HERALD - Hyperspectral retinal imaging for non-invasive detection of amyloid-β in patients with Alzheimer disease

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ABSTRACT
The Herald project aims at developing a method to detect amyloid beta in the retina as a sign of onset of Alzheimer's Disease, using an affordable hyperspectral camera combined with conventional ophthalmological imaging devices. The hyperspectral retinal imaging set-up consists of a compact hyperspectral snapshot camera and a standard fundus camera. Hyperspectral images were taken of 39 subjects, both AD patients and controls. Using these images, a classifier that differentiates AD subjects from controls was trained and evaluated. Adding OCT images slightly increased the performance of the classifier. This is a first step towards application in clinical practice.

Keywords: hyperspectral imaging; retinal biomarkers; Alzheimer's Disease; amyloid-beta (Aβ).

1. INTRODUCTION

Dementia is a devastating disease with important public health challenges. The number of patients will almost double to 82 million by 2030. Roughly 75% of the patients with dementia have Alzheimer's disease (AD). There is no cure yet for Alzheimer's Disease. Processes leading to irreversible cognitive decline, already start long before the first symptoms appear. Detecting subjects with early signs of the disease is therefore key in the development of drugs that target these early processes. Today, AD diagnosis is made too late and individuals are not sufficiently stratified in early stages of the disease. This likely explains why many clinical trials for new AD drugs have failed so far.
Accumulation of amyloid beta (Aβ) is an essential marker for clinical AD diagnosis. Aβ concentrations can be determined using brain Positron Emission Tomography (PET) and cerebrospinal fluid (CSF) sampling. However, these methods have large infrastructure costs, limited resolution, and are potentially harmful.[1] The need for better and more convenient AD identification and monitoring using affordable and non-invasive diagnostic tools is urgent.[2]
Non-invasive hyperspectral imaging of the retina is a possible solution. Anatomically and developmentally, the retina is an extension of the brain. It can be visualized directly at very high resolution, and provides a unique window to the central nervous system without the need for expensive, invasive and/or potentially harmful examinations.[3] Pilot data show that aggregates of retinal Aβ can be detected based on their hyperspectral signature. Unfortunately, few data exist about the possible translation of these findings to humans. In addition, applicability in humans is limited because no practical and affordable hyperspectral imaging system exists. The HERALD project aims for a breakthrough in the detection of retinal Aβ in early stages using a low-cost non-invasive technique for use in clinical practice. The technique combines a convenient and affordable hyperspectral camera mounted on a conventional fundus cameras, with advanced image analysis. To attain higher diagnostic accuracy a bimodal set-up including standard Optical Coherence Tomography (OCT) images is also assessed.
A HSRI setup with a snapshot camera was realized for clinical study and an image analysis pipeline developed, and they were used in a clinical study with 39 subjects (AD/controls). A Linear Discriminant Analysis (LDA) classifier was trained and evaluated to differentiate AD subjects from controls. Discriminatory information was mainly derived from hyperspectral data, but the model also benefits from additional information from OCT images.

2. STATE OF THE ART

The retina offers a unique opportunity to overcome limitations associated with current diagnostic technologies. The number of studies supporting the hypothesis that Aβ accumulates in the retina of AD patients, is increasing.
Post mortem studies in both animal and human retinas, and in vivo studies, have shown that HSRI can detect retinal Aβ aggregates.[4,5,6] HSRI is a label-free imaging technique that can detect a decrease in the spectral reflectance at wavelengths between 460 and 570 nm. There are strong indications that the observed spectral effects observed in these wavelengths are caused by the

presence of soluble Aβ in the retina, leading to increased Rayleigh scattering.[4,6]
It has recently been shown that machine learning methods using HSRI data are capable of distinguishing between amyloid-PET positive cases and controls in a clinical setting.[5] This and other studies used wavelength scanning HSRI techniques (i.e. Metabolic Hyperspectral Retinal Camera). Although these techniques allow for a high spectral resolution, they require long acquisition times, leading to motion artefacts in between consecutive monochromatic images, and have a high equipment cost. An alternative imaging system simultaneously captures a conventional two-dimensional retinal image and a spectral image along one dimension. However, it provides spectral information along a single horizontal line only.[6] Applicability of this type of imaging in clinical practice is thus limited because no practical and affordable hyperspectral imaging system exists.
Studies using OCT imaging have demonstrated the thinning of retinal layers, more specifically a thinning of the retinal nerve fibre layer (RNFL).[7] However, the diagnostic accuracy of RNFL changes alone, is probably insufficient due to its low specificity. The highest diagnostic accuracy can likely be achieved by combining several ATN biomarkers, where "A" stands for Aβ status, "T" for tau, and "N" for neurodegeneration biomarkers.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

The aim of the HERALD project is to force a breakthrough in the sensitive and convenient detection of retinal Aβ by giving proof-of-concept for a low-cost non-invasive technique for early diagnosis of Alzheimer's Disease (AD). As an accurate, non-invasive diagnostic tool to identify patients in the early, asymptomatic stage of the disease HSRI can cause a breakthrough in (pre)clinical research on disease-modifying drugs, i.e. for screening patients for inclusion in clinical trials. Further on it can be used (at population-level) for identifying patients at risk that need further neurological examinations and for longitudinal follow-up of disease progression in AD patients.
HERALD focuses on the development of a hyperspectral retinal imaging (HSRI) set-up that can be used on a routine basis in clinical practice. The proposed solution consists of a convenient and affordable hyperspectral camera mounted on a conventional fundus camera that is found in every ophthalmologist's office. The HERALD project is the first to demonstrate the use of a hyperspectral snapshot camera allowing to acquire spatial and spectral information in one capture without the need for wavelength or spatial scanning thus enabling instantaneous acquisition of a spectral image of the retina which is crucial to avoid eye movements. This comes however at the cost of lower spatial and spectral resolution, which poses additional challenges on image acquisition and processing, image analysis and development and training of a predictive model. In contrast to some other studies due attention is given to a stringent training and evaluation approach, i.e. a nested leave-one-out cross-validation (LOOCV) approach, to account for the small sample size and for overfitting. The area under the curve (AUC) generated in this nested LOOCV is an unbiased estimate.[8]
It is broadly acknowledged that combining several biomarkers increases diagnostic accuracy. OCT devices are commonly available at ophthalmological and neurological departments. Therefore, the potential of a bimodal model including OCT images next to HSRI was also assessed, and it was investigated whether bimodal retinal image analysis could allow for better differentiation between AD patients as compared to cognitively intact elderly (CIE).

4. PROJECT RESULTS

HSRI set-up

A HSRI set-up was realized consisting of a XIMEA SNm4x4 VIS hyperspectral snapshot camera connected with a C-mount to a TL-230T relay lens (Topcon Corporation, Japan), installed on a Topcon TRC-50DX fundus camera (Topcon Corporation, Japan) (Figure 1).
The XIMEA camera contains a hyperspectral sensor from IMEC, a 4x4 mosaic pattern of pixel-size spectral filters integrated on a standard CMOS sensor (1088x2048 pixels). This allows acquiring spatial and spectral information (460-620nm, 16 spectral bands, 10 nm bandwidth) in one capture (272x512 pixels), without wavelength or spatial scanning.[9] Image acquisition settings were optimized during extensive testing. The acquisition of one hyperspectral image takes 0.2ms and implies exposure to one flash of low to moderate intensity.

Fig. 1. Left : Ximea SNm4x4 camera. Right : Ximea mounted on TopCon fundus camera

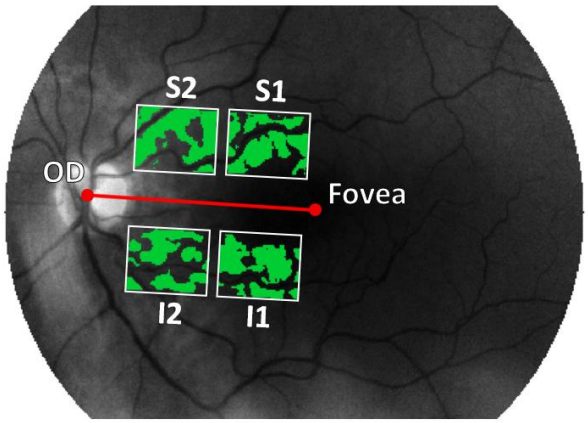

Fig. 2. Retinal image with four rectangular regions of interest: superior regions S1 and S2, inferior regions I1and I2. Green zones : remaining pixels after removing the retinal blood vessels. OD : optic disc.

Image processing pipeline

Relative reflectance (eliminating the spectral profile of the light source and correcting for electrical pixel noise) was computed for each hyperspectral image and subsequently corrected for cross-talk, leakage, harmonics and camera-specific acquisition artefacts. This resulted in a spectrum of relative reflectance values at 14 wavelengths for each hyperspectral pixel (two spectral bands omitted due to their large signal-to-noise-ratio).
Pixels corresponding to retinal blood vessels were removed from the hyperspectral images by applying a difference of Gaussians (DoG) filter. Four regions of interest (ROIs, roughly 40 x 40 pixels) relative to centre of the optic disc (OD) and fovea, were defined to standardise analysis between subjects (Figure 2). Average spectra of each ROI were standardized to reduce inter-subject variability related to differences in illumination.

Clinical study

A clinical study was carried out with 39 participants, 17 participants with AD and 22 cognitively intact elderly (CIE) controls. Hyperspectral images were taken with the HSRI setup. OCT measurements were performed with RT-vue XR Avanti (Optovue). RNFL thickness was measured over 360° and per quadrant.

Training and evaluation of classification model

Figure 3(a) shows average mean spectra for AD and CIE in the four ROIs after standardisation. These spectra and the RNFL thickness measures served as input to train Linear Discriminant Analysis (LDA) classifiers to distinguish AD from CIE subjects. Two input configurations for the classifier were considered: hyperspectral data only (14 input features, one for each wavelength) and hyperspectral data combined with the five RNFL measures. The performance of the different ROIs, with and without the RNFL measures, was compared using nested leave-one-out cross-validation (LOOCV). Figure 3(b) shows the final ROC curve generated for predictions in the nested LOOCV loop. An AUC of 0.74 with a 95%-confidence interval of [0.60-0.89] was obtained. Discriminatory information was mainly derived from hyperspectral data, but the model also benefits from the additional information from the OCT images.
Fifteen out of 22 CIE subjects had a probability of having AD close to zero, and 9 out of 17 AD patients had a score near 1. There were no significant differences in non-retinal parameters between AD patients with high and low probability scores.

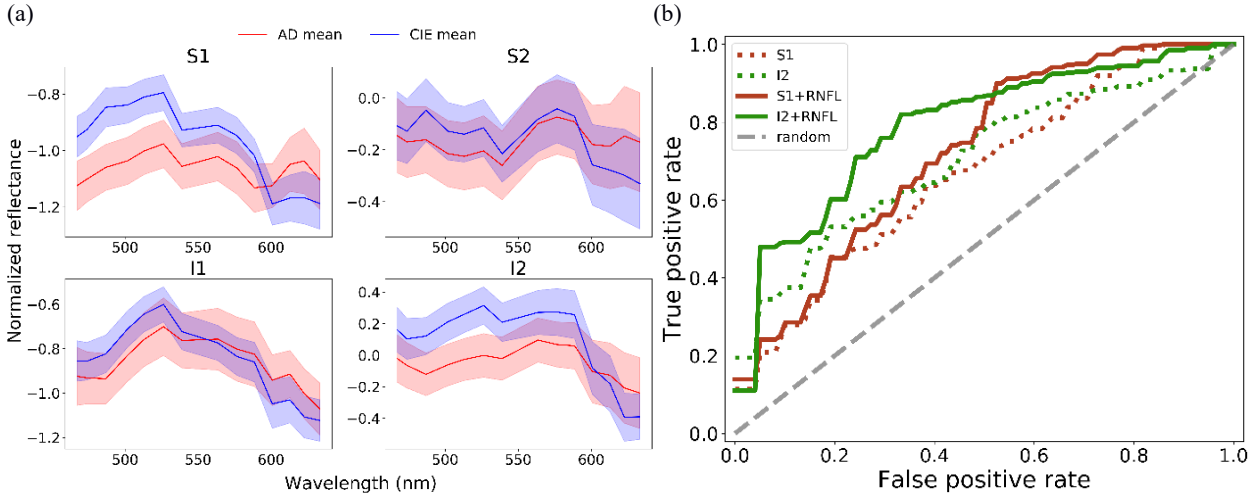

Fig. 3. Mean spectra for AD and CIE in the four ROIs after standardisation. Shaded areas indicate the mean +/- the standard error of the mean. S1, S2 : superior regions, I1, I2 : inferior regions. (b) Average Receiving Operating Characteristic (ROC) curves over all inner loop cross-validation runs for the two evaluated configurations in regions S1 and I2.

Conclusion

The results of this study support the idea that non-invasive HSRI, combined with a machine learning approach, can contribute to a classification model for the detection of AD. It further supports the idea that this can be achieved with a low-cost, compact and easy to use snapshot camera mounted on top of a standard fundus camera, found in every ophthalmologist's office. Performance of the current machine learning model improved with the addition of standard OCT-based RNFL thickness measures, demonstrating the added value of the bimodal imaging approach.
Parallel work on mouse retinas has been submitted for publication.[10] It confirms that HSRI can be used to quantify retinal Aβ.
This opens the way towards routine application in clinical practice.

FUTURE PROJECT VISION

4.1. Technology Scaling

In HERALD Phase 1 a TRL level of 3 is achieved. In the Phase 2 the ambition is to raise it to TRL level 7 : fully functional, validated hyperspectral camera, standardized image acquisition protocols and classification and quantification algorithms. Main steps to achieve this :

- Refine user requirements from the clinical side and from the side of pharmaceutical, diagnostic or medtech companies;
- Optimisation of the HSRI set-up – seamless integration with fundus camera – full system calibration for different human eye optical properties;
- Further development of image analysis and algorithms: improved segmentation; quantification of Aβ deposits in the retina of AD patients; classification of AD stages; prediction of progression; retinal Aβ as a proxy for cerebral Aβ;
- Clinical validation of HSRI biomarker and classification and prediction model in collaboration with other clinical research partners in order to get access to a wide range of patients – prospective longitudinal study, to evaluate the prognostic value and the potential to follow AD disease progression – extended multi-center cross-sectional trial;
- Demonstration units deployed at various end-users, i.e. clinical AD centres and pharmaceutical companies (clinical trials).

4.2. Project Synergies and Outreach

The consortium will be reinforced with:

- an engineering company with expertise in optical system engineering, and hardware and software integration;
- a medical image analysis company for co-development of image processing and algorithms;
- academic clinical partners specialized in AD for carrying out clinical trials and interpreting data and model results;
- potentially pharmaceutical companies and medical device manufacturers.

There is potential for clustering with other ATTRACT Phase 1 project, i.e. COSMIC, MOMENTO or SPECTA. Based upon their final results, complementarity and possible collaboration will be assessed, while keeping in mind not to dilute the core objectives of HERALD.

Dissemination of phase 1 results will be essential to involve possible end-users in refining user requirements and specifications, involvement in clinical trials and for the commercialisation strategy. Dissemination will target clinical AD centres, pharmaceutical, medtech and diagnostics companies. First efforts have already lead to promising contacts with pharmaceutical (i.e. Janssen Pharmaceutica and MSD) and diagnostics companies (i.e. Oxurion).

4.3. Technology application and demonstration cases

The HSRI system and the algorithms and models for diagnosis and monitoring of AD will be the core for mid-term and long-term applications for diverse customer segments (Figure 4). The first demonstrated applications will be targeted research on AD, and selection of patients in clinical trials of pharmaceutical companies. In a later (post-project) stage medical specialists can use them for diagnostic guidance, and finally, it can become a broadly used screening tool. The multi-centre clinical trials aim at both technological improvements and clinical validation in relevant settings. Demonstration units will further be dispatched to technological frontrunners for use in clinical practice or clinical trials of pharmaceutical companies. These applications will bring about a research platform for use by research organisations, improvement in clinical trials for disease-modifying drugs, opportunities for medtech and diagnostic companies and, obviously, improved health and well-being through early detection and therapy for AD.

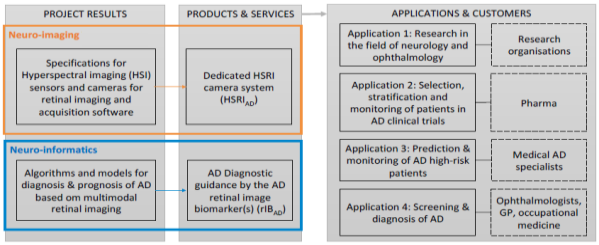

Fig. 4. Products and services, based on the HERALD project results, and mid-term and long-term applications for diverse customer segments.

Further collaboration will be sought with Euro-BioImaging, the European landmark research infrastructure for biological and biomedical imaging.

4.4. Technology commercialization

Commercialisation actions will run in parallel with R&D activities. Pharmaceutical companies, such as Janssen or MSD, expressed clear interest in using AD retinal biomarkers for selection and follow-up of participants in their clinical trials. Other companies, like Eli Lilly and Novartis, will be attracted by the clinical network of the HERALD consortium. Imaging diagnostics companies, such as Oxurion, showed interest in the diagnostic services pipeline. Involvement of pharmaceutical, diagnostic or medtech companies to refine user requirements will lay the foundation for future commercialisation. A business plan and a value network map will be drafted, regulatory requirements assessed, and a market evaluation carried out. The tech transfer departments of the partners will be involved from the project proposal stage onwards.

HERALD will be presented to potential investors through networks of tech transfer departments and involved companies, on major Life Sciences events, such as the Biofit conference and on relevant trade fairs. Access to potential investors through Intermediates (e.g. Nine-Sigma) will be investigated.

4.5. Envisioned risks

The main risk is for the hyperspectral camera to provide sufficient resolution for proper classification of AD subjects in different stages of the disease. However, the project partners have close lines of collaboration with IMEC, and hence to the latest hyperspectral sensors and cameras. Even if the clinical trials in the project are not yet fully convincing in terms of diagnosis, they will still provide a clear case for investment in improved sensors.

Recruitment of participants is always a difficult task. The project tackles this by tapping into existing patient cohorts of the clinical partners.

4.6. Liaison with Student Teams and Socio-Economic Study

The academic partners will assist MSc. Level student teams to work on alternative applications for retinal biomarkers. At KU Leuven this will be embedded in the Mission Lucidity R&D Hub on neurodegenerative diseases, led by experienced researchers. VITO also often involves MSc. Level students in exploratory research. The consortium will also support the expert-driven socio-economic study of the ATTRACT initiative and ecosystem through interviews with key staff, overviews of the value network mapping exercises, and facilitation of contacts with the technology end users (clinical and industry).

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