid set-up. The output is precompensated with a pair of dispersive mirrors (Mosaik) and expanded. A half wave plate (HWP) and a polarizing beam splitter (pBS) allow for arbitrary splitting between reference and sample arm. The longer wavelength portion is sent into the OCT system. The signal is collected by a collimator and send through a single mode fiber to the spectrometer. 5 day-old zebrafish (right): Top to bottom: Coronal histological cross-section (a), corresponding OCT cross-section (b) and averaged OCT volume (c) corresponds to the macroscopic visualization (d). Adult zebrafish osteons (e), consisting of SH- (f), TH (g) and CARS signal to identify collagen, dielectric interfaces and phosphate respectively.

A spectral domain OCT system is specially designed for ultrabroad bandwidth with minimized chromatic aberrations (SNR~95 dB, axial and transversal resolution < 3µm and 18 µm respectively, signal roll-off of ~6 dB at 0.9 mm). Data acquisition is programmed in Labview including real time view, resampling of spectral data to spatial domain data and dispersion compensation. A 3D translation stage allows for raster scanning and stepwise acquisition of the sample. Tests are performed in zebrafish. 3D large volume OCT screening reveals all major organs. In cooperation with the Weizmann Institute of Science additional molecular information in adult zebrafish osteons is revealed with Second Harmonic (SH), Third Harmonic (TH) and CARS imaging (cf. Fig. 3) to identify collagen, boundaries and phosphate.

3. Results

Functional and morphological OCT and CARS imaging is demonstrated. The signal is amplified and digitized within the A/D converter (Alazartech, ATS 660) and recorded by NI Labview software as an additional channel to the SD OCT acquisition module. The feasibility of the hybrid platform is demonstrated in the fingerprint region (500 – 1500 cm^{-1}) with Polybeads of various diameters (cf. Fig. 4). The time to obtain the FTCARS spectra is limited by the scanning speed of the linear-stage which is currently 20Hz. The speed for the SD OCT imaging is limited by the line rate of the camera (27 kHz).

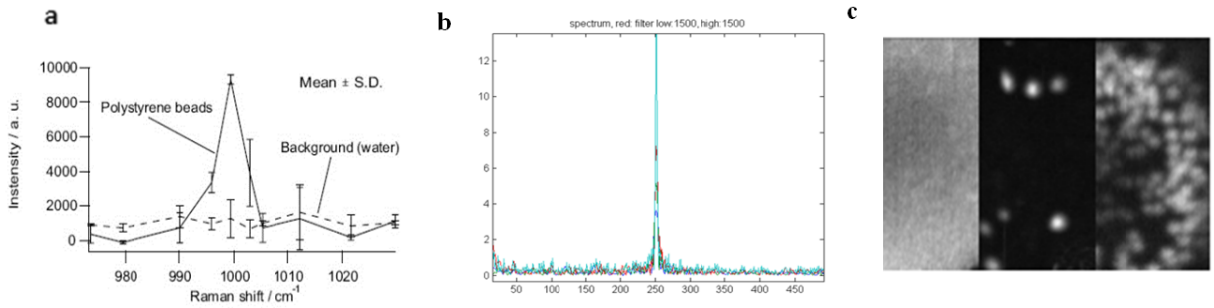

Fig. 4 FTCARS-OCT: Polybeads with 3/45/90 µm diameter – (a) Raman shift around 1000 cm^{-1}. (b) reveals the FFT from the acquired CARS signals from Polybeads with varying diameters in blue, green and red – the x axis shows arbitrary units and is not calibrated. (c) shows the corresponding OCT images.

4. Conclusion

In conclusion a multimodal FTCARS and spectral domain OCT using a single ultrafast Ti:Sapphire laser was developed and preliminary imaging is demonstrated. The relevance of this cost-effective noninvasive multimodal imaging platform providing morphological and molecular information will be evaluated in a preclinical environment.