NEW INSTRUMENTATION FOR BLOOD-RETINAL BARRIER PERMEABILITY ANALYSIS

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Abstract: **The aim of the work herein presented is to assess the blood-retinal barrier (BRB) of the human eye *in vivo*. In order to quantitatively evaluate the BRB function, a confocal scanning laser ophthalmoscope instrument was used. The instrument was changed from the basic instrument to increase confocality and to change the starting plane of acquisition after focusing on the patient retinal surface. After a complete scan, a volumetric image of 32 confocal planes of 256 by 256 pixels of 1 byte in width is processed in four steps. The full procedure described permits us to compute the total amount of fluorescein that penetrates into the human vitreous after intravenous administration of dye. A single scan is required for the whole procedure. This new system allows for better identification of the central leaking sites in diabetes, showing well their relationship with the retinal vasculature.**

Keywords: **Image Processing, Blood-Retinal Barrier, Diabetic Retinopathy, Fluorometry**

Introduction

The aim of the work herein presented is to assess the blood-retinal barrier (BRB) of the human eye *in vivo*.

The human eye is a complex organ composed of several structures. Among them, the most important, from the point of view of the current work, are: cones and rods that transform light intensity into electrical signals to be interpreted as an image, the vitreous, a gel that keeps the form of the eye and the BRB, whose function is to prevent the passage of toxic components from the blood stream into the vitreous. The income of toxic components into the vitreous would damage cones and rods, consequently affecting vision.

The permeability of the BRB is affected by diabetes. Diabetes, in the US alone, increases at a ratio of 100 thousand new cases per year, while in the EU, 10% of the population are expected to develop diabetes by the year 2010 [1].

Diabetic retinopathy (DR) is a retinal disease directly related to diabetes. It affects persons between the ages of 24 to 74 and has a large impact on the active working population [2]. These figures show the socio-economic impact of this disease.

The BRB function is usually assessed by injecting fluorescein, due to its non-toxic properties, into the blood circulation and by photographically registering its passage in the retina. This is known as fluorescein angiography (FA). The assessment is made on the evaluation of this photographic register, it being in this way a qualitative assessment of the BRB function.

The new method herein proposed is based on a confocal scanning laser ophthalmoscope (CSLO). This device permits the measurement of the fluorescence levels in the human eye fundus by scanning the region of interest (ROI), in a selected plane, rejecting most of the light coming from both the anterior and posterior planes (confocality). Accordingly, a set of 32 confocal planes can be scanned in an area of the eye *fundus*. The scanned area is normally the 20° central-area, i.e., centred on the fovea. This is the most important area in retina since the detailed and colour vision is located in the fovea while the rest of the retina contains mainly rods, which allow for night-vision and do not perceive colour.

The method presented is known as the Retinal Leakage Analyzer, RLA.

Materials and Methods

In order to quantitatively evaluate the BRB function, a CSLO instrument from Heidelberg, the Heidelberg Retina Angiograph (HRA, Heidelberg Engineering, Dossenheim, Germany) was used. The instrument permits us to gather volumetric information for the 20° central area, in 32 confocal planes along 7 mm of depth, in 1.6 seconds.

The instrument available at our laboratory contains changes from the basic instrument in order to increase confocality and to change the starting plane of acquisition after focusing on the patient's retinal surface.

After a complete scan, a volumetric image of 32 confocal planes of 256 by 256 pixels of 1 byte in width each are exported for further processing. Each pixel is an 8-bit quantization of fluorescence levels.

This volumetric data has two types of information: the fluorescence distribution among the different confo-

cal planes at different retinal locations; the eye fundus reference with vessel distribution in the retina.

To model this instrument, the first step was the characterization of the energy transference models from the CSLO source to the eye fundus.

Point Spread Function (PSF): To compute the confocality and to check the independence of the output profile to the level of fluorescence, we computed the transfer function using a step signal. Nevertheless, special attention was given to the fact that the final optical path of the entire system is the human eye, i.e., the instrument is built for human eye scanning and thus takes into account the total eye power.

To calibrate the fluorescence measurement a *cuvette* was used and filled with a known concentration of fluorescein, and an apparatus composed of a 66.6 diopters lens with an artificial pupil of 8 mm was placed in front of the lens. In this way, the amount of light collected and the distance between planes is close to the normal human eye.

As a result of this step, it was shown that the output profile shape is independent of the fluorescein concentration and is shaped like a Lorentzian function.

To register the image planes correctly in the volume, a second step was used. This second step is to preprocess the gathered data in order to compensate for saccadic eye movements during the acquisition.

Image Alignment: due to both the voluntary and non-voluntary saccadic eye movements during the acquisition period of time, each plane may be shifted from the previous one. In order to compute a profile along the z-axis for any location in the eye fundus, one has to compensate for this displacement.

The method should be robust for decreasing in image details and not influenced by differences in image intensity. The chosen method is based on the shift property of the Fourier transform and known "Phase Correlation" [3].

After computing the shift for the whole set of confocal planes, the common scanned area is considered for further processing.

The third step of this process is to build the z-profile, i.e., the profile of fluorescence intensity along the z-axis for each pixel and then de-convolve the obtained profile with the achieved PSF. This is obtained by an iterative procedure that uses the difference between the expected outputs and the real signal, in order to update the guessed input. The process stops when the maximum number of iterations is reached or when the error signal is less than a pre-defined maximum error.

The fourth step is the determination of the retina-vitreous interface from the de-convolved signal. From this interface into the vitreous side an exponential decay profile is fitted to data by means of least squared error algorithm.

Finally, from the parametric curve the amount of fluorescein that crossed the BRB and got into the vitreous is computed, after calibrating the system to convert from fluorescence intensity to concentration equivalents of fluorescein (Eq. ng/ml).

The full procedure herein described permits us to compute the total amount of fluorescein that penetrates into the human vitreous after intravenous administration. A single scan is required for the whole procedure.

Normalizing these values by the amount of fluorescein present in the blood stream allows us to computing the BRB permeability index to fluorescein. This index is used for comparisons between different patients.

Results

The modified Heidelberg confocal scanning laser ophthalmoscope was tested in a series of normal and diabetic eyes, including two eyes three days after focal laser treatment. This new RLA-system allows for identification of the central leaking sites in diabetes, showing well their relationship with the retinal vasculature.

Discussion

We modelled an instrument based on the Heidelberg confocal scanning laser, to study retinal diseases.

We developed a new method to perform localized retinal fluorescein leakage using a confocal scanning ophthalmoscope, modified to obtain increased confocality.

When compared with previous instrumentation for fluorometry, the results show increased resolution and that the procedure needs only a single scan to be made.

From our previous experience, this instrumentation represents a major step forward in transferring these new methodologies of BRB analysis into clinical practice.

Conclusions

This new RLA-system, based on the Heidelberg confocal scanning laser ophthalmoscope, maps localized alterations of the BRB with improved resolution and with simultaneous imaging of the retina.

The system permits to assess the BRB in a quantitative manner, thus becoming independent of the external factors that may influence the evaluation made based on traditional fluorescein angiographies.

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