Radim Kolář

METHODS FOR IMAGE ANALYSIS
AND PATTERN RECOGNITION
- APPLICATION TO EARLY
GLAUCOMA DIAGNOSIS
METHODS FOR IMAGE ANALYSIS AND PATTERN RECOGNITION - APPLICATION TO EARLY GLAUCOMA DIAGNOSIS

METODY PRO ANALÝZU OBRAZŮ A ROZPOZNÁVÁNÍ - APLIKACE PRO VČASNOU DIAGNOSTIKU GLAUKOMU

ZKRÁCENÁ VERZE HABILITAČNÍ PRÁCE
Obor Biomedicínské inženýrství
Klíčová slova:
analýza obrazů, registrace obrazů, diagnostika glaukomu, sítnice

Key Words:
image analysis, image registration, glaucoma diagnosis, retina

Originál habilitační práce je dostupný na Vědeckém oddělení děkanátu
FEKT VUT v Brně, Údolní 53, 602 00, Brno

Autor:
Radim Kolář
Ústav biomedicínského inženýrství
Fakulta elektrotechniky a komunikačních technologií
Vysoké učení technické v Brně
Kolejní 2906/4, 612 00, Brno

© Radim Kolář, 2009
ISSN 1213-418X
Radim Kolář was born in 1975 in Brno. In 1988, he obtained Ing. (MSc.) degree in Cybernetics, automation and control and in 2002, Ph.D. degree in Biomedical engineering, both at the Brno University of Technology.

Since 2001, Radim Kolář has been working as a lecturer at the Department of Biomedical Engineering of Brno UT. He is engaged in teaching signal processing, biosignal analysis, and biomedical instrumentation. His research interest includes image processing, analysis and registration, particularly in ophthalmology and ultrasound.
# CONTENTS

1  INTRODUCTION..........................................................................................................................5

2  GLAUCOMA DIAGNOSIS BASED ON AUTOFLUORESCENT RETINAL IMAGES........5
   2.1 Introduction..............................................................................................................................5
   2.2 Data ACQUISITION................................................................................................................6
   2.3 Image registration...................................................................................................................6
   2.4 Registration results................................................................................................................7
   2.5 Conclusion on registration......................................................................................................7
   2.6 Application of registration result..........................................................................................8
      2.6.1 Pixel level image fusion.................................................................................................8
      2.6.2 Image segmentation..........................................................................................................10
   2.7 Conclusion on autofluorescent image analysis......................................................................15

3  RETINAL NERVE FIBRE LAYER IN COLOUR FUNDUS IMAGES........................................15
   3.1 Introduction............................................................................................................................15
   3.2 Data.........................................................................................................................................16
   3.3 Methods for texture analysis................................................................................................17
   3.4 Features selection..................................................................................................................18
      3.4.1 Max-Relevance and Min-Redundancy..........................................................................18
      3.4.2 Application of mRMR approach to feature selection ....................................................19
   3.5 Classification........................................................................................................................20
      3.5.1 Classifiers.......................................................................................................................20
      3.5.2 Classifiers – setting..........................................................................................................20
      3.5.3 Classification strategies.................................................................................................20
      3.5.4 Results of classification.................................................................................................20
   3.6 Conclusion on texture analysis...............................................................................................21

4  SUMMARY..................................................................................................................................22

REFERENCES................................................................................................................................23

APPENDIX....................................................................................................................................27

ABSTRAKT.....................................................................................................................................28
1 INTRODUCTION

This thesis describes two different methods for early glaucoma diagnosis. Glaucoma is the second most frequent cause of permanent blindness in industrial developed countries [45]. It is caused by an irreversible damage of the optical nerve connected with degeneration of the retinal ganglia cells, axons (neural fibers) and gliocells (providing nutrition for the axons). If not diagnosed in early stage, the damage of the optical nerve becomes permanent, which in the final stage may lead to blindness. The number of people with open-angle and angle-closure glaucoma in 2020 is estimated to 79.6 million [45]. Development of new diagnostic approaches that would enable early detection of glaucoma development is therefore very desirable. One important view is that these new methods are to be applicable in routine diagnostic process; they should be robust and fast, particularly for screening programs.

Both methods, presented here, are based on different imaging techniques. First method uses advanced laser scanning principle to acquire retinal images, which produces high resolution image data. Proposed methodology, which uses these images, has not been established widespread in routine clinical work yet. The second method uses images recorded by the digital ophthalmoscope, which is basic device in many ophthalmologic clinics and ambulatory care centers. This makes the proposed methodology possibly applicable in a wide sense.

One of the main aims of this work is to show several image processing approaches applied to practical problems. These techniques cover basic and advanced methods - from image filtering, texture analysis, image registration (connected with optimization) to image segmentation and image fusion. Another important part uses several approaches from pattern recognition field - methods for feature selection/extraction and classification.

2 GLAUCOMA DIAGNOSIS BASED ON AUTOFLUORESCENT RETINAL IMAGES

2.1 INTRODUCTION

This section describes the physiological background of examination focused on the retinal pigment epithelium (RPE), which is a single layer of cells between the neurosensory retina and the choroid. This layer is an important structure for maintenance of the outer retina, it participates on vitamin A circulation and one of the main issues is to phagocytize the outer discs of the photoreceptors, which enables its proper function [19]. With age or due to various diseases RPE function may by incomplete and accumulation of the intralysosomal molecules, the lipofuscin, takes place. Therefore, it is believed that accumulation of lipofuscin correlates with reduced RPE metabolical activity. Excessive accumulation of lipofuscin within the RPE may play a major role in the pathogenesis of age-related macular degradation [23, 44, 53], Best or Stargardt disease [19] and other diseases, leading to significant loss of vision due to degradation of photoreceptor layer. Lipofuscin is a mixture of many substances and appears to contain at least ten different fluorophores, which are able to emit visible light after illumination by laser beam of appropriate wavelength.

Lipofuscin granules in the retinal pigment epithelium have been identified as the dominant fundus fluorophore by spectrometric investigation, which mainly arise from degradation of outer photoreceptor segments and consists of various components, as lipids, proteins and retinoids. In age-related macular degeneration and hereditary retinal diseases, imaging of fundus autofluorescence is already established as an important diagnostic procedure [23, 44, 53].

Increased autofluorescence has also been demonstrated in the parapapillary region of patients with primary open angle glaucoma and ocular hypertension. The higher level of autofluorescence in these cases is predominantly observed at the border of atrophic zone alpha, which is characterized by an irregular hypo- and hyperpigmentation, associated with thinning of the
chorioretinal tissue layer and might indicate progressive chorioretinal atrophy [30, 51, 52]. Histological and electron microscopic investigations demonstrate an accumulation of lipofuscin in lysosomes of the retinal pigment epithelium in this region [28, 29]. Therefore, the detection of increased parapapillary autofluorescence, especially in case of ocular hypertension and early glaucoma, may offer an important early diagnostic tool.

2.2 DATA ACQUISITION

For exciting lipofuscin in clinical settings nowadays the confocal laser scanning devices are used. The Heidelberg Retina Angiograph (HRA) is a device, designed primarily for angiographic examinations, but it enables the detection of fundus autofluorescence level and distribution in vivo as well [4, 5]. During examination, the retina is illuminated by laser beam point-by-point in raster manner. In reflection mode, this beam (λ = 820 nm) is reflected as it penetrates retinal structures and reflected portion of light is detected by photo-sensitive device. In autofluorescent mode the wavelength of laser light is λ = 488 nm. During scanning, lipofuscin is excited and emits photons of longer wavelength (above 500 nm). After separation of incident and reflected light by barrier filter, these photons are detected. The emitted intensity depends on the amount of lipofuscin accumulated in retinal pigment epithelium (RPE).

The acquisition of IR image has two main reasons. It has higher contrast of the optic nerve head (ONH), which enables its easier segmentation. The second reason is that the focus level and illumination must be set appropriately to individual requirements during AF scanning. For that reason an IR image is acquired before AF image sequences are taken.

2.3 IMAGE REGISTRATION

The image registration techniques play an important role in imaging techniques of data from different types of (medical) imagining devices. General view of registration methods applied to various types of data (CT, PET, MRI, etc.) can be found in several review papers [8, 39, 42, 56]. Specific methods for the retinal image registration are also described in several application papers [9, 10, 55].

Image registration can be treated as the optimization process of finding the parameter vector \( \alpha_0 \) of the spatial transformation \( T_\alpha \) aligning the content of the images:

\[
\alpha_0 = \arg \left\{ \max_{\alpha} C(f, T_\alpha(g)) \right\}, \quad (1)
\]

where \( f \) denotes the reference (fixed) image, \( g \) is the transformed (moving) image and \( C \) is an optimization criterion, which evaluates the registration quality.

Based on the prior knowledge of image properties, all parts of the registration process should be chosen carefully to ensure registration robustness. The components of the registration framework for AF – IR images were selected based on detail image analysis and based on requirements of practicability:

- Preprocessing – noise suppression by anisotropic diffusion filtering.
- Criterion metrics – normalized cross-correlation using gradient images was used as a trade off between mean squared differences (fast and simple) and mutual information (computationally demanding).
- Spatial transformation – using shift, rotation and scaling.
- Optimization strategy – controlled random search (CRS) was used to avoid local maxima.
- Interpolation, needed to match the discretization grids of both images – fast nearest neighbor method was used.
The used registration approach utilizes a multiscale approach, widely used to improve speed, accuracy and robustness [56]. The basic idea is that registration is first performed at a coarse scale (low spatial resolution). The spatial mapping determined at the coarse level is then used to initialize registration at the next finer scale. This process is repeated recursively at finer levels until it reaches the finest possible scale.

For AF and IR images we set only one coarse level with decimal subsampling, where only translations $t_x$ and $t_y$ are considered as the optimization parameters. At the next finest scale, all the transformation parameters are considered.

### 2.4 REGISTRATION RESULTS

The proposed approach with the above parameters was tested on the database of 131 ophthalmologic image pairs (AF and IR images). These images were obtained in low ($512 \times 512$ pixels) or high ($1024 \times 1024$ pixels) resolution mode. High resolution images were decimated to low resolution, taking every second pixel, to speed up the computation. The CRS optimization was run 5 times in each case of 30 image pairs (randomly selected from 131 image pairs) to test the sensitivity to random initialization. No visible changes among these particular results were observed. Unfortunately, verification of registration algorithms accuracy is not trivial, because the correct geometrical alignment is not known. The verification was based on the visual inspection of the edge images and the mosaic images. An example of the registration results are shown in Figure 1 together with the edge and mosaic images, used for visual evaluation.

Each registered pair was classified according to [47] as:

1. **Excellent (class 1)** – the best quality with no visible discrepancy between both images.
2. **Good (class 2)** – small misalignment between the images in the range of 1 to 5 pixels.
3. **Moderate (class 3)** – higher misalignment between the images in the range of 6 to 15 pixels.
4. **Poor (class 4)** – registration with significant misalignment.

<table>
<thead>
<tr>
<th>Expert</th>
<th>Class 1</th>
<th>Class 2</th>
<th>Class 3</th>
<th>Class 4</th>
<th>Mean Score</th>
<th>Class 1+2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>107 (81.7%)</td>
<td>15 (11.4%)</td>
<td>6 (6.4%)</td>
<td>3 (2.3%)</td>
<td>1.27</td>
<td>122 (93.1%)</td>
</tr>
<tr>
<td>B</td>
<td>98 (74.8%)</td>
<td>21 (16.0%)</td>
<td>4 (3.1%)</td>
<td>8 (6.1%)</td>
<td>1.41</td>
<td>119 (90.8%)</td>
</tr>
</tbody>
</table>

Table 1. Registration results

### 2.5 CONCLUSION ON REGISTRATION

To discuss the results of the registration phase, let us concentrate to the Table 1. One can see that even considering only the class 1, the percentage of good registration is satisfactory. Class 2 and 3 includes images where the misalignment errors were visible at the periphery of the images and were in the range of several pixels. For the application, which will be described in next section, mainly the areas around the ONH are important for analysis of the AF zones. Therefore, it is important to register precisely the central part (ONH with its surroundings). In that sense, at least class 2 can also be considered well registered and the percentage of successfully registered images is thus 93.1% and 90.8%, respectively (including Class 1 and 2).

By detailed analysis of images in class 3, it can be concluded that the rare wrong registration results arise from blurred IR images, images with small overlap and for images with small but complex distortions, where more generic flexible registration is needed. Investigating the images with high registration errors in the class 4 we can conclude that significant misalignment is found in pairs of images where the IR image is blurred and has a low contrast and the AF image is also blurred or dark. Also, in some cases, a visible strong flexible distortion is present.
2.6 APPLICATION OF REGISTRATION RESULT

Two clinical applications of AF – IR image registration will be shortly described in this section: pixel level image fusion and segmentation.

2.6.1 Pixel level image fusion

Pixel level image fusion means merging the information from several images into a single image. There are many different areas of image fusion, namely thermal and visual image fusion [50], remote sensing [17, 43] and medical imaging [1] and also multimodal retinal images [32]. The aim of the AF – IR image fusion process is to provide a single image with extended information content for the glaucoma diagnosis process. Generally, image fusion is particularly useful for reducing the workload of human operators, because it offers different information content in one image. The greatest benefit of the image fusion is achieved if the component images contain complementary information, which applies to AF – IR image pairs. The AF image contains information about the zones with high autofluorescent activity and more visible periphery blood vessels while the IR image carries the information about the optical disc border, its structure and...
blood vessels inside ONH. The important point is the knowledge of the mutual positions of the autofluorescence zones with respect to optic disc border.

Without fusion, the physician must move his or her eye between images and it may be difficult to recognize the relationship among patterns and objects, particularly ONH border and AF zones. To prevent this, two pixel-wise fusion processes were tested. These fusion methods are very fast, using artificial color mapping applied on the two mentioned gray-scale component images.

**HVS fusion method**

This approach is similar to the method used in [32], where the fusion was used for color fundus and angiography images. This scheme arises from the biologically motivated pixel-wise fusion methods [1] (HVS stands for the Human Visual System). For AF – IR fusion application, the scheme is shown in Figure 2. First, the image normalization to 256 levels is performed. Consecutively, the red channel is computed as a difference between IR and AF image, which enhances the information present in IR image. The blue channel is the negative of the previous combination. The green channel is the AF image itself, because the zones with higher autofluorescency play an important role in early diagnosis of glaucoma and the fusion is thus performed with visually emphasized the AF components.

![AF – IR fusion scheme](image)

**TV-IR fusion method**

This scheme is based on a method used for grayscale video (TV) and infrared (IR) images as in [50]. The common component of the images is computed as the morphological intersection:

\[
\text{Common component } f \cap g = \text{Min}(f[i,j], g[i,j]).
\]

The characteristic components \(f^*\) or \(g^*\) of each image remain after subtraction of the common component:

\[
f^* = f - f \cap g, \quad \text{and} \quad g^* = g - f \cap g.
\]

The characteristic component can be emphasized in the fused image \(h\) by subtracting each of them from the other image, so that the RGB components are then defined as in [50]:

\[
h = \begin{pmatrix}
R \\
G \\
B
\end{pmatrix} = \begin{pmatrix}
f - g^* \\
g - f^* \\
0
\end{pmatrix}.
\]

This definition has been modified in the similar way as in the HVS method, to enhance the AF zones in the fused image:

\[
h = \begin{pmatrix}
R \\
G \\
B
\end{pmatrix} = \begin{pmatrix}
f - g^* \\
f \\
g - f^*
\end{pmatrix}.
\]
where $f$ represents the AF image, $g$ represents the IR channel. For all channels the normalization to 256 levels is performed before displaying.

**Image fusion results**

The RGB results of the image fusion are shown in Figure 3, where the original (registered) AF and IR images are shown together with the corresponding fusion results.

As for the preliminary evaluation of the fusion, it can be seen that the results of the fusion process are very similar for HVS and TV-IR fusions methods. Considering the aim of the fusing, i.e. human analysis of the AF zones with respect to ONH border, the TV-IR scheme seems to be more convenient, because of higher contrast of AF zones. This was observed in most cases of the used database and the usefulness of fusion confirmed by medical expert.

![Figure 3. Example of AF - IR fusion results: a) AF image, b) IR image, c) HVS method, d) TV-IR method](image)

2.6.2 Image segmentation

The second application that utilizes results of the AF-IR registration is focused on segmentation. As written in Section 2.1, increased autofluorescence has been observed in patients
with primary open angle glaucoma and ocular hypertension. Hence, there is an assumption that certain geometric parameters of these AF pattern can assist the glaucoma diagnosis [51, 52]. Standard HRA software allows assessment of local autofluorescence changes, but the image analysis requires manual outlining of interesting structures, which is time consuming and results are subjective to a certain degree. Intraobserver variability has been shown to be relatively low, but interobserver variability is rather wide even if the assessment is performed by experienced ophthalmologists [34]. Therefore, there is an effort to decrease these variabilities using a (semi)automatic approach for segmentation of zones with increased autofluorescence in the measured scene.

The main aim is to detect the areas with lipofuscin accumulation and to show their position with respect to optical disc. Once the positions of the pixels that belong to the particular zones are known, several geometric parameters can be determined (area, distance from optical disc and other shape parameters [34]). Two segmentation applications will be described in the next paragraphes. One is focused on ONH segmentation and the other on AF zones segmentation.

**Optic disc segmentation in IR images**

The detection of the ONH border is substantial for determining, because the mutual distance between AF zones and ONH border is an important diagnostic parameter [52]. This detection is performed in IR image, because ONH border is not clearly visible in AF image and is almost impossible to detect it here by eye. If the physician sees this border in AF image clearly, he can diagnose it more accurately.

The simplified flowchart for ONH detection in IR image is depicted in Figure 4. The center of the ONH is roughly indicated interactively. Then, after image smoothing by an averaging filter with Gaussian mask, the optic nerve head border is approximated by a circle \( C \) with the radius \( R \), which satisfies:

\[
E = \min_R \frac{1}{N} \sum_{(x,y) \in C} I(x,y). 
\]  

(6)

The summation is computed from \( N \) image pixels \( I(x,y) \) lying on the circumference. Looking for such circle with radius \( R \), along which the sum of the pixel values is minimal can be substantiated as follows: when examining the IR images, it can be seen that the ONH is always darker then its surrounding. Although the inner region of the ONH is always lighter then the region near its border, this is excluded by the limits for radius \( R \). The circle with the minimal \( E \) will therefore approximate the ONH border. The radius extent to be examined is determined by the possible ONH size; regarding the resolution, the radius range from 20 pixels to 60 pixels was used (for \( 512 \times 512 \) pixels images). These values refer to the retinal morphology of the ONH [22].

The active contour [38] is consequently used for fine adjustment of the ONH border. The active contour is a deformable \( v(s) = [x(s),y(s)], \ s \in [0,1] \), which moves through the spatial domain of an image to minimize function:
\[
J = \int_{0}^{1} \alpha |v'(s)|^2 + \beta |v''(s)|^2 + E_{\text{ext}}(v(s)) \, ds.
\] (7)

Parameters \(\alpha\) and \(\beta\) are weighting values for first and second derivative and they control contour's tension and rigidity, respectively. External force \(E_{\text{ext}}\) is a potential function derived from the image so that it takes smaller values at the boundaries, which should be detected.

Many possibilities exist for computing the external forces, because this formulation allows user-based design of this force. The most popular is gradient vector flow, based on edge map or simple image gradient, which was also used in this case. The internal forces were determined by cubic B-splines, which ensures smoothness (continuous first and second derivatives) and flexibility (due to local control by number of control points [8]).

The practical implementation include these steps:

1. Computation of external forces.
2. Initialization of active contour by circle with minimal \(E\) value.
3. Computation of control points from active contour.
4. Analysis of the external force field in spline's close surrounding (in a perpendicular direction to active contour).
5. Moving the active contour points according to gradient value.

Figure 5. Two pairs of corresponding IR (left) and AF (right) images with detected ONH contour.
6. Modification of control points.
7. Evaluation of stopping criteria.

Results of ONH segmentation

The example of active contour segmentation is presented in Figure 5. The registration results are used to transform the ONH coordinates from IR image to AF image. It can be seen that optic nerve head border in AF image is almost impossible to detect. This results help physician to see AF zone with respect to ONH border.

Quantitative assessment of this result was not possible because provided data were not labeled in this sense. The assessment was done only on the subjective level by experienced opthalmologist.

AF zones segmentation in AF images

The segmentation of AF zones was also designed and implemented as a semiautomatic. It is based on the interactive indication of point (a seed) inside each AF area. This method can be divided into four steps, which are described bellow:

1. Image preprocessing.
3. Applying region growing method.
4. Fine adjustment of the detected contour.

As can be seen from the original image, the AF images are quite noisy, although this image is a result of averaging several images obtained by HRA. Therefore, a simple median filter is used as a preprocessing step. The size of window $5 \times 5$ pixels was determined based on analyzing the minimal size of AF zone in images from the database.

The second step is region growing method. Even though the principle of this method is simple [24], it must be adjusted for a particular application. For AF area detection, the fact that the pixels inside this area have higher intensity than surrounding pixels was used. Therefore, the decision rule (i.e. if actual pixel belongs into the region or not) is based on the pixel intensity value and the mean instantaneous value of the actual region that is changing during the growing process. The whole algorithm is depicted in Figure 6.

It must be noted that some AF zones are hard to detect, because of their low contrast, which may cause region overflowing. Therefore, the algorithm was set to underestimate the AF area to prevent this overflowing. To achieve accurate result, the active contour were used as a next step.

After region growing has finished, the active contour is employed again to adjust the AF zone contour. The important modification for AF zone segmentation is the computation of external force field $E_{ext}$. The weighted sum of smoothed image gradient and distance transform gradient was used in this application:

$$E_{ext}(x, y) = k \left| \nabla (G_w(x, y) * I(x, y)) \right|^2 + (1 - k) \left| \nabla D(x, y) \right|. \quad (7)$$

Field $D(x, y)$ represents the distance transform of this image and $k$ is parameter that influences the sensitivity of active contour. It was observed that combination of these gradients gives satisfactory result, better than the only simple image gradient. The value of the parameter $k$ has been found 0.1. The illustration of active contour is depicted in Figure 7, where the left figure shows the result after region growing. Contour is placed in original image, where the white region represents the AF area and blue arrows the external force according to Equation (7). The middle image shows several iterations of the active contour and the right image the final contour.
Figure 6. Flowchart for AF zone detection in AF images
Figure 7. Illustration of the contour evolution: a) result of region growing, b) several iterations of the active contour, c) final contour

2.7 CONCLUSION ON AUTOFLUORESCENT IMAGE ANALYSIS

Registration methods have generated great interest in theoretical and applied science during recent ten years. It has become standard tool (almost as a preprocessing step) in many medical applications. New imaging modalities call for new modification of present approaches. For example images from nowadays PET/CT scanners need a multimodal registration approach with high accuracy. These methods have become also very popular in commercial areas as tools for image mosaicing and panorama creating.

This Chapter described only one particular multimodal application based on pixel-value registration. A robust framework for registration of AF and IR images was proposed and experimentally verified, including the image preprocessing. The parameters of this approach were optimized for routine use in ophthalmologic clinics. This framework is the part of custom made software, which is currently used at the Department of Ophthalmology, Friedrich-Alexander University of Erlangen-Nurnberg (Germany). A reasonable tradeoff between the speed of computation and registration accuracy was achieved. The computation time for a single registration is about 1 minute at Intel, Pentium M, 1.7 MHz. The computation time for fusion and for ONH and AF zone segmentation is negligible.

3 RETINAL NERVE FIBRE LAYER IN COLOUR FUNDUS IMAGES

3.1 INTRODUCTION

The diagnostic value of observing retinal nerve fiber (RNF) layer has been proved for glaucoma diagnosis. The diffuse or focal RNF layer atrophy is indicated as a texture changes in colour or greyscale photographs. Therefore, there has been a high effort to use colour or greyscale retinal images to evaluate a RNF layer thickness. But until now, there is no routinely used method for RNF quantification (based only on colour photography) although an increasing effort in this field is noticeable [33, 41, 49]. This part of thesis deals with analysis of structure created by retinal
nerve fibre layer in colour fundus images. The main aim of this analysis is to find a set of parameters (features), which will describe changes in this structure. Therefore, techniques from the texture analysis, features selection and classification will be used in this chapter.

3.2 DATA

As mentioned above, the RNF create a texture in color fundus image. This texture can be analyzed in order to evaluate the RNF quality, e.g. thinning and losses. The neural fibres are locally oriented approximately in parallel, which causes their lightly stripy appearance (albeit barely visible - Figure 8). This local orientation of a (roughly) periodic structure is probably the most prominent feature of the neural layer. The second feature is the higher level of brightness, caused by higher reflectivity of RNF in green-blue part of the visible spectra. One example of annotated retinal image from image database, presenting the most prominent structures is shown in Figure 9a and corresponding schematic plot of the main retinal structures in Figure 9b. RNF runs from the ONH to macula with the highest concentration in radial direction.

Figure 8. Small part of greyscale retinal image with RNF, which appears as stripy structure.

Figure 9. a) Annotated retinal image of glaucomatous eye, b) Schematic plot of the main retinal structures
The images were taken by colour fundus camera Canon CF-60UDi with digital camera Canon D20 in JPEG format with very low compression. The original images were recorded as a colour images in RGB colour-space. But the mean value from green and blue channels was computed, because the red component doesn't carry any information from RNF reflection.

The database of images used in this study contains 30 retinal images: 16 glaucomatous images with RNF layer loss (or losses) and 14 images of healthy eye (with no suspicious finding). The lack of convenient images is unfavorable, because of statistical evaluation. However, the design of convenient features can be made. To overcome this difficulty, the following data preparation was made.

Several small square image samples (41 × 41 pixels) were selected from each retinal image for texture analysis. All these samples were transformed to 64 grey-levels and adjusted whole extent of this colourmap to eliminate the non-uniform illumination:

1. Class A - image samples with tissues containing the RNF (304 samples) from patients with glaucoma.
2. Class B - image samples from area without RNF (176 samples) from patients with glaucoma.
3. Class C - image samples from control group - samples selected from healthy eyes of patients without glaucoma (308 samples).

Because the occurrence of RNF losses is mainly detectable in a close surrounding from ONH, these samples were selected from distance not exceeding ONH diameter. This also ensures that the RNF layer thickness is not affected by natural RNF decrease with distance.

3.3 METHODS FOR TEXTURE ANALYSIS

Texture analysis covers wide range of applications in image processing area. Many reviews or texture evaluation papers can be found concerning this topic [18, 40]. Approaches to texture analysis are usually categorized into four groups [40]:

First, structural approaches describe texture by defined primitives and the order of the placement of these primitives. Bundle of papers, using this approach for the texture synthesis, exists. Nevertheless, only several applications for the texture analysis in medical applications can be found, e.g. in mammographic images [37] or microscopic images [13]. The different way for texture analysis is used by statistical approaches. These methods represent the texture by non-deterministic properties that govern specific probability distribution and by relation between the grey levels. These methods include particularly first-order and second-order statistics. A number of papers for various applications using this approach can be found, e.g. method based on co-occurrence matrix [48], grey level run length matrix [2] or statistics based on the thresholded image. Model-based analysis uses appropriate model for texture description, e.g. fractals [10, 11], Markov random fields [16], Gibbs Random Fields [16] or autoregressive random fields [25]. The model selection usually follows the nature of image texture or is selected ad hoc. The transform methods use some transform to different space, usually wavelet (packet) domain [31], or Fourier domain [35]. This class also encompasses Gabor filters based methods [14] and steerable pyramids [20].

Several methods were employed to cover all these categories, to demonstrate their different approach (see list of features in Appendix, Table 2). In more details:

1. structural approach uses adaptive thershold and consecutive morphological analysis of binary structures.
2. statistical approach uses first, second and higher order statistics features.
3. model-based approach uses two different fractal model to estimate the fractal dimension.
4. transform-based approach – uses discrete two-dimensional Fourier transform to compute different spectral features.
All these methods provide a set of features for each class (A, B, and C). The next task is to evaluate the feature's usefulness for classification.

3.4 FEATURES SELECTION

Features reduction and features selection is an extensive task in pattern analysis. Many methods have been published to cope with features selection for small or large feature sets [54]. From all these methods, two general approaches can be identified: filters and wrappers [21].

**Filter methods** select features on the basis of their relevance or discriminant powers with regard to their classes. Simple methods based on correlation, mutual information, different metrics and statistical tests have been used [54]. **Wrapper methods** use a specific method, which estimates classification accuracy or its change due to each feature and judges about features usefulness. Wrapper methods typically require extensive computation to search the best features [36].

3.4.1 Max-Relevance and Min-Redundancy

Different approaches can be used by filter-based methods. Specific objective function is defined to select the best features. This function has to describe feature from two points of view: the feature's separability measure (various types of metric, like Euclidean, Bhattacharyya etc.) and the correlation criteria (measured for example by correlation coefficient or mutual information).

Suppose a feature space \( F \) with features \( x_i, i=1,2,...,P \) and target classes \( c \). Furthermore suppose its subset \( S \subseteq F \). The aim of features selection method is to find \( S \) with \( M \leq P \) features \( x_i, i=1,2,...,M \) according to defined criteria.

Max-Relevance and Min-Redundancy scheme is based on two concepts: a good feature should have maximum relevance to target class \( c \) and minimum redundancy to already selected features. These two properties can be described by mutual information. Consider two random variables \( x \) and \( y \), which have probabilistic density function \( p(x), p(y) \) and \( p(x,y) \). Their mutual information is defined as:

\[
I(x,y) = \int \int p(x,y) \log \frac{p(x,y)}{p(x)p(y)} \, dx \, dy. \tag{8}
\]

For discrete variables an approximation of \( I(x,y) \) has to be estimated. It can be computed using Parzen windows technique, commonly used in medical image registration [26].

The Max-Relevancy scheme selects such features \( x_i \), which have largest mutual information \( I(x_i,c) \) with the target class \( c \). The features are selected in such a way that the mean value \( D \) of selected mutual information is maximized:

\[
\max_S D(S,c); \quad D(S,c) = \frac{1}{P} \sum_{x \in S} I(x,c). \tag{9}
\]

The Min-Redundancy scheme selects features according to their redundancies. When two features are highly dependent on each other, their selection does not change so much the respective classification accuracy. Therefore, this scheme for the features selection is based on the minimization of function \( R(S) \):

\[
\min_S R(S); \quad R(S) = \frac{1}{P} \sum_{x_i,x_j \in S} I(x_i,x_j). \tag{10}
\]

The Max-Relevance and Min-Redundancy (mRMR) is a combination of these two constraints. The final criteria can be constructed using difference [15]:

\[
\max_S \left[ D(S,c) - R(S) \right] \tag{11}
\]
The features selection works in an iterative manner. First, the feature with the highest mutual information is selected. The rest features are selected incrementally, e.g. previously selected features stay in a subset $S_{i-1}$ and the new feature $x_i$ is selected in a such way, that it maximizes criterion in Equation 11 to create $S_i = S_{i-1} + x_i$.

3.4.2 Application of mRMR approach to feature selection

There are four possibilities how to compute the optimal order using different combinations of classes. The following approach was proposed here to evaluate the mean order:

- Compute the order of each feature according to criteria (Equation 11) for classes A-C, B-C and A-B.
- Compute the order of each feature according to criteria (Equation 11) for classes A-B-C.
- Compute the mean order of each feature using previous results.

The purpose of this scheme is to consider all interesting combinations of classes in mRMR approach and to evaluate the best features for each discrimination. The mean order provides features useful for discrimination between classes A, B and C. The results of this approach are presented in Figure 10.

![Figure 10. Values of the mean order computed from different combinations of mRMR approach.](image)

Several interesting results can be formulated. Feature number 23 (Short Run Length Entropy) is on the first place according to this criteria for all class combination. Similarly, the feature number 7 (Texture Entropy) is the second most relevant feature. Next features are not so clearly ordered. According to mean order, features number 3, 14 and 31 follow. These five best features were checked, whether they also leads to good classification results or not.
3.5 CLASSIFICATION

3.5.1 Classifiers

Two different classifiers were used for testing during this work:

1. Ho-Kashyap (H-K) classifier – this classifier involves perceptron-based and least-mean-squares (LMS) error-based learning during training for linear separation. This classifier finds either linearly separated hyperplane (if exists) or linear hyperplane according to LMS rule.


These two classifiers represent two different approaches: a simple linear classification and generally non-linear classification. The reason to use these two extreme cases is to find out the more convenient classifier for this task. Only results for Ho-Kashyap classifier will be presented here in Subsection 3.5.4.

3.5.2 Classifiers – setting

A few words should be written about parameters of each classifier, because they may influence the error. There are several parameters for each classifier to be set beforehand. Below, these parameters are summarized for the tested data:

1. Ho-Kashyap classifier: maximum number of iteration $N_{iter} = 1000$ should be set sufficiently to be able to achieve the convergence criterion if possible. Learning rate (fixed) $\eta=0.1$ and convergence criterion $\theta = 0.001$.

2. Support Vector Machine: $\nu$-SVM type with parameter $\nu = 0.5$ and radial basis function (RBF) kernel. Several modifications of these parameters were tested during work on this topic. Specifically, the type of kernel was changed from polynomials of degree 1, 2 and 3 to sigmoid kernel and RBF. The last kernel gave lowest classification error. The value of parameter $\nu$ was changed from 0.2 to 0.8, with only slightly change in the classification error. The final value $\nu=0.5$ was selected based on these experiments.

3.5.3 Classification strategies

Cross-validation technique is an approach to estimate how well the trained classifier performs on unseen data [6]. There are two methods, which are used very often for this purpose:

1. Repeated random sub-sampling validation (RRSCV) - during predefined number of iteration, $N_I$ samples are used for training and $N_T$ samples for testing. Training and testing subsets were randomly selected during each iteration. The advantage of this method is that the training and validation subset is not dependent on the number of iterations. The disadvantage is that some observations may never be selected for training/validation, whereas others may be selected more than once. The following parameters were used for our test: $N_I = 200$, $N_T = 100$ and $I = 100$.

2. Leave-one-out cross validation (LOOCV) [27] – is based on selection of all samples from training set, except one sample. This one sample is used for testing and remaining samples for training. In such a way, all samples are successively used for testing.

3.5.4 Results of classification

Comparing the results from mRMR approach, the best features comes from statistic-based methods (see list of features in Appendix, Table 2). These are particularly Texture Entropy (TE)
and Short Run Length Entropy (SRLE) and also Standard deviation (\(\sigma\)) and Texture Homogeneity (H). The convenient feature from other methods is mainly Relative number of level crossing pixels \(N_R\).

<table>
<thead>
<tr>
<th>Feature index</th>
<th>LOOCV</th>
<th>RRSCV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B - C</td>
<td>A - C</td>
</tr>
<tr>
<td>23</td>
<td>2.98%</td>
<td>15.82%</td>
</tr>
<tr>
<td>23, 7</td>
<td>1.74%</td>
<td>6.98%</td>
</tr>
<tr>
<td>23, 7, 3</td>
<td>1.24%</td>
<td>5.27%</td>
</tr>
<tr>
<td>23, 7, 3, 14</td>
<td>1.24%</td>
<td>5.46%</td>
</tr>
<tr>
<td>23, 7, 3, 14, 31</td>
<td>1.24%</td>
<td>5.28%</td>
</tr>
</tbody>
</table>

Table 2. Classification errors for different combination of classes, LOOCV and RRSCV strategies and for five first features selected by mRMR approach. Ho – Kashyap classifier was used.

### 3.6 CONCLUSION ON TEXTURE ANALYSIS

The plots of various features in a feature space can be shown according to selected features. Only plot for two features are presented to show the clusters for class A, B, and C. These features are two most relevant feature from mRMR scheme: Texture Homogeneity (H) and Short Run Length Entropy (SRLE). The plot for class B and C is shown in Figure 11. Two clusters are visible for class C, which represents healthy patients (without glaucoma) and B, with RNF losses in patients with glaucoma. Figure 12 shows same situation, but class A is also plotted (class representing patients with glaucoma without RNFL loss). It can be seen that the feature's values are moving according to tissue state - from healthy to glaucomatous (depicted by black arrow). But quite high overlap is visible, particularly for class A and other classes.

In spite of successfulness of classification, it must be emphasized that all presented results are based only on few images. Although these results are promising, the analysis must be extended to more images, which were not at our disposal during writing this text. However, the approach used here creates a background and knowledge base for simple extension if more data will be analyzed.
4 SUMMARY

This text summarizes several image processing areas and shows their applications to retinal image processing from two imaging modalities. It might be helpful for those, who are interested mainly in texture analysis and image registration. These two topics are discussed in more detail as well as several topics from image segmentation and pattern recognition, which provides bases for practical applications. Both parts are organized to more theoretical sections and are completed by specific applications. Although these parts are focused on different image data and different kinds of analysis, they are linked with the same aim: to diagnose glaucoma in its early stage.

The basic registration problem is described in Chapter 1: the design of the whole registration framework, selection of individual steps from pre-processing, through metric selection, image interpolation to optimization task has been described. The registration problem was described generally with focus on AF - IR retinal image registration.

Chapter 2 of this text is focused mainly on the texture analysis. Over 30 texture parameters have been presented with the aim to describe retinal nerve fibers properties in close surrounding to optic nerve head. Several parameters were designed exactly to the texture uniqueness. All parameters were tested with various methods to describe their usefulness for texture classification. It was shown that statistical based approaches offers most relevant features. Although, this second part is focused on analysis of the retinal textures, it can serve as a guide for various applications. As a part of this analysis, two classifiers were tested and compared. It was concluded that despite the popularity of SVM classifier, the simple linear classifier is sufficient for this task.

All these methods have been used to design methodology for computer aided early glaucoma diagnosis, which is an important part in glaucoma diagnosis, because it is an irreversible damage of the optic nerve head. The glaucoma illness was also discussed within this text to provide a short insight to this disease.
REFERENCES


Reproducibility of parapapillary autofluorescence measurement in glaucoma diagnostics, Proceedings of DOG 2005, pp. 482


Table 3. List of features obtained by various texture analysis methods.

<table>
<thead>
<tr>
<th>Feature index</th>
<th>Symbol</th>
<th>Name</th>
<th>Comments, method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>μ</td>
<td>Mean value</td>
<td>First-order statistic</td>
</tr>
<tr>
<td>2</td>
<td>σ</td>
<td>Standard deviation</td>
<td>First-order statistic</td>
</tr>
<tr>
<td>3</td>
<td>E</td>
<td>Entropy</td>
<td>First-order statistic</td>
</tr>
<tr>
<td>4</td>
<td>γ₁</td>
<td>Skewness</td>
<td>First-order statistic</td>
</tr>
<tr>
<td>5</td>
<td>γ₂</td>
<td>Kurtosis</td>
<td>First-order statistic</td>
</tr>
<tr>
<td>6</td>
<td>E_{co}</td>
<td>Texture entropy</td>
<td>Second-order statistic</td>
</tr>
<tr>
<td>7</td>
<td>CON</td>
<td>Texture contrast</td>
<td>Second-order statistic</td>
</tr>
<tr>
<td>8</td>
<td>H</td>
<td>Texture homogeneity</td>
<td>Second-order statistic</td>
</tr>
<tr>
<td>9</td>
<td>COR</td>
<td>Texture correlation</td>
<td>Second-order statistic</td>
</tr>
<tr>
<td>10</td>
<td>P_{max}</td>
<td>Maximum probability</td>
<td>Second-order statistic</td>
</tr>
<tr>
<td>11</td>
<td>U</td>
<td>Uniformity of energy</td>
<td>Second-order statistic</td>
</tr>
<tr>
<td>12</td>
<td>DE</td>
<td>Difference entropy</td>
<td>Second-order statistic</td>
</tr>
<tr>
<td>13</td>
<td>SA</td>
<td>Sum average</td>
<td>Second-order statistic</td>
</tr>
<tr>
<td>14</td>
<td>SE</td>
<td>Sum entropy</td>
<td>Second-order statistic</td>
</tr>
<tr>
<td>15</td>
<td>IC</td>
<td>Information measure of correlation</td>
<td>Second-order statistic</td>
</tr>
<tr>
<td>16</td>
<td>SRE</td>
<td>Short runs emphasize</td>
<td>Higher-order statistic</td>
</tr>
<tr>
<td>17</td>
<td>LRE</td>
<td>Long runs emphasize</td>
<td>Higher-order statistic</td>
</tr>
<tr>
<td>18</td>
<td>GLN</td>
<td>Grey level non-uniformity</td>
<td>Higher-order statistic</td>
</tr>
<tr>
<td>19</td>
<td>RLN</td>
<td>Run length non-uniformity</td>
<td>Higher-order statistic</td>
</tr>
<tr>
<td>20</td>
<td>RP</td>
<td>Run percentage</td>
<td>Higher-order statistic</td>
</tr>
<tr>
<td>21</td>
<td>LGRE</td>
<td>Low grey level runs emphasize</td>
<td>Higher-order statistic</td>
</tr>
<tr>
<td>22</td>
<td>HGRE</td>
<td>High grey level runs emphasize</td>
<td>Higher-order statistic</td>
</tr>
<tr>
<td>23</td>
<td>SRLE</td>
<td>Short run length entropy</td>
<td>Higher-order statistic</td>
</tr>
<tr>
<td>24</td>
<td>USRE</td>
<td>Ultra short run length entropy</td>
<td>Higher-order statistic</td>
</tr>
<tr>
<td>25</td>
<td>N_{R}</td>
<td>Relative number of level crossing pixels</td>
<td>Structural approach</td>
</tr>
<tr>
<td>26</td>
<td>N_{LS}</td>
<td>Number of broken structures</td>
<td>Structural approach</td>
</tr>
<tr>
<td>27</td>
<td>L_{μ}</td>
<td>Mean length of broken components</td>
<td>Structural approach</td>
</tr>
<tr>
<td>28</td>
<td>L_{MED}</td>
<td>Median length of broken components</td>
<td>Structural approach</td>
</tr>
<tr>
<td>29</td>
<td>β₁</td>
<td>1D Fractal spectral parameter 1</td>
<td>1D Fractal model</td>
</tr>
<tr>
<td>30</td>
<td>C₁</td>
<td>1D Fractal spectral parameter 2</td>
<td>1D Fractal model</td>
</tr>
<tr>
<td>31</td>
<td>β₂</td>
<td>2D Fractal spectral parameter 1</td>
<td>2D Fractal model</td>
</tr>
<tr>
<td>32</td>
<td>C₂</td>
<td>2D Fractal spectral parameter 2</td>
<td>2D Fractal model</td>
</tr>
<tr>
<td>33</td>
<td>P_{frequency1}</td>
<td>Spectral parameter 1</td>
<td>Discrete Fourier Transform</td>
</tr>
<tr>
<td>34</td>
<td>P_{frequency2}</td>
<td>Spectral parameter 2</td>
<td>Discrete Fourier Transform</td>
</tr>
<tr>
<td>35</td>
<td>P_{frequency3}</td>
<td>Spectral parameter 3</td>
<td>Discrete Fourier Transform</td>
</tr>
<tr>
<td>36</td>
<td>P_{frequency4}</td>
<td>Spectral parameter 4</td>
<td>Discrete Fourier Transform</td>
</tr>
<tr>
<td>37</td>
<td>P_{frequency5}</td>
<td>Spectral parameter 5</td>
<td>Discrete Fourier Transform</td>
</tr>
<tr>
<td>38</td>
<td>P_{frequency6}</td>
<td>Spectral parameter 6</td>
<td>Discrete Fourier Transform</td>
</tr>
</tbody>
</table>
ABSTRAKT

Tato práce se zabývá metodami zpracování obrazových dat, které mohou sloužit pro včasnou diagnostiku glaukomu. Obě metody využívají různých postupů z oblastí zpracování a analýz obrazů a jsou založeny na zcela odlišných zobrazovacích modalitách. Konkrétně se jedná o dvě oblasti.

První je založená na využití tzv. autofluorescenčních (AF) a infračervených obrazů (IR), které lze zaznamenávat pomocí laserového skenovacího oftalmoskopu, za využití různých vlnových délek. V případě AF snímků získáváme informaci o množství lipufuscinu, akumulovaném ve vrstvách sítnice. Tato látka je příznakem probíhajících degenerativních změn.

Analýza snímků je založena na fúzi obrazových dat. Fúzí se má v širším smyslu na mysli také využívání dalších informací z obou obrazů, například o poloze či velikosti objektů. Fúze je tedy založena na správné registraci (slicování) AF – IR obrazů. Návrhem komplexního registračního postupu se zabývá první část této práce. Uvažuje se také předzpracování obrazů. Celý registrační postup je pak testován na množině 131 obrazů a výsledky jsou vyhodnoceny na základě subjektivního hodnocení.

Aplikace výsledků registrace je pak úkázana na dvou příkladech. První je fúze jasových hodnot za účelem vytvoření barevného snímku. Druhá aplikace spočívá v segmentaci optického disku v AF snímku, kdy se využívá informace z IR snímku. Popsána je i semiautomatická segmentace oblastí ze zvýšenou autofluorescencí.

Druhá část této práce se zabývá texturní analýzou barevných snímků sítnice, které jsou pořízeny fundus kamerou. Texturní analýza je provedena za účelem detekce výpadků ve vrstvě nervových vláken, která by měla být nezávislá na iluminaci sítnice a na směru svazků nervových vláken. Za tímto účelem je aplikováno několik přístupů pro texturní analýzu, z nichž je výsledkem řada příznaků. Kvalita příznaků je testována pomocí dvou metod. První je založena na vyhodnocení chyby klasifikace a druhá metoda využívá sdruženého kritéria maximální relevance a minimální relevance. Na základě těchto metod je vybráno několik příznaků, které jsou vhodné pro klasifikaci retinální tkáně z hlediska vrstvy nervových vláken. Výsledkem této analýzy je, mimo jiné, i zjištění, že pro analýzu vrstvy nervových vláken jsou vhodné zejména metody založené na statistikách (prvního, druhého či vyššího řádu).