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Mini Review

New Methods for Spatial Analysis of Thickness in Optical Coherence Tomography

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Abstract

The retina is the most accessible alive neuronal tissue and is relevant not only for ophthalmology but also for neuroscience, because of the increasing evidence that it shows degeneration processes similar to those found in the brain of patients with Alzheimer's disease and Amyotrophic Lateral Sclerosis. Optical Coherence Tomography (OCT) provides retinal scanning noninvasively, in few seconds, at low cost, and with a spatial resolution thousand times finer than what today is feasible in the living brain. But OCT data have some features derived from its 3D structure that render unsuited classical data analysis methods. Current proposal is to systematically incorporate to their analysis new techniques including geometric corrections of each OCT to eliminate noise inherent to the technique, spatial normalization of all OCTs to achieve their anatomical correspondence, and advanced statistical techniques-based on theories of random fields or random permutations- to precisely locate thickness changes in total retina or in its layers. These methodologies are already available and have proven their usefulness when applied to OCT data to reveal the localization, size and shape of lesions caused in ten retinal layers by Alzheimer's disease.

Keywords: *Optical Coherence Tomography (OCT); Alzheimer's Disease; Amyotrophic Lateral Sclerosis*

Introduction

The retina is the most accessible alive neuronal tissue and is relevant not only for ophthalmology but also for neuroscience, because of the increasing evidence that it shows degeneration processes similar to those found in the brain of patients with Alzheimer's disease and Amyotrophic Lateral Sclerosis. Optical coherence tomography (OCT) is the technique enabling its direct observation: it provides 3D scanning of selected retinal pieces non-invasively and with a spatial resolution one thousand times finer than most techniques for observation of the working brain, such as CT, MRI, PET or MEG; and data can be obtained in few seconds, in contrast with a lot of minutes required for the brain. The investment in OCT is about ten times less expensive that imaging techniques used in brain studies, because the cost of the equipment is much lower, it only requires the space of a standard table and magnetic or radiation shielding of the room is not needed.

Alzheimer's disease (AD) and Amyotrophic Lateral Sclerosis (ALS) are two relevant neurodegenerative diseases which are being investigated in our research group and are an increasing health issue for elderly people and healthcare services in all developed countries. There is wide agreement about the relevance of early disease detection, leading to interest in early, convenient and affordable biomarkers. The brain is the main tissue affected in AD and the retina is the only neuronal tissue that can be accessed non-invasively. Increasing evidence suggests that retinal analysis can provide insights into brain pathology; Mutlu., *et al.* found that a thinner ganglion cell layer (GCL)

of patients with mild AD, nerve fiber layer (NFL) and inner plexiform layer (IPL) are associated with smaller grey matter, white matter and hippocampal volume [1]. Ong *et al*. found that thinner retina is associated with smaller grey matter volume only in the temporal lobe, whereas thinner GC-IPL complex is associated with smaller grey matter and white matter volumes in the temporal lobe, as well as smaller grey matter volume in the occipital lobe [2]; Casaletto., *et al.* found retinal and GCL thinning to be related to medial temporal lobe atrophy [3]; Salobrar-Garcia *et al.* [4] brought evidence that peripapillary total retinal thinning correlates with AD development in patients with early-stage AD; more recently, our group has found that retinal thinning in the macular area appears at a very early stage of AD [5], together with a 40% decrease in contrast sensitivity [6]; all these findings converge to demonstrate that the volume of brain structures involved in AD is related to retinal thickness and visual function, and suggest that AD-associated neuronal damage and amyloid deposits may occur in the retina before they can be detected in the brain, implying that retinal analyses could allow AD detection during the asymptomatic preclinical period [7,8].

Concerning ALS, Rojas *et al.* [9], based also in OCT retinal scanning, found that that (1) comparing ALS baseline with control baseline, the thickness of macular temporal and inferior areas of the inner macular ring appears significantly increased; (2) in ALS follow-up vs. ALS baseline, there is a significant macular thinning in the inner and outer macular ring inferior areas; (3) in ALS follow-up with respect to ALS baseline, there is a significant thinning of the peripapillary retinal nerve fiber layer (pRNFL) in the superior and inferior quadrants; and (4) ALS patients showed a moderate correlation between some OCT pRNFL parameters and Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) score.

OCT provides a huge amount of structural data of the retina and its different layers, but such data have specific characteristics that should not be overlooked at the time of their analysis. Three of them, with their implications for analysis, are now reviewed: measurement errors, that require being corrected before analysis; anatomical mismatching among OCTs of different subjects, that requires previous co-registering or spatial normalization; and strong spatial autocorrelation -thickness of any retinal point is similar to that of its neighbours- which discourages the use of classical statistical methods in favour of new approaches. These problems and the proposed solutions are now described.

Correction of OCT errors

When the retina or any of its layers is not perpendicular to the direction of the scanning light beam (A-scan direction), then measured thickness T' exceeds the true thickness value T. There are at least three types of retinal tilt causing this problem and that can be labelled according to their origin [10]. The first is global tilt, that makes the retinal OCT image to appear tilted and which is mainly caused by pupil eccentricity at the entry of the OCT beam [11]; this tilt is constant over the scanned area and reached 10.0º in our study, a value quite close to 11º reported by Antony *et al* [12]. A second type is spherical tilt, which is due to natural curvature of the eye: it increases radially, from zero near the centre of the scanned area up to 7.0º at its corners. The third type is local tilt, which is due to thickness variation of retinal layers that leads them to appear wavy; it varies from point to point of the scanned area and reached 14.4º in the NFL very close to the fovea centre of a subject of our study. The three types of tilt are combined as explained in Jañez-Escalada *et al.* [10] to get the true thickness value T from the measured thickness T'.

Spatial normalization for anatomical matching among subjects

OCT data from different subjects need to be averaged when two or more groups are compared. But spatial maps of thickness from different subjects should not be directly averaged, because in practice the retinal pieces scanned differ from one subject to another both in anatomical and functional sense. Certainly the scanned regions from any pair of subjects may be centred at the same anatomical point (the fovea or the optic disc), but they differ in their rotation because the head tilt of each subject is not sufficiently controlled during OCT acquisition; the inclination of their maculopapilar axes is different too; and real size of the scanned area always differ from the nominal 6 x 6 mm^2, as a consequence of eye size, its elongation and lateral magnification. The variability brought by these factors, if not corrected,

adds noise to the data and increases the risk of the effects investigated not reaching statistical significance. Luckily that variability can be removed by spatial normalization: all OCTs should be moved, rotated and scaled so that their macular and papillary centres get overlapped [10].

New statistical data analysis to identify the true size and shape of regions affected by disease

Up to now, when the researcher or the practitioner wonder about the retinal regions affected by a disease, answers they got are frequently less informative than expected. OCT provides reflectivity measurements for many thousands of retinal points and one would expect the damaged regions be delimited with similar precision. But the answers obtained only refer to the presence or absence of effects in very few regions whose position, size and shape are predefined: 9 concentric circular sectors for ETDRS, 12 circle sectors for clock grid, or nxn squares for rectangular grids. Thus, the fine spatial resolution of the OCT is finally lost and reduced more than one thousand times, which means giving up most knowledge of the position, size and shape of damaged regions. As explained elsewhere [10], this is a consequence of using data analysis methods which are not well suited for OCT data. Most research and clinical OCT based studies, including most of those cited previously, draw their conclusions through classical data analysis (Student's t-test, ANOVA, etc.) using Bonferroni 's correction for multiple comparisons; nevertheless this type of correction is inappropriate for OCT data because is designed for independent comparisons whereas OCT data a very correlated; the consequence is that the threshold for statistical significance gets higher than it should be; as a result, many statistically significant differences may be missed, thus slowing down knowledge progress and increasing the number of contradictory results. The issue has been solved for OCT data [10] by developing a new data analysis methodology based on statistical parametric maps [13], whose critical values for statistical significance are provided by Random Field Theory (RFT) [14,15] in a parametric approach or by Random Permutations Theory (RPT) in a non-parametric one [16].

Usefulness and results of the methods proposed

The methods proposed above have already shown their usefulness [10]. In a sample of only 19 patients in a very early stage of AD and 24 controls, ten retinal layers have been automatically segmented and studied: nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), inner segments/outer segments layer (IS/OS), outer segment layer (OSL), outer segment PR/RPE complex (OPR) and retinal pigment epithelium layer (RPE).

Results from the application of the above methodology revealed that all 10 layers showed AD-related thinning over a relevant percent of their surface, and thinning reached statistical significance at various locations in NFL, GCL, IPL, INL, OSL and total retina. All layers except INL and OS also showed thickened regions, though their thickening did not reach statistical significance. In neural layers thinned regions showed a statistically significant increase in area over that of thickened ones, whereas the opposite was observed in the RPE. Volume lost in thinned regions of neural layers was greater than volume gained in thickened regions, the difference being statistically significant; the opposite was again observed in the RPE. Across different retinal layers, thinned regions appeared to co-localize while thickened regions appeared to "avoid" co-localization, in both cases to a statistically significant extent.

A key difference between the proposed methods and those commercially available is that commercial methods provide the mean thickness of each retinal layer in big and geometrically predetermined regions. Our method, in contrast, explores a 6 x 6 mm^2 square at the pixel level and without prior constraints, thus avoiding the risk that geometrically predetermined regions may allow cancelling positive and negative effects occurring within the same region and consequently masking clinically relevant differences. Our spatially unconstrained analysis of retinal layers allows to identify where each layer is truly affected by the disease. Tilt corrections and spatial normalization enable more efficiency in research through noise removal from data. RFT-based statistical analysis allows precise identification of the location, extension and real shape of damaged retinal regions. This theoretical approach is well established in brain research from some decades ago, but our work appears to have been its first systematic application to OCT studies of retinal thickness. In summary, RFT- and RPT-based statistical methods have four main advantages over classical ones: (1) they allow search for thickness changes at pixel reso-

lution, giving much finer spatial resolution than previous studies based on predefined geometric grids [17,18]; (2) they provide precise information about the spatial localization, shape and size of thinned (and thickened) regions in each layer; (3) they avoid masking and biasing results as a consequence of the shape and location of predefined search regions, such as ETDRS or rectangular grids; and (4) they are better suited for multiple comparisons than Bonferroni correction because they take into account the spatial autocorrelation inherent in OCT data. Thus, they may contribute to solving existing controversies in research and to providing more accurate and informative analyses of changes over time in normal and pathological retinas in clinical practice.

The methods described here may be also useful for other types of retinal studies and other disease contexts. They are now being extended to compare successive OCTs of the same person, what may expand their usefulness to clinical practice.

Conclusion

OCT based research and clinical practice may benefit from three methodological advances: a) to apply geometric corrections of global, spherical, and local tilts to reduce the noise inherent to each OCT data set; b) to perform spatial normalization of all OCTs before calculating indices of centrality and variability in each group of subjects or in each sequence of OCTs of the same person; and c) to apply the statistical analysis techniques based on theories of Random Field or Random Permutations to get precise knowledge about the location, size and true shape of the retinal regions affected by the disease.

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