Introduction. Age-related macular degeneration (AMD) is the commonest cause of blindness in industrial countries among persons over the age of 50 and its prevalence is likely to increase in absolute numbers globally as a consequence of population ageing [1]. About 180 million people worldwide are known to be visually impaired due to AMD [2]. Sever form of the disease, called exudative AMD is marked by rapid and severe vision loss caused by abnormal choroidal neovascularisation and contributes to 90% of severe vision loss. Anti-choroidal neovascularization treatment is done by injections of drugs controlling and/or blocking vascular endothelial growth factor and preventing choroidal neovascularization. To monitor the effect of such treatment macular thickness is measured by means of optical coherence tomography, expensive procedure, sometimes of limited access for patients. Eye fundus images instead are taken during most of patient examinations. Experts ophthalmologists, comparing eye fundus images taken before and after the treatment, can recognize some changes in AMD lesion area. However, quantitative evaluation of changes is not possible because of big differences in eye fundus positioning and illumination while taking the pictures. Anyway, fundus images of the same eye, but after certain time, always represent nearly the same structure, consisting of the unique blood vessels network, optical nerve position and other elements. So it should be possible to align eye fundus images taken before and after the treatment to each other for further evaluation of changes. One can expect that quantitative estimates of changes will reflect treatment effect. The aim of this study was to elaborate imaging method for eye fundus images alignment and quantitative evaluation of changes in AMD lesion area caused by anti-choroidal neovascularization treatment.

Methods and materials. Pairs of eye fundus images taken before and after anti-choroidal neovascularization treatment of 10 AMD patients in Eye Clinics of Lithuanian University of Health Sciences were used for this study. The images were taken using Visucam NM Digital Camera (Carl Zeiss Meditec
AG, Germany) at 0.5 × 7.4 magnification and 5 Mpix resolution. Uneven background illumination was cancelled by subtracting background illumination profile calculated by mathematical morphology operation “opening” using structure element “disc” with radius of 200 pixels (bigger in size than any structural element in the image) [3]. Combined spatial transform (rotation, scaling and translation) was used to align two analysed pictures. Two reference areas not affected by AMD and containing well-contrasted elements (e.g. bifurcation of blood vessels) on the opposite sides of the images were selected by the operator and used as position references. Combined spatial transform of the images is done according to automatic alignment of these two areas in both images by maximizing cross correlation between them.

A three-dimensional array representing differences between pixel values of aligned images represents changes in AMD lesion area. Principal Component Analysis (PCA) of this array is used to construct new variables, giving optimal representation of changes in lesion area. The array is transformed into two-dimensional array, concatenating all rows of differences in pixel values of every colour into one-dimensional arrays:

\[
X = \begin{bmatrix}
x_{11} & x_{12} & \cdots & x_{1m} \\
x_{21} & x_{22} & \cdots & x_{2m} \\
\vdots & \vdots & \ddots & \vdots \\
x_{n1} & x_{n2} & \cdots & x_{nm}
\end{bmatrix},
\]

(1)

Spatial correlation of original representation of difference in images \(X\) can be estimated as:

\[
R_x = \frac{1}{3N} X \cdot X^T.
\]

(2)

The eigenvector equation for \(R_x\) is:

\[
R_x \cdot \Psi = \Psi \cdot \Lambda,
\]

(3)

where \(\Lambda\) denotes the eigenvalue matrix with the eigenvalues sorted in descending order, and \(\Psi\) is the corresponding eigenvector matrix. The matrix \(\Psi\) defines an orthonormal transformation, which is applied to the original data \(X\):

\[
Y = \Psi^T \cdot X
\]

(4)

to obtain the transformed representation, rows of which contain principal components of \(X\). Principal components are transformed back to two-dimensional arrays for visualization of changes in AMD lesion area. Area covered by difference is determined as count of pixel values in principal components, exceeding empirically determined threshold level (1/3 of maximal pixel level in this area) multiplied by area covered by single pixel.

**Results.** Example of two images, representing eye fundus of AMD patient before and after treatment is shown on Fig. 1. Difference in macular area is barely noticeable, however visual acuity improvement in this case was substantial: from 0.2 till 0.94. Difference in two images of AMD lesion area is presented in Fig.2. Upper row images show original RGB representation and bottom row images show three principal components. As we see, noticeable differences are in first two original images (R and G, marked by arrows).
PCA concentrates these differences into one, marked by two arrows into second principal component PC2. Estimated area, covered by this difference in PC2 was 0.384 mm².

Fig. 1. Eye fundus images representing AMD lesion area before and after anti-choroidal neovascularization treatment.

Dependency of difference in visual acuity in regard to area of difference in lesion of all 10 patients is plotted in Fig.3. Actual values are shown in the table, right to the graph. We did not find significant correlation between changes in AMD lesion area and changes in visual acuity.

**Discussion.** Interactive alignment of eye fundus images taken before and after the anti-choroidal neovascularization treatment reveals new possibilities for investigation of treatment effect by visualization of changes in AMD lesion area. PCA transforms original representation of changes into optimal space concentrating concurrent changes into one or another principal component. Arbitrary changes reflected in certain colors are highly expected due to the pathogenesis of the disease (blood, lipoprotein-derived debris, etc.). Visualization of such components will have diagnostic value, which we are planning to investigate. We did not find significant correlation between area of difference in lesion and visual activity, probably, due to limited amount of investigated patients. Having the elaborated method we are planning to involve more patients and continue the study.

**Conclusion.** Principal Component analysis of differences in automatically aligned eye fundus images, taken before and after anti-choroidal neovascularization treatment, can reveal areas of changed eye fundus structures reflecting treatment effect.
Fig. 2. Difference in lesion area of images shown on Fig. 1. Upper row: original RGB representation; Bottom row: representation by three principal components.

Fig. 3. Dependency of changes in visual acuity and area of difference in AMD lesion represented in principal components PC1 and PC2.

References
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Evaluation of Anti-Choroidal Neovascularization Treatment Effect in Age-Related Macular Degeneration Patients by Means of Multivariate Analysis of Eye Fundus Images

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Computer aided alignment and of eye fundus images of AMD patients taken before and after anti-choroidal neovascularization treatment opens possibility of quantitative evaluation of changes in eye fundus structures. Principal Component Analysis of the differences in these images can reveal diagnostically valuable details about treatment effect.