FREQUENCY SHIFTER BASED *EN-FACE* OCT SYSTEM AT 1060 nm

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A highly efficient power optical coherence tomography configuration is implemented using a Multiwave Photonics broadband source centred at 1060 nm wavelength, FWHM = 50 nm and a Mach Zehnder interferometer. The interferometer contains a fibre acousto-optic modulator in each arm. The system has been used to acquire *en-face* images as well as cross section optical coherence tomography images from skin and embryos based on T-scans (transversal reflectivity profiles).

Introduction

Optical coherence tomography (OCT) is a relatively new non-contact optical imaging method that is capable of producing cross-sectional images of biological tissues with superior spatial resolution (10 μm) to depths of a few millimeters[1,2] .OCT has been a technology in continuous expansion and has been used for *in vivo* and *in vitro* imaging of a variety of transparent and scattering biological tissue such as intraocular structures (retina and anterior eye segment)[3] , skin[4], teeth[5], muscle[6], and gastro-intestinal tissue[7]. Various types of innovative OCT systems have been geared towards specific applications that extend the capabilities and imaging contrast of typical systems such as Doppler OCT for blood flow imaging[8], and polarisation sensitive OCT for mapping depth resolved polarisation proprieties of tissue[9].

At the core of the OCT technique is the method of low coherence or white light interferometry[10]. Phase sensitive interferometry was developed long before the advent of OCT for different applications in the optical sensing field. It can be used to determine with high accuracy the magnitude of various measurements, such as displacement, temperature, pressure and strain, that induce phase shifts between the reference and sample arm of the two beams in the interferometer.

In many applications it is desirable to obtain *en-face* images in real time, which are slices of the tissue with perpendicular orientation on the optical axis. *En-face* imaging operate at fixed depth and in this case a path imbalance modulator is needed in order to create a carrier for the image signal. It is known[11] that the X and Y scanners can be used to introduce a path modulation, similar to a path modulation created by the longitudinal scanner in longitudinal imaging OCT set-ups. When the beam scans the target, the OCT signal is modulated by the fringe pattern. As the pattern is not regular, the transversal resolution varies across the target and different frequencies result in contrast to OCT longitudinal imaging case where the carrier frequency is constant. A phase modulator at a frequency much larger than the signal bandwidth is desirable to insure a constant transversal resolution over the target.

Experimental setup

In this work, we present an OCT system, which incorporates an acousto-optic modulator in each arm of the Mach Zehnder interferometer12 (Fig. 1). The system has been used to acquire optical coherence tomography *en-face* images from skin and embryos based on T-scans (transversal reflectivity profiles).

The acousto-optic modulator placed in the reference arm is driven at a fixed frequency of 40 MHz. The modulator placed in the object arm is driven by a RF Function Generation. at a frequency between 40.1 to 41.5 MHz. In this way, the *en-face* OCT signal is frequency shifted by 100 kHz to 1.5 MHz. The system implements a dual channel OCT and confocal microscope. The interferometer is fed by a broadband fibre source centred at 1060 nm wavelength (Multiwave Photonics) with 50 nm bandwidth (FWHM). The dual channel configuration has been designed and constructed to acquire live images from scattering biological samples.

In the sample arm, a circulator leads light from the frequency shifter to the sample and collects the backscattered and retroreflected light from the sample, which is then sent to one input of the balanced receiver (AC Photonics).

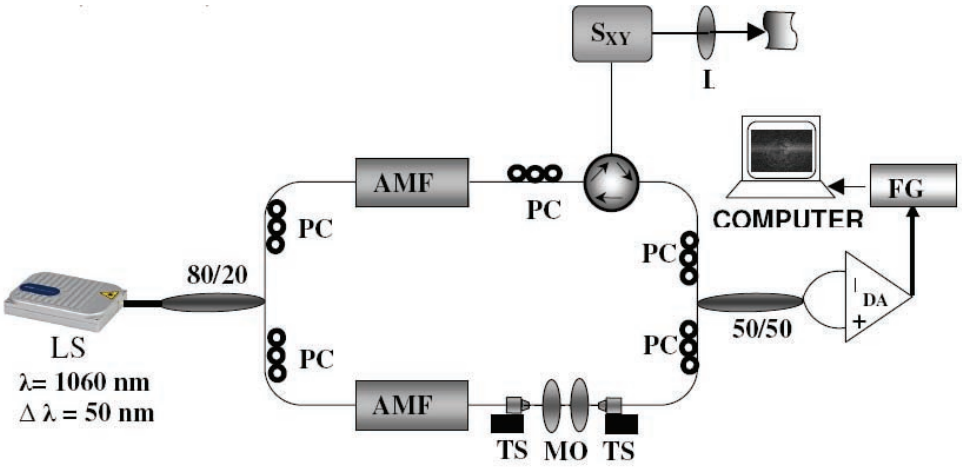

Fig. 1. Components of the imaging system: LS: Laser source (Multiwave Photonics); 80/20: coupler, 50/50-couplers; PC: polarisation controller; AOFS: acousto-optic frequency shifter; TS: 3D translation stages, MO: X10 microscope objectives; FG: frame grabber.

The laser beam is scanned over the sample by an *xy*-galvano scanner unit (General Scanning Inc.) which consists of a pair of scanning mirrors with a maximum scanning frequency of around 1 kHz. TTi function generators drive the pair of scanning mirrors, which determine fast scanning along the *x*-and slow scanning along the *y*-direction. In combination with the focusing lens in front of the sample with a focal length of 30 mm, large en-face scan areas can be acquired 3x3 mm2. Light retroreflected from the sample is sent via the circulator to a balanced coupler where it interferes with the reference beam. The balanced detection receiver is made of a 50/50 single mode coupler and two InGaAs photodetectors followed by a differential amplifier. In our configuration, up to 1 mW of power is delivered to the sample.

Good depth resolutions require dispersion compensation between the reference and sample arms to ensure a narrow correlation function peak. We use a pair of lenses in the reference arm to compensate for the dispersion due to the lens in the sample arm.

System characterisation

The system has been used to acquire *en-face* OCT images from skin and embryos based on T-scans (transversal reflectivity profiles). When the two galvanometer scanner are driven to generate a raster, *en-face* images are obtained. In the confocal channel, a lateral resolution better than 10 microns is obtained. In order to evaluate the lateral resolution achievable in both OCT and confocal chanels, a United States Air Force (USAF) resolution target was imaged (Fig. 2) with the dual imaging system.

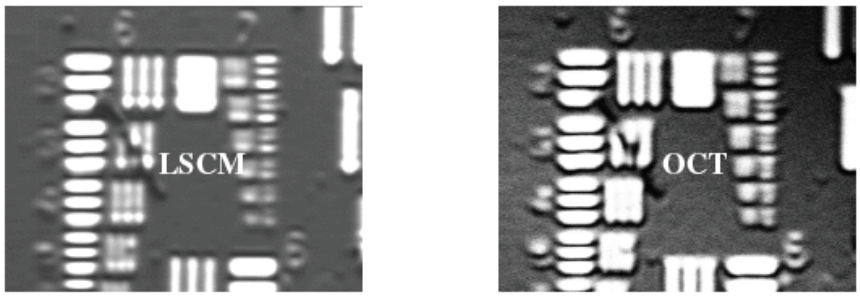

Fig. 2. *En-face* confocal (left) and OCT (right) showing the smallest group of elements in the USAF

The autocorrelation function of the source + OCT system is shown for different values of the carrier frequency . Due to the polarisation mode dispersion of the circulator and the non Gaussian spectrum profile of the source, autocorrelation function profile exhibits a FWHM ~ 30 microns, larger than 19 microns, value expected for the given FWHM of the source spectrum.

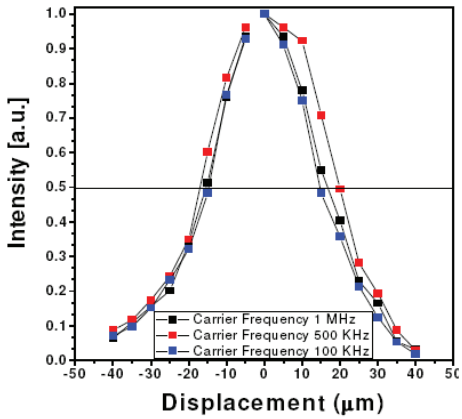

Fig. 3. Autocorrelation function of the OCT system for different carrier frequencies

Images

The dechorionated embryo was placed on the glass slide with its dorsal side up then imaged with the OCT system. The larvae at 3rd instar stage were selected and immobilized by laying them on the double-sided adhesive tape with their dorsal side up. The posterior side was imaged and the C-scan (*en-face*) OCT images Fig. 4 obtained.

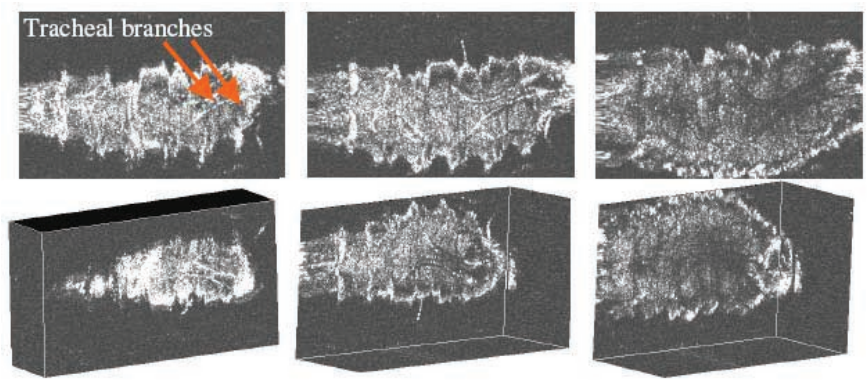

Fig. 4. Top raw: *En-face* OCT images collected from an embryo (Drosophila Megalogaster) from different depths. Bottom raw: 3D images. The size of the images was 2.15 x 1.1 mm (frequency of the signal driving the horizontal and vertical scanners were fx=700 Hz, respectively fy=1.69 Hz) The depth range in the 3D images is 700 μm measured in air.

En-face (C-scan) OCT imaging proved capable of differentiating coetaneous structures in skin4. We illustrate similar capability with *in vivo* measurements using our transversal OCT imaging. We collected images from the fingertip of a volunteer (Fig. 5), placed at 3 cm away from the last lens of the interface optics. We set the scanning rate to 700 Hz a line and 1.69 s for the frame rate and used 0.8 mW power towards the skin. A glass window held in a mount was used as support for the fingertip. 25 OCT transversal images were collected by moving the reference translation stage TS in the Figure 1 in steps of 10 microns measured in air. As shown by snap-shots of movie in Fig. 5, the finger-print ridges are visible touching the glass plate interface. The stratum corneum and the epidermis are clearly distinguishable.

Using special 3D software (OTI, voxel) we created 3D images of the images finger. In the reconstructed voxel profile, longitudinal cuts from the current depth to larger depths are shown. The 3D images display different perspectives of the sweat ducts in the tissue. The transversal distribution of sweat ducts is clearly visible in Fig. 5 as a dotted pattern.

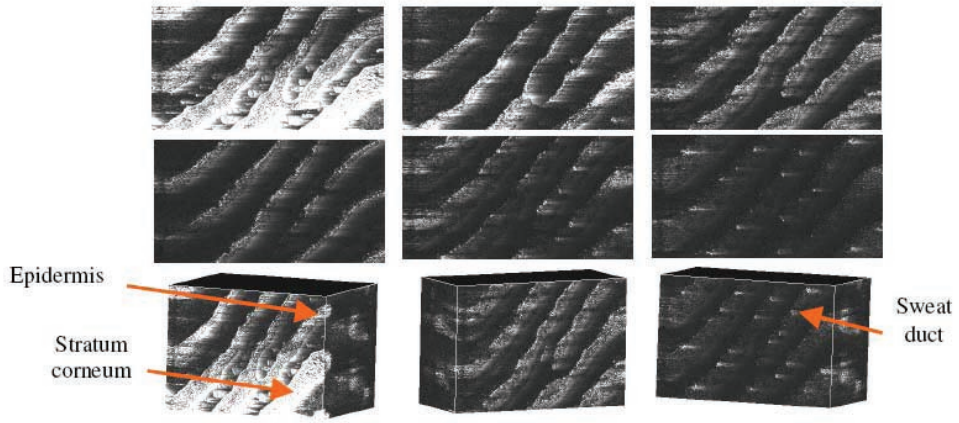

Fig. 5. Top and middle raws: *en-face* OCT images from the finger of a volunteer at different depths. Bottom raw: 3D images, 2 mm depth range measured in air. The size of the images is: 3 x 2 mm, frequency of the driving signal of the horizontal scanner, fx=700 Hz, frequency of the driving signal of the vertical scanner fy=1.69 Hz)

Conclusions

En-face OCT images as well as cross section images from drosophila melanogaster in second larval stage were achieved at 1 Hz frame rate. *En-face* OCT images can be assembled to display 3D views to explore the volume of the embryos. At this wavelength, good penetration is achievable, of up to 2 mm in the embryo structure.

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