Ultrahigh Resolution and Spectroscopic OCT Imaging of Cellular Morphology and Function

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Abstract: Optical coherence tomography (OCT) is a promising optical microscopy technique which enables ultrahigh-resolution, spectroscopic, *in vivo* imaging in transparent and non-transparent biological specimens. This is achieved by exploiting the short temporal coherence of ultrabroad bandwidth light sources to image morphological features at subcellular resolution at depths beyond that of conventional bright-field and confocal microscopes. Extraction of spatially resolved spectroscopic information is feasible to improve image contrast and to obtain functional or biochemical properties of the investigated tissue. The potential for using OCT to image the morphological expression of genes involved in normal and abnormal development has been shown in common developmental biology animal models. *In vivo* imaging of single cell morphology, mitosis, and migration, as well as preliminary spectroscopic imaging, is demonstrated.

OCIS codes: (170.4500) optical coherence tomography; (170.7160) ultrafast technology; (170.6510) spectroscopy, tissue diagnostics.

Introduction

Developmental and cellular biology are research fields that have burgeoned within recent years due to advances in molecular biology techniques. Optical coherence tomography (OCT) can produce high resolution cross-sectional images of biological tissue *in vivo* and in real time [1-4]. OCT has been demonstrated for high resolution *in vivo* imaging of developmental processes, including morphological abnormalities and functional parameters [5-9]. With the advent of molecular biology and genetic techniques that can site-specifically modify the genome of animal models, OCT has been shown to be a useful tool to image and trace the morphologic and functional expression of the genetic code.

The axial resolution of OCT (typically 10-15 μm) is mainly limited by the bandwidth of the low-coherence light source, usually a superluminescent diode. This resolution provides more detailed structural information of architectural tissue morphology than any other conventional technique, but is insufficient to identify individual cells or to assess subcellular structures such as nuclei or mitotic figures. The first sub-10-μm-resolution was achieved by using broadband fluorescence from organic dye [10] and from Ti:sapphire [11] lasers, but due to their low brightness, biological imaging could not be performed. Recent developments of femtosecond Kerr-lens mode locking of solid-state lasers has enabled the generation of low-coherence light for OCT with a single transverse mode and powers of more than 100 mW [12,13]. High image resolutions of 5 μm with 2-3 mm imaging penetration depths in scattering tissue using a Cr^{4+}:forsterite laser emitting light at 1300 nm has permitted microscopic visualization of dynamic morphological and functional changes that occur during embryonic development [8].

An ultrahigh resolution OCT system using a state-of-the-art, ultrabroad bandwidth, Kerr-lens modelocked Ti:Al_2O_3 laser with specially designed double-chirped mirrors has been recently developed [14]. This laser source emits sub-two-cycle pulses with bandwidths of up to 350 nm centered at 800 nm. *In vivo* subcellular imaging with longitudinal resolutions of 1 µm and transverse resolutions of 3 µm has been achieved. To our knowledge, this represents the highest axial resolution demonstrated for *in vivo* OCT imaging [15]. Since scattering is the predominant mechanism of attenuation at near-infrared wavelengths (800 nm), the imaging depth in non-transparent tissue is limited to 0.5 to 1.0 mm [16]. This depth is less compared to imaging with wavelengths in the 1300 nm region, but is sufficient to image superficial layers of biological tissues. In addition, the spectral region at 800 nm is important because it overlaps absorption features of several important biological chromophores, e.g. melanin, oxy- and deoxyhemoglobin, and may enable the functional imaging of hemoglobin oxygen saturation. Spectroscopic OCT may also be used to enhance image contrast, enabling the differentiation of tissue pathologies via their wavelength-dependent properties or functional state. This "spectroscopic staining" would be somewhat analogous to histological staining.

Ultrahigh-resolution OCT is capable of *in vivo* subcellular imaging, which may ultimately have a role in the early diagnosis of human malignancies. Neoplasias are most responsive to medical intervention at early stages, prior to undergoing metastasis. When these disorders arise from known premalignant states, and if a detection method exists, high risk populations can be screened to reduce patient morbidity and mortality. The ability to perform spectroscopic functional imaging, in addition to real-time cellular and subcellular resolution imaging, could represent a powerful tool for the early identification of neoplasms.

Cellular Morphology and Function

Imaging embryonic morphology that results from cellular differentiation is important for the understanding of genetic expression, regulation, and control. The high-resolution and high-speed imaging capabilities of OCT make it well suited as an imaging modality for biological microscopy and for the investigation of clinical disease at the cellular level. The *Xenopus laevis* (African frog tadpole) developmental animal model was selected because its development is well-characterized. This model also provides a variety of cell-types with high mitotic indices. A Cr^{4+}:forsterite laser was used for imaging mesenchymal cells (Fig. 1) *in vivo* with axial and transverse resolutions of 5 and 9 µm, respectively [12]. Cells as small as 15 µm in diameter could be imaged with this OCT system [8]. The size of most malignant cells in humans varies dramatically, showing an approximate correlation with the degree of differentiation. As a general rule, cell dimensions found in human neoplasias are typically in the range of 10-40 µm and therefore, similar in size to the cells imaged in this animal model.

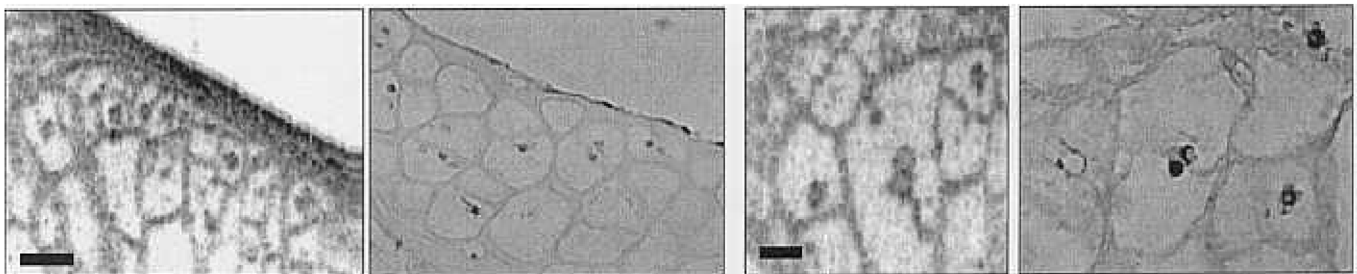

Fig. 1. OCT image correlation with corresponding histology of *Xenopus laevis* mesenchymal cells. Reproduced with permission from Ref. 8. Copyright 1999, Nature Medicine.

Mitosis is the process by which a parent cell replicates DNA and physically divides into two daughter cells [17]. Abnormal mitotic activity can result in unregulated growth, poor differentiation, and the growth of neoplasms. The ability to assess the mitotic stage of cells, *in vivo*, and to determine their state of differentiation will provide a key diagnostic for the early detection of neoplasms in humans. A sequence of OCT images following the mitotic activity of a single cell was acquired and shown in Figure 2. A number of mesenchymal cells are observed within each image. Cell nuclei and cell membranes are readily apparent as regions of high backscatter compared with the low backscattering cytoplasm. A number of cells in Figure 2A show sub-nuclear morphology, such as the regions of increased optical backscatter within the nucleus. One possibility is that these are regions of varying chromatin concentration, indicative of high mitotic activity.

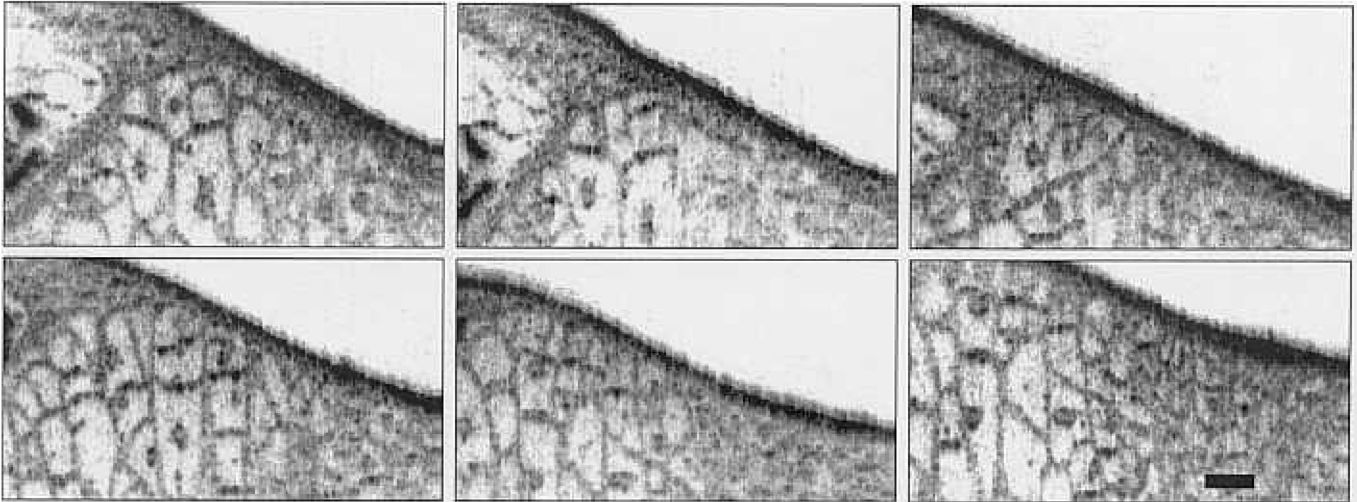

Fig. 2. OCT tracking of cellular mitosis. Reproduced with permission from Ref. 8. Copyright 1999, Nature Medicine.

Cell migration, like mitosis, has a positive role in development as well as a negative role in the spread of neoplasms. The capability of monitoring cell migration through an organism would be a powerful tool in a variety of developmental and molecular models and may have clinical applications in oncology. In developing embryos, neural crest cells originate from the newly formed neural tube and migrate to differentiate into cardiovascular and epidermal tissue. The ability of OCT to track individual cell movement has been demonstrated with neural crest cells in the *Xenopus* model [8]. In neoplasms, tumor cells will migrate through tissue, contributing to the growth and spread of the tumor. When a tumor metastasizes, tumor cells typically spread over great distances, usually through the circulation. Tumor cell migration occurs when the cell enters and exits the blood stream.

Although previous studies have demonstrated *in vivo* cellular OCT imaging of tissue morphology, most have imaged tissue at ~5-10 μm resolution, which does not allow clear differentiation of subcellular structure. Using a state-of-the-art femtosecond Kerr-lens modelocked Ti:sapphire laser as a low-coherence light source, an ultrahigh-resolution, spectroscopic OCT system has been developed [15,18]. By carefully matching dispersion (especially higher orders), minimizing chromatic aberrations, and optimizing optics, fibers, electronics, and data acquisition, a fiber-optic OCT system has been developed. This system is able to support a 260 nm spectral bandwidth and therefore enabling *in vivo* imaging with axial resolutions of 1 μm in biological tissues.

Figures 3 and 4 demonstrate the feasibility of this novel system for *in vivo* subcellular imaging of a *Xenopus laevis* (African frog tadpole). An area of 0.75 x 0.5 mm (1700 x 1000 pixels) has been imaged and is shown in Figure 3. A three-dimensional sequence consisting of eighteen tomograms spaced

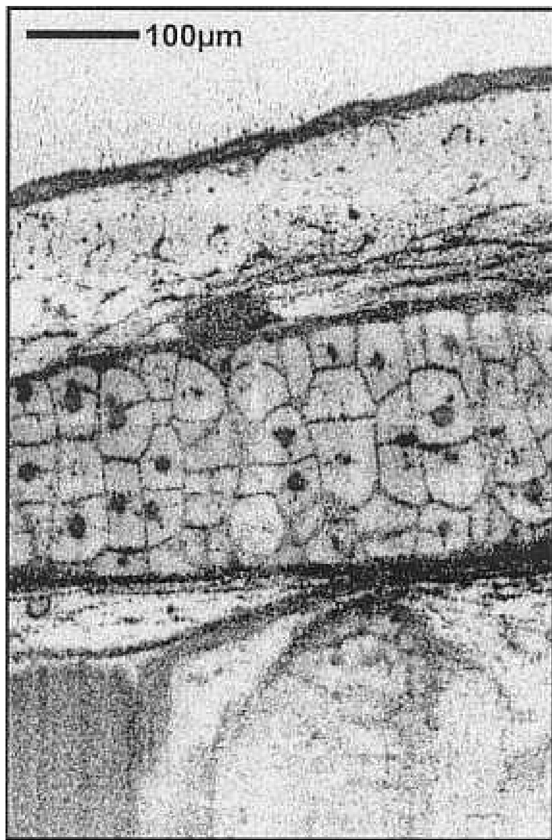

Fig. 3. ***In vivo*** **subcellular imaging at ultrahigh resolutions. Reproduced with permission from Ref. 15. Copyright 1999, Optics Letters.**

2 μm apart and covering approximately half of a *Xenopus laevis* cell is depicted in Figure 4. Each tomogram represents an area of 70 x 50 μm and consists of 170 x 100 pixels with 0.4 x 0.5 μm pixel spacing. A resolution of 1 x 3 μm (longitudinal x transverse) could be achieved in both figures. Cell membranes and cell nuclei of pleomorphic mesenchymal cells in different stages of mitosis (see two cells indicated by arrows in Figure 3), in addition to tissue morphology like the neural olfactory tract, can be visualized. To overcome depth-of-field limitation and maintain high transverse resolution at varying depths through the image, a zone-focus and image-fusion technique was used. Multiple tomograms were recorded with the focus set to different depths within the specimen, each being in focus over a depth range comparable to the confocal parameter of approximately 40 μm. The in-focus regions from each images were then fused into a single tomogram with greatly extended depth-of-field. This technique is similar to C-mode scanning used in high-frequency ultrasound imaging and has been used for the first time for OCT imaging. In Figure 4, the back surface of the cell (at 0 μm), cell membranes and nuclei, as well as intracellular morphology (especially at 20 μm to 26 μm), can be clearly visualized.

In standard OCT imaging only the envelope of the interference signal is detected. Spectral information of the investigated tissue can be obtained by measuring the full interference signal and using digital signal post processing, for example, the Morlet-Wavelet transform or the short-time Fourier transform. This extension of OCT is closely related to classical Fourier transform infrared spectroscopy and has the advantage that the spectroscopic information can be acquired at multiple wavelengths across the available bandwidth of the light source in a single measurement. As a first approach, the "center of mass" of the spectra was calculated and represented in a multidimensional map. Hue, saturation, luminance (HSL) color space (not RBG) were used to map the backscattering intensity into the saturation and the spectral center of mass into the hue, keeping luminance constant [18]. This permits the intensity and spectral shift of the backscattered light to be visualized. Spectroscopic OCT can enhance image contrast, providing additional information on tissue pathology, and may enable micron-scale, cross-sectional, functional imaging of tissue. Figure 5 shows a conventional ultrahigh-resolution OCT image (left) and a spectroscopic OCT image (right) of the same site in a *Xenopus laevis* tadpole *in vivo*. Red hue indicates a long wavelength enhancement of the backscattered light, while green hue is a short wavelength enhancement. The spectroscopic OCT image is consistent with the fact that longer wavelengths penetrate deeper than shorter wavelengths. The shallower structures have a green hue while deeper structures have a red hue. Melanocytes appear bright red in the spectroscopic OCT image, indicating that they are strongly scattering and red-shift light. The pigmented layer below the cell layer appears red in the spectroscopic OCT image. A melanocyte that is difficult to identify in the conventional OCT image is visible in the upper middle of the spectroscopic image.

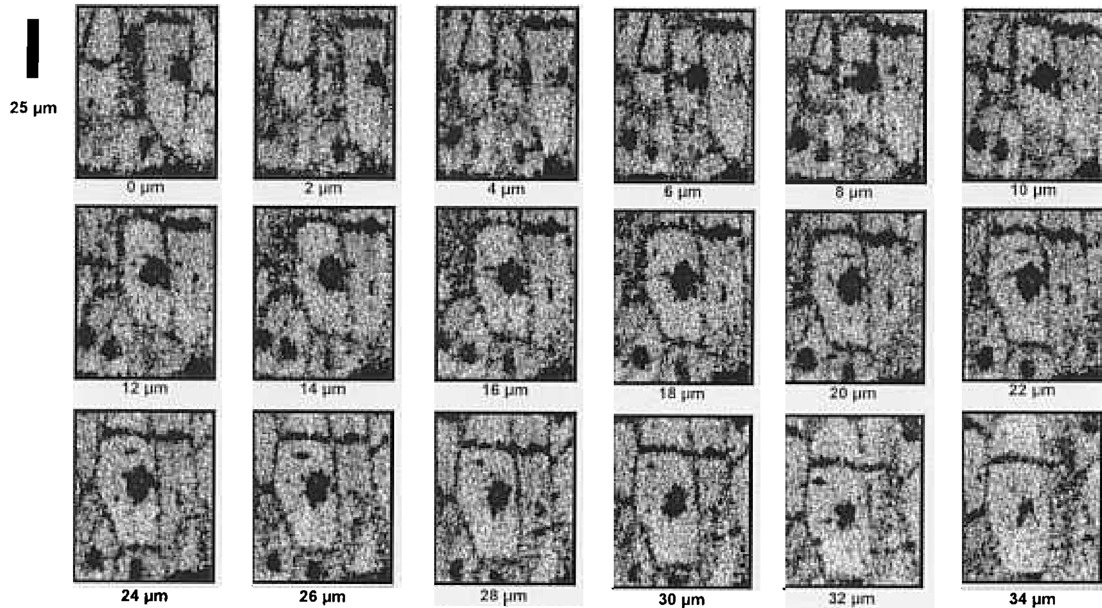

Fig. 4. Eighteen *in vivo* tomograms spaced 2 µm apart containing a 3-D volume of a *Xenopus* mesenchymal cell.

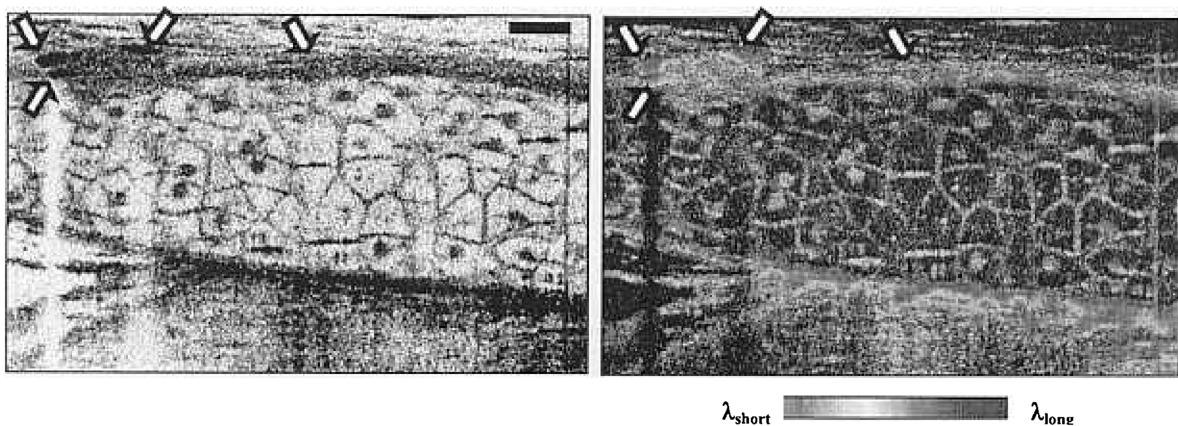

Fig. 5. Conventional (left) and spectroscopic (right) *in vivo* OCT tomogram of *Xenopus* cells. Bar represents 50 µm. Reproduced with permission from Ref. 18. Copyright 2000, Optics Letters.

Conclusions

Subcellular functional imaging is demonstrated in a living, nontransparent, organism with ultrahigh-resolution and spectroscopic OCT. These results suggests a feasibility for assessing neoplastic changes in humans. The observations of greatest clinical relevance were the ability to identify active cell division and assess nuclear-to-cytoplasmic ratios, two important markers of malignant transformation. A change in the backscattering intensity from nuclei was also noted as a function of time. This is postulated to represent a change in the concentration or packing of chromatin, which may be of diagnostic relevance. An extension of OCT yields not only dramatic improvements in OCT resolution, but also spectroscopic imaging in order to obtain functional or biochemical properties of the investigated tissue. At the same time, contrast is enhanced in a manner somewhat analogous to staining in histopathology.

The future potential of OCT in developmental and cellular biology is extremely promising. Visualizing and studying many developmental processes, those known as well as those yet undiscovered, may only be possible with the unique imaging capabilities of this technology. Ultimately, OCT can help contribute to the scientific understanding of how a single fertilized egg develops into a complex, multi-system organism. Beyond this scientific investigation, OCT can contribute to the understanding of developmental processes which have the long-term potential to improve clinical therapies and patient care.

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