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Mariana Gomes Miranda

Human *versus* Artificial Intelligence: Validation of a Deep Learning Model for Retinal Layer and Fluid Segmentation in Optical Coherence Tomography Images from Patients with Age-Related Macular Degeneration

MARÇO, 2024

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Mestre Tânia Filipa Fernandes de Melo

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Human *versus* Artificial Intelligence: Validation of a Deep Learning Model for Retinal Layer and Fluid Segmentation in Optical Coherence Tomography Images from Patients with Age-Related Macular Degeneration

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☒ Não procedi à utilização de ferramentas de *chatbox* generativo baseadas em *large language models* para nenhuma das tarefas no contexto do meu trabalho de Dissertação ou Monografia

☐ Procedi à utilização de ferramentas de *chatbox* generativo baseadas em *large language models* no contexto do meu trabalho de Dissertação ou Monografia, encontrando-se todas as interacções (*prompts* e respostas) transcritas em anexo bem como a indicação das aplicações utilizadas.

Neste sentido, confirmo que a eventual utilização de ferramentas de *chatbox* generativo baseadas em *large language models* no contexto do meu trabalho de Dissertação ou Monografia foi exclusivamente descrita na sequência de *prompts* e respostas transcritos em anexo e nas aplicações indicadas.

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"When will you realize, Vienna waits for you?"

Billy Joel, 1977

Dedicatória

À minha Orientadora, Professora Ângela, a minha profunda gratidão e admiração. O meu sincero agradecimento por ter definido as margens deste percurso e, por nele, ter sempre oferecido uma carinhosa orientação médica e científica.

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Aos meus pais e ao meu irmão, por serem o meu carinhoso porto de abrigo, a cada regresso a casa. O mais especial e ternurento agradecimento. Por tudo aquilo que me ensinaram. Pelo colossal amparo e proteção. Pela paciência dedicada e compreensivo carinho. Pelo constante incentivo de persistência. Pelo enorme esforço que sempre fizeram para que eu pudesse voar a tentar. E por terem estado, continuamente, de braços abertos a cada queda. A vocês, devo aquilo que sou e a mais ninguém nutro tão grandiosa admiração.

À Daniela, amiga de sempre e madrinha de curso, por me ter acompanhado durante todo este meu percurso, antes mesmo de ele ter começado. Por ser a minha maior referência e por me ter sempre mostrado o encanto por detrás de todos os desafios. Pelo mais querido, constante e sincero apoio e por nunca me ter deixado desistir. Obrigada por teres crescido comigo e por estares sempre presente, a cada passo.

À Ana, à Bia, à Bea, à Raquel, à Xica, à Marta, à Matilde e ao Ricardo, umagradecimentoapertadinho, por serem a mais bonita família de coração que me podia ter acolhido dentro destas paredes amarelas. Porque me ensinaram que a vida é mais bonita quando rodeada de tanto amor. Obrigada pela companhia, pelo amparo, pelos abraços e pelas gargalhadas. Obrigada por toda a motivação e inspiração. É com um orgulho desmedido que vos vejo a cruzar esta meta final. Vocês serão para sempre o mais bonito *glimmer* deste percurso. E é sobre isto. Todas estas memórias, que ficam em cada canto desta casa. Isto será tudo o que eu levarei destes 6 anos.

O meu obrigada.

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Article

Human *versus* Artificial Intelligence: Validation of a Deep Learning Model for Retinal Layer and Fluid Segmentation in Optical Coherence Tomography Images from Patients with Age-Related Macular Degeneration

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Abstract: Artificial intelligence (AI) models have received considerable attention in recent years for their ability to identify optical coherence tomography (OCT) biomarkers with clinical diagnostic potential and predict disease progression. This study aims to externally validate a deep learning (DL) algorithm by comparing its segmentation of retinal layers and fluid with a gold standard method of manually adjusting the automatic segmentation of the Heidelberg Spectralis HRA + OCT software. A total of sixty OCT images of healthy subjects and patients with intermediate and exudative age-related macular degeneration (AMD) were included. A quantitative analysis of retinal thickness and fluid area was performed and the discrepancy between the methods was investigated. The results showed a moderate to strong correlation between the metrics extracted by both software, in all groups, and an overall near-perfect area overlap was observed, except for the inner segment ellipsoid (ISE) layer. The DL system detected a significant difference in outer retinal thickness across disease stages and accurately identified fluid in exudative cases. In more diseased eyes, there was significantly more disagreement between the methods. This DL system appears to be a reliable method for accessing important OCT biomarkers in AMD. However further accuracy testing should be conducted to confirm its validity in real-world settings to ultimately aid ophthalmologists in OCT imaging management and guide timely treatment approaches.

Keywords: age-related macular degeneration; artificial intelligence; retinal layer segmentation; fluid segmentation; deep learning; machine learning; optical coherence tomography; diagnosis; external validation.

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1. Introduction

Age-related macular degeneration (AMD) is a leading cause of moderate to severe visual impairment (MSVI) [1] and irreversible vision loss in adults aged 50 years and older, in high-income countries [2]. It is estimated to be responsible for 8.7% of global blindness cases and, due to population aging, its prevalence is expected to increase [3].

AMD primarily affects the macula, the region of the retina responsible for central vision. This metabolic-inflammatory-vascular disease [4-6] is associated with ageing, genetic predisposition [7], and environmental risk factors [8] and is characterised by the deposition of lipid-rich extracellular metabolites within and/or beneath the retinal

pigment epithelium (RPE), known as drusen [9]. AMD can be classified into three stages: early, intermediate, and late. Early AMD is defined by the presence of small drusen, while intermediate AMD is associated with medium-sized drusen and retinal pigmentary abnormalities. Late AMD presents in two major forms: geographic atrophy (GA) and neovascular AMD (nAMD) [10]. The latter is caused by macular neovascularization (MNV), which leads to the accumulation of subretinal fluid (SRF), sub-RPE fluid (sRPEF), and/or intraretinal fluid (IRF).

Optical coherence tomography (OCT) is currently the gold standard for AMD management [11]. Cross-sectional scans of the retina at the micron scale are acquired, where structural features – imaging biomarkers – are identifiable. Central retinal thickness was one of the earliest described, however, others such as drusen volume and hyperreflective foci quantification [12,13], fluid volume and pigment epithelial detachment (PED) [14,15] have also been recognized for their insight into disease activity.

Since the rise of artificial intelligence (AI) in medical imaging, retinal OCT has been at the forefront of ophthalmology research [16]. Deep learning (DL) models are currently the state-of-the-art among AI technologies and they have shown to be capable of assisting in AMD classification, diagnosis and prognosis [17,18], in ongoing monitoring of treatment efficacy [19], and in predicting disease progression [20].

Automated scan analysis using these algorithms is a faster, cost-effective, and fatigue-free process. However, it may have some limitations that affect quality and accuracy of the results. Therefore, it is crucial to test recently developed and trained methods, such as the BioImagingLab/INESC TEC model [21], on new datasets and externally validate them for their applicability in real-world clinical practice.

Thus, the present study aims to evaluate the performance of a DL algorithm in identifying and quantifying retinal layers' thickness and fluid areas in OCT scans of healthy and AMD eyes. Firstly, quantitatively compare the DL system's segmentation with the gold standard of manual adjustment of Heidelberg Spectralis HRA + OCT automatic segmentation. Subsequently, study whether there is a clear relationship between the thickness layer values extracted and the disease stage, as well as the DL method accuracy in detecting fluid in nAMD. Finally, investigate if the discrepancies between the segmentation methods are related to the disease severity and to the subjective difficulty degree, perceived by the human eye.

2. Materials and Methods

2.1. Study Design

A validation study was conducted on normal eyes and eyes with AMD. The study was single-centred, observational, retrospective, and cross-sectional.

The Ethics Committee of the Centro Hospitalar Universitário of São João approved the study protocol for access to and analyse the patients' data. Informed consent from patients was not applicable, since all clinical data and OCT images were anonymised by investigators at the Ophthalmology Service. A code number, independent of participants' personal data, was generated to protect the patients' identity. This clinical study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

2.2. Setting

OCT scans were collected from subjects with normal eyes and patients diagnosed with intermediate and exudative AMD who presented for routine clinical care at the Ophthalmology Service of the Centro Hospitalar Universitário of São João, a tertiary referral hospital, between January 2010 and December 2023.

2.3. Study population

A total of 60 fovea-centred cross-sectional OCT B-scans were included from 60 different patients, comprising 20 healthy controls, 20 with intermediate AMD (iAMD), and 20 with exudative AMD (eAMD). Each patient was exclusively assigned to one of the three groups, and only one eye per subject was studied.

A randomized numerical sequence was automatically generated, and each patient was assigned a number at baseline within their designated group. Inclusion and exclusion criteria were applied until a total of 20 participants were obtained. If the random sequence resulted in the selection of an eye from a patient whose contralateral eye was already included, only one – the one with better visual acuity (whether right or left) – was chosen.

Male and female subjects were evenly distributed, with a 10:10 ratio per group. Controls were required to be 50 years of age or older at the time of image acquisition while AMD patients were included if they were 50 years of age or older at the time of diagnosis. Additionally, an adequate follow-up duration of at least one year after the disease diagnosis was required.

Exclusion criteria for the study included images from patients diagnosed with diabetic retinopathy, high myopia (refractive error ≤ − 6.0 dioptres), and/or chorioretinal diseases other than AMD. Patients with OCT scans with poor technical quality due to excessive background noise, imaging artifacts, poor image centration, or reduced visualization of the retinal layers were also excluded.

In December 2023, 798 participants were assessed for eligibility, including 160 images from healthy controls, 176 scans from individuals diagnosed with iAMD and 630 OCTs from patients with eAMD. Of the 160 eyes from controls, 30 were excluded as they were under 50 years of age at the time of image acquisition and 3 were excluded due to inadequate quality of the OCT B-scans. Out of the 176 scans from patients diagnosed with iAMD, 9 were excluded due to poor image quality, 3 due to a diagnosis of diabetic retinopathy, 4 participants had a follow-up duration of less than one year, and in 1 case, the initial consultation did not align with the moment of diagnosis, making it challenging to ascertain whether therapeutic anti-VEGF injections were administered prior to the first accessible acquisition of the OCT scan. Lastly, out of the 630 eAMD scans, 28 were excluded because of poor image quality, 4 did not complete the one-year follow-up, 5 had diabetic retinopathy, and 4 patients had received a diagnosis prior to the acquisition of the first assessable OCT scan. It is important to note that the retina.pt database contains data from both eyes of each patient. Some cases of unilateral eAMD diagnosis occurred, and the numerically sequenced chosen eye belonged to the contralateral eye, which was either healthy or, more frequently, had intermediate AMD. This scenario was identified in 84 cases, which were also selectively excluded from the third group analysis, as represented in **Figure 1**.

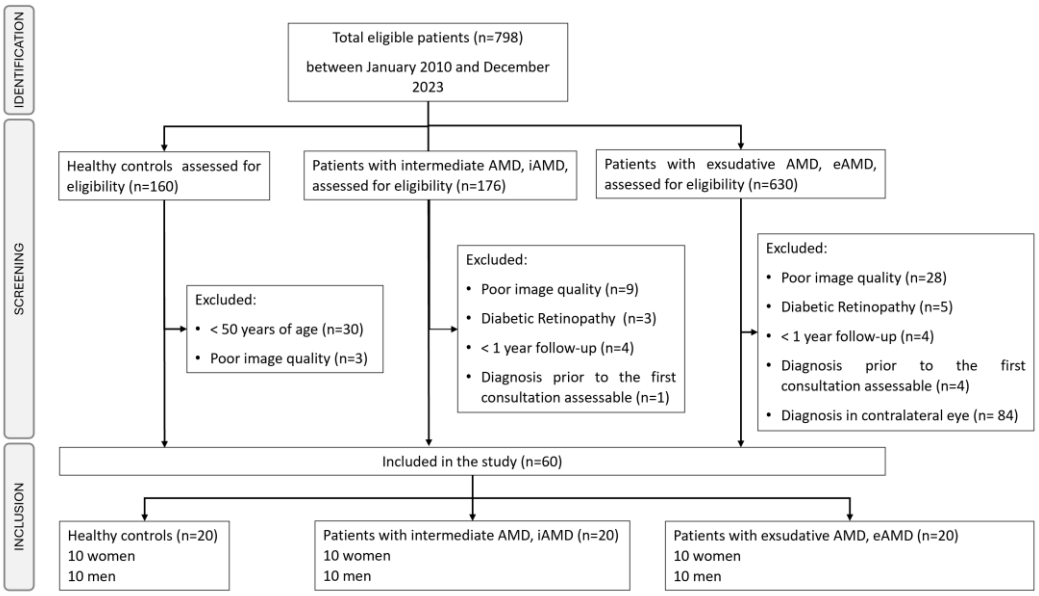


Figure 1. Study flow diagram: patient identification and selection.

2.4. Identification and Image Data Collection

Controls and patients with iAMD were recruited from the electronic health records of the Centro Hospitalar Universitário of São João, applying predefined filters: "Controlos" and "DMI Intermédia", respectively, which mean "Controls" and "Intermediate AMD". Participants with eAMD were selected from the online database retina.pt under the category "DMI Avançada (Membrana Neovascular Coroideia)" which translates to "Late AMD (Choroidal Neovascular Membrane)".

The OCT images were selected from 6, 18, 19 or 25-line horizontal volume scans acquired using the Automatic Real-Time (ART) function, always centred on the fovea. Enhanced Depth Imaging (EDI) volume scans were not used due to the reduced quality of retinal layers since this modality typically provides enhanced visualization of the choroid instead. All images had a maximum image height of 496 pixels and variable length, with dimensions of 512 (high speed mode) or 1024 (high resolution mode) pixels. No standardization of image size was conducted as these variations do not introduce bias or affect image processing.

The OCT B-scan obtained by the investigators consistently corresponded to the initial image acquired during the first medical consultation. The Heidelberg Spectralis propriety software has the capability to conduct retinal segmentation directly within its interface and provides tools to manually adjust each boundary. All original and segmented images were exported as TIF files. Additionally, images of nAMD were imported into a different platform, the MATLAB system, to complement fluid segmentation. Finally, the original images were imported into the DL software, which performed fully automated segmentation and compared the segmentation results with the gold standard at INESC TEC - Institute for Systems and Computer Engineering, Technology and Science.

2.5. Algorithms Description

Retinal layer segmentations were performed using two distinct methods.

2.5.1. Reference Standard

The first software used was the Spectral-Domain Optical Coherence Tomography (SD-OCT, Heidelberg Engineering GmbH, Heidelberg, Germany, Spectralis™ Acquisition Software Version 6.16.8.0) provided with the Heidelberg Spectralis HRA+ OCT. This is the proprietary system available to assist ophthalmologists during clinical practice at the Centro Hospitalar Universitário of São João. The software automatically detects ten

retinal boundaries. However, during adjusted automated segmentation, three boundaries were omitted from the automated segmentation, as exemplified in **Figure 2.** This led to the identification of eight final boundaries, thereby enabling a comparative analysis with the segmentation performed by the DL software.

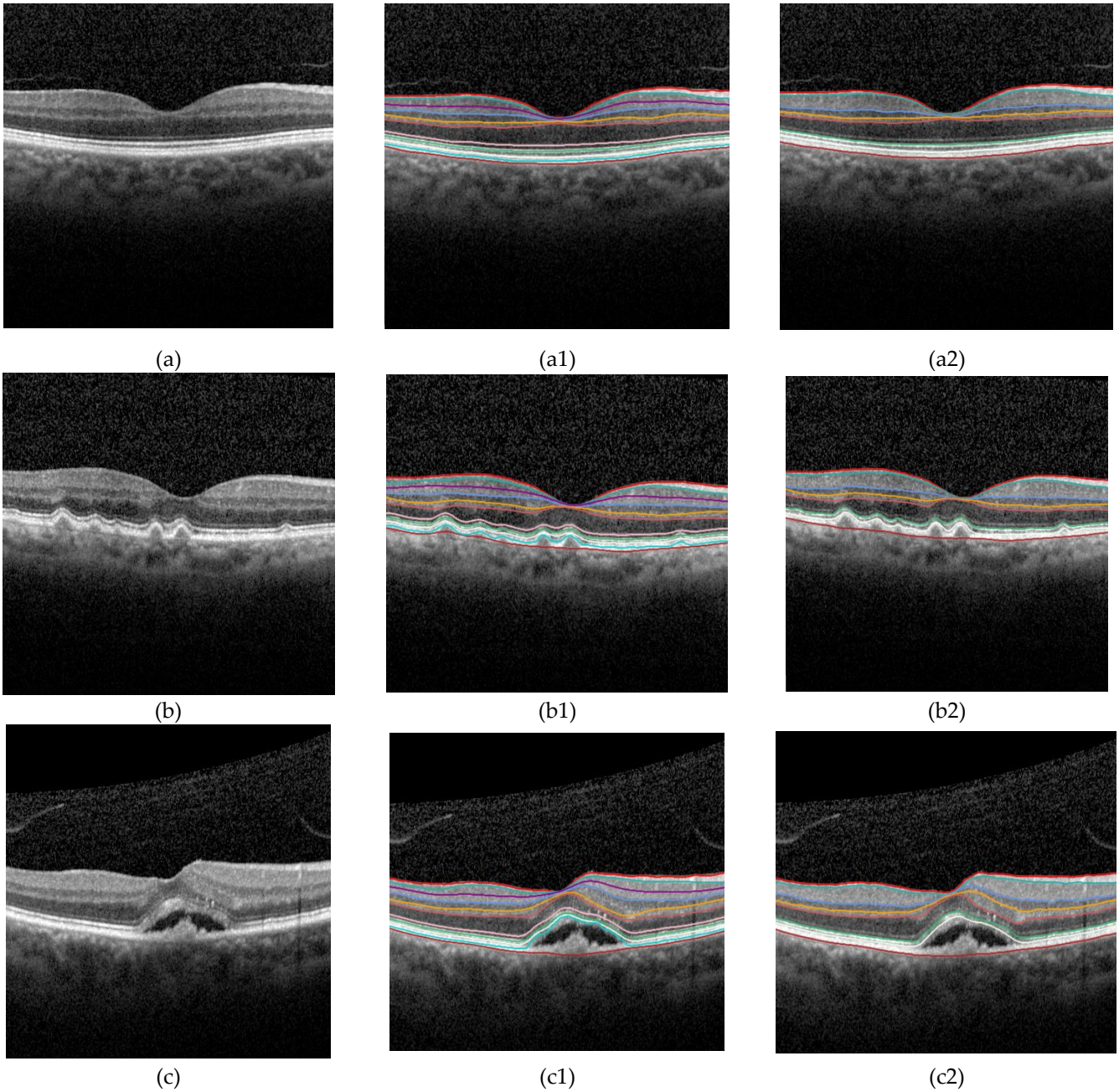


Figure 2. Representative examples of retinal boundaries segmentation on OCT B-scan for **(a)** controls, **(b)** iAMD, and **(c)** eAMD patients: **(a1, b1, c1)** Automated segmentation generated by the Heidelberg System, retinal layers are as follows, from the upper boundary to the lower boundary: internal limiting membrane (ILM), retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), external limiting membrane (ELM), ellipsoid zone (PR1), interdigitation zone (PR2), retinal pigment epithelium (RPE), and Bruch's membrane (BM); **(a2, b2, c2)** Adjusted automated segmentation, followed by the omission of three layers: ganglion cell layer (GCL), external limiting membrane (ELM), and retinal pigment epithelium (RPE).

Only for the reference standard (RS) method, fluid segmentation was completed using MATLAB R2023b Update 6 (23.2.0.2485118). All total retinal fluid, including subretinal fluid (SRF), sub-RPE fluid (sRPEF), and/or intraretinal (IRF) was manually segmented with some automatic suggestions as well.

This first method described was determined as the gold standard for validating the DL model for retinal layers and fluid segmentation.

2.5.2. DL software

The DL software tested was the BioImagingLab/INESC TEC model. The AI algorithm itself identifies the corresponding eight boundaries, starting from the superior limit: internal limiting membrane (ILM), nerve fiber layer/ganglion cell layer (NFL/GLC), inner plexiform layer/inner nuclear layer (IPL/INL), inner nuclear layer/outer plexiform layer (INL/OPL), outer plexiform layer/outer nuclear layer (OPL/ONL), inner segment myeoid/inner segment ellipsoid (ISM/ISE), outer segment/retinal pigment epithelium (OS/RPE), and Bruch's membrane (BM).

Thus, the seven retinal layers studied in all groups and the fluid detected in nAMD images are represented in **Figure 3**.

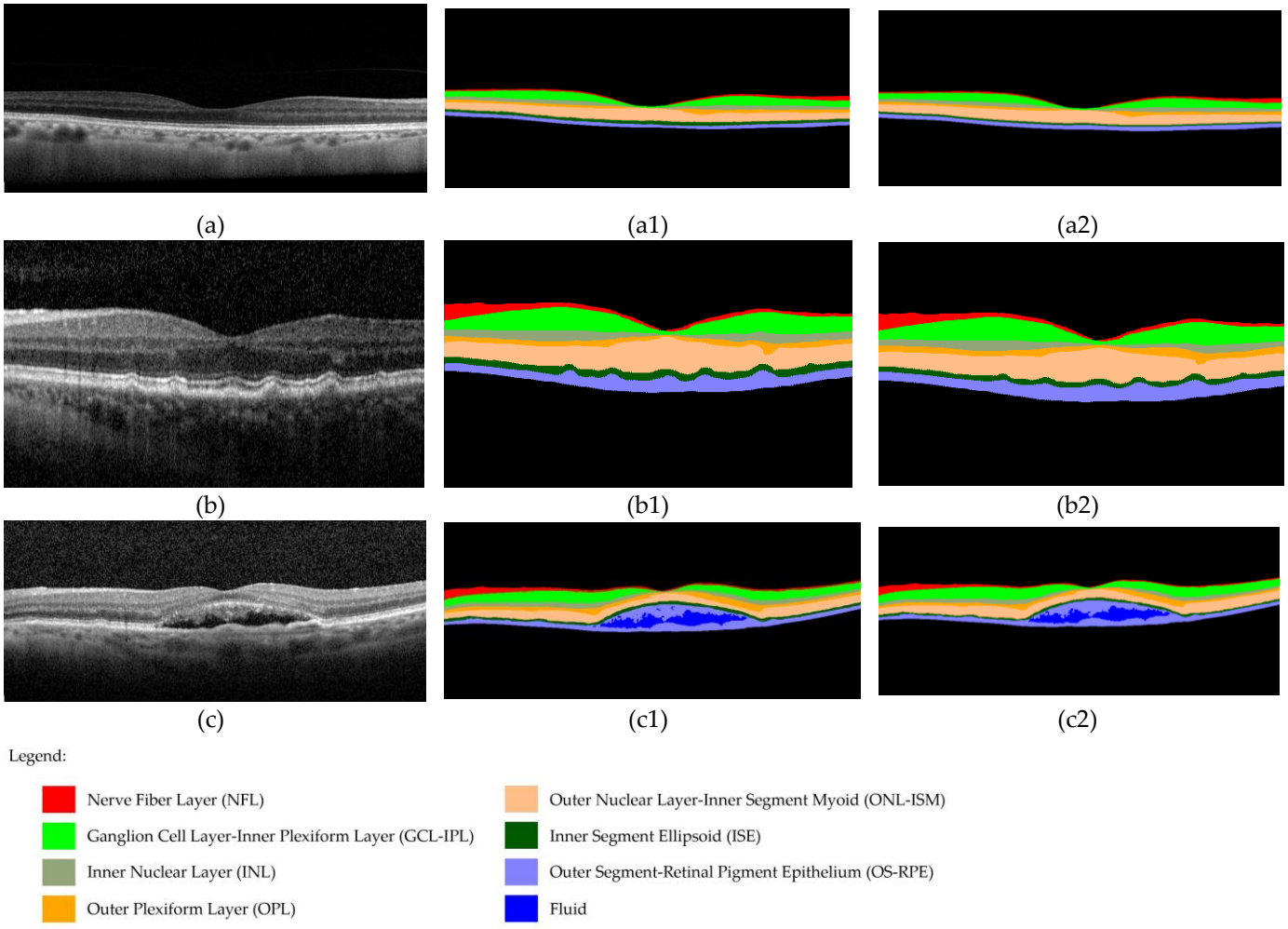


Figure 3. Example of **(a)** control eye imaging, **(b)** iAMD eye imaging and **(c)** eAMD eye imaging. Representative examples of retinal layers and fluid segmentation using: **(a1, b1, c1)** the RS method and **(a2, b2, c2)** the DL method.

2.6. Image Annotations

The adjusted automated segmentation was carried out by two examiners. Initially, the retinal boundaries were performed by a medical student. The annotations automatically generated by the Heidelberg Spectralis software were reviewed and refined through manual adjustments. This involved rearrangement when the system appeared to fail, the annotation was considered inaccurate, or in cases of doubt, the average of both suggestions was represented. The segmentation was then corrected, if necessary, and validated by another observer, an independent medical expert in the field.

During the annotation process, the student assigned a value to each OCT image to reflect the perceived difficulty of segmentation. This scale was determined subjectively, taking into account the human perception of image quality of the OCT scan and was based on a five-level scale: 1 = very easy (no difficulty in any boundary), 2 = easy (difficulty in segmenting a region of the image into one or two boundaries), 3 = medium (difficulty in segmenting a region of the image into more than two boundaries), 4 = difficult (difficulty in segmenting one or two boundaries throughout the entire image), 5 = very difficult (difficulty in segmenting more than two boundaries throughout the entire image).

The standard fluid segmentation also underwent revision and validation by the same ophthalmologist.

Finally, fully automated segmentation of retinal layers and fluid was generated by the AI system for the same 60 images.

2.7. Outcomes

The primary outcomes were the agreement between DL and RS evaluations and the accuracy of DL software in detecting boundaries and quantifying the thickness of the outer retina (ISE and OS-RPE) and fluid area in nAMD. The secondary outcome was the variance of disagreement observed between the methods across different image categories: disease stage (objective score) and values of human-perceived difficulty of segmentation (subjective score).

2.8. Statistical Analysis

The data were recorded and analysed using Microsoft Excel for Microsoft 365 MSO (Version 2401 Build 16.0.17231.20236) and IBM SPSS Statistics 29.0.0.0 (241). A p-value of less than 0.05 was assumed for statistical significance.

All metrics were measured in pixels. The thickness of the segmented retinal layers was converted to microns (μm) using a vertical scaling factor of 3.87 μm/pixel for all images. The fluid area was converted to square millimetres (mm^2) using, additionally, one of two different horizontal scaling factors: 11.35μm/pixel for images taken in high-speed mode and 5.7 μm/pixel for images captured in high-resolution mode.

Data normality was evaluated through the implementation of the Kolmogorov–Smirnov test and Shapiro-Wilk tests.

To evaluate the performance of the DL system, Paired Sample T-Test was applied to compare the means of retinal layer thickness and fluid area between the DL and RS methods. This was done to determine whether the observed differences were statistically significant. Additionally, the Pearson Correlation Coefficient was calculated to assess the level of correlation between the variables measured by the two methods. The correlation strength (correlation coefficient value, r) was categorized as weak ($0 < r < 0.3$ or $-0.3 < r < 0$), moderate ($0.3 \leq r < 0.7$ or $-0.7 < r \leq -0.3$), or strong ($0.7 \leq r < 1$ or $-1 < r \leq -0.7$). Finally, the Dice score, also known as the Dice Similarity Coefficient, was obtained to evaluate the similarity between the segmentation results and the gold standard. The Dice values range from 0 (no overlap) to 1 (perfect overlap).

To further evaluate accuracy, the relationship between the thickness of the outer retina extracted by the DL system and the disease stage was investigated through a one-way ANOVA test. Furthermore, a Bland-Altman plot analysis with linear regression was

employed to evaluate the concordance between the AI software and RS assessment in quantifying fluid area.

Finally, the differences in pairs of measures of retinal thickness and fluid area between software were compared using one-way ANOVA tests. The comparison was made with respect to the degree of disease severity (controls, iAMD, and eAMD), as well as the subjective classification assigned during RS annotation (very easy, easy, medium, difficult, and very difficult).

3. Results

A total of 60 images from 60 patients were included in this study, with 20 images each from healthy controls (mean age ± standard deviation (SD): 63.15 ± 8.00 years, range: 50-79 years), iAMD (mean age ± SD: 71.80 ± 7.72 years, range: 59-83 years), and eAMD (mean age ± SD: 73.80 ± 9.99 years, range: 53-93 years) for a comparative quantitative analysis.

The mean retinal thickness was 307.22 ± 82.99 μm (range: 277.34-332.50 μm) in controls, 319.12 ± 109.79 μm (range: 279.00-395.74 μm) in iAMD, and 332.74 ± 156.91 μm (range: 259.79-384.90 μm) in eAMD. The combined area of SRF, sRPEF, and IRF represented a mean total fluid area of 0.13 ± 0.10 mm^2 (range: 0.02-0.43 mm^2).

3.1. Performance Evaluation of the DL Software

The assessment of the AI algorithm's performance involved comparing the automated layer segmentation and the thickness values obtained through the DL system with the measurements obtained from the RS method (**Table 1**).

The Paired Sample T-Test assessment revealed that in the control group, there was no significant difference between the RS evaluation and the segmentation generated by the DL system in the GCL-IPL layer. However, significant differences were observed in all other layers ($p < 0.05$). In the iAMD group, only the segmentation of the GCL-IPL and ONL-ISM layers showed no significant difference between the two methods, while significant differences were noted in all other layers ($p < 0.05$). When comparing pairs of metrics for eAMD, the segmentation of all retinal layers had significant differences, although no significant difference was found in fluid areas between the RS and DL measurements.

All layers in the control group had strongly positive Pearson correlation coefficients ($0.7 \leq r < 1$), except for the ISE and OS-RPE. In the iAMD group, there was a strongly positive ($0.7 \leq r < 1$) correlation for INL and ONL-ISM layers. Similarly, in eAMD, the NFL, ONL-ISM, ISE, OS-RPE layers, and fluid segmentation also showed the same positive correlation range. All other pairs of layer thickness in iAMD and eAMD showed a moderate correlation ($0.3 \leq r < 0.7$), except for two layers in each group. Specifically, ISE and OS-RPE in controls, NFL and ISE in iAMD, and GCL-IPL and INL in eAMD had a correlation coefficient that was not statistically significant.

Finally, the segmented area showed a near-perfect overlap between methods in all three groups, as revealed by the Dice coefficient. The mean Dice coefficient for the layers was 0.947 for controls, 0.946 for iAMD, and 0.936 for eAMD. In addition, the fluid had a Dice coefficient of 0.976.

Table 1. Retinal layer thickness and fluid area obtained from healthy controls, iAMD, and eAMD groups using both RS and DL software. The following layers were considered: ganglion cell layer-inner plexiform layer (GCL-IPL), inner nuclear layer (INL), inner segment ellipsoid (ISE), nerve fiber layer (NFL), outer nuclear layer-inner segment myoid (ONL-ISM), outer plexiform layer (OPL), and outer segment-retinal pigment epithelium (OS-RPE). DL: deep learning; eADM: exudative AMD; iAMD: intermediate AMD; SD: standard deviation.

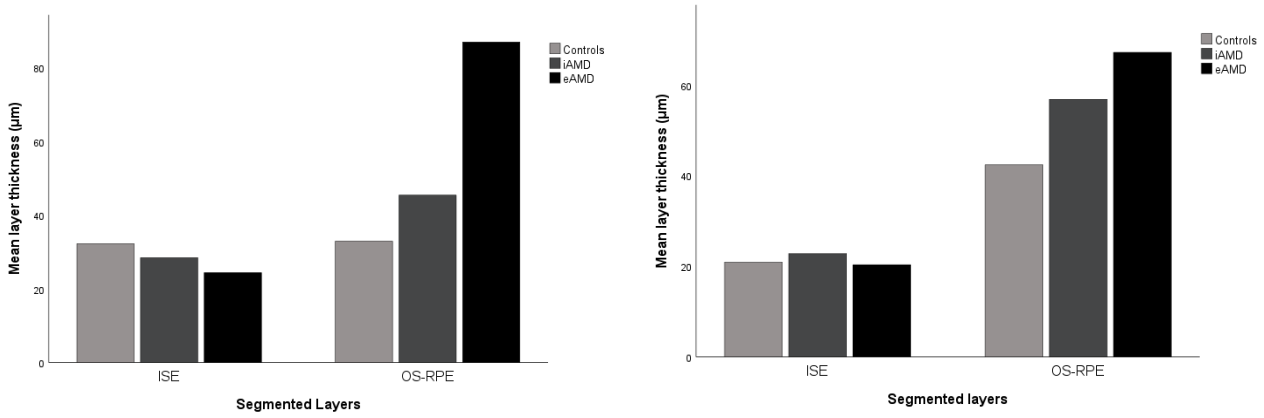
	Reference Standard (Mean ± SD)	DL Software (Mean ± SD)	Paired t-test (p value)	Pearson Correlation Coefficient (r)	Dice Coefficient
Healthy Controls (n=20)					
NFL	20.10 ± 10.61	18.23 ± 11.24	< 0.001	0.924	0.989
GCL-IPL	71.43 ± 23.32	70.78 ± 24.40	0.101	0.956	0.995
INL	33.00 ± 10.21	32.00 ± 11.38	< 0.001	0.980	0.995
OPL	27.47 ± 9.55	26.21 ± 8.88	< 0.001	0.971	0.978
ONL-ISM	89.82 ± 20.20	96.83 ± 20.22	< 0.001	0.957	0.962
ISE	32.28 ± 5.29	20.91 ± 3.74	< 0.001	0.395†	0.783
OS-RPE	32.96 ± 3.78	42.43 ± 3.18	< 0.001	0.227†	0.928
iAMD (n=20)					
NFL	21.96 ± 12.39	19.91 ± 12.61	< 0.001	0.382†	0.978
GCL-IPL	71.11 ± 23.28	72.03 ± 26.46	0.711	0.496	0.972
INL	33.00 ± 10.07	29.14 ± 11.25	< 0.001	0.952	0.951
OPL	27.24 ± 9.37	32.33 ± 11.34	0.002	0.695	0.923
ONL-ISM	89.49 ± 21.93	88.34 ± 22.96	0.274	0.930	0.989
ISE	28.48 ± 6.01	22.84 ± 6.83	< 0.001	0.148†	0.881
OS-RPE	45.46 ± 21.15	56.91 ± 23.94	< 0.001	0.477	0.927
eAMD (n=20)					
NFL	22.08 ± 12.71	19.54 ± 12.69	< 0.001	0.814	0.938
GCL-IPL	69.96 ± 25.27	77.76 ± 29.75	0.019	-0.061†	0.964
INL	34.64 ± 13.01	30.06 ± 12.44	0.002	0.388†	0.938
OPL	31.92 ± 12.33	28.29 ± 15.53	0.003	0.645	0.919
ONL-ISM	71.26 ± 20.54	80.82 ± 24.27	< 0.001	0.889	0.922
ISE	24.42 ± 7.61	20.36 ± 18.32	0.003	0.781	0.878
OS-RPE	87.08 ± 62.10	67.28 ± 47.26	< 0.001	0.733	0.992
Fluid	0.125*	0.127*	0.192	0.999	0.976

* The standard deviation (SD) for the fluid area was not extracted.
† The results were not statistically significant.

3.2. Accessing DL software accuracy

3.2.1. Retinal layer thickness segmentation

The one-way ANOVA test showed a statistically significant difference in the mean thickness of outer retina in the adjusted automated segmentation ($p < 0.001$) and in the AI automatic segmentation ($p = 0.026$) across different disease severities. **Figure 4** provides a visual representation of this relationship, particularly in the OS-RPE layer, which showed a mean thickening of 27.06 μm and 12.43 μm with disease, using the RS and the DL methods, respectively.



(**a**) (**b**)

Figure 4. Mean outer retinal thickness measured by **(a)** the RS and **(b)** the DL systems across three different disease stages: controls, iAMD, and eAMD. eAMD: exudative AMD; iAMD: intermediate AMD; ISE: inner segment ellipsoid; OS-RPE: outer segment-retinal pigment epithelium.

3.2.2. Fluid segmentation

In order to compare the results of the DL system with RS for fluid segmentation, a Bland-Altman plot with the differences in the fluid area detected in eAMD eyes is presented in **Figure 5**. The mean difference obtained was 0.001 ± 0.004 mm^2. All measurements, except for one potential outlier, were within the range of ±1.96SD (0.007-0.009). No apparent trend was observed, suggesting that the DL software tended to either overestimate or underestimate fluid area, depending on the magnitude of exudation.

The results of the linear regression analysis did not exhibit statistical significance (p=0.164), which corroborated the absence of proportional bias.

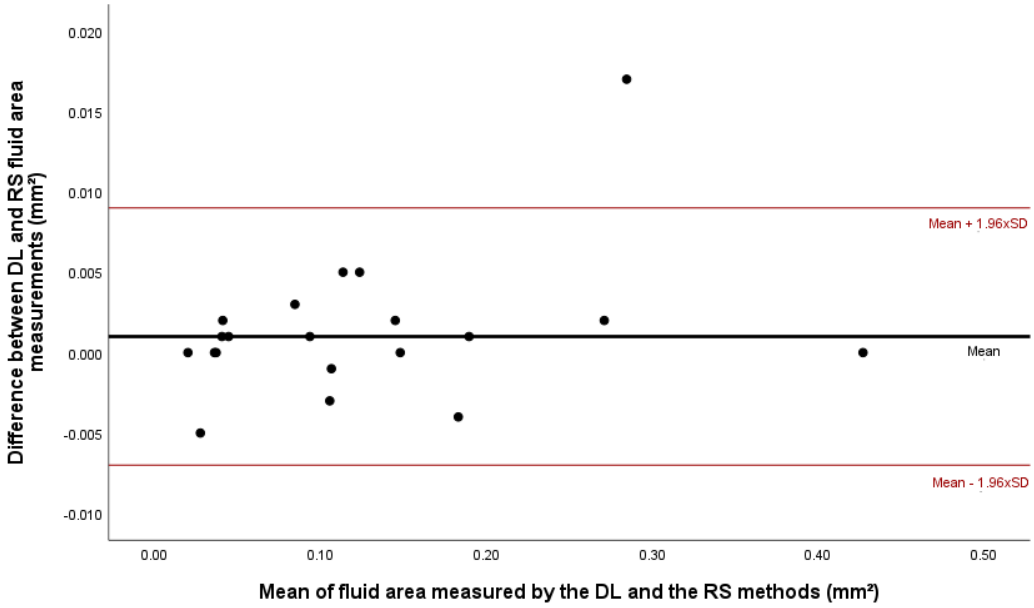
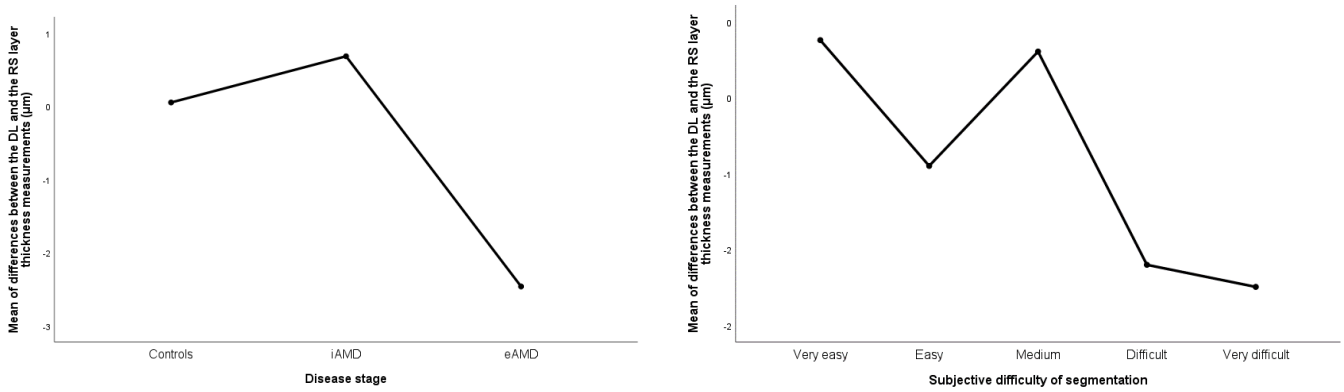


Figure 5. Bland-Altman plot analysis comparing the fluid area measures obtained using the DL software and the RS method. DL: deep learning; RS: reference standard; SD: standard deviation.

3.3. Disagreement between methods in layers' segmentation

A one-way ANOVA test revealed statistically significant differences between both methods for all three stages of disease severity ($p < 0.001$). However, there was no statistically significant difference across the levels of perceived difficulty of segmentation (p=0.625). Representative graphs illustrating the variance across the objective and

subjective scales are found in **Figure 6.** It was observed that the disagreement increased, as expected, with the disease progression. In iAMD, the DL method gave rise to higher values for the layers' thickness compared to the RS (positive difference), whereas in eAMD, the DL system resulted comparatively in lower values (negative difference).



(**a**) (**b**)

Figure 6. Plots representing the mean differences between the obtained DL and RS layer thickness measurements across **(a)** disease stage and **(b)** subjective difficulty perception of segmentation. DL: deep learning; eAMD: exudative AMD; iAMD: intermediate AMD; RS: Reference Standard.

4. Discussion

This article evaluates the performance of a DL algorithm in the automatic detection and segmentation of retinal layers and fluid in three OCT data groups: healthy controls and two different stages of AMD, intermediate and exudative.

In the absence of an established gold standard, it is common practice to compare automated AI segmentations with manual annotations, which are typically conducted by independent masked retinal experts, as seen in other published studies [22,23]. However, this approach may not be completely reliable and precise, due to interobserver and intraobserver variability, which introduces some degree of subjectivity [24]. Manual segmentation may also be influenced by limitations in imaging resolution detectable by the human eye. Therefore, even though differences may still exist between human perception and machine interpretation, their merged contribution was considered reasonable. Thus, this study innovated by combining human expertise with non-AI automatic suggestions to establish a gold standard.

Additionally, the study included real-world OCT images from routine clinical practise rather than images acquired proposedly for investigation purposes. Efforts were made to retain scans of all levels of complexity, in order to create balanced groups, ensuring a representative sample to truly test the system.

Firstly, the objective was to determine whether there was agreement in segmentation between the two systems. Significant differences were observed in almost all pairs of metrics, and in six cases, the Pearson correlation coefficient was not statistically significant. Upon further investigation of the scatter plots between RS and DL measures, outliers were found in all cases except for the ISE layer pairs in controls. Therefore, the non-significant results could be attributed to the presence of outliers in a small sample size. Additionally, in general, there was a moderate to strong correlation in all groups and the overlap segmentation area of layers and fluid between both methods was consistently near-perfect. Manual segmentation is a time-consuming and exhaustive task that requires knowledge, a learning curve, and skill. AI-based detection methods could help to overcome the mentioned disadvantages [25]. These results may emphasize the potential this DL method holds in the future as a valuable tool to assist clinics in medical practice, offering a faster, less fatiguing and more systematic approach.

Secondly, software accuracy was specifically evaluated by examining the extracted values of outer retinal thickness. This measurement is expected to be higher in recently diagnosed and treatment-naïve patients. In iAMD, the increase may be caused by basal laminar deposits beneath the RPE, i.e. drusen formation, and macula elevation. In eAMD, the thickening may be due to the accumulation of fluid and/or detachment of RPE. Thinning of some retinal layers may also occur as a consequence of RPE and photoreceptor degeneration, but it is expected to develop over a longer course of the disease [26]. In this study, the outer retina was defined as consisting of the last two retinal layers measured, ISE and OS-RPE, as they were considered the most representative layers for this analysis. The results showed a statistically significant difference, particularly due to an increase in the thickness of the OS-RPE layer, as expected. If this biomarker is correlated with the disease stage, it supports the research in confirming its potential for disease classification.

During the evaluation of the software's capacity to detect and measure fluid, one outlier was identified. The authors confirmed it resulted from a vertical shift difference between the fully automatic segmented image (maintaining the original OCT scan position) and the one resulting from the adjusted automated segmentation. This discrepancy likely occurred during the acquisition of the latter image due to a system error, which did not occur in any other case. Despite this outlier, the overall interpretation of results indicates that the DL system's performance was not affected by the degree of exudation and it had the capability to accurately detect and quantify fluid area. Patients with AMD might suffer insidious or sudden painless loss of central or pericentral vision (scotomas) and perception distortion (metamorphopsia) [27], as well as reduced visual acuity in low luminance conditions and impaired dark adaptation [28]. These symptoms can notably impact daily activities and quality of life [29]. Thus, to ensure timely clinical approaches and treatment decisions, it is essential to distinguish between nonexudative and exudative AMD based on OCT evaluation. In nonexudative AMD, there are currently limited effective therapies for managing atrophy. Disease progression may be slowed with dietary anti-oxidant supplementation [30], and only recently, innovational therapies for atrophic disease were approved [31]. Therefore, OCT findings consistent with nAMD may be more determinant of prognosis. If this software tool could aid ophthalmologists in detecting and quantifying retinal fluid, a critical hallmark for initiating anti-vascular endothelial growth factor (anti-VEGF) injections [32,33], which are highly effective, particularly in difficult cases with lower fluid areas appearing in OCT scans, it could potentially improve patient management.

Finally, a more in-depth investigation was conducted to understand the sources of discrepancies, exploring both objective and subjective difficulty levels. The study found an association between a late disease stage and increased disagreement between methods. However, no statistical difference was found across values of human perception of difficulty in segmentation. The investigation into retinal pathology at different stages of AMD allowed to understand whether the severity of the disease could impact the capacity of the system to accurately recognise and segment retinal layers. This analysis identified areas where the system encountered reasonable higher difficulty, providing insights to guide future technical improvements in the DL algorithm.

Furthermore, the validation of this AI method could consider its application in real-world settings, in clinical practice or even serve as an educational tool for new medical professionals, contributing to their learning curve and skill development in interpreting OCT images accurately.

This study had several limitations. Firstly, the enrolled sample size was relatively small, particularly given the single-centre nature of the study. Future research could benefit from a larger population to better assure the generalizability of the findings. Secondly, the absence of 3D volume measurements for both retinal layers and fluid due to the need for multiple 2D scans and segmentation of each image individually. Nevertheless, further investigation should consider seeking into volumetric analyses, as previous studies have shown their valuable insights [34,35]. Thirdly, this study was limited to diagnostic

evaluation, as longitudinal follow-up data were not collected. Gathering such data could provide a more comprehensive understanding of the predictive capabilities of the developed segmentation system, including its prognostic value [36,37]. Lastly, the study only focused on AMD pathology. It would be important to further include other retinal diseases such as diabetic retinopathy, central serous chorioretinopathy, epiretinal membrane , and glaucoma, as done in other approaches [38-41]. Testing the method on a wider range of retinal pathologies would be a valuable challenge and a step towards simulating real-world clinical conditions.

In the future, there is the prospect of training this system in disease classification using biomarkers, such as those studied in this paper, ultimately enabling an autonomous diagnosis, that could potentially precede human visual discernment. In a study setting with a considerable sample size, it would be interesting to test the accuracy of the system in classifying diseases and diagnosing based on retinal thickness. This could be done by calculating sensitivities, specificities, and predictive values. Another aspect to explore would be the discriminative capacity of the DL system in distinguishing between the presence and absence of fluid in OCT scans. This could be accomplished by determining a cut-off value and measuring area under a receiver operating characteristic (ROC) curve (AUC).

In summary, upon completed validation of this segmentation system, several potential clinical applications may arise. Formal implementation could enable early and more precise diagnosis, thereby facilitating timely therapeutic interventions, particularly in exudative cases, thus improving patients' prognosis and quality of life.

5. Conclusions

In conclusion, this study contributes to the current knowledge of AI algorithms in OCT imaging. This DL algorithm, when compared to a Reference Standard, demonstrated a moderate to strong correlation between metrics, an overall high overlap on area segmentation, the ability to detect outer retinal thickening across disease progression and proved to have high precision for detecting fluid in exudative cases. Moreover, an increased discrepancy between methods was observed in more advanced disease stages.

Overall, these systems hold the potential to overachieve the precision of the human eye. They could serve as reliable tools that could, initially, complement existing methods and, eventually, function autonomously to detect, segment, measure, classify, diagnose and predict prognosis. Lastly, their integration into patient care and therapeutic assessment could improve clinical outcomes in the ophthalmology field.

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Informed Consent Statement: Patient consent was not necessary for the image analysis due to complete anonymization of all clinical data and OCT images.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Causes of blindness and vision impairment in 2020 and trends over 30 years, and prevalence of avoidable blindness in relation to VISION 2020: the Right to Sight: an analysis for the Global Burden of Disease Study. *Lancet Glob Health* **2021**, *9*, e144-e160, doi:10.1016/s2214-109x(20)30489-7.
2. Bourne, R.R.A.; Jonas, J.B.; Bron, A.M.; Cicinelli, M.V.; Das, A.; Flaxman, S.R.; Friedman, D.S.; Keeffe, J.E.; Kempen, J.H.; Leasher, J.; et al. Prevalence and causes of vision loss in high-income countries and in Eastern and Central Europe in 2015: magnitude, temporal trends and projections. *Br J Ophthalmol* **2018**, *102*, 575-585, doi:10.1136/bjophthalmol-2017-311258.
3. Wong, W.L.; Su, X.; Li, X.; Cheung, C.M.; Klein, R.; Cheng, C.Y.; Wong, T.Y. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health* **2014**, *2*, e106-116, doi:10.1016/s2214-109x(13)70145-1.
4. Jabbehdari, S.; Handa, J.T. Oxidative stress as a therapeutic target for the prevention and treatment of early age-related macular degeneration. *Surv Ophthalmol* **2021**, *66*, 423-440, doi:10.1016/j.survophthal.2020.09.002.
5. Chen, M.; Xu, H. Parainflammation, chronic inflammation, and age-related macular degeneration. *J Leukoc Biol* **2015**, *98*, 713-725, doi:10.1189/jlb.3RI0615-239R.
6. Mullins, R.F.; Schoo, D.P.; Sohn, E.H.; Flamme-Wiese, M.J.; Workamelahu, G.; Johnston, R.M.; Wang, K.; Tucker, B.A.; Stone, E.M. The membrane attack complex in aging human choriocapillaris: relationship to macular degeneration and choroidal thinning. *Am J Pathol* **2014**, *184*, 3142-3153, doi:10.1016/j.ajpath.2014.07.017.
7. Fritsche, L.G.; Igl, W.; Bailey, J.N.; Grassmann, F.; Sengupta, S.; Bragg-Gresham, J.L.; Burdon, K.P.; Hebbring, S.J.; Wen, C.; Gorski, M.; et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet* **2016**, *48*, 134-143, doi:10.1038/ng.3448.
8. Heesterbeek, T.J.; Lorés-Motta, L.; Hoyng, C.B.; Lechanteur, Y.T.E.; den Hollander, A.I. Risk factors for progression of age-related macular degeneration. *Ophthalmic Physiol Opt* **2020**, *40*, 140-170, doi:10.1111/opo.12675.
9. Curcio, C.A. Soft Drusen in Age-Related Macular Degeneration: Biology and Targeting Via the Oil Spill Strategies. *Invest Ophthalmol Vis Sci* **2018**, *59*, Amd160-amd181, doi:10.1167/iovs.18-24882.
10. Ferris, F.L., 3rd; Wilkinson, C.P.; Bird, A.; Chakravarthy, U.; Chew, E.; Csaky, K.; Sadda, S.R. Clinical classification of age-related macular degeneration. *Ophthalmology* **2013**, *120*, 844-851, doi:10.1016/j.ophtha.2012.10.036.
11. Elsharkawy, M.; Elrazzaz, M.; Ghazal, M.; Alhalabi, M.; Soliman, A.; Mahmoud, A.; El-Daydamony, E.; Atwan, A.; Thanos, A.; Sandhu, H.S.; et al. Role of Optical Coherence Tomography Imaging in Predicting Progression of Age-Related Macular Disease: A Survey. *Diagnostics (Basel)* **2021**, *11*, doi:10.3390/diagnostics11122313.
12. Schlanitz, F.G.; Baumann, B.; Kundi, M.; Sacu, S.; Baratsits, M.; Scheschy, U.; Shahlaee, A.; Mittermüller, T.J.; Montuoro, A.; Roberts, P.; et al. Drusen volume development over time and its relevance to the course of age-related macular degeneration. *Br J Ophthalmol* **2017**, *101*, 198-203, doi:10.1136/bjophthalmol-2016-308422.
13. Waldstein, S.M.; Vogl, W.D.; Bogunovic, H.; Sadeghipour, A.; Riedl, S.; Schmidt-Erfurth, U. Characterization of Drusen and Hyperreflective Foci as Biomarkers for Disease Progression in Age-Related Macular Degeneration Using Artificial Intelligence in Optical Coherence Tomography. *JAMA Ophthalmol* **2020**, *138*, 740-747, doi:10.1001/jamaophthalmol.2020.1376.
14. Metrangolo, C.; Donati, S.; Mazzola, M.; Fontanel, L.; Messina, W.; D'Alterio, G.; Rubino, M.; Radice, P.; Premi, E.; Azzolini, C. OCT Biomarkers in Neovascular Age-Related Macular Degeneration: A Narrative Review. *J Ophthalmol* **2021**, *2021*, 9994098, doi:10.1155/2021/9994098.
15. Lai, T.T.; Hsieh, Y.T.; Yang, C.M.; Ho, T.C.; Yang, C.H. Biomarkers of optical coherence tomography in evaluating the treatment outcomes of neovascular age-related macular degeneration: a real-world study. *Sci Rep* **2019**, *9*, 529, doi:10.1038/s41598-018-36704-6.

16. Dahrouj, M.; Miller, J.B. Artificial Intelligence (AI) and Retinal Optical Coherence Tomography (OCT). *Semin Ophthalmol* **2021**, *36*, 341-345, doi:10.1080/08820538.2021.1901123.
17. Leng, X.; Shi, R.; Wu, Y.; Zhu, S.; Cai, X.; Lu, X.; Liu, R. Deep learning for detection of age-related macular degeneration: A systematic review and meta-analysis of diagnostic test accuracy studies. *PLoS One* **2023**, *18*, e0284060, doi:10.1371/journal.pone.0284060.
18. Bhuiyan, A.; Wong, T.Y.; Ting, D.S.W.; Govindaiah, A.; Souied, E.H.; Smith, R.T. Artificial Intelligence to Stratify Severity of Age-Related Macular Degeneration (AMD) and Predict Risk of Progression to Late AMD. *Transl Vis Sci Technol* **2020**, *9*, 25, doi:10.1167/tvst.9.2.25.
19. Schmidt-Erfurth, U.; Reiter, G.S.; Riedl, S.; Seeböck, P.; Vogl, W.D.; Blodi, B.A.; Domalpally, A.; Fawzi, A.; Jia, Y.; Sarraf, D.; Bogunović, H. AI-based monitoring of retinal fluid in disease activity and under therapy. *Prog Retin Eye Res* **2022**, *86*, 100972, doi:10.1016/j.preteyeres.2021.100972.
20. Yim, J.; Chopra, R.; Spitz, T.; Winkens, J.; Obika, A.; Kelly, C.; Askham, H.; Lukic, M.; Huemer, J.; Fasler, K.; et al. Predicting conversion to wet age-related macular degeneration using deep learning. *Nat Med* **2020**, *26*, 892-899, doi:10.1038/s41591-020-0867-7.
21. Melo, T.; Carneiro, Â.; Campilho, A.; Mendonça, A.M. Retinal layer and fluid segmentation in optical coherence tomography images using a hierarchical framework. *J Med Imaging (Bellingham)* **2023**, *10*, 014006, doi:10.1117/1.Jmi.10.1.014006.
22. Liu, J.; Yan, S.; Lu, N.; Yang, D.; Lv, H.; Wang, S.; Zhu, X.; Zhao, Y.; Wang, Y.; Ma, Z.; Yu, Y. Automated retinal boundary segmentation of optical coherence tomography images using an improved Canny operator. *Sci Rep* **2022**, *12*, 1412, doi:10.1038/s41598-022-05550-y.
23. Schlegl, T.; Waldstein, S.M.; Bogunovic, H.; Endstraßer, F.; Sadeghipour, A.; Philip, A.M.; Podkowinski, D.; Gerendas, B.S.; Langs, G.; Schmidt-Erfurth, U. Fully Automated Detection and Quantification of Macular Fluid in OCT Using Deep Learning. *Ophthalmology* **2018**, *125*, 549-558, doi:10.1016/j.ophtha.2017.10.031.
24. Camacho, P.; Dutra-Medeiros, M.; Salgueiro, L.; Sadio, S.; Rosa, P.C. Manual Segmentation of 12 Layers of the Retina and Choroid through SD-OCT in Intermediate AMD: Repeatability and Reproducibility. *J Ophthalmic Vis Res* **2021**, *16*, 384-392, doi:10.18502/jovr.v16i3.9435.
25. Srivastava, O.; Tennant, M.; Grewal, P.; Rubin, U.; Seamone, M. Artificial intelligence and machine learning in ophthalmology: A review. *Indian J Ophthalmol* **2023**, *71*, 11-17, doi:10.4103/ijo.IJO_1569_22.
26. Acton, J.H.; Smith, R.T.; Hood, D.C.; Greenstein, V.C. Relationship between retinal layer thickness and the visual field in early age-related macular degeneration. *Invest Ophthalmol Vis Sci* **2012**, *53*, 7618-7624, doi:10.1167/iovs.12-10361.
27. Bjerager, J.; Schneider, M.; Potapenko, I.; van Dijk, E.H.C.; Faber, C.; Grauslund, J.; Pfau, K.; Huemer, J.; Muttuvelu, D.V.; Rasmussen, M.L.R.; et al. Diagnostic Accuracy of the Amsler Grid Test for Detecting Neovascular Age-Related Macular Degeneration: A Systematic Review and Meta-analysis. *JAMA Ophthalmol* **2023**, *141*, 315-323, doi:10.1001/jamaophthalmol.2022.6396.
28. Finger, R.P.; Schmitz-Valckenberg, S.; Schmid, M.; Rubin, G.S.; Dunbar, H.; Tufail, A.; Crabb, D.P.; Binns, A.; Sánchez, C.I.; Margaron, P.; et al. MACUSTAR: Development and Clinical Validation of Functional, Structural, and Patient-Reported Endpoints in Intermediate Age-Related Macular Degeneration. *Ophthalmologica* **2019**, *241*, 61-72, doi:10.1159/000491402.
29. Taylor, D.J.; Hobby, A.E.; Binns, A.M.; Crabb, D.P. How does age-related macular degeneration affect real-world visual ability and quality of life? A systematic review. *BMJ Open* **2016**, *6*, e011504, doi:10.1136/bmjopen-2016-011504.
30. Chapman, N.A.; Jacobs, R.J.; Braakhuis, A.J. Role of diet and food intake in age-related macular degeneration: a systematic review. *Clin Exp Ophthalmol* **2019**, *47*, 106-127, doi:10.1111/ceo.13343.
31. Chew, E.Y. Complement inhibitors for the treatment of geographic atrophy. *Lancet* **2023**, *402*, 1396-1398, doi:10.1016/s0140-6736(23)01844-5.

32. Brown, D.M.; Kaiser, P.K.; Michels, M.; Soubrane, G.; Heier, J.S.; Kim, R.Y.; Sy, J.P.; Schneider, S. Ranibizumab versus verteporfin for neovascular age-related macular degeneration. *N Engl J Med* **2006**, *355*, 1432-1444, doi:10.1056/NEJMoa062655.
33. Martin, D.F.; Maguire, M.G.; Ying, G.S.; Grunwald, J.E.; Fine, S.L.; Jaffe, G.J. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med* **2011**, *364*, 1897-1908, doi:10.1056/NEJMoa1102673.
34. Alex, V.; Motevasseli, T.; Freeman, W.R.; Jayamon, J.A.; Bartsch, D.G.; Borooah, S. Assessing the validity of a cross-platform retinal image segmentation tool in normal and diseased retina. *Sci Rep* **2021**, *11*, 21784, doi:10.1038/s41598-021-01105-9.
35. Pawloff, M.; Gerendas, B.S.; Deak, G.; Bogunovic, H.; Gruber, A.; Schmidt-Erfurth, U. Performance of retinal fluid monitoring in OCT imaging by automated deep learning versus human expert grading in neovascular AMD. *Eye (Lond)* **2023**, *37*, 3793-3800, doi:10.1038/s41433-023-02615-8.
36. Banerjee, I.; de Sisternes, L.; Hallak, J.A.; Leng, T.; Osborne, A.; Rosenfeld, P.J.; Gregori, G.; Durbin, M.; Rubin, D. Prediction of age-related macular degeneration disease using a sequential deep learning approach on longitudinal SD-OCT imaging biomarkers. *Sci Rep* **2020**, *10*, 15434, doi:10.1038/s41598-020-72359-y.
37. Russakoff, D.B.; Lamin, A.; Oakley, J.D.; Dubis, A.M.; Sivaprasad, S. Deep Learning for Prediction of AMD Progression: A Pilot Study. *Invest Ophthalmol Vis Sci* **2019**, *60*, 712-722, doi:10.1167/iovs.18-25325.
38. Midena, E.; Toto, L.; Frizziero, L.; Covello, G.; Torresin, T.; Midena, G.; Danieli, L.; Pilotto, E.; Figus, M.; Mariotti, C.; Lupidi, M. Validation of an Automated Artificial Intelligence Algorithm for the Quantification of Major OCT Parameters in Diabetic Macular Edema. *J Clin Med* **2023**, *12*, doi:10.3390/jcm12062134.
39. Ko, J.; Han, J.; Yoon, J.; Park, J.I.; Hwang, J.S.; Han, J.M.; Park, K.H.; Hwang, D.D. Assessing central serous chorioretinopathy with deep learning and multiple optical coherence tomography images. *Sci Rep* **2022**, *12*, 1831, doi:10.1038/s41598-022-05051-y.
40. Jin, K.; Yan, Y.; Wang, S.; Yang, C.; Chen, M.; Liu, X.; Terasaki, H.; Yeo, T.H.; Singh, N.G.; Wang, Y.; Ye, J. iERM: An Interpretable Deep Learning System to Classify Epiretinal Membrane for Different Optical Coherence Tomography Devices: A Multi-Center Analysis. *J Clin Med* **2023**, *12*, doi:10.3390/jcm12020400.
41. Mariottoni, E.B.; Datta, S.; Shigueoka, L.S.; Jammal, A.A.; Tavares, I.M.; Henao, R.; Carin, L.; Medeiros, F.A. Deep Learning-Assisted Detection of Glaucoma Progression in Spectral-Domain OCT. *Ophthalmol Glaucoma* **2023**, *6*, 228-238, doi:10.1016/j.ogla.2022.11.004.

APÊNDICES

Índice

Section/Topic	Item	Checklist Item	Page
Title and abstract			
Title	1	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	**Page 1** "Human *versus* Artificial Intelligence: Validation of a Deep Learning Model for Retinal Layer and Fluid Segmentation in Optical Coherence Tomography Images from Patients with Age-Related Macular Degeneration"
Abstract	2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	**Page 1** "Artificial Intelligence (AI) models (…) OCT imaging management and guide timely treatment approaches."
Introduction			
Background and objectives	3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	**Page 1/2, paragraphs 1-2/1-3** "Age-related macular degeneration (AMD) is a leading cause (…) applicability in real-world clinical practice."
	3b	Specify the objectives, including whether the study describes the development or validation of the model or both.	**Page 2, paragraph 4** "Thus, the present study aims to evaluate the performance (…) subjective difficulty degree, perceived by the human eye."
Methods			
Source of data	4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	**Page 2, paragraph 5** "The study was single-centred, observational, retrospective, and cross-sectional."
	4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	**Page 2, paragraph 7** "OCT scans were collected from subjects with normal eyes and patients diagnosed with intermediate and exudative AMD (…) between January 2010 and December 2023."
Participants	5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	**Page 2, paragraphs 5 and 7** "The study was single-centred (…)" "(…) who presented for routine clinical care at the Ophthalmology Service of the Centro Hospitalar Universitário of São João, a tertiary referral hospital (…)"
	5b	Describe eligibility criteria for participants.	**Page 3, paragraphs 3-4** Inclusion criteria: "Male and female subjects (…) after the disease diagnosis was required." Exclusion criteria: "Exclusion criteria for the study (…) or reduced visualization of the retinal layers were also excluded."
	5c	Give details of treatments received, if relevant.	Not applicable. It was not relevant considering the objectives of the study.
Outcome	6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	**Page 7, paragraph 3** "The primary outcomes were (…) and fluid area in nAMD." "The secondary outcome was (…) of segmentation (subjective score)."
	6b	Report any actions to blind assessment of the outcome to be predicted.	Not applicable in this study approach.
Predictors	7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	**Pages 6, paragraphs 1-2** "(…) the retinal boundaries (…) throughout the entire image)." **Pages 7, paragraph 1** "The standard fluid segmentation (…) by the same ophthalmologist."
	7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	Not applicable in this study approach.
Sample size	8	Explain how the study size was arrived at.	**Page 3, paragraphs 2 and 5** "A randomized numerical sequence was automatically generated (…) until a total of 20 participants were obtained." "In December 2023, 798 participants were assessed for eligibility (…) from the third

			group analysis, as represented in Figure 1."
Missing data	9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	Not applicable. No missing data were observed.
Statistical analysis methods	10c	For validation, describe how the predictions were calculated.	**Page 5, paragraph 4** "Thus, the seven retinal layers studied in all groups and the fluid detected in nAMD images are represented in Figure 3." **Page 7, paragraph 5** "The thickness of the segmented retinal layers was converted to microns (µm) (…) fluid area was converted to square millimetres (mm^2) (…)".
	10d	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	**Page 7, paragraphs 7-8** "To evaluate the performance of the DL system (…) to 1 (perfect overlap)." "To further evaluate accuracy, (…) quantifying fluid area."
	10e	Describe any model updating (e.g., recalibration) arising from the validation, if done.	Not applicable. There was no update of the model during this research.
Risk groups	11	Provide details on how risk groups were created, if done.	Not applicable.
Development vs. validation	12	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	Not applicable.
Results			
Participants	13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	Not applicable. This study was not longitudinal.
	13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	**Page 3, paragraphs 3-5** "Male and female subjects were evenly distributed (…) as represented in Figure 1." **Page 7, paragraph 10** "A total of 60 images from 60 patients (…) for a comparative quantitative analysis."
	13c	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	Not applicable.
Model performance	16	Report performance measures (with CIs) for the prediction model.	**Page 7, paragraph 4** "A p value of less than 0.05 was assumed for statistical significance." **Page 7, paragraph 7** "(…) Paired Sample T-Test was applied (…) Pearson Correlation Coefficient (…) the Dice score (…)". **Page 9/10, paragraphs 3-4/1** "(…) a statistical significant difference in the mean thickness of outer retina layers (…) segmentation (p = 0.026) across different disease severities." "(…)The mean difference obtained (…) absence of proportional bias." **Page 10, paragraph 2** "(…) there was no statistically significant difference across the levels of perceived difficulty of segmentation (p=0.625). (…)"
Model-updating	17	If done, report the results from any model updating (i.e., model specification, model performance).	Not applicable. There was no update of the model during this research.
Discussion			
Limitations	18	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	**Page 12, paragraph 3** "This study had several limitations. (…) simulating real-world clinical conditions."
Interpretation	19a	For validation, discuss the results with reference to performance in the development data, and any other validation data.	Not applicable.

	19b	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	**Pages 11/12, paragraphs 4-6/1** "Firstly, the objective was to (…) future technical improvements in the DL algorithm."
Implications	20	Discuss the potential clinical use of the model and implications for future research.	**Page 12, paragraph 2** "Furthermore, the validation of this AI method (…) learning curve and skill development in interpreting OCT images accurately." **Page 12, paragraphs 4-5** "In the future, there is the prospect (…) thus improving patients' prognosis and quality of life."
Other information			
Supplementary information	21	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Not applicable.
Funding	22	Give the source of funding and the role of the funders for the present study.	**Page 13** "This research received no external funding."

We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

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    - **Back matter**: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, **References**.
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Images gathered at different times or from different locations should not be combined into a single image unless it is stated that the resultant image is a product of time-averaged data or a time lapse sequence. If juxtaposing images are essential, the borders should be clearly demarcated in the figure and described in the legend.

We encourage the inclusion of the following with the final revised version of the manuscript for publication:

In the Methods section, specify the type of equipment (microscopes/objective lenses, cameras, detectors, filter model, and batch number), the acquisition software used, and the magnification or the scale bar in the figure caption. Although we appreciate that there is some variation between instruments, equipment settings for critical measurements should also be listed.

We encourage the deposition of unprocessed image files in a publicly available database (a **link** to the downloadable table from data availability instructions should be shared here) (including relevant metadata for acquisition information, including time and space resolution data (xyzt and pixel dimensions); image bit depth; experimental conditions such as temperature and imaging medium; and fluorochromes (excitation and emission wavelengths or ranges, filters, and dichroic beam splitters) if any).

Processing software should be named in the Methods section and any manipulations should be indicated in the relevant figure legends (such as type of deconvolution, three-dimensional reconstructions, surface and volume rendering, “gamma changes”, filtering, thresholding, and projection).

Comprehensive guidelines on data management and the ethical handling of digital images can obtained from The Office of Research Integrity: **http://ori.hhs.gov/ images/ddblock/data.pdf**

MDPI is a member of the Committee on Publication Ethics and takes the responsibility to uphold strict ethical policies and standards very seriously.

Supplementary Materials, Data Deposit and Software Source Code

MDPI Research Data Policies

MDPI is committed to supporting open scientific exchange and enabling our authors to achieve best practices in sharing and archiving research data. We encourage all authors of articles published in MDPI journals to share their research data including, but not limited to protocols, analytic methods, raw data, processed data, code, software, algorithms, and study material. The data should be FAIR – findable, accessible, interoperable, and reusable – so that other researchers can locate and use the data.

We recommend that data and code should be deposited in a trusted repository that will allow for maximum reuse (see the Data Preservation section below). If this is not possible, authors are encouraged to share the specific reason in the Data Availability Statement and make this material available upon request to interested researchers. In addition, research materials necessary to enable the reproduction of an experiment should be indicated in the Materials and Methods section. Individual journal guidelines can be found at the journal 'Instructions for Authors' page. Data sharing policies concern the minimal dataset that supports the central findings of a published study. Generated data should be publicly available and cited in accordance with journal guidelines.

MDPI data policies are informed by **TOP Guidelines**.

Where ethical, legal, or privacy issues are present, data should not be shared. The authors should clarify the availability status of the data upon submission and make any limitations or exceptions clear in the Data Availability Statement. Authors should ensure that the data shared is in accordance with consent provided by participants on the use of confidential data. Authors should ensure that the publication of such data does not compromise the anonymity of the participants or breach local data protection laws.

In situations where access is restricted to protect confidential or proprietary information, authors will be requested to clearly explain the restrictions on the dataset and make the data available upon request, with permission for the purposes of peer review.

MDPI recognizes that some institutions and funding agencies only require the retention of research data for a finite period after a project's completion or publication. However, there are no such limits specified within the MDPI Data Availability Policy and, therefore, we encourage the authors to archive their research data through appropriate data repositories or provide us with minimal datasets within Supplementary Material.

Data availability statements

Data availability statements are required for all articles published with MDPI. During the peer review and editorial decision process, authors can be asked to share existing datasets or raw data that have been analyzed in the manuscript, and whether they will be made available to other researchers following publication. Authors will also be asked for the details of any existing datasets that have been analyzed in the manuscript.

Below are the recommended Data Availability Statements:

Data availability status	Recommended Data Availability Statement
Data available in a publicly accessible repository	The original data presented in the study are openly available in [repository name, e.g., FigShare] at [DOI/URL] or [reference/accession number].
Data available on request due to restrictions (e.g., privacy, legal or ethical reasons)	The data presented in this study are available on request from the corresponding author due to (specify the reason for the restriction).
3rd Party Data	Restrictions apply to the availability of these data. Data were obtained from [third party] and are available [from the authors/at URL] with the permission of [third party].
Embargo on data due to commercial restrictions	The data that support the findings will be available in [repository name] at [URL / DOI link] following an embargo from the date of publication to allow for commercialization of research findings.
Restrictions apply to the datasets:	The datasets presented in this article are not readily available because [include reason, e.g., the data are part of an ongoing study or due to technical/ time limitations].

	Requests to access the datasets should be directed to [text input].
Data derived from public domain resources:	The data presented in this study are available in [repository name] at [URL/DOI], reference number [reference number]. These data were derived from the following resources available in the public domain: [list resources and URLs]
Data sharing is not applicable (only appropriate if no new data is generated or the article describes entirely theoretical research.	No new data were created or analyzed in this study. Data sharing is not applicable to this article
Data is contained within the article or supplementary material:	The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.
Dataset available on request from the authors.	The raw data supporting the conclusions of this article will be made available by the authors on request.

Data preservation

MDPI acknowledges that researchers, institutions, journals, and data repositories have a shared responsibility to ensure long-term data preservation, and MDPI encourages authors to select data repositories with this goal in mind.

MDPI encourages authors to commit to preserving their datasets on their laboratory or institutional servers, for at least five years after publication. If, during that time, the repository to which the data were originally submitted disappears or experiences data loss, we may ask the authors to upload the data to another repository and publish a correction or update to the original publication.

If authors remove their data from the original public repository or change access criteria in a manner that is inconsistent with the publication, we may ask authors to notify the editorial office as soon as possible.

How to choose an appropriate data repository

MDPI encourages the submission of data to community-recognized data repositories where possible. We recommend the authors visit **re3data.org** or **fairsharing.org** to help identify registered and certified data repositories relevant to their subject area if no community resource is available. If the authors' institution has its generalist data repository this can be used to host authors' data as long as the repository can mint **DataCite DOIs**, and allows for data to be shared under open terms of use (for example the **CC0 waiver**).

Data repository criteria

The following criteria should be considered when selecting an appropriate repository, ensuring that platforms:

- Ensure long-term persistence and preservation of datasets in their published form;
- Provide stable identifiers for submitted datasets (DOIs in most cases);
- Allow public access to data without barriers, such as logins or paywalls;
- Support open licenses (CC0 and CC-BY, or their equivalents, are required in most cases);
- Provide confidential review of submitted datasets without the requirement for reviewers to provide identifying information.

Data citation

Authors are encouraged to formally cite any datasets stored in external repositories that are mentioned within their manuscript, including the main datasets that are the focus of the submission, as well as any other datasets that have been used in the work. For previously published datasets, authors should cite both the related research articles and the datasets themselves. Appropriate citation of data is checked and enforced by *Journal Editorial* staff before publication.

Computer Code and Software

For work where novel computer code was developed, authors should release the code either by depositing in a recognized, public repository or uploading as supplementary information to the publication. The name and version of all software used should be clearly indicated.

Supplementary Material

Additional data and files can be uploaded as "Supplementary Files" during the manuscript submission process. The supplementary files will also be available to the referees as part of the peer review process. Any file format is acceptable, however we recommend that common, non-proprietary formats are used where possible. For more information on supplementary materials, please refer to **https://www.mdpi.com/authors/layout#_bookmark83**.

Unpublished Data

Restrictions on data availability should be noted during submission and in the manuscript. "Data not shown" should be avoided: authors are encouraged to publish all observations related to the submitted manuscript as Supplementary Material. "Unpublished data" intended for publication in a manuscript that is either planned, "in preparation" or "submitted" but not yet accepted, should be cited in the text and a reference should be added in the References section. "Personal Communication" should also be cited in the text and reference added in the References section. (see also the MDPI reference list and citations style guide).

Remote Hosting and Large Data Sets

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult databib.org or re3data.org. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal **Data** also accepts submissions of data set papers.

Deposition of Sequences and Expression Data

New sequence information must be deposited to the appropriate database prior to submission of the manuscript. Accession numbers provided by the database should be included in the submitted manuscript. Manuscripts will not be published until the accession number is provided.

- *New nucleic acid sequences* must be deposited into an acceptable repository such as **GenBank**, **EMBL**, or **DDBJ**. Sequences should be submitted to only one database.
- *New high throughput sequencing (HTS) datasets* (RNA-seq, ChIP-Seq, degradome analysis, …) must be deposited either in the GEO database or in the NCBI's Sequence Read Archive.
- *New microarray data* must be deposited either in the GEO or the ArrayExpress databases.The "Minimal Information About a Microarray Experiment" (MIAME) guidelines published by the Microarray Gene Expression Data Society must be followed.
- *New protein sequences* obtained by protein sequencing must be submitted to UniProt (submission tool SPIN).

All sequence names and the accession numbers provided by the databases should be provided in the Materials and Methods section of the article.

References in Supplementary Files

Citations and References in Supplementary files are permitted provided that they also appear in the reference list of the main text.

Research and Publication Ethics

Research Ethics

Research Involving Human Subjects

When reporting on research that involves human subjects, human material, human tissues, or human data, authors must declare that the investigations were carried out following the rules of the Declaration of Helsinki of 1975 (**https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/**), revised in 2013.

According to point 23 of this declaration, an approval from the local institutional review board (IRB) or other appropriate ethics committee must be obtained before undertaking the research to confirm the study meets national and international guidelines. As a minimum, a statement including the project identification code, date of approval, and name of the ethics committee or institutional review board must be stated in Section 'Institutional Review Board Statement' of the article.

Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

For non-interventional studies (e.g. surveys, questionnaires, social media research), all participants must be fully informed if the anonymity is assured, why the research is being conducted, how their data will be used and if there are any risks associated. As with all research involving humans, ethical approval from an appropriate ethics committee must be obtained prior to conducting the study. If ethical approval is not required, authors must either provide an exemption from the ethics committee or are encouraged to cite the local or national legislation that indicates ethics approval is not required for this type of study. Where a study has been granted exemption, the name of the ethics committee which provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation regarding why ethical approval was not required.

A written informed consent for publication must be obtained from participating patients. Data relating to individual participants must be described in detail, but private information identifying participants need not be included unless the identifiable materials are of relevance to the research (for example, photographs of participants' faces that show a particular symptom). Patients' initials or other personal identifiers must not appear in any images. For manuscripts that include any case details, personal information, and/or images of patients, authors must obtain signed informed consent for publication from patients (or their relatives/guardians) before submitting to an MDPI journal. Patient details must be anonymized as far as possible, e.g., do not mention specific age, ethnicity, or occupation where they are not relevant to the conclusions. A **template permission form** is available to download. A blank version of the form used to obtain permission (without the patient names or signature) must be uploaded with your submission. Editors reserve the right to reject any submission that does not meet these requirements.

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If the study reports research involving vulnerable groups, an additional check may be performed. The submitted manuscript will be scrutinized by the editorial office and upon request, documentary evidence (blank consent forms and any related discussion documents from the ethics board) must be supplied. Additionally, when studies describe groups by race, ethnicity, gender, disability, disease, etc., explanation regarding why such categorization was needed must be clearly stated in the article.

Ethical Guidelines for the Use of Animals in Research

The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs [1]':

- Replacement of animals by alternatives wherever possible,
- Reduction in number of animals used, and
- Refinement of experimental conditions and procedures to minimize the harm to animals.

Authors must include details on housing, husbandry and pain management in their manuscript.

For further guidance authors should refer to the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures [2], American Association for Laboratory Animal Science [3] or European Animal Research Association [4].

If national legislation requires it, studies involving vertebrates or higher invertebrates must only be carried out after obtaining approval from the appropriate ethics committee. As a minimum, the project identification code, date of approval and name of the ethics committee or institutional review board should be stated in Section 'Institutional Review Board Statement'. Research procedures must be carried out in accordance with national and institutional regulations. Statements on animal welfare should confirm that the study

complied with all relevant legislation. Clinical studies involving animals and interventions outside of routine care require ethics committee oversight as per the American Veterinary Medical Association. If the study involved client-owned animals, informed client consent must be obtained and certified in the manuscript report of the research. Owners must be fully informed if there are any risks associated with the procedures and that the research will be published. If available, a high standard of veterinary care must be provided. Authors are responsible for correctness of the statements provided in the manuscript.

If ethical approval is not required by national laws, authors must provide an exemption from the ethics committee, if one is available. Where a study has been granted exemption, the name of the ethics committee that provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation on why the ethical approval was not required.

If no animal ethics committee is available to review applications, authors should be aware that the ethics of their research will be evaluated by reviewers and editors. Authors should provide a statement justifying the work from an ethical perspective, using the same utilitarian framework that is used by ethics committees. Authors may be asked to provide this even if they have received ethical approval.

MDPI endorses the ARRIVE guidelines (**arriveguidelines.org/**) for reporting experiments using live animals. Authors and reviewers must use the ARRIVE guidelines as a checklist, which can be found at **https://arriveguidelines.org/sites/arrive/files/documents/ARRIVE%20Compliance%20Questionnaire.pdf**. Editors reserve the right to ask for the checklist and to reject submissions that do not adhere to these guidelines, to reject submissions based on ethical or animal welfare concerns or if the procedure described does not appear to be justified by the value of the work presented.

1. NSW Department of Primary Industries and Animal Research Review Panel. Three Rs. Available online: **https://www.animalethics.org.au/three-rs**
2. Home Office. Animals (Scientific Procedures) Act 1986. Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes. Available online: **https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388535/CoPanimalsWeb.pdf**
3. American Association for Laboratory Animal Science. The Scientific Basis for Regulation of Animal Care and Use. Available online: **https://www.aalas.org/about-aalas/position-papers/scientific-basis-for-regulation-of-animal-care-and-use**
4. European Animal Research Association. EU regulations on animal research. Available online: **https://www.eara.eu/animal-research-law**

Research Involving Cell Lines

Methods sections for submissions reporting on research with cell lines should state the origin of any cell lines. For established cell lines the provenance should be stated and references must also be given to either a published paper or to a commercial source. If previously unpublished *de novo* cell lines were used, including those gifted from another laboratory, details of institutional review board or ethics committee approval must be given, and confirmation of written informed consent must be provided if the line is of human origin.

An example of Ethical Statements:

The HCT116 cell line was obtained from XXXX. The $MLH1^{+}$ cell line was provided by XXXXX, Ltd. The DLD-1 cell line was obtained from Dr. XXXX. The DR-GFP and SA-GFP reporter plasmids were obtained from Dr. XXX and the Rad51K133A expression vector was obtained from Dr. XXXX.

Research Involving Plants

Experimental research on plants (either cultivated or wild) including collection of plant material, must comply with institutional, national, or international guidelines. We recommend that authors comply with the **Convention on Biological Diversity** and the **Convention on the Trade in Endangered Species of Wild Fauna and Flora**.

For each submitted manuscript supporting genetic information and origin must be provided. For research manuscripts involving rare and non-model plants (other than, e.g., *Arabidopsis thaliana, Nicotiana benthamiana, Oryza sativa*, or many other typical model plants), voucher specimens must be deposited in an accessible herbarium or museum. Vouchers may be requested for review by future investigators to verify the identity of the material used in the study (especially if taxonomic rearrangements occur in the future). They should include details of the populations sampled on the site of collection (GPS coordinates), date of collection, and document the part(s) used in the study where appropriate. For rare, threatened or endangered species this can be waived but it is necessary for the author to describe this in the cover letter.

Editors reserve the rights to reject any submission that does not meet these requirements.

An example of Ethical Statements:

Torenia fournieri plants were used in this study. White-flowered Crown White (CrW) and violet-flowered Crown Violet (CrV) cultivars selected from 'Crown Mix' (XXX Company, City, Country) were kindly provided by Dr. XXX (XXX Institute, City, Country).

Arabidopis mutant lines (SALKxxxx, SAILxxxx,…) were kindly provided by Dr. XXX, institute, city, country).

Clinical Trials Registration

Registration

MDPI follows the International Committee of Medical Journal Editors (ICMJE) **guidelines** which require and recommend registration of clinical trials in a public trials registry at or before the time of first patient enrollment as a condition of consideration for publication.

Purely observational studies do not require registration. A clinical trial not only refers to studies that take place in a hospital or involve pharmaceuticals, but also refer to all studies which involve participant randomization and group classification in the context of the intervention under assessment.

Authors are strongly encouraged to pre-register clinical trials with an international clinical trials register and cite a reference to the registration in the Methods section. Suitable databases include **clinicaltrials.gov**, **the EU Clinical Trials Register** and those listed by the World Health Organisation **International Clinical Trials Registry Platform**.

Approval to conduct a study from an independent local, regional, or national review body is not equivalent to prospective clinical trial registration. MDPI reserves the right to decline any paper without trial registration for further peer review. However, if the study protocol has been published before the enrolment, the registration can be waived with correct citation of the published protocol.

CONSORT Statement

MDPI requires a completed CONSORT 2010 **checklist** and **flow diagram** as a condition of submission when reporting the results of a randomized trial. Templates for these can be found here or on the CONSORT website (**http://www.consort-statement.org**) which also describes several CONSORT checklist extensions for different designs and types of data beyond two group parallel trials. At minimum, your article should report the content addressed by each item of the checklist.

Dual Use Research of Concern

MDPI follows the practical framework defined in **Guidance for Editors: Research, Audit and Service Evaluations** and introduced by the Committee on Publication Ethics (COPE). Research that could pose a significant threat, with broad potential consequences to public health or national security, should be clearly indicated in the manuscript, and potential dual-use research of concern should be explained in the cover letter upon submission. Potential areas of concern include but are not limited to biosecurity, nuclear and chemical threats, and research with a military purpose or application, etc. For these manuscripts to be considered for peer review, the benefits to the general public or public health must outweigh the risks. The authors have a responsibility to comply with relevant national and international laws.

Sex and Gender in Research

We encourage our authors to follow the **'Sex and Gender Equity in Research – SAGER – guidelines'** and to include sex and gender considerations where relevant. Authors should use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Article titles and/or abstracts should indicate clearly what sex(es) the study applies to. Authors should also describe in the background, whether sex and/or gender differences may be expected; report how sex and/or gender were accounted for in the design of the study; provide disaggregated data by sex and/or gender, where appropriate; and discuss respective results. If a sex and/or gender analysis was not conducted, the rationale should be given in the Discussion. We suggest that our authors consult the full **guidelines** before submission.

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Potential disputes over borders and territories may have particular relevance for authors in describing their research or in an author or editor correspondence address, and should be respected. Content decisions are an editorial matter and where there is a potential or perceived dispute or complaint, the editorial team will attempt to find a resolution that satisfies parties involved.

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Diagnostics is a member of the Committee on Publication Ethics (**COPE**). We fully adhere to its **Code of Conduct** and to its **Best Practice Guidelines**.

The editors of this journal enforce a rigorous peer review process together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, image manipulation, inappropriate authorship credit, and the like, do arise. The editors of *Diagnostics* take such publishing ethics issues very seriously and are trained to proceed in such cases with a zero tolerance policy.

Authors wishing to publish their papers in *Diagnostics* must abide to the following:

- Any facts that might be perceived as a possible conflict of interest of the author(s) must be disclosed in the paper prior to submission.
- Authors should accurately present their research findings and include an objective discussion of the significance of their findings.
- Data and methods used in the research need to be presented in sufficient detail in the paper, so that other researchers can replicate the work.
- Raw data should preferably be publicly deposited by the authors before submission of their manuscript. Authors need to at least have the raw data readily available for presentation to the referees and the editors of the journal, if requested. Authors need to ensure appropriate measures are taken so that raw data is retained in full for a reasonable time after publication.
- Simultaneous submission of manuscripts to more than one journal is not tolerated.
- The journal accepts exact translations of previously published work. All submissions of translations must conform with our **policies on translations**.
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Irregular manipulation includes: 1) introduction, enhancement, moving, or removing features from the original image; 2) grouping of images that should obviously be presented separately (e.g., from different parts of the same gel, or from different gels); or 3) modifying the contrast, brightness or color balance to obscure, eliminate or enhance some information.

If irregular image manipulation is identified and confirmed during the peer review process, we may reject the manuscript. If irregular image manipulation is identified and confirmed after publication, we may correct or retract the paper.

Our in-house editors will investigate any allegations of publication misconduct and may contact the authors' institutions or funders if necessary. If evidence of misconduct is found, appropriate action will be taken to correct or retract the publication. Authors are expected to comply with the best ethical publication practices when publishing with MDPI.

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Authors should not copy references from other publications if they have not read the cited work.

Authors should not preferentially cite their own or their friends', peers', or institution's publications.

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In accordance with COPE guidelines, we expect that "original wording taken directly from publications by other researchers should appear in quotation marks with the appropriate citations." This condition also applies to an author's own work. COPE have produced a discussion document on **citation manipulation** with recommendations for best practice.

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During the submission process, please suggest three potential reviewers with the appropriate expertise to review the manuscript. The editors will not necessarily approach these referees. Please provide detailed contact information (address, homepage, phone, e-mail address). The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last three years. Proposed reviewers should be from different institutions to the authors. You may identify appropriate Editorial Board members of the journal as potential reviewers. You may suggest reviewers from among the authors that you frequently cite in your paper. For detailed information regarding the qualifications and responsibilities of the reviewers, please visit **https://www.mdpi.com/reviewers**.

Extensive English Editing

It is the authors' responsibility to submit their work in correct English. The APC includes only minor English editing, conducted by native English speakers. The APC does not include extensive English editing. If extensive editing is required, your paper could be returned to you at the English editing stage of the publication process. This could delay the publication of your work. You may have your work reviewed by an experienced English-speaking colleague or use a paid language-editing service before submitting your paper for publication. We offer rapid English editing, completed in 1 day, here: **Author Services**.

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Diagnostics accepts submissions that have previously been made available as preprints provided that they have not undergone peer review. A preprint is a draft version of a paper made available online before submission to a journal.

MDPI operates ***Preprints***, a preprint server to which submitted papers can be uploaded directly after completing journal submission. Note that *Preprints* operates independently of the journal and posting a preprint does not affect the peer review process. Check the *Preprints* **instructions for authors** for further information.

Expanded and high-quality conference papers can be considered as articles if they fulfill the following requirements: (1) the paper should be expanded to the size of a research article; (2) the conference paper should be cited and noted on the first page of the paper; (3) if the authors do not hold the copyright of the published conference paper, authors should seek the appropriate permission from the copyright holder; (4) authors are asked to disclose that it is conference paper in their cover letter and include a statement on what has been changed compared to the original conference paper. *Diagnostics* does not publish pilot studies or studies with inadequate statistical power.

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MDPI follows the International Committee of Medical Journal Editors (**ICMJE**) guidelines which state that, in order to qualify for authorship of a manuscript, the following criteria should be observed:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or reviewing it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Those who contributed to the work but do not qualify for authorship should be listed in the acknowledgments. More detailed guidance on authorship is given by the **International Committee of Medical Journal Editors (ICMJE)**.

Any change to the author list should be approved by all authors including any who have been removed from the list. The corresponding author should act as a point of contact between the editor and the other authors and should keep co-authors informed and involve them in major decisions about the publication. We reserve the right to request confirmation that all authors meet the authorship conditions.

For more details about authorship please check **MDPI ethics website**.

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Editorial independence is of utmost importance and MDPI does not interfere with editorial decisions. All articles published by MDPI are peer reviewed and assessed by our independent editorial boards, and MDPI staff are not involved in decisions to accept manuscripts. When making an editorial decision, we expect the academic editor to make their decision based only upon:

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All authors must disclose all relationships or interests that could inappropriately influence or bias their work. Examples of potential conflicts of interest include but are not limited to financial interests (such as membership, employment, consultancies, stocks/shares ownership, honoraria, grants or other funding, paid expert testimonies and patent-licensing arrangements) and non-financial interests (such as personal or professional relationships, affiliations, personal beliefs).

Authors can disclose potential conflicts of interest via the online submission system during the submission process. Declarations regarding conflicts of interest can also be collected via the **MDPI disclosure form**. The corresponding author must include a summary statement in the manuscript in a separate section “Conflicts of Interest” placed just before the reference list. The statement should reflect all the collected potential conflicts of interest disclosures in the form.

See below for examples of disclosures:

Conflicts of Interest: Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stocks in Company Y. Author C has been involved as a consultant and expert witness in Company Z. Author D is the inventor of patent X.

If no conflicts exist, the authors should state:

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Editorial Procedures and Peer Review

Pre-check

Immediately after submission, the journal’s Managing Editor will perform the technical pre-check to assess:

- Overall suitability of the manuscript to the journal/section/Special Issue;
- Manuscript adherence to high-quality research and ethical standards;
- Standards of rigor to qualify for further review.

The academic editor (i.e., the Editor-in-Chief in the case of regular submissions, the Guest Editor in the case of Special Issue submissions, or an Editorial Board member in the case of a conflict of interest and of regular submissions if the Editor-in-Chief allows) will be notified of the submission and invited to perform an editorial pre-check. During the editorial pre-check phase, the academic editor will assess the suitability of the submission with respect to the scope of the journal, as well as the overall scientific soundness of the manuscript, including the relevance of the references and the correctness of the applied methodology. Academic editors can decide to reject the manuscript, request revisions before peer review, or continue with the peer review process and recommend suitable reviewers.

Peer Review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer review. A single-blind review is applied, where authors' identities are known to reviewers. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

In the case of regular submissions, in-house assistant editors will invite experts, including recommendations by an academic editor. These experts may also include *Editorial Board Members* and Guest Editors of the journal. Potential reviewers suggested by the authors may also be considered. Reviewers should not have published with any of the co-authors during the past three years and should not currently work or collaborate with any of the institutions of the co-authors of the submitted manuscript. For more details about potential conflicts of interest, please check here, **https://www.mdpi.com/reviewers#_bookmark9**.

Optional Open Peer Review

The journal operates optional open peer review: *Authors are given the option for all review reports and editorial decisions to be published alongside their manuscript. In addition, reviewers can sign their review, i.e., identify themselves in the published review reports.* Authors can alter their choice for open peer review at any time before publication, but once the paper has been published changes will only be made at the discretion of the *Publisher* and *Editor-in-Chief*. We encourage authors to take advantage of this opportunity as proof of the rigorous process employed in publishing their research. To guarantee impartial refereeing, the names of referees will be revealed only if the referees agree to do so, and after a paper has been accepted for publication.

Editorial Decision and Revision

All the articles, reviews and communications published in MDPI journals go through the peer review process and receive at least two reviews. The in-house editor will communicate the decision of the academic editor, which will be one of the following:

- *Accept after Minor Revisions*:
  The paper is in principle accepted after revision based on the reviewer's comments. Authors are given five days for minor revisions.

- *Reconsider after Major Revisions*:
  The acceptance of the manuscript would depend on the revisions. The author needs to provide a point by point response or provide a rebuttal if some of the reviewer's comments cannot be revised. A maximum of two rounds of major revision per manuscript is normally provided. Authors will be asked to resubmit the revised paper within a suitable time frame, and the revised version will be returned to the reviewer for further comments. If the required revision time is estimated to be longer than 2 months, we will recommend that authors withdraw their manuscript before resubmitting so as to avoid unnecessary time pressure and to ensure that all manuscripts are sufficiently revised.

- *Reject and Encourage Resubmission*:
  If additional experiments are needed to support the conclusions, the manuscript will be rejected and the authors will be encouraged to re-submit the paper once further experiments have been conducted.

- *Reject*:
  The article has serious flaws, and/or makes no original significant contribution. No offer of resubmission to the journal is provided.

All reviewer comments should be responded to in a point-by-point fashion. Where the authors disagree with a reviewer, they must provide a clear response.

Author Appeals

Authors may appeal a rejection by sending an e-mail to the Editorial Office of the journal. The appeal must provide a detailed justification, including point-by-point responses to the reviewers' and/or Editor's comments using an **appeal form**. Appeals can only be submitted following a "reject and decline resubmission" decision and should be submitted within three months from the decision date. Failure to meet these criteria will result in the appeal not being considered further. The *Managing Editor* will forward the manuscript and related information (including the identities of the referees) to a designated *Editorial Board Member*. The Academic Editor being consulted will be asked to provide an advisory recommendation on the manuscript and may recommend acceptance, further peer review, or uphold the original rejection decision. This decision will then be validated by the *Editor-in-Chief*. A reject decision at this stage is final and cannot be reversed.

Production and Publication

Once accepted, the manuscript will undergo professional copy-editing, English editing, proofreading by the authors, final corrections, pagination, and, publication on the **www.mdpi.com** website.

Please read detailed Editorial Process **here**.

Promoting Equity, Diversity and Inclusiveness within MDPI Journals

Our Managing Editors encourage the Editors-in-Chief and Associate Editors to appoint diverse expert Editorial Boards. This is also reflective in our multi-national and inclusive workplace. We are proud to create

equal opportunities without regard to gender, ethnicity, sexual orientation, age, religion, or socio-economic status. There is no place for discrimination in our workplace and editors of MDPI journals are to uphold these principles in high regard.

Resource Identification Initiative

To improve the reproducibility of scientific research, the **Resource Identification Initiative** aims to provide unique persistent identifiers for key biological resources, including antibodies, cell lines, model organisms and tools.

We encourage authors to include unique identifiers - RRIDs- provided by the **Resource Identification Portal** in the dedicated section of the manuscript.

To help authors quickly find the correct identifiers for their materials, there is a single **website** where all resource types can be found and a 'cite this' button next to each resource, that contains a proper citation text that should be included in the methods section of the manuscript.

363/2023

SÃO JOÃO

DELIBERAÇÃO DO CONSELHO DE ADMINISTRAÇÃO

Após apreciação e pareceres favoráveis da Comissão de Ética e do Centro de Epidemiologia Hospitalar, considerando que se encontram reunidos os requisitos e demais trâmites previstos no circuito para submissão de projetos de investigação na ULS de São João e em conformidade com as disposições legais em vigor, o Conselho de Administração – ao abrigo das competências previstas no Artigo 71.º dos Estatutos dos hospitais, centros hospitalares, institutos portugueses de oncologia e unidades locais de saúde, aprovados pelo Decreto-Lei n.º 52/2022, de 4 de agosto – delibera:

1. Aprovar a realização do projeto de investigação:
   - "Human versus Artificial Intelligence: Validation of a Deep Learning Model for retinal layer and fluid segmentation in Optical Coherence Tomography Images".
   - Serviço(s) onde decorrerá o projeto de investigação: Oftalmologia.
   - Investigador(a) principal: Mariana Gomes Miranda
2. Remeta-se à Comissão de Ética para os procedimentos adequados e demais trâmites convenientes.

CONSELHO DE ADMINISTRAÇÃO DA ULS DE SÃO JOÃO, EPE • REUNIÃO DE 25 DE JANEIRO DE 2024

Presidente do Conselho de Administração

Prof.ª Doutora Maria João Baptista

Diretor Clínico	Enfermeiro Diretor	Vogal Executiva	Vogal Executiva
Prof.º Doutor Roberto Roncon	--- AUSENTE --- Enfermeiro Paulo Emílio Mota	Dra. Sofia Leal	Dra. Fernanda Oliveira

› Comissão de Ética
› Centro de Epidemiologia Hospitalar
› Direção Clínica

CE 363/2023

MEC-IN011-1

Centro de Epidemiologia Hospitalar

Tomei conhecimento. Nada a opor. À DC.

22 de Janeiro de 2024

A Diretora do Centro de Epidemiologia Hospitalar

(Prof.ª Doutora Ana Azevedo)

n.º 363 / 2023

SÃO JOÃO

DIRECÇÃO CLÍNICA

23 JAN 2024

PEDIDO DE AUTORIZAÇÃO

Realização de Investigação

Exmo. Senhor Presidente do Conselho de Administração
do Centro Hospitalar Universitário de São João

Nome do Investigador Principal:

Mariana Gomes Miranda

Título da Investigação:

Human versus Artificial Intelligence: Validation of a Deep Learning Model for retinal layer and fluid segmentation in Optical Coherence Tomography Images

Pretendendo realizar no(s) Serviço(s) de:

Oftalmologia

a investigação em epígrafe, solicito a V. Exa., na qualidade de Investigador/Promotor, autorização para a sua efetivação.

Para o efeito, anexo toda a documentação referida no dossier da Comissão de Ética do Centro Hospitalar Universitário de São João/ Faculdade de Medicina da Universidade do Porto respeitante à investigação, à qual enderecei pedido de apreciação e parecer.

Com os melhores cumprimentos.

O Investigador/ Promotor

Porto, 25 *de* setembro *de* 2023 .

Mariane Gomes Miranda
assinatura

Centro Hospitalar São João
Centro de Epidemiologia Hospitalar
16, 1, 2024

SÃO JOÃO

Encarregado de Proteção de Dados
Data Protection Officer

Entrada 23/11/2023

CES-IM005-1

Parecer da Comissão de Ética do Centro Hospitalar Universitário de São João

Título do Projeto: Human versus Artificial Intelligence: Validation of a Deep Learning Model for retinal layer and fluid segmentation in Optical Coherence Tomography Images

Nome da Investigadora Principal: Mariana Gomes Miranda, estudante MIMED/FMUP, que dispõe da competência científica para a realização do estudo. Serão co-investigadoras Tânia Filipa Fernandes de Melo (Estudante de Doutoramento da FEUP e INESC TEC) e a Dra. Joana Inês Santos de Oliveira (CHUSJ).

Onde decorre o Estudo: No Serviço de Oftalmologia do CHUSJ. Apresentou a declaração do Prof. Doutor Fernando Falcão Reis, Diretor de Serviço. A Profissional de Ligação será a Prof.ª Doutora Ângela Carneiro. O Serviço dispõe das condições para a realização do estudo.

Objetivos do Estudo:

Trata-se de um estudo retrospetivo, observacional e sem intervenção que tem como objetivos:

1. Estudar o impacto que as previsões de um sistema automático tem na performance de um médico no processo de segmentação das camadas da retina e de fluído, em imagens de OCT.;
2. Comparar as anotações humanas e as anotações resultantes do modelo de IA, na segmentação das camadas da retina e de fluído, em imagens de OCT de casos saudáveis e com patologia - Degenerescência Macular da Idade (DMI) em estadios diferentes: DMI intermédia e DMI exsudativa;
3. Estudar a possível correlação entre as métricas extraídas dos dados da segmentação (espessura das camadas da retina e área do fluído) com a severidade da DMI e a sua potencialidade como futuros biomarcadores de estadiamento da doença;
4. Investigar se as discrepâncias entre a segmentação humana e a do sistema de IA se relacionam com a severidade da doença e se as situações de erro do sistema IA se associam às situações de uma maior dificuldade percecionada pelo humano, durante o processo de anotações.

Este estudo é realizado no âmbito do Mestrado Integrado em Medicina da FMUP, sob orientação da Prof.ª Doutora Ângela Carneiro e coorientação da Dra. Joana Oliveira

Conceção e Pertinência do estudo:

O estudo é pertinente e adequado porquanto consta no protocolo submetido à CES: "É inquestionável, no enquadramento atual da Inteligência Artificial, a potencialidade futura de esta vir a ser introduzida em especialidades médicas como a Oftalmologia. Em especial, apoiando na análise de meios complementares de diagnóstico de imagem, abundantes nesta área, como é o OCT (Tomografia de Coerência Ótica). Assim, é importante a validação destes sistemas com o conhecimento e experiência médica dos oftalmologistas. E considerando as vantagens de rapidez, constância e facilidade na avaliação automática realizada, se, por fim, for, no mínimo, comparavelmente eficaz, fiável e reproduzível, será de interesse a sua implementação como ferramenta de apoio aos clínicos no diagnóstico e prognóstico de patologias retinianas".

Serão incluídos 60 participantes: controlos saudáveis – 20; DMI intermédia – 20; DMI exsudativa – 20.

Estão definidos os critérios de inclusão no estudo que não levantam questões do foro ético. Serão recolhidos de forma anonimizada e pertinente e adequada aos objetivos do estudo os seguintes dados: sexo; idade do diagnóstico da DMI (ou da primeira aquisição OCT, nos controlos saudáveis); período de

tempo de seguimento desde o diagnóstico; diagnósticos (como critérios de exclusão) de Retinopatia Diabética/Edema Macular Diabético e/ou Erros Refrativos (Miopia Grave, ≤−6.0 dioptrias).

Benefício/risco:
Apenas serão recolhidas imagens e simples informações clínicas, previamente adquiridas na prática clínica e com total anonimização de todos os dados.

Confidencialidade dos dados:
Todos os dados (imagens de OCT scans) e informações médicas recolhidas serão totalmente anonimizadas no serviço de Oftalmologia pelos investigadores que a eles acederem, antes do seu estudo e processamento. Tal será concretizado através de um processo de codificação com atribuição de um número aleatório (e independente dos dados) a cada participante, de forma a ser difícil o retorno a esta informação num processo inverso.
Apresentou um pedido de reutilização de registos clínicos para Investigação e Desenvolvimento ao RAI, e uma 'avaliação sobre o impacto da proteção de dados' para o EPD.

Respeito pela liberdade e autonomia do sujeito de ensaio: Não aplicável

***Curriculum* da investigadora**: Adequado à investigação.

Data previsível da conclusão do estudo: junho de 2024. Este estudo não tem encargos financeiros para o CHUSJ.

Conclusão: Proponho um parecer favorável à realização do estudo.

Porto, 20 de outubro de 2023

O Relator da CE, Doutor Pedro Brito

Questionário eletrónico

n.º 363 / 2023

SÃO JOÃO

SUBMISSÃO DE PROJETO DE INVESTIGAÇÃO PARA PARECER E AUTORIZAÇÃO

Preenchimento em formato digital obrigatório

Caro/a Investigador/a

Este questionário vai ser objeto de apreciação pela Comissão de Ética (CE), que emitirá o competente parecer; pelo Responsável de Acesso à Informação (RAI), que emitirá um despacho de autorização ou indeferimento, relativamente à reutilização da informação a que pretende ter acesso; pelo Encarregado de Proteção de Dados (EPD), que emitirá um parecer, mediante a 'avaliação do impacto sobre a proteção de dados' (que deve anexar a este pedido); pelo Centro de Epidemiologia Hospitalar; sendo enviado, num último momento, para decisão institucional do Conselho de Administração do CHUSJ. Os respetivos despachos de parecer e autorização serão exarados no final deste documento, e enviados posteriormente, de modo a poder iniciar, nesse momento, a investigação a que se propõe.

IDENTIFICAÇÃO DO ESTUDO

Título do projeto:

Human versus Artificial Intelligence: Validation of a Deep Learning Model for retinal layer and fluid segmentation in Optical Coherence Tomography Images

Data prevista para início: 01 / 11 / 2023 Data prevista para o término: 01 / 03 / 2024

EQUIPA DE INVESTIGAÇÃO

1. Investigador principal

Nome: Mariana Gomes Miranda

Afiliação institucional: ☐ CHUSJ ☒ FMUP Outro:

Serviço/ Departamento: Serviço de Oftalmologia/Departamento de Cirurgia e Fisiologia da Faculdade de Medicina

Grupo profissional: Estudante de Mestrado Integrado em Medicin Cédula Profissional n.º:

Contacto telefónico: 917925687 Endereço eletrónico institucional: up201705176@edu.med.up.pt

Formação em Boas Práticas Clínicas (GCP): ☒ Não ☐ Sim

2. Co-investigadores

Nome: Tânia Filipa Fernandes de Melo

Afiliação institucional: FEUP e INESC TEC

Grupo profissional: Estudante de Doutoramento Cédula Profissional n.º:

Contacto telefónico: 913768924 Endereço eletrónico institucional: tania.f.melo@inesctec.pt

Nome: Joana Inês Santos de Oliveira

Afiliação institucional: Centro Hospitalar Universitário São João

Grupo profissional: Médico Cédula Profissional n.º: 67845

Contacto telefónico: 913111707 Endereço eletrónico institucional: u016825@chsj.min-saude.pt

(acrescentar nº de investigadores, se apropriado ao projeto de investigação)

3. Promotor *(se aplicável)*:

CARACTERIZAÇÃO DA INVESTIGAÇÃO

1. Metodologia da investigação

☐ Qualitativa ☒ Mista (qualitativa+quantitativa) ☐ Outra. Qual? _____

Se quantitativa:

☐ Experimental ☒ Observacional ☒ Sem intervenção ☐ Com intervenção

Se experimental ou observacional com intervenção, qual o tipo de intervenção?

☐ Algoritmo de decisão diagnóstica/terapêutica ☐ Comunicação

☐ Outra. Qual? _____

2. Aleatorização dos braços de intervenção: ☐ Não ☐ Sim

3. Se observacional, qual o desenho?

☐ Coorte prospetivo ☐ Coorte retrospetivo ☐ Caso-controlo

☒ Transversal ☐ Ecológico ☐ Outro. Qual? Retrospetivo

REALIZAÇÃO DA INVESTIGAÇÃO

Local onde se realiza a investigação: ☒ CHUSJ ☐ FMUP ☐ Outro

Serviço/ Departamento: Serviço de Oftalmologia/Departamento de Cirurgia e Fisiologia da Faculdade de Medicina

Existem outros Centros onde se realizará a investigação? ☐ Não ☒ Sim. Quais? INESC TEC

ENTIDADE(S) QUE TUTELA(M) A INVESTIGAÇÃO

1. CHUSJ – Serviço: Oftalmologia
2. FMUP – Departamento: Cirurgia e Fisiologia da Faculdade de Medicina da Universidade do Porto
3. Outra instituição. Qual? INESC TEC

ORIENTADOR *(se aplicável)*

Nome: Ângela Maria Veloso Guimarães Carneiro

Afiliação: Serviço de Oftalmologia, CHU Endereço eletrónico institucional: u009516@chsj.min-saude.pt

PROFISSIONAL DE LIGAÇÃO *(se aplicável - ver anexo)*

Nome: Ângela Maria Veloso Guimarães Carneiro Serviço: Oftalmologia do CHUSJ

ENQUADRAMENTO DA INVESTIGAÇÃO

Em trabalho académico? ☐ Não ☒ Sim Conferidor de grau? ☐ Não ☒ Sim

Síntese dos objetivos:

1. Estudar o impacto que as previsões de um sistema automático tem na performance de um médico no processo de segmentação das camadas da retina e de fluído, em imagens de OCT.
2. Comparar as anotações humanas e as anotações resultantes do modelo de IA, na segmentação das camadas da retina e de fluído, em imagens de OCT de casos saudáveis e com patologia - Degenerescência Macular da Idade (DMI) em estadios diferentes: DMI intermédia e DMI exsudativa.
3. Estudar a possível correlação entre as métricas extraídas dos dados da segmentação (espessura das camadas da retina e área do fluído) com a severidade da DMI e a sua potencialidade como futuros biomarcadores de estadiamento da doença.
4. Investigar se as discrepâncias entre a segmentação humana e a do sistema de IA se relacionam com a severidade da doença e se as situações de erro do sistema IA se associam às situações de uma maior dificuldade percecionada pelo humano, durante o processo de anotações.

Fundamentação ética *(incluir informação sumária sobre o estado da arte, ganhos em conhecimento/inovação, ponderação geral sobre benefícios/risco)*:

É inquestionável, no enquadramento atual da Inteligência Artificial, a potencialidade futura de esta vir a ser introduzida em especialidades médicas como a Oftalmologia. Em especial, apoiando na análise de meios complementares de diagnóstico de imagem, abundantes nesta área, como é o OCT (Tomografia de Coerência Ótica). Assim, é importante a validação destes sistemas com o conhecimento e experiência médica dos oftalmologistas. E considerando as vantagens de rapidez, constância e facilidade na avaliação automática realizada, se, por fim, for, no mínimo, comparavelmente eficaz, fiável e reproduzível, será de interesse a sua implementação como ferramenta de apoio aos clínicos no diagnóstico e prognóstico de patologias retinianas.

PARTICIPANTES PREVISTOS PARA A INVESTIGAÇÃO

Estão definidos critérios de inclusão / de exclusão de doentes? ☐ Não ☒ Sim

Onde e como serão recrutados os participantes no estudo?

Controlos e DMI Intermédia: SPECTRALIS® HRA+OCT/Software Heilderberg Eye Explorer; DMI Exsudativa: plata

Qual é o tamanho amostral? 60 participantes

Está prevista a recolha de material biológico específico para a investigação?

☒ Não ☐ Sim. Identifique e justifique:

BENEFÍCIO/RISCO DECORRENTE DA PARTICIPAÇÃO

Descreva os benefícios previsíveis:

Sem qualquer benefício direto e a curto prazo para os participantes. Será potencial este benefício, futuramente, se

Descreva os riscos/incómodos previsíveis:

Não se prevê qualquer risco ou incómodo aos participantes: apenas serão recolhidas imagens e simples informações clínicas, previamente adquiridas na prática clínica e com total anonimização de todos os dados.

CONSENTIMENTO INFORMADO, ESCLARECIDO E LIVRE

Prevê a obtenção de consentimento informado? ☐ Sim ☒ Não

Se não, justifique: Serão recolhidas, retrospetivamente, apenas imagens de OCT scans e simples informações clín

Prevê informação escrita para os participantes? ☐ Sim. **Enviar modelo preenchido.**

☒ Não. Justifique: Serão recolhidas, retrospetivamente, apenas imagens de OCT scans e simples informações clí

O modelo para obtenção de consentimento é o modelo institucional do CHUSJ? ☐ Sim ☐ Não

PROTEÇÃO DE DADOS PESSOAIS

Necessita consultar registos clínicos? ☐ Não ☒ Sim

Está previsto o tratamento de dados pessoais? ☐ Não ☒ Sim

Se sim, de que forma é garantida a pseudonimização dos dados recolhidos? *(codificação, uso de filtros, siglas...)*

Todos os dados (imagens de OCT scans) e informações médicas recolhidas serão totalmente anonimizadas no serviço de Oftalmologia pelos investigadores que a eles acederem, antes do seu estudo e processamento. Tal será concretizado através de um processo de codificação com atribuição de um número aleatório (e independente dos dados) a cada participante, de forma a ser difícil o retorno a esta informação num processo inverso.

Descreva o património informacional a que pretende ter acesso *(v.g.: nome, idade, data nascimento, idade, morada, diagnóstico, história clínica, tratamento...)*:

- Sexo;
- Idade do diagnóstico da DMI (ou da primeira aquisição OCT, nos controlos saudáveis);
- Período de tempo de seguimento desde o diagnóstico;
- Diagnósticos (como critérios de exclusão) de Retinopatia Diabética/Edema Macular Diabético e/ou Erros Refrativos (Miopia Grave, ≤−6.0 dioptrias).

Está prevista a criação de um Banco de Dados? ☒ Não ☐ Sim

Está previsto o registo de som ou de imagem dos participantes? ☒ Não ☐ Sim

O estudo envolve investigação genética? ☒ Não ☐ Sim

PROPRIEDADE INTELECTUAL

De quem será a propriedade intelectual da investigação e seus resultados?

☐ Investigador ☐ Promotor ☒ Serviço/CHUSJ ☐ Todos

DIVULGAÇÃO DOS RESULTADOS

Está prevista a divulgação dos resultados da investigação? ☐ Não ☒ Sim

Se sim, estão definidos critérios de publicação? ☒ Não ☐ Sim. Quais? _____

CONTRAPARTIDAS PARA OS PARTICIPANTES

Estão previstas contrapartidas para os participantes? ☒ Não ☐ Sim

Pela participação? ☒ Não ☐ Sim

Pelas deslocações? ☒ Não ☐ Sim

Pelas perdas salariais? ☒ Não ☐ Sim

Por outras perdas e/ou danos? ☒ Não ☐ Sim

EXAMES COMPLEMENTARES DE DIAGNÓSTICO

Estão previstos exames complementares de diagnóstico, para além dos inerentes à rotina assistencial?

☒ Não ☐ Sim. Quais? _____

Por quem serão suportados estes custos?

_____

PROTOCOLO FINANCEIRO

Existe protocolo financeiro com o CHUSJ? ☒ Não ☐ Sim

SEGURO

Este estudo prevê intervenção clínica que implique a existência de um seguro para os participantes?

☒ Não ☐ Sim *(se sim, junte cópia da respetiva Apólice)*

Data previsível para fim das credenciais de acesso: 01 / 03 / 2024

DOCUMENTOS ANEXOS *(em suporte digital)*

- [x] Pedido de autorização ao Presidente do Conselho de Administração do CHUSJ
- [x] Protocolo do estudo
- [ ] Caderno de recolha de dados (CRF)
- [x] Declaração Diretor(es) Serviço(s)
- [x] Informação Orientador
- [x] Profissional de ligação
- [ ] Informação aos participantes
- [ ] Modelo de consentimento a utilizar
- [ ] Instrumentos de avaliação *(escalas, inquéritos)* _____
- [x] Curriculum/a vitae *(investigador/es)*
- [ ] Questionário para Encarregado de Proteção de Dados (EPD)
- [ ] Termo de Responsabilidade do Centro Académico Clínico *(para investigadores da FMUP que não pertençam ao CHUSJ)*
- [ ] Protocolo financeiro
- [ ] Outros: _____

TERMO DE RESPONSABILIDADE

Aceitação dos termos e condições de reutilização

Cumulativamente com as obrigações decorrentes da *Lei n.º 26/2016, de 22 de agosto*, maxime dos *n.º 2 e 3 do artigo 21 e o n.º 1 e 2 do artigo 12*, ao submeter o presente pedido, concordo e fico ainda juridicamente vinculado aos seguintes termos e condições:

- Comprometo-me a manter confidencial toda a informação à qual vou ter acesso;
- Após explicação do RAI do CHUSJ, embora a Lei 26/2016, de 22 de agosto, imponha como requisito a anonimização sem possibilidades de reversão, tal desiderato, é não só uma impossibilidade matemática já comprovada, como ainda resulta num prejuízo para a investigação, face à quantidade e à qualidade da informação a retirar à fonte, razão pela qual, concordando com o RAI, assumimos como compromisso a pseudonimização, o que impõe uma avaliação e gestão do risco, num quadro ético-jurídico que aceitamos e nos comprometemos a colaborar e respeitar;
- Não vou elaborar registos, suscetíveis de identificar ou tornar identificável a identidade das pessoas a quem os mesmos dizem respeito;
- Comprometo-me a consultar os processos clínicos nos termos e locais que me forem indicados para o efeito;
- Tomei conhecimento, que a violação de qualquer dos compromissos aqui assumidos, poderá resultar no apuramento de responsabilidades disciplinares, civis e penais, e ainda, à impossibilidade futura de aceder a informação de saúde para fins de investigação.
- Independentemente de requerer a Certidão de Reutilização, DAta REuse Certificate for Research (DARE), comprometo-me a citar as fontes, sempre que publicitar, no todo ou em parte, resultados da presente investigação.

COMPROMISSO DE HONRA E DECLARAÇÃO DE INTERESSES

Eu, Mariana Gomes Miranda ,

abaixo assinado, na qualidade de Investigador Principal, declaro por minha honra que as informações prestadas neste questionário são verdadeiras. Mais declaro que, durante o estudo, serão respeitadas as recomendações constantes na Declaração de Helsínquia (1960, e sucessivas emendas), da Organização Mundial de Saúde, da Convenção de Oviedo e das 'Boas Práticas Clínicas' (GCP/ICH) no que se refere à experimentação que envolve seres humanos. Aceito, também, a recomendação da CE de que o recrutamento para este estudo se fará junto de doentes que não tenham participado em outro estudo, nos últimos três meses. Comprometo-me a entregar à CE o relatório final da investigação, assim que concluído.

Data: 25 / 09 / 2023 Mariana Gomes Miranda
assinatura

COMISSÃO DE ÉTICA CHUSJ/FMUP

Parecer da CE, emitido na reunião plenária de 20 / 10 / 2023

A Comissão de Ética para a Saúde APROVA por unanimidade o parecer do Relator, pelo que nada tem a opor à realização deste projecto de investigação.

• Centro Hosp. Univ. São João •
Comissão de Ética
Manuel Vaz Silva
Presidente

RAI 23016506

RAI - Responsável pelo Acesso à Informação no Centro Hospitalar Universitário de São João (Art. 9º, Lei 26/2016 de 22 de Agosto)

SÃO JOÃO HOSPITAL
Gabinete de Apoio ao RAI
Recebido em 21/11/2023
A funcionária

SÃO JOÃO
AUTORIZADO
RAI - Responsável pelo Acesso à Informação do Centro Hospitalar de São João (Art. 9º, Lei 26/2016, de 22/8)
21/11/2023

CENTRO DE EPIDEMIOLOGIA HOSPITALAR

SÃO JOÃO

ENCARREGADO DE PROTEÇÃO DE DADOS (EPD)
UNIDADE LOCAL DE SAÚDE SÃO JOÃO, E.P.E.
Paulo Alexandre Mota da Silva
Encarregado de Proteção de Dados do CHUSJ
epd@chsj.min-saude.pt

Ref.ª CES CHUSJ: **363 / 2023**

Título do Projeto	Human versus Artificial Intelligence: Validation of a Deep Learning Model for retinal layer and fluid segmentation in Optical Coherence Tomography Images
Responsável pelo tratamento	Mariana Gomes Miranda
Instituição	Centro Hospitalar Universitário São João (CHUSJ) Faculdade de Medicina da Universidade do Porto (FMUP) Instituto de Engenharia de Sistemas e Computadores, Tecnologia e Ciência (INESTEC)
Investigador	Interno **Externo**
Contacto telefónico	917925687 **Endereço Electrónico** up201705176@edu.med.up.pt
Profissional de Ligação	Ângela Maria Veloso Carneiro (Assistente Hospitalar Graduada de Oftalmologia)
Amostra	60
Análise de Risco	Tolerável **Baixo** Elevado Muito Elevado

Parecer do EPD:

Data: 12/01/2024

Finalidade: Considerando que uma das principais patologias responsáveis por cegueira em países desenvolvidos ser a Degenerescência Macular da Idade (DMI), é, assim, proposto um projeto de estudo que permita a validação de um sistema com IA na segmentação automática das camadas da retina e de fluído em imagens OCT, tendo por base participantes saudáveis e doentes em dois estádios de evolução da DMI diferentes (DMI intermédia e DMI exsudativa). Este projeto irá procurar responder a quatro principais questões, sendo estas: (1) qual é o impacto que as previsões de um sistema automático, acessível no dia a dia, na prática clínica (Software Heilderberg Eye Explorer) têm na performance dos médicos no processo de segmentação da retina?; (2) que resultados obtemos na comparação das anotações realizadas pelo sistema de IA com as anotações humanas, em casos controlo e em situações patológicas de DMI?; (3) existe uma clara relação entre os valores dos biomarcadores extraídos (espessura das camadas da retina e área do fluído) e o estadiamento da doença?; (4) as discrepâncias entre as anotações humanas e as do método de IA estão relacionadas, por um lado, com o estadio/severidade da doença e/ou, por outro, com a dificuldade percecionada pelo humano durante o processo de anotações?.

Licitude: fundamento previsto no artigo **9(2)(j)**, com as garantias do **89(1)** do **RGPD**, e artigo **31(1)** da **LERGPD.**

Categorias de dados pessoais: variáveis identificadas com detalhe na AIPD, datada de 23/11/2023, **ponto 13**, tendo presente o **princípio da minimização dos dados.**

Conservação: os dados serão alvo de **pseudonimização**, armazenados em **local seguro**, em área restrita com acesso limitado ao profissional de ligação e Investigador Principal, com acesso a ficheiros protegido por **palavra-passe**, efetuando-se a conservação até a conclusão da investigação, durante o prazo máximo de **seis (6) meses**. Os dados recolhidos **serão destruídos** após a finalização do estudo.

Comunicação de Dados: **há partilha** de dados pessoais (pseudonimizados).

Face ao exposto, e observadas as recomendações, entende-se que a presente AIPD apresenta os elementos necessários para assegurar que o **tratamento é realizado em conformidade com o RGPD.**

Recomendações:

1. Garantir medidas de segurança adicionais no transporte dos dados com recurso a dispositivos electrónicos de armazenamento (Laptop, Disco Externo), nomeadamente através de medidas de cifragem e autenticação;
2. Reforçar as medidas de segurança previstas para a conservação dos documentos em formato de papel que impeçam o acesso à informação a pessoas não autorizadas, bem como o seu manuseamento indevido;
3. Garantir medidas de segurança adicionais (ex. transformar os dados com recursos a técnicas de generalização, perturbação e/ou como último recurso, a supressão de dados) para os dados que, ainda que pseudonimizados, contém elementos informativos que no seu conjunto possibilitam a re-identificação dos participantes, tendo presente a amostra em estudo (doentes seguidos na consulta de Oftalmologia), em particular na fase de disseminação ou publicação dos resultados (incluindo repositórios científicos).

4. A chave de descodificação que permite a re-identificação dos titulares dos dados (CHUSJ) deve ficar sempre na posse do Elo de Ligação CHUSJ e protegida de acessos indevidos / terceiros;
5. Em caso de necessidade de extensão de prazo e/ou de qualquer alteração dos pressupostos atinentes ao presente parecer o Investigador Principal deverá solicitar a reapreciação do projeto de investigação junto do EPD.

Revisão AIPD:

☐ Data da próxima revisão: ___/___/_____ ☒ Não carece de revisão.

Anexos:

1. Processo CES n.º 363/2023
2. Parecer CES (20/10/2023)
3. AIPD (23/11/2023)
4. Protocolo de Investigação

Encarregado de Proteção de Dados
Assinado por: **PAULO ALEXANDRE MOTA DA SILVA**
Data: 2024.01.12 11:42:25+00'00'
Localização: CHUSJ