Characterization of the retinal changes of the 3×Tg-AD mouse model of Alzheimer’s disease

Hugo Ferreira1 · João Martins1,2 · Ana Nunes1 · Paula I. Moreira2,3 · Miguel Castelo-Branco1,3 · António Francisco Ambrósio2,3,4 · Pedro Serranho1,5 · Rui Bernardes1,3 ©

Received: 31 January 2020 / Accepted: 12 February 2020 © The Author(s) 2020

Abstract
Alzheimer’s disease (AD) is a progressive neurodegenerative disorder whose diagnosis remains a notable challenge. The literature suggests that cerebral changes precede AD symptoms by over two decades, implying a significantly advanced stage of AD by the time it is usually diagnosed. In the study herein, texture analysis was applied to computed optical coherence tomography ocular fundus images to identify differences between a group of the transgenic mouse model of the Alzheimer’s disease (3×Tg-AD) and a group of wild-type mice, at the ages of one and two-months-old. A substantial difference between groups was found at both time-points across all neuroretina’s layers. Here, the inner nuclear layer stands out both in the level of statistically significant differences and on the extension of these differences which span through the imaged area. Also, the progression of AD is suggested to be spotted by texture analysis as demonstrated by the significant difference found in the inner plexiform and the outer nuclear layers from the age of one to the age of two-months-old. These findings demonstrate the potential of the use of the retina and texture analysis to the diagnosis of AD and monitor AD progression. Besides, the differences between groups found in this study suggest that the 3×Tg-AD model may be inappropriate to study early changes associated with the AD and other animal models should be tested following the same path and rationale. Moreover, these results also suggest that the human genes present in these transgenic mice may have an impact on the neurodevelopment of offspring which would justify the significant changes found at the age of one-month-old.

Keywords Optical coherence tomography · Retina · Biomarkers · Texture analysis · Alzheimer’s disease · Mouse model · 3×Tg-AD · Early diagnosis

1 Introduction
Diagnosis of Alzheimer’s disease (AD) remains a major challenge. Simple diagnostic to identify early biomarkers for tracking the onset and progression of AD would be of utmost importance. In the USA alone, 5.6 million people aged 65 and older lived with AD in 2019, that is, one in ten people (10%) in this age-group [1]. With the increase of life expectancy for the world’s population, 64.2 years in 1990, 72.6 years in 2019 and 77.1 years expected in 2050 [2], the prospect is to an increase in the population with AD in the near future, even though epidemiological evidence suggests a potential reduction in dementia incidence [3].

Based on Pittsburgh Compound-B (PiB) studies, it is hypothesised that cerebral changes precede AD symptoms by over two decades [4, 5]. This substantial period implies a significantly advanced stage of AD by the time it is usually diagnosed. Furthermore, a definite diagnosis can only be performed post-mortem [6]. These challenges make it difficult to access the first pathophysiological changes caused by AD and to track their progress during the prodromal stages of the disease.

An alternative diagnostic approach that gained momentum in recent years is the use of the retina as a window into the brain [7, 8]. The supporting rationale is that the
retina and the brain have the same embryonic origin [5], which makes the retina the only part of the central nervous system (CNS) directly accessible through non-invasive optical means. This hypothesis allows performing non-invasive studies, in vivo and in situ, at any time along the lifespan of the person or animal being studied.

Conflicting reports exist on the findings of one of the hallmarks of AD, the accumulations of Amyloid-β (Aβ), in the retina [9]. Other biomarkers of AD were studied based on the hypothesis that AD is a vascular disorder [10]. Alternative biomarkers were also put forward, like the thinning of the retinal nerve fibre layer (RNFL) in the eyes of AD patients [11, 12]. Here, a particularly confounding factor is the fact that RNFL thinning is not exclusive to AD [13].

We have put forward alternative biomarkers based on the analysis of computed fundus images for individual layers of the retina [14]. Also, we recently demonstrated that texture biomarkers allow not only to distinguish AD patients from age-matched healthy controls correctly but also to distinguish them from age-matched Parkinson’s disease patients [15], thus demonstrating the potential of texture biomarkers for the study of neurodegenerative disorders [16].

To address the fundamental question of whether the retina holds vital information towards AD diagnosis and, if so, how early can changes be identified, an ongoing project is imaging triple-transgenic mouse model of AD (3×Tg-AD) mice since they express three major genes associated with familial AD, namely APPswe, PS1M146V, and tauP301L, and develop the pathological hallmarks of disease in an age-dependent manner [17, 18]. These animals are being imaged from the age of one-month-old, every month up to the age of four-months-old, and at the ages of eight-, twelve-, sixteen-, and twenty-four-months-old. Preliminary results using this mouse model [19, 20] suggest that texture analysis can be successfully used for this purpose.

As part of the research project mentioned above, in this work, we identify the texture features that present statistically significant differences between the mouse model of AD and the healthy control groups, at the ages of one and two-months-old, and report the image processing steps towards the computation of these features.

2 Materials and methods

2.1 Sample and study groups

The mouse model of AD used in this work is the 3×Tg-AD, while the healthy control mice used for comparison are wild-type (WT) mice (C57BL6/129S background). This study was approved by the Animal Welfare Committee of the Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra. All procedures involving mice were conducted as per the Association for Research in Vision and Ophthalmology statement for animal use, and in agreement with the European Community Directive Guidelines for the care and use of non-human animals for scientific purposes (2010/63/EU), transposed into the Portuguese law in 2013 (DL113/2013).

Optical coherence tomography (OCT) data was gathered (when possible) from both eyes of 35 WT and 35 3×Tg-AD mice. This would provide 70 eye scans per group. However, some were excluded from the study due to poor image quality, yielding a total of 68 and 65 eye scans, respectively for the WT and the 3×Tg-AD groups, for the age of one-month-old and, 60 and 64 eye scans at the age of two-months-old.

2.2 Data acquisition and characterization

Mice were imaged by the Micron IV OCT System (Phoenix Research Labs, Pleasanton, CA, USA). All eye volume scans, made of 512 non-compressed B-scans TIFF files (of 512 A-scans each), were taken at the same retinal location, horizontally centred on the optic disc and vertically above the optic disc’s border.

OCT B-scan images allow distinguishing between several retinal layers in a way that each visible retinal layer corresponds to histological reports of the retina [21, 22]. However, from a computational point-of-view, retinal layer segmentation from OCT data is hampered by the low signal-to-noise ratio (SNR), common in this data. The main reason is speckle noise, intrinsic to low-coherence interferometry techniques, which cannot wholly be removed by digital means. To this end, some pre-processing techniques are applied in order to improve the quality of the segmentation.

2.3 OCT data pre-processing

Data used in this work was pre-processed in two steps, aiming to centre and align the location of the retina within each B-scan. This normalisation allows us to know, a priori, the rough location of the different retinal layers, which will stretch horizontally along the B-scan. Mice, as opposed to humans, do not possess macula, leading to homogeneous layer thickness throughout the imaged retina.

Two average A-scans (avgA) are computed for each B-scan, one for the left and another for the right halves of the B-scan. Each is then low-pass filtered to achieve only two local maxima (Fig. 1), one due to the hyper-reflectivity of the RNFL and one due to the hyper-reflectivity of the retinal pigmented epithelium (RPE), respectively the left and right peaks shown in Fig. 1.
Fig. 1 Example of an average A-scan low-pass filtered (vitreous, retina and choroid - from left to right). Two distinct peaks are present, one due to the retinal nerve fibre layer (RNFL - left peak) and one due to the retinal pigment epithelium (RPE - right peak).

One then determines the midpoints between the two peaks for each of the left and right half average A-scans and determines the line that crosses both of them, as illustrated in orange in Fig. 2 (left). Then each A-scan of the B-scan is shifted (in the depth direction) so that this line becomes horizontal in a predefined depth position (in red, in Fig. 2), as illustrated in Fig. 2 (right).

2.4 Retinal interface detection

Many segmentation methods for OCT data have been reported [23, 24], most for OCT data of human retinas. However, these do not perform well when applied to mouse retinas because of the low contrast between retinal layers when compared to humans. Furthermore, because the mouse retina does not have a macula/foveal depression, retinal layers present a homogeneous thickness, and all retinal layers are present in the entire data volume of the OCT.

Taking advantage: 1) of the homogeneous thickness of each retinal layer across each B-scan; 2) that consecutive layers present distinct intensity levels, and; 3) of the horizontal distribution of the layers following the pre-processing steps, we compute a local metric defined by:

$$R(i, j) = \frac{C^u(i, j)}{C^d(i, j)},$$  
(1)

at each B-scan’s pixel. To speed up the process and because the location of the retina within the B-scan is approximately known, after the pre-processing, this process can be run only in the region of interest within the B-scan.

The metric defined in Eq. 1 is the ratio of the convolution of the B-scan image ($I$) with the two kernels, of size $M \times N$, defined in Eqs. 2 and 3, respectively, $\omega^u$ and $\omega^d$, with $C^\gamma = I * \omega^\gamma, \gamma = \{u, d\}$.

$$\omega^u(s, t) = \begin{cases} 1 & 0 \leq t \leq \frac{N}{2} \text{ and } \frac{M}{N}t \leq s \leq -\frac{M}{N}(t - N), \\ 0, & \text{otherwise} \end{cases}$$
(2)

$$\omega^d(s, t) = \begin{cases} 1 & \frac{N}{2} \leq t \leq N \text{ and } -\frac{M}{N}(t - N) \leq s \leq \frac{M}{N}t, \\ 0, & \text{otherwise} \end{cases}$$
(3)

where Eq. 3 is the 180-degree rotation of Eq. 2, as illustrated in Fig. 3.

Filtering each B-scan with any of these kernels produces a scaled version of a low-pass filtered B-scan with the scaling parameter cancelling out by the ratio. The ratio $R(1)$ presents a maximum for transitions from hyper- to hypo-reflective layers, and a minimum for transitions from hypo- to hyper-reflective layers, in the top to bottom direction. This is illustrated in Fig. 4. The size of the kernel used was adjusted to the thickness of the layers at hand, and the triangular shape allows avoiding the influence of immediate neighbouring A-scans at the interface location, accommodating small local variations of the interface to the horizon.
2.5 Segmentation

The histology of the mouse retina is well known and well documented in the literature [21, 22]. Therefore, the number of retinal layers and their characteristics, including their average thickness, and the relation between the thickness of different layers is well known. Also, the layered structure of the retina is evident in the B-scan images, as shown in Fig. 4. At the interfaces between layers, there is a transition in the intensity level from one layer to the next, which matches with local maximum/minimum of R, as defined in Eq. 1.

The segmentation of each B-scan is processed in three steps. First, two interfaces are determined, one corresponding to the transitions between the vitreous and the RNFL – the inner limiting membrane (ILM) –, and the interface between the outer segment of the photoreceptors (OS) and the RPE – the OS-RPE –, as the RNFL and the RPE are the most hyper-reflective layers in the retina.

The second step uses the interface OS-RPE as a starting point to search for the location of the interface between the outer nuclear layer (ONL) and the inner segment of the photoreceptors (IS) – ONL-IS – as the one presenting a local maximum in the R mapping (1) as shown in Fig. 4 (centre). Having determined the ILM and the ONL-IS interfaces, the region of the retina for analysis within this work, the neuroretina, is now well established.

Finally, the remaining interfaces between the layers of the neuroretina – the RNFL, the ganglion cell layer (GCL), the inner plexiform layer (IPL), the inner nuclear layer (INL), the outer plexiform layer (OPL), and the ONL–, can now be estimated resorting to the knowledge of the relation between the thickness of these layers, drawn from histological studies [21, 22], by mapping their relations to the region of the B-scan within the established limits.

The result of this approach can be seen in Fig. 5, where the mapping mentioned above determined the additional interfaces.

2.6 Texture features of OCT data

Texture provides information on the spatial distribution of pixels’ intensity in a region or a whole image, for both grayscale and colour [25]. In general, two types of texture can be considered: pattern texture and random texture. While the first exhibits a visible regularity, the latter does not [25].

For each of the segmented layers, a mean-value fundus (MVF) image [14] was computed as the average of the A-scan values between the boundaries of the layer, i.e. none of the remaining A-scan values is taken into account.

MVF images were split into squared blocks of 20 pixels each, to capture local texture information, yielding $24 \times 24$ blocks per image, therefore cropping MVF images from their original $512 \times 512$ pixels size to $480 \times 480$ pixels, discarding pixels from the borders of the image. Also, the number of grayscale levels of the images was reduced from 65536 (16 bits) to 16 (4 bits).

The Gray-Level Co-occurrence Matrix (GLCM) [26] was used to determine texture information for each block.
Fig. 5 Result of the segmentation process. Solid yellow lines show the segmented interfaces. Dashed lines show the interfaces as determined based on the average layer thickness for mice – drawn from known histology [21, 22].

Furthermore, four directions (0°, 45°, 90° and 135°) with symmetry ON (i.e., 180° apart angles are considered the same) and scale (pixel distance) of one pixel, led to four GLCMs per block. For each of these, 20 features were determined: a) Inverse Difference Moment/Energy, b) Contrast/Inertia, c) Correlation, d) Angular Second Moment/Uniformity, e) Sum Average, f) Sum of Squares, g) Sum Variance, h) Sum Entropy, i) Difference Variance, j) Difference Entropy, k) Information Measure of Correlation 1 (IMC1), l) Information Measure of Correlation 2 (IMC2), m) Entropy, n) Dissimilarity, o) Autocorrelation, p) Maximum Probability, q) Cluster Prominence, r) Cluster Shade, s) Inverse Difference Normalized (INN) and t) Inverse Difference Moment Normalized (IDN). These are defined in [26] (a to m), [27] (n), [28] (o and p), [29] (q and r), and [30] (s and t). Each feature is considered as the maximum across the four directions to decouple features’ values from the orientation, resulting in 20 features per block.

Blocks were then aggregated into quadrants by defining each quadrant feature’s value as the average of 12 × 12 blocks. In this way, a total of 480 features (20 features × 4 quadrants × 6 layers) characterise each retina.

2.7 Statistical analysis

The Kolmogorov-Smirnov normality test was applied to the 480 features to test normality in each group. Since the results indicated that the distributions are not normal for most features, we used the non-parametric Mann-Whitney test to determine differences between WT and 3×Tg-AD groups, considering significance levels of 5%, 1%, and 0.1%.

All data processing was performed using Matlab R2018a (The MathWorks Inc., Natick, MA, USA), and the statistical treatment was made using IBM SPSS 25.

3 Results

The number of features with significant statistical differences between groups, per layer and time-point, are presented in Table 1.

Please note that the number of features disclosed takes into account the same feature if it is present - that is, showing statistically significant differences between

| Table 1 | Number of significant features per layer, at the ages of one and two-months-old |
|---------|---------------------------------|
|         | Number of features | Two months old | Number of significant features | Two months old |
|         | p ≤ 0.001 | p ≤ 0.01 | p ≤ 0.05 | p ≤ 0.001 | p ≤ 0.01 | p ≤ 0.05 |
| RNFL | 19 (23.8%) | 29 (36.3%) | 35 (43.8%) | 29 (36.3%) | 32 (40.0%) | 39 (48.8%) |
| GCL | 27 (33.8%) | 38 (47.5%) | 50 (62.5%) | 40 (50.0%) | 52 (65.0%) | 61 (76.3%) |
| IPL | 25 (31.3%) | 38 (47.5%) | 47 (58.8%) | 45 (57.5%) | 59 (73.8%) | 66 (82.5%) |
| INL | 48 (60.0%) | 61 (76.3%) | 69 (86.3%) | 59 (73.8%) | 67 (83.8%) | 71 (88.8%) |
| OPL | 33 (41.3%) | 48 (60.0%) | 55 (68.8%) | 19 (23.8%) | 25 (31.3%) | 35 (43.8%) |
| ONL | 33 (41.3%) | 41 (51.3%) | 47 (58.8%) | 46 (57.5%) | 53 (66.3%) | 64 (80.0%) |
| Total | 185 (38.5%) | 255 (53.1%) | 303 (63.1%) | 239 (49.8%) | 288 (60.0%) | 336 (70.0%) |

The total number of features per layer is 80
Table 2 discloses all texture features presenting statistically significant differences between groups at both time-points, i.e. at the age of one and two-months-old, and at the same locations (same quadrants). While it is quite notorious the widespread differences across the several layers and the imaged area, as shown by the number of quadrants involved per textures feature and layer, the INL stands out again because of the highest level of significance and the extension of the differences spreading through the entire imaged area of the retina.

Also of notice, is the steady increase in the number of statistically significant differences from the first to the second time-points in all layers but the OPL.

Furthermore, even though the total number of features presenting statistically significant differences increased...
from 306 to 330, ~8% increase, the notorious fact is that there was a significant increase at the highest significance level ($p$-value ≤ 0.001) with 185 at the age of one-month-old and 239 at the age of two-months-old, an increase of almost 30%.

Table 3 further details these findings by presenting the number of features at each time-point, the number of those that showed significant differences at both time-points ($T_{01} \cap T_{02}$) (detailed in Table 2) and those that showed at only one of the time-points, $T_{01} - (T_{01} \cap T_{02})$ and $T_{02} - (T_{01} \cap T_{02})$, respectively those present only at the age of one-month-old and those present at the age of two-months-old.

As in Tables 1 and 2, Table 3 shows that the INL is the layer where texture features consistently present statistically significant differences at both time-points, while the RNFL is the one presenting the least consistent differences.

From the progression point of view, two layers stand out in Table 3, the IPL and the ONL. Data in Table 3 demonstrates that in these layers only 2 out of 47 and 9 out of 47 features, respectively for the IPL and ONL, ceased to show statistically significant differences at the age of two-months-old while having shown at the age of one-month-old. On the other hand, 21 and 26 features show differences at the age of two-months-old without having done so at the age of one-month-old, being strong candidates for biomarkers of progression and showing these may be the layers undergoing the most notorious changes. These features are disclosed in Table 4, where it is notorious that the majority of these features present statistically significant differences at the highest significance level.

### 4 Discussion

The need for the early detection of changes unfolding in the CNS due to Alzheimer’s disease is of utmost, and increasing, importance as life expectancy is steadily increasing [1] and the prevalence in people aged 65 and older reached the two figures in 2019 in the USA [2]. Furthermore, it is hypothesised that AD has advanced over two decades when detected by state-of-the-art means [4, 5], hampering the access to the first pathophysiological changes caused by AD and tracking their progress. While the use of the retina as a window into the CNS has been used, mostly resorting to thickness measurements, these studies should be taken into account carefully as the generally claimed thinning of the retina is not exclusive to AD.

In this study, we address changes in the retina of the triple-transgenic mouse model of AD, at the ages of one and two-months-old, by comparing those with a WT group at the same age. We performed a texture analysis of the MVF image for each of the inner six retinal layers (from the RNFL to the ONL) to identify differences in the retina between two mice groups. These images were then split into $24 \times 24$ blocks, that were individually analysed by computing local texture metrics (GLCM). We followed a prior work on humans that demonstrated the ability of texture analysis in distinguishing between AD patients, Parkinson’s patients, and healthy controls [15], and previous results in a smaller study involving this mouse model of AD [20].

| Layer | Feature | Quadrants | Layer | Feature | Quadrants |
|-------|---------|-----------|-------|---------|-----------|
| RNFL  | Biophysical | □□□□□□□ | ONL   | Biophysical | □□□□□□□ |
|       | Histogram | □□□□□□□ |       | Histogram | □□□□□□□ |
| GCL   | Correlation | □□□□□□□ |       | Correlation | □□□□□□□ |
| IPL   | Aggregation | □□□□□□□ |       | Aggregation | □□□□□□□ |
| INL   | Texture | □□□□□□□ |       | Texture | □□□□□□□ |
| OPL   | Scale of Variance | □□□□□□□ |       | Scale of Variance | □□□□□□□ |
| ONL   | Sum | □□□□□□□ |       | Sum | □□□□□□□ |
| Total | □□□□□□□ |       | Total | □□□□□□□ |}

This table follows the same display scheme described in Table 2.
A massive distinction between WT and 3×Tg-AD was found across the different layers of the retina and spreading over the imaged area. The most relevant differences between groups were found at the INL with a significant number of statistically significant differences at both time-points, showing that texture features are consistent in showing the differences between groups. Also, the progression of AD is clear from changes within the IPL and ONL layers between the to time-points.

It is of particular importance to notice that this study demonstrates the potential use of texture for the detection and progression assessment of AD, as suggested by found differences at each time-point and the significant differences found at the IPL and ONL from the age of one to the age to two-months-old.

The limitation of the present study arises from the substantial differences found at the first time-point, at the age of one-month-old, where the two groups already present clear differences spread over the retina, which precludes the identification of changes associated with the onset of the AD. Another limitation is due to the estimation of layer interfaces based on the relative thickness known from the histology of mice. Nevertheless, these interfaces were visually assessed through a substantial sampling over the B-scans, and none showed to be incorrect requiring any correction. Furthermore, tests were conducted by artificially changing the location of the interfaces to assess the consistency of the findings to those changes that kept demonstrating the same differences between groups.

The differences between groups found in this study suggest that the 3×Tg-AD model may be inappropriate to study early changes associated with the AD and other animal models should be tested following the same path and rationale. Moreover, these results also suggest that the human genes present in these transgenic mice may have an impact on the neurodevelopment of offspring, which would justify the significant changes found at the age of one-month-old.

Further work on this subject will focus on a new mouse model of AD and on its study of early changes in the retina and the brain. Nonetheless, the found differences shed light on changes unfolding in the retina associated with the AD, its progression, and allow to state with confidence that texture biomarkers from OCT data convey information on CNS changes associated with the AD using a non-invasive, in vivo and in situ optical imaging system, the OCT. In this study, we provide evidence suggesting the neuroretina, the visible part of the CNS, presents distinct characteristics for WT (controls) and 3×Tg-AD mice, that these differences are present early in life (at the age of one-month-old), and that differences progress from the age of one to the age of two-months-old.

**Funding Information** This study was funded by The Portuguese Foundation for Science and Technology (FCT) through PTDC/EMDEMD/28039/2017, PEst-UID/NEU/04539/2019, UID/Multi/04621/2013 and UID/04950/2017, and by FEDERCOMPETE through POCI-01-0145-FEDER-028039 and POCI-01-0145-FEDER-007440.

**Compliance with Ethical Standards**

**Conflict of interests** The authors declare that they have no conflict of interest.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

**References**

1. Alzheimer Association, Alzheimer’s and Dementia, pp 1–88, 2019.
2. United Nations, World population prospects 2019, 141, 2019.
3. Shah H, Albanese E, Duggan C, Ruidan I, Langa KM, Carrillo MC, Chan KY, Joannette Y, Prince M, Rossor M, Saxena S, Snyder HM, Sperling R, Varghese M, Wang H, Wortmann M, Duñ T. Lancet Neurol. 2016;15(12):1285. https://doi.org/10.1016/S1474-4422(16)30235-6.
4. Jack CR, Lowe VJ, Weigand SD, Wiste HJ, Senjem ML, Knopman DS, Shiung MM, Gunter JL, Boeve BF, Kemp BJ, Weiner M, Petersen RC. Brain. 2009;132(5):1355. https://doi.org/10.1093/brain/awp062.
5. Harper DJ, Augustin M, Lichtenegger A, Gesperger J, Himmel T, Muck M, Merkle CW, Eugui P, Kummer S, Woehrer A, Glössmann M, Baumann B, pp 1–27, 2019.
6. Frisoni GB, Boccardi M, Barkhof F, Blennow K, Cappa S, Chiotis K, Demonet JF, Garibotto V, Giannakopoulos P, Gietl A, Hansson O, Herholz K, Jack CR, Nobili F, Nordberg A, Snyder HM, Ten Kate M, Varghese M, Wang H, Wortmann M, Duan T. Lancet Neurol. 2016;15(12):1285. https://doi.org/10.1016/S1474-4422(16)30235-6.
an early diagnosis of Alzheimer’s disease based on biomarkers. 
https://doi.org/10.1016/S1474-4422(17)30159-X. 2017.
7. London A, Benhar I, Schwartz M. Nat Rev Neurol. 2013;9(1):44. 
https://doi.org/10.1038/nrneurol.2012.227.
8. Svetozarskiy SN, Kopishinskaya SV. Sovremennye Tehnol Med. 
2015;7(1):116. https://doi.org/10.17691/stm2015.7.1.14.
9. Ong SS, Murali Doraismawy P, Lad EM. Controversies and 
future directions of ocular biomarkers in Alzheimer disease. 
https://doi.org/10.1001/jama.neurol.2018.0602. 2018.
10. De La Torre JC. Is Alzheimer’s disease a neurodegenerative or 
a vascular disorder? Data, dogma, and dialectics. 
https://doi.org/10.1016/S1474-4422(04)00683-0. 2004.
11. Chan VT, Sun Z, Tang S, Chen LJ, Wong A, Tham CC, Wong 
TY, Chen C, Ikram MK, Whitson HE, Lad EM, Mok VC, 
Cheung CY. Ophthalmology. 2019;126(4):497. https://doi.org/10. 
1016/j.ophtha.2018.08.009.
12. den Haan J, Verbraak FD, Visser PJ, Bouwman FH. Alzheimer’s 
and dementia: Diagnosis, Assessment and Disease Monitoring. 
2017;6:162. https://doi.org/10.1016/j.dadm.2016.12.014.
13. lui Cheung CY, Ikram MK, Chen C, Wong TY. Progr Retinal 
Eye Res. 2017;57:89. https://doi.org/10.1016/j.preteyeres.2017. 
01.001.
14. Guimarães P, Rodrigues P, Lobo C, Leal S, Figueira J, Serranho 
P, Bernardes R. Comput Med Imaging Graph. 2014;38:381. 
https://doi.org/10.1016/j.compmedimag.2014.02.003.
15. Nunes A, Silva G, Duque C, Januário C, Santana I, Ambrósio 
AF, Castelo-Branco M, Bernardes R. PLoS ONE. 2019;14(6):1. 
https://doi.org/10.1371/journal.pone.0218826.
16. Ferreira H, Martins J, Nunes A, Moreira PI, Castelo-Branco 
M, Ambrósio AF, Serranho P, Bernardes R. XV Mediterranean 
Conference on Medical and Biological Engineering and Computing – MEDICON 2019. MEDICON 2019. IFMBE Proceedings. pp 1816–1821. In: Henriques J and Neves N, editors. Cham: Springer; 2020. https://doi.org/10.1007/978-3-030-31635-8.
17. Oddo S, Caccamo A, Kitazawa M, Tseng BP, LaFerla FM. 
Neurobiol Aging. 2003;24(8):1063. https://doi.org/10.1016/j.neurobiolaging.2003.08.012.
18. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, 
Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM. 
Neuron. 2003;39(3):409. https://doi.org/10.1016/S0896-6273(03) 
00434-3.
19. Nunes A, Ambrósio AF, Castelo-Branco M, Bernardes R. 
Proceedings - 2018 IEEE 18th International Conference on 
Bioinformatics and Bioengineering. BIBE. 2018;2018:41–46. 
https://doi.org/10.1109/BIBE.2018.00016.
20. Bernardes R, Silva G, Chiquita S, Serranho P, Ambrósio AF. 
2017.
21. Dysli C, Enzmann V, Sznitman R, Zinkernagel MS. Transl Vis 
Sci Technol. 2015;4(4):9. https://doi.org/10.1167/tvst.4.4.9.
22. Kim KH, Puoris’hau M, Maguluri G, Umino Y, Cusato K, Barlow 
RB, de Boer J, J Vis. 2008;8:17.1. https://doi.org/10.1167/8.1.17.
23. Roy AG, Conjeti S, Karri SPK, Sheet D, Katouzian A, 
Wachinger C, Navab N. Biomed Opt Express. 2017;8(8):3627. 
https://doi.org/10.1364/boe.8.003627.
24. Baghaie A, Yu Z, D’Souza RM. Quant Imaging Med Surg. 
2015;5(4):603. https://doi.org/10.3978/j.issn.2223-4292.2015.07.02.
25. Meyer-Base A. Patter recognition in medical imaging. Amster- 
dam: Elsevier Inc.; 2004. https://doi.org/10.1016/B978-0-42-493 
290-6.X5000-7.
26. Haralick RM, Shanmugam K, Dinstein I. IEEE Trans Syst Man 
Cybern. 1973;SMC-3(6):610. https://doi.org/10.1109/TSMC.197 
3.4309314.
27. Soh LK, Tsatsoulis C. IEEE Trans Geosci Remote Sens. 
1999;37(2):780. https://doi.org/10.1109/36.752194.
28. Haralick RM. Proc IEEE. 1979;67(5):786. 
https://doi.org/10.1109/PROC.1979.11328.
29. Conners RW, Trivedi MM, Harlow CA. Comput Vis Graph 
Image Process. 1984;25(3):273. https://doi.org/10.1016/0734-189 
8490197-x.
30. Clausi DA. Can J Remote Sens. 2002;28(1):45. https://doi.org/ 
10.5589/m02-004.
Publisher's note Springer Nature remains neutral with regard to 
jurisdictional claims in published maps and institutional affiliations.