patología
Revista latinoamericana

Patología 2012;50(3):179-181

Artículo original

Optical digital biopsy: A new method of tissue and cell identification with ophthalmic applications

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The concept of "optical digital biopsy" comprises of two essential components: firstly to capture a high resolution in vivo digital image and then secondly, to unmask the digital image by post processing means so as to achieve tissue and cell identification. Because of optical clarity and anatomic location, both anterior segment and the posterior segment structures of the eye lend itself to imaging. Particularly retina and retinal pigment epithelium (RPE) are amenable to assessment by optical coherence tomography (OCT), a widely used non-invasive technique for high resolution and cross-sectional tomographic imaging.[1,2] With rapid improvements in technology and shift to Fourier-domain (FD) OCT, it is possible to capture retinal images 40 to 50 times faster and with higher resolution (axial resolution of less than 6 microns) than standard time domain (TD) OCT.[3]

Since 1995, we have been exploring the possibility of "digital staining" for certain substances and structures such as proteins, pigments, immunoglobulin deposits, and synaptic interconnections in the retina (unpublished data). We have been able to perform specific "digital staining" of fibrous, muscle, adipose or vascular tissue component. This has led us to realize that high resolution digital images can be unmasked to reveal tissue and cellular composition both in normal and pathological conditions (optical digital biopsy).

As a first step to ophthalmic applications of "optical digital biopsy", we started with the high resolution images of histological slides of normal and diseased retina stained with hematoxylin and eosin (HE), and through digital post processing of these images, we were able to obtain images that resembled those generated by SD OCT of retina cellular layers (Figure 1) and drusen deposits in the Bruchs's membrane (Figure 2). The next step was to reverse the process, that is to start with high resolution retinal SD OCT images and through digital post processing of these images to unmask and identify tissue and cellular compositions generating virtual histopathology (optical digital biopsy).

We report our initial observations on the technique of "optical digital biopsy" and its applications in a variety of diseases involving the vitreous, retina, retinal pigment epithelium, and choroid.

MATERIAL AND METHODS

Cleveland Clinic and University of Buenos Aires Institutional Review Board approval, was obtained. The final ophthalmic diagnosis was based on clinical history, ophthalmoscopic examination, angiographic studies, ultrasonography, and biopsy (when indicated). We evaluated 52 cases of a variety of diseases involving the vitreous, retina, retinal pigment epithelium, and choroid (Table 1).

SD-OCT examination

All patients underwent SD-OCT examinations through a dilated pupil by a trained operator. 3D OCT-1000 (OCT-

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Received: May 2012; Accepted: September 2012.

This article must be quoted as: Zárate JO, Pelayes D, Singh AD. Optical digital biopsy: A new method of tissue and cell identification with ophthalmic applications. Patología Rev Latinoam 2012;50(3):179-181.

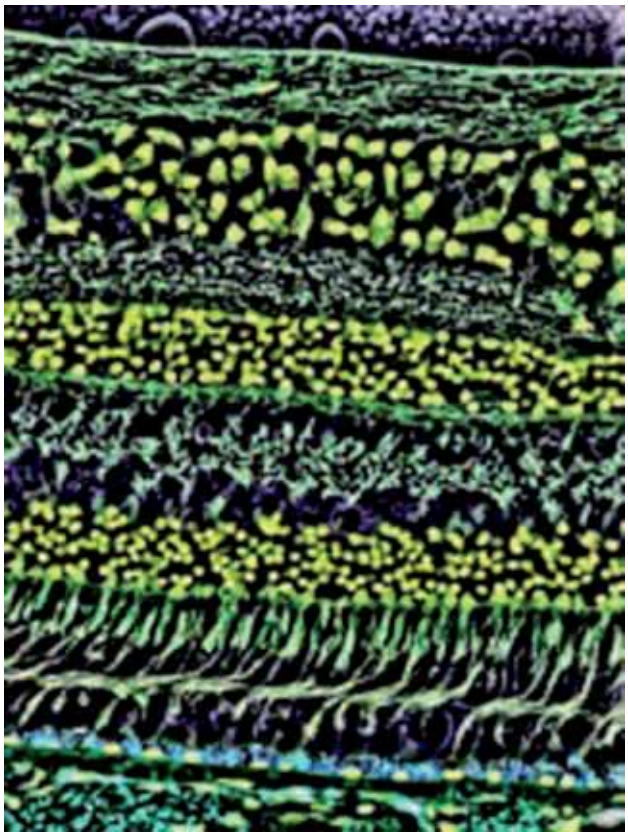

Figure 1. SD OCT of retina cellular layers. For color images from this paper see Annex 1.

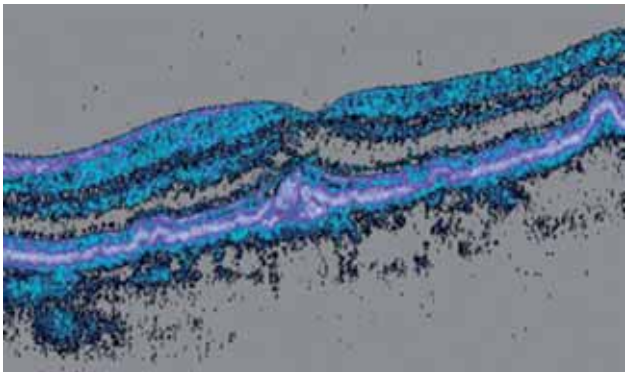

Figure 2. Drusen deposits in the Bruchs's membrane.

1000; Topcon Inc., Paramus, NJ) was used in this study. The axial resolution of the 3D OCT-1000 is less than 6 microns with the data-acquisition speed of 18,000 A scans/second. For the study, patients were imaged using a "3D-scan" (128 B-scans × 512 A-scans, covering a retinal area of 6.0 × 6.0 mm).

Table 1. Evaluation of 52 cases of a variety of diseases involving the vitreous, retina, retinal pigment epithelium, and choroid

Disease	*Number*
Vitreous-retinal lesions (diabetic)	10
Drusen	10
Choroideal melanomas	8
choroideal and retinal vascular lesions	8
macular holes	8
vitreoretinal lymphoid lesion	6
Choroideal nevus	2

Digital Processing

The digital processing of the SD OCT images was performed by using multiple sequential standardized steps categorized into 3 basic algorithms based upon presumptive clinical diagnosis: Degenerative lesion (I), Neoplastic lesion (II), and Vascular lesions (III). The algorithms involve changes in color balance (red, green and blue), changes in hue and saturation, adjusting the color levels and its curve (principal, red, magenta, blue, cyan, green and yellow) and subsequent corrections to highlight structures with high resolving power so as to establish cell and tissue characteristics. The automatic settings that exist within the commercially available imaging programs are essential to keep images as pure as possible but may be altered to enhanced cellular morphology by overcoming pixilation to obtain cell rounding.

RESULTS

In all cases was reached a diagnosis morphological injury. In 45 it was possible to make a true histopathological diagnosis. Choroideal cellular melanocitic nevi 5, Choroideal melanoma 25, choroideal melanoma with linphocitic infiltration 1, choroideal melanocytoma 4, choroideal metastatic tumours 2, drusen 5, lymphoproliferative diseases 5 and others 5.

In 10 cases were subject to descriptive morphological diagnosis: retinochoroideal inflamation pattern 5, pigmentary epithelium detachment 2, and epiretinal fibrovascular membrane 3.

In 5 cases, the method could not speak clearly enough of structural changes, because the cellular and tissue image were not morphological representative of lesions.

In 5 malignancies could, with the method, trapping the DNA of the cells that compose it.[4]

DISCUSSION

For a long time we tried to infer tissue morphology of non-invasive methods.[5,6,7] The concept of "optical digital biopsy" comprises of two essential components: firstly to capture a high resolution in vivo digital image and then secondly, to unmask the digital image by post processing means so as to achieve tissue and cell identification.

The first essential component of "optical digital biopsy" is widely used SD spectral domain OCT that generates high resolution images. The novelty of our concepts lies in the unmasking algorithms that we have created leading to identification of tissue and cell composition.

The quality of a digital image depends on many factors, some of which are purely subjective. But three inter related factors are the most important, and that can be measured: the amount of detail, contrast level of the detail that determines the definition, and the image resolution (pixel/μ). Other variables such as gamma tonal range of the tonal scale, the Acutance and digital noise were also evaluated. We also considered the so-called contrast transfer function, tonal rendition or spatial frequency response (modulation transfer function). The stepwise application of software algorithms is based upon suspected diagnosis for optimal resolution so as to identify tissue and cellar composition.

In summary, we report a novel methodology for the processing in vivo OCT digital images that can discern normal and pathologic structures in a variety of vitreo retinal and choroidal diseases by generating virtual histopathology. Optical digital biopsy (CCF-019620 us pat) is a non invasive technique by which tissue and cellular composition can be achieved. Refinements of technique and additional validation studies are necessary before optical digital biopsy is clinically applied.

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