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The Perspectives of Applying OCT Technology to the Investigations of Selected Biological Materials and Processes

1. Introduction

Several well-recognized imaging technologies are currently used to provide structural information about microscopic specimens. These include magnetic resonance imaging, computer tomography, ultrasound, and confocal microscopy. High resolution magnetic resonance imaging has been used to image the mouse embryonic cardiovascular system as well as to produce in vivo cross-sectional images of early *Xenopus laevis* development with resolutions of 12 μm. Because the static and gradient magnetic fields required to obtain these resolutions are orders of magnitude greater than those found in most clinical systems, this modality represents a technically challenging option that requires considerable skill from its operator in order to achieve high resolution images. High resolution computed tomographic imaging of fixed insect specimens revealed internal microstructure with 8–12 μm resolution yet required an elaborate microfocusing instrument and image reconstruction algorithms. Ultrasound backscatter microscopy using high frequencies (40–100 MHz) is capable of 50 μm resolutions to depths of 4–5 mm and has been applied to the analysis of early embryonic development in the mouse. To effectively image with ultrasound, probes require contact with the tissue. The invention of the confocal microscope and laser-scanning confocal microscopy has advanced the understanding of biological systems and their development largely due to the ability to selectively visualize biological specimens, cells, and subcellular constituents. Transverse resolutions of 0,5 μm with 1 μm optical sections are possible. Although confocal microscopy is superb for optically sectioning a specimen, imaging depths are limited to less than 500 μm in nontransparent tissue [3].

Optical Coherence Tomography (OCT) is 3D imaging technology, which perfectly fills apparent gap in depth/resolution feature space existing between confocal microscopy and ultrasound methods (Fig. 1). The OCT system producers offer the maximum transverse scan

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dimensions of 10×10 mm at the lateral resolution of 10 μm. The *xy*-limits can be easily extended up to 10 cm by applying an appropriate motorized stage with stepper motors. With imaging depth up to 3 mm at the axial resolution 5–12 μm this technique gives the opportunity to analyze sub-surface layers of many biological materials or full cross-sections of thin materials. Optical coherence tomography (OCT) enables noninvasive three-dimentional imaging technique of the internal microstructure of biological and industrial matherials. Since late 1980s, when it was invented OCT technology was successful applied in medicine, where it established a new standard of real time diagnostic method. The main advantage of this technique is its ability to in situ imaging of tissues with a resolution approaching that of histology, but without the need for tissue excision and postprocessing. This method could significantly improve early medical diagnosis and as a consequence of its innovation and painless it started to be named optical biopsy. Most of all the OCT tomography was introduced in ophthalmology [4]. For example it enables observation, imaging and digitalization of high quality cross-sections of anterior and posterior eye sections. As it is important for patients the examination is noninvasive, non-destructive, quick and it consist almost all in image acquisition. Further proceedings and measurements are carried out on the image of eye parts with the use of automatic methods of different OCT parameter analysis. There have been numerous recent developments in OCT technology and considerable interest in this topic in other fields of medicine – e.g. pullmonology [7], urology, cardiology, angiology [12] etc.

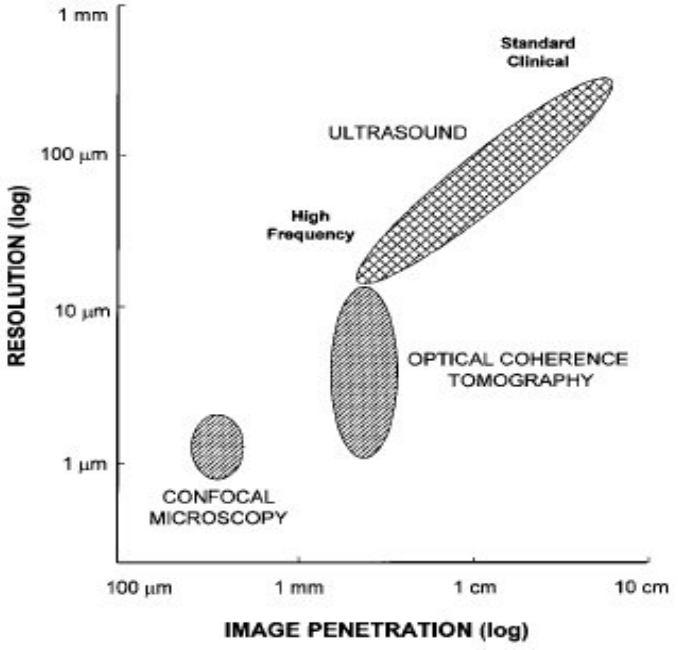

Fig. 1. Resolution and penetration of ultrasound, OCT, and confocal microscopy [3]

Recently many pilot studies using OCT tomography were started also in biological sciences. There are important tasks and urgent challenges for environmental and human preservation in such experimental and bioengineering fields as plant physiology, agriculture or biotechnology. These domains need modern, more precise methods of tissue penetration for exactly and broader possibilities of proper concluding.

The authors briefly present in this paper the fundamentals of Optical Coherence Tomography. Additionally the advantages and perspectives of applying OCT technology to the investigations of selected biological materials and processes are discussed.

2. The fundamentals of OCT

In optical coherence tomography, measurements of distance and microstructure are performed using light that is backreflected and backscattered from microstructural features within the material or tissue. OCT performs imaging by measuring the echo time delay and magnitude of backreflected or backscattering light using interferometry. The most common detection method is based upon a Michelson interferometer with a scanning reference delay arm. Backreflected or backscattered light from the object being imaged is correlated with light that travels a known reference path delay. The interferometer measures the field autocorrelation of the light. In contrast to conventional microscopy, in OCT the mechanisms that govern the axial and transverse image resolution are independent. The axial resolution in OCT imaging is determined by the coherence length of the light source, and high axial resolution can be achieved independently of the beam-focusing conditions. The coherence length is the spatial width of the field autocorrelation produced by the interferometer. The envelope of the field autocorrelation is equivalent to the Fourier transform of the power spectrum. Thus, the width of the autocorrelation function, or the axial resolution, is inversely proportional to the width of the power spectrum. Typically, OCT imaging is performed with low numerical aperture focusing to have a large depth of field, and low coherence interferometry is used to achieve axial resolution. The axial image resolution is determined by the coherence length, and the transverse resolution by the spot size. In contrast to conventional microscopy, this mode of operation achieves high axial resolution independently of the available numerical aperture. Most OCT imaging is performed with low NA focusing, where the confocal parameter is much longer than the coherence length. Depending upon the coherence length of the light, the depth of field can be shorter than the coherence length. In this case the depth of field can be used to differentiate backscattered or backreflected signals from different depths. This regime of operation has been referred to as Optical Coherence Microscopy (OCM). This mode of operation can be useful for imaging scattering systems because the coherence gating effect removes the contributions from scattering in front and in back of the focal plane more effectively than confocal gating [3].

Most of OCT systems used Time-Domain optical interferometry in which the optical path length difference between the reference mirror and the sample in the Michelson or Mach-Zehnder interferometer is modulated in time. Time-Domain or TD-OCT had opened

up the potential of optical biopsy but there are performance limitations for further extension of the applications. First, imaging speed is relatively slow because of mechanical delay modulation. Second, even when higher frequency scanning is possible, detection sensitivity drops because of detection bandwidth in return. Fourier-Domain OCT is a break-through technology which enables high sensitivity and high speed imaging at the same time. FD-OCT relies on analyzing the individual frequency components of backscattered light from the sample or tissue. There are two methods within FD-OCT. One is Spectral-Domain OCT (SD-OCT) which uses a low coherence light source and a spectrometer, where frequency components are spatially analyzed on the CCD array. The fast readout speed of CCD provides high imaging speed, and high signal-to-noise ratio (SNR) gives 20–30 dB advantage over conventional TD-OCT [13].

The second method is Swept-Source OCT (SS-OCT) (Fig. 2) which uses a continuous and repetitively tunable ("swept") light source where frequency components are analyzed in time with a single photodetector. Each wavelength scan generates depth information, lateral scanning of the laser beam then enables a cross section image to be constructed. This technique has a theoretical sensitivity benefit equal to that of SD-OCT, while overcoming the disadvantages of SD-OCT such as fringe washout, and allowing the use of longer wavelengths, over 1 μm to 1.5 μm range. Advanced data acquisition and digital signal processing techniques are employed in the SS-OCT system to enable real-time video rate OCT imaging. This OCT system enables the generation of images similar to confocal microscopy by summing signals in the axial direction. High-speed 3D OCT imaging provides comprehensive data that combines the advantages of surface microscopy and structural OCT imaging in a single system.

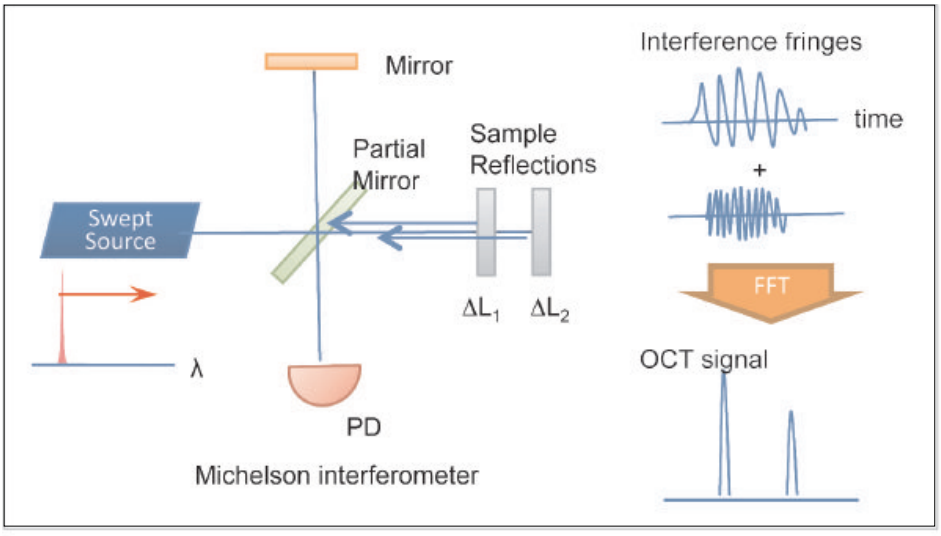

Fig. 2. Swept – Source OCT principle [13]

2.1. Sweep linearity

The OCT signal is processed by time-sampling the backscattered light as the swept source sweeps the wavelength followed by Fourier transform (FFT). Ideally, the sweep should be linear in k space ($k = 2\pi/\lambda$). But actual sweep curves of most of the proposed swept sources are non-linear in time, because of the intrinsic tuning mechanism. For examples, the use of Galvano mirror or fiber Fabry-Perot filter imposes sinusoidal sweep due to its driving characteristics. If simply applying FFT on the time-sampled interferogram when this non-linearity is present, the resolution of the signal is blurred and the signal power also decays. So, in general, most SS-OCT systems implement either nonlinear sampling with the use of an optical clock having another set of interferometer and detector, or the post processing approach; the so called “wavelength rescaling process”.

2.2. Swept rate (scanning speed)

Wavelength swept rate, or scanning speed of the swept source is directly reflected on the imaging speed like the readout speed or refresh rate of the CCD in SD-OCT. Swept rate corresponds to A-line rate in OCT. Increasing A-line rate makes it possible to accommodate more A-lines per frame or increase the frame rate. In practical applications the ability to produce video rate images is of critical importance. This not only removes imaging artifacts that are created by undesired movement, but also enables a large area/volume measurement without compromising resolution, in a short amount of time. Depending on the applications, swept rate of 10 kHz to 100 kHz range are required.

2.3. Wavelength range

The choice of wavelength band in OCT is dependent on the water absorption and scattering property of the sample or tissue of interest. In general, 800 nm range is used for retinal imaging because of low absorption in vitreous humor, and recently 1060 nm range gains attentions because of large penetration in retinal tissue as well as low dispersion property in tissues.

2.4. Coherence length

Coherence length is defined as the optical round trip delay or twice of the depth range (Fig. 3) here fringe visibility drops half or the Fourier-transformed OCT signal drops 6 dB compared to the signal power at zero delay [13].

2.5. Polarization Sensitive Optical Coherence Tomography (PS-OCT)

Polarization Sensitive Optical Coherence Tomography (PS-OCT) is a cross-sectional birefringence imaging tool for a wide range of biological and industrial materials. PS-OCT is an extension of OCT that is based on measuring the polarization properties of light from birefringent samples. Birefringence is where a material decomposes light into two

polarization states. It only occurs if the material is anisotropic. Materials that exhibit birefringence properties including tissues such as tendons, muscles, teeth, bones, blood vessels and skin. In samples such as these, PS-OCT provides additional contrast of the birefringent material in a sample over conventional OCT structural images. The real-time, high-resolution imaging capability of PS-OCT makes it well suited for studying glaucoma and other eye diseases, dental diseases, burn depths in the skin, and vascular imaging to guide plaque excision. Birefringence is also created in isotropic materials that have undergone a deformation such that the isotropy is lost in one direction. In such cases, PS-OCT is very useful for nondestructive detection of stress birefringence in industrial materials such as plastics, thin-films, semiconductors, and liquid crystals. Thorlabs has developed a real-time, fiber based swept source PS-OCT imaging system which provides simultaneous cross-sectional imaging of the intensity and phase retardation of light backscattered from birefringent samples. This system utilizes a standard Thorlabs 1300 nm SS-OCT Imaging System (OCS1300SS) with the PS-OCT add-on module. The modularity of the Thorlabs OCT systems enables incorporation of PS-OCT imaging capability at any time. The provided software enables easy display of OCT structural or PS-OCT birefringence images with a single button [14].

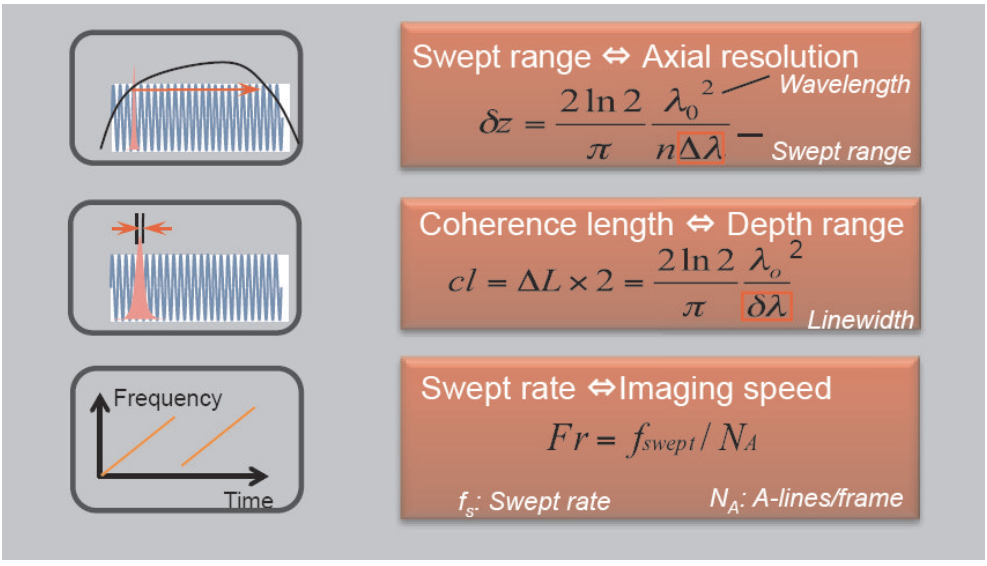

Fig. 3. Important parameters in Swept – Source OCT [13]

3. The variants of measurement stands

The probe in OCT laboratory measurement system is doubly connected to an interferometer module (laser output, probe I/O) and indirectly to a PC computer equipped with software reconstructing and archiving acquired image data [16]. The probe includes an infrared light illuminator connected to a laser source and objective lenses with digital cam-

era coupled with the computer via USB or Firewire link. An articulated probe mount is typically included with an OCT system for manual probe positioning over the region of interest. To perform an automatic image analysis an OCT microstand with the probe can be equipped with a motorized *xy*-stage extending scanning area up to 5–10 cm (Fig. 4).

Another variant of the measurement station is a microscope specifically designed for use in the OCT system instead of a handheld probe, connected with an interferometer module (Fig. 5).

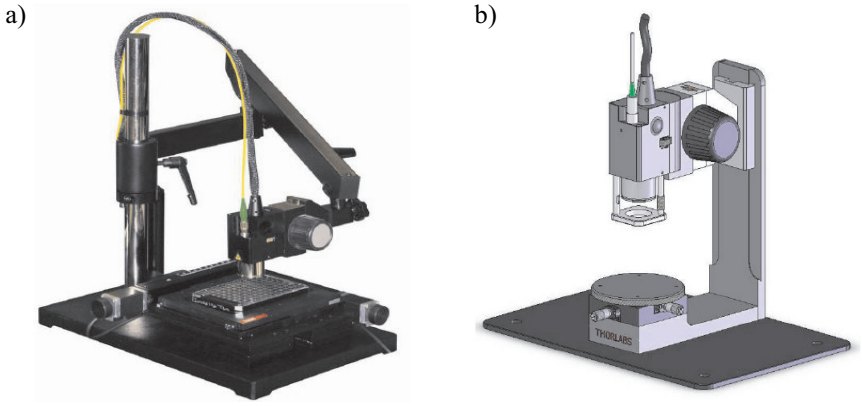

Fig. 4. OCT probe stand types offered by ThorLabs: a) articulated probe mount; b) OCT microstand [16]

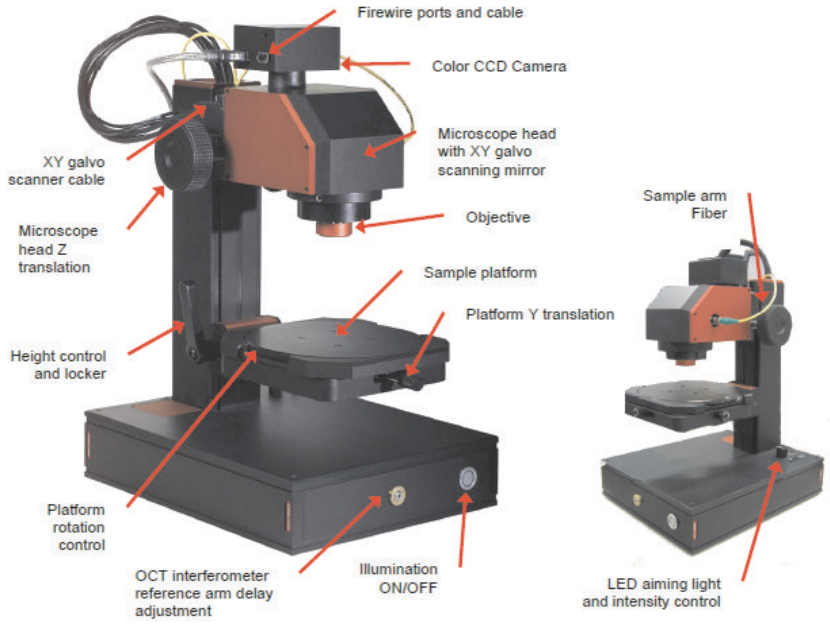

Fig. 5. OCT microscope [16]

For the purposes of processing biological materials the authors suggest the selection of Nikon or Olympus microscopes with add-on OCT module. It is the solution offered by Thorlabs company to the 1325 nm Swept Source OCT engine [15, 16]. These microscope systems are designed specially for imaging small animals and thick specimens. Additionally their OCT beam path is collinear with the optical microscopy path and the microscopes allows users for fast switching between OCT and optical or sub-micron fluorescence images, which can be both registered and overlayed. This variant also makes possible flow imaging with the included Doppler imaging function. It can also be equipped with motorized stage extending the *xy*-ranges of imaging to 5–10 cm (e.g. MAX201B-NIK).

Fig. 6. The illustration of an OCT microscope laboratory system;
OCT microscope probe mounted on an Olympus BX41 research microscope [17]

OCT Microscope Module		
Optical Source Specifications (Thorlabs SL1325-P32)		
Center Wavelength	1325 nm	
3 dB Spectral Bandwidth	100 nm	
Axial Scan Rate	32 kHz	
Imaging Specifications		
OCT Objective	**Thorlabs LSM03**	**Thorlabs LSM02**
Magnification	5X	10X
Transverse Resolution	20.0 µm	13.0 µm
Field of View	9.4 x 9.4 mm	4.7 x 4.7 mm
Axial Resolution Air (Water)	12.0 µm (9.0 µm)	
Max Imaging Depth	3.0 mm	
Max Imaging Width	10.0 mm	
2D Imaging Speed [frames/second (area)]	60 (for 628 x 582 pixels)	
3D Imaging Time [seconds (volume)]	<10 (1024 x 1024 x 512 pixels)	
Sample Illumination Power	3.0 mW	

Fig. 7. The technical specification of Olympus OCT microscope module shown in Figure 6 [17]

Type:	Swept-source Fourier-Domain OCT
Light source:	HSL-2000-11 MDL* (Wider sweep range)
Laser peak power:	15 mW
Laser centre wavelength:	1305+/- 15 nm
Laser wavelength sweep range (>3 mW):	150 nm
Axial optical resolution (in tissue):	< 10 µm
Pixel size (isotropic, in tissue):	< 4.5 µm
Lateral optical resolution:	< 7.5 µm
Max frame width:	5 mm
Min frame width:	0.1 mm
A-line rate:	10 kHz
Frame rate:	> 6 fps (5 mm / 1,250 A-lines) > 20 fps (1 mm / 250 A-lines) > 30 fps (0.2 mm / 50 A-lines)
3D image acquisition:	Yes, with motorized stage option
Image formats:	TIFF, TIFF stack, raw
Visual channels:	2 MPixel, colour

Fig. 8. The technical specification of Michelson Diagnostics EX1301 OCT microscope module [11]

The technical specification of well known OCT microscope modules are presented in Figures 7 and 8.

4. OCT applications in biology

4.1. Review of existing applications

OCT was originally developed and demonstrated in ophthalmology [2] for high-resolution tomographic imaging of the retina and anterior eye at the light wavelength 800 nm. Since 2000 OCT has been applied for imaging other than the eye, nontransparent tissues of plants and animals [2, 4, 9]. The achievable imaging depth up to 3 mm (or even 6 mm for special solutions) is limited by optical attenuation resulting from light scattering and absorption. OCT imaging in non-transparent tissues is typically performed at the wavelengths from 850 to 1550 nm [2, 4, 9]. Nowadays high resolution OCT using short-coherence-length has 2D cross-sectional image acquisition rates 25 to 40 fps (frames per second) [11, 15]. Because of very low power scattered in material (2–3.5 mW) during the process of scanning OCT is suitable to in vivo imaging of living cells and tissues. It does not require the addition of fluorophores, dyes, or stains in order to improve contrast in images. Instead, it relies on the contrast generated by variations in optical scattering and the index of refraction.

Understanding of organo- and morphogenesis processes is limited by our ability to visualize and quantify the cellular, morphological and functional changes in situ and over time. OCT is used for imaging early developmental transformations taking place in orga-nisms. For its high resolution, noninvasive and real time capabilities OCT imaging it is

a perfect tool for monitoring the growth and development of biological tissues. In developmental biology animal models from insects, fish, and amphibians to small birds and mammals can be monitored as biological models utilized for studying a variety of human diseases. Typical non-mammal models are: fruit flies (*Drosophila melanogaster*), fish (zebrafish, *Brachydanio rerio*; medaka, Oryzias), and amphibians (African frog, *Xenopus laevis*). Small animals like chicken (*Gallus domesticus*), mouse (*Mus musculus*) or rat (*Rattus norvegicus*) are preferred because of their development and organ functions similar to humans. OCT can applied to image arterial pathology in vitro and is able to differentiate plaque morphology with superior resolution to ultrasound. OCT combined with catheter/endoscope-based delivery performs in vivo imaging in animal bodies [3, 4].

OCT has been successfully applied as an investigative tool in tissue engineering [4]. An example can be a bioreactor with hydrogel tube (4 mm outside diameter) is shown in Figure 9.

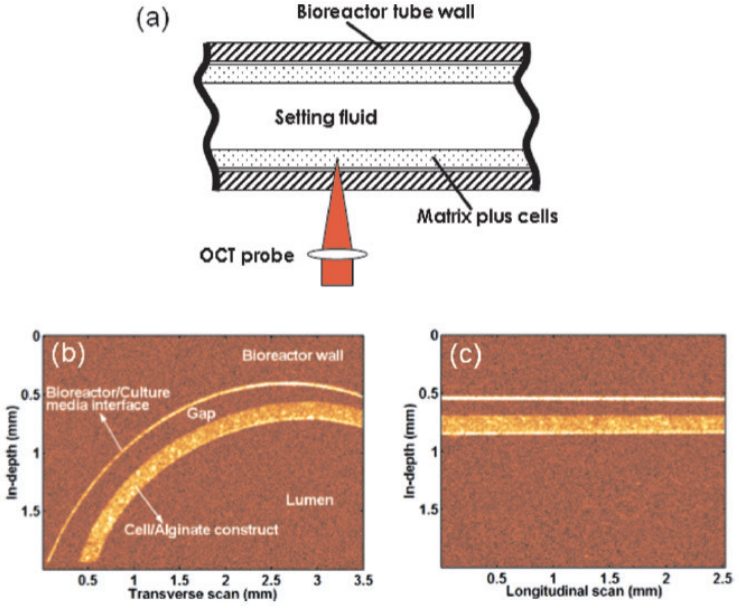

Fig. 9. OCT imaging of a bioreactor with its engineered tissue [4]:
a) schematic of the bioreactor; b) transverse OCT scan; c) longitudinal scan

From the OCT images, it can be seen that the bioreactor contains an inner lining of living cells within a hydro-gel and a distinct inner lumen containing culture medium. The distribution of the cells within the hydro-gel can be visualized, with the cells appearing as bright spots in the OCT images.

Real-time *in vivo* cross-sectional imaging of an African Frog during its tadpole stage was studied with OCS1300SS OCT microscope system (Fig. 10). Accompanying software enables 4D reconstruction (3D + time). This enables to study of the individual development of this animal [9, 14, 15].

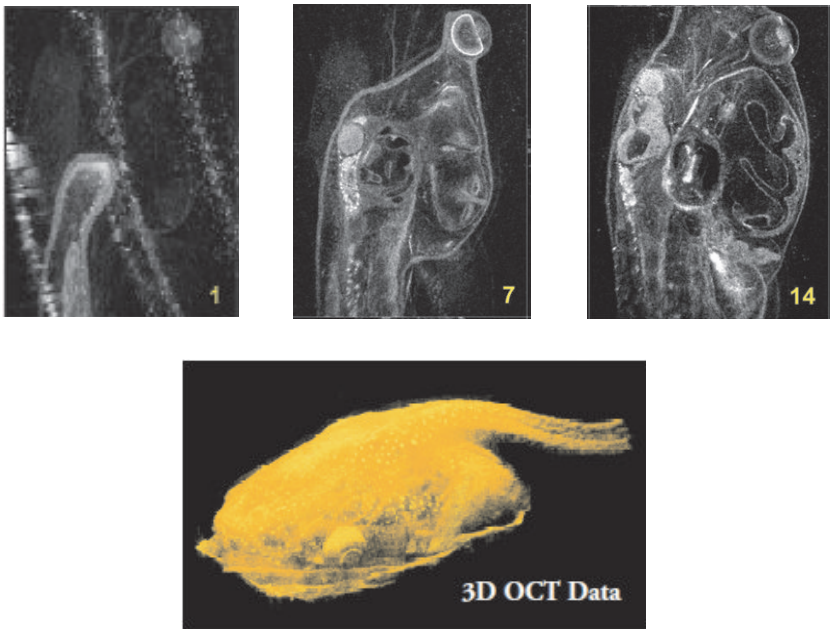

Fig. 10. The selected *en face* images of an African frog tadpole and a 3D image reconstruction. All images are 6 mm × 8 mm and were taken from the posterior to the anterior of the tadpole in 100 μm increments [15]

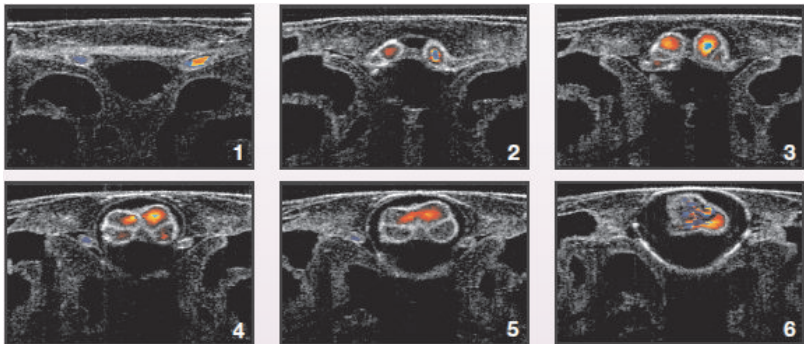

Fig. 11. *In vivo* cross-sectional SS-OCT images of a beating tadpole heart superimposed with Doppler blood flow images [10, 15]

At the University of Toronto the cardiovascular system of living tadpoles was studied using swept source Thorlabs OCT Imaging System (OCS1300SS) with Doppler imaging (Fig. 11) [10, 14, 15]. The series of images below show *in vivo* cross-sectional SS-OCT images of a beating tadpole heart superimposed with Doppler blood flow images. This allows detailed visualization of the complex cardiac motion and hemodynamics in the beating heart.

At MIT and Massachusetts General Hospital modified OCT SS system coupled with video microscope has been assembled, in which OCT scans can be directed to a region pointed by video microscope [1, 14, 15]. In Figure 12 it can be seen that OCT scan enables identification of the scull (S), surface vasculature (V) and meningeal layers with dura mater (D). The blue color shows functionality active cortex regions.

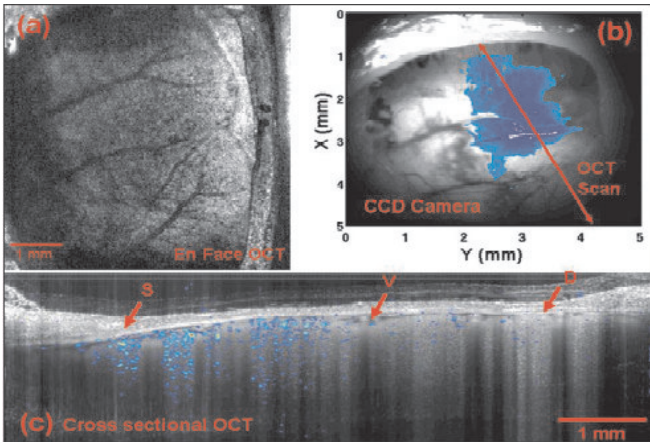

Fig. 12. OCT images representing the functionally active region of a rat brain; images were taken by researchers at MIT and Massachusetts General Hospital using a modified Thorlabs Swept Source OCT system. The OCT scan is directed to the region using a video microscope [1, 15]

4.2. Possible applications

The authors have been personally faced with three problems in biology of plants which could be perfectly solved with OCT imaging [5, 6]:

- the estimation of white roots discolouration regions formed as a result of plant abiotic stress,
- the observation of root systems and the recognition of topological and morphological changes in the same situation as above,
- the measurement of leaf blades regions with pathological changes induced by different infections of plants.

The analysis of plant root discolourations plays an important role in the diagnosis of plant health state, the detection of possible diseases and growth distortions. Plant roots respond to heavy metal stress not only with the restriction of growth, but also with the appearance of toxicity symptoms such as discolourations, usually greyish or brownish. Additionally, after histochemical or vital staining, blue, yellow or red discolourations can be observed (Fig. 13). The root images are acquired in two dimensions by appropriate scanner device, after they when put in a shallow dish of water or placed directly on a scanner glass and covered with specific background. So, the root images are two-dimensional and

shoot only from one side. We never know what discolourations could be found on the other side and what shape or volume the pathological regions have inside of the roots. Traditionally the volumetric data of infection level are obtained by histochemical methods, which are expensive and time consuming. The results of 2D image processing are relatively fast and can be statistically significant for large enough tested populations of the roots. However to compare with the infection level obtained by chemical treatment we at least should know statistical dependency between visible discolouration spot areas and their volumes.

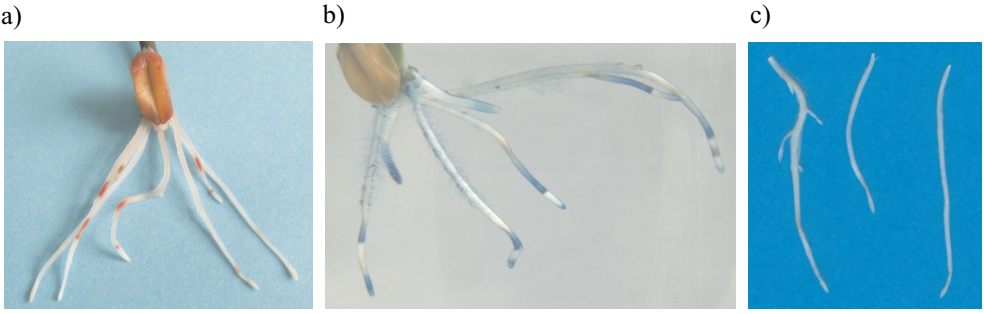

Fig. 13. Example wheat root images with discolourations: a) Ni-treated root system after staining for lignin detection; b) Ni-treated root system after staining with Evans blue; c) separated wheat roots with dark discolourations [5]

Applying OCT device gives a chance to estimate this statistical dependency for the populations of certain type discolourations. The primary wheat roots from 7-days hydroponic cultures have diameters of order 0.5–0.6 mm, so their discolourations across the root can form 3D regions of 40–50 pixels in the directions transversal to the root medial axis (assuming average resolution 12 μm).

Fig. 14. The exemplary fibrous root system (wheat) 7-days grown in water culture

The flat images of spatially distributed root systems obtained in a scanner device give no chance of their proper identification unless main roots are manually separated (Fig. 14) [6]. Some root branches can be obscured by the others. Applying an OCT system and

xy-direction motorized stage would enable to find the root system regions with ambiguous run of root branches. The regions can be properly recognized in three dimensions by OCT scanning.

Plant leaves in reaction to abiotic (e.g. heavy metals, acid rain) and biotic stress (bacterial or fungal infection) response by concentration of hydrogen peroxide (H_2O_2) at the places where this stress is located. Concentration of H_2O_2, after its extraction from tissues, can be estimated using biochemical methods but they do not allow localization of H_2O_2 in plant organs. After special staining the signs of H_2O_2 appear as brown spots of different size and intensity on a brownish leaf blade. The localization of spots can play an important role in plant stress analysis. It can be found by the segmentation of 2D leaf blade images obtained by scanning or taking photos of leaf's upper side. This method gives no information about the spot shape and size inside of the leaf blade, but this knowledge is necessary to compare image processing results with biochemical techniques. The simplest way to obtain volumetric data is to apply OCT over all leaf surface (slower) or only in particular places where the spots appear in the leaf (faster) (Fig. 15). The second approach can drop the H_2O_2 spots visible only from one side.

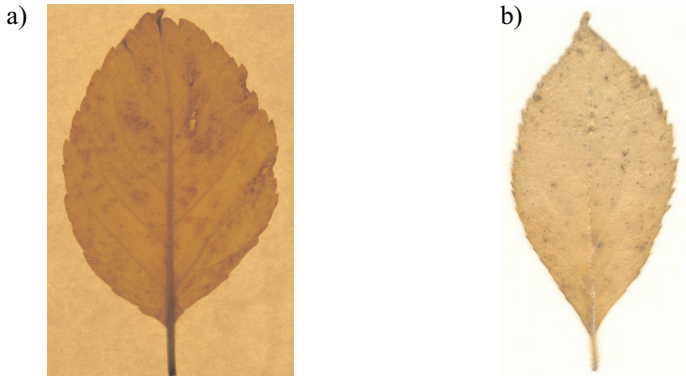

Fig. 15. Example apple-tree leaves stained with diaminobenzidine for H_2O_2 detection: a) the photo of a wet leaf blade; b) the scanned image of a dry leaf blade

In all cases discussed above it would be worth to experiment with polarization sensitive OCT because it is known that tissues can exhibit birefringence properties what provides additional contrast to OCT images and better conditions for image segmentation.

5. Conclusions

Optical coherence tomography (OCT) can produce high resolution cross-sectional images of biological issues in vivo and in real time. OCT has been demonstrated for high resolution in vivo imaging of developmental processes, including morphological abnorma-

lities and functional parameters. The authors have been personally faced with three problems in biology of plants which could be perfectly solved with OCT imaging:

– the estimation of white roots discolouration regions formed as a result of abiotic stress,
– identification of root systems and their topological and morphological changes in the same situation as above,
– the measurement of leaf blades regions with pathological changes induced by different biotic and abiotic stressors.

OCT fills a niche between confocal microscopy and imaging modalities such at magnetic resonance imaging (MRI) and ultrasound. It promises to become a powerful and unique investigative tool in many fields of experimental biology. For example high image resolutions (2–10 μm) with 2–3 mm imaging penetration depths in scattering tissue permit the microscopic visualization of dynamic changes which are studied in developmental biology and histobiology.

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