DATA ACQUISITION AND ANALYSIS FOR FLUORESCENCE TARGETED BIOPSY

**Summary.** Biopsy is the main criterion for cancer diagnosis, especially in early histopathological stage. The success of such examination strongly depends on a proper choice of the tissue acquisition. In this paper a semi-automated method of the ROI extraction in photodynamical images is presented.

**Keywords:** cancer diagnosis, photodynamic images

AKWIZYCYJA I ANALIZA DANYCH DLA CELÓW BIOPSJI Z WYKORZYSTANIEM OBRAZÓW FOTODYNAMICZNYCH

**Streszczenie.** Biopsja jest głównym kryterium w początkowym stadium w diagnostyce raka. Skuteczność zależy od poprawnego wyboru miejsca akwizycji tkanki. Artykuł prezentuje proces akwizycji i analizy danych, której celem jest półautoma-tyczne wyznaczenie potencjalnego obszaru biopsji.

**Słowa kluczowe:** wczesna diagnostyka raka, obrazy fotodynamiczne

1. Introduction

Laser induced fluorescence (LIFE) is a fast and non – invasive method of diagnostics of precancerous and cancerous diseases [1,2]. It is based on differences in optical properties between healthy and neoplastic tissues. Autofluorescence imaging system (Xillix Onco LIFE CCD Camera) shows groups of neoplastic tissues as shades of the red colour on the red colour scale and healthy tissues are visible as shades of green on green colour scale. The fluorescence of endogenic fluorophores is excited by the broadband blue light. Most of the falling light is reflected. The rest of the light penetrates the tissue and is dispersed. The non-absorbed light passes the tissue. The energy dose of the absorbed light is re-emitted as
fluorescence. The Xillix camera acquires two images: one of the green autofluorescence and the second one of the dispersed red light, reflected from the tissue. The image of the dispersed red light changes very little between healthy and pathological tissues while the green autofluorescence intensity decreases significantly. When a combined image is built, the colour changes in the pathological region [2, 3] (Fig. 2).

2. Methodology

In this study a semi-automated analysis of photodynamic images is presented. The input data consist of the series of autofluorescence and fluorescence images, which have been acquired from Xillix Onco Life Systems. The aim of this programme is to analyse photodynamic images, captured and saved during patient examination, and mark the ROI.

The advantages of the Onco LIFE system are of particular importance. They are real-time high resolution images in white and fluorescence modes of imaging, possibility of shifting from the white light imaging to fluorescence imaging with one button, ability of picture-in-picture viewing which enables simultaneous display of white light and fluorescence images.

At the very beginning quantification of colour value is performed. Numerical Colour Value (NCV) is defined as the ratio of red to green colour components in each pixel [8]. Histogram [5] of the NCV matrix is displayed and can be used by a clinician to adjust the threshold value which allows to extract regions of interest. In the separate image a set of points corresponding to the selected regions is superimposed on the original image.

Within the ROI the greatest value of the NCV is found and marked in the image. It shows the point of the probably highest histological stage to be confirmed by the histological procedure.

The set of images is loaded from the database located on the local disk or on a remote server [4]. All images from this set are displayed and presented to the user, who can pick one of them for a detailed analysis. Such an analysis consists of following steps:

- Image loading, and initial calculations such as: calculating the NCV for each image pixel, finding the maximum of the NCV values within the whole image. After this step the NCV value of any image pixel in obtained by pointing it with the cursor on the Original Image tab (Fig 1).

- Calculating the grayscale representation of the image, which allows to adjust the threshold used for the background removal. This is done with a commonly used formula:

\[ Y = 0,3 \cdot R + 0,59 \cdot G + 0,11 \cdot B \]  

(1)

where: R, G, B are colour components of the saturated image [9].
• Background removal, performed using a simple threshold segmentation. Pixels for which the grayscale level is greater than a specified level are removed from the image. The background has to be removed because it is characterised by a high red to green ratio. The selection of the threshold is done manually by picking the intensity from a pixel selected on the boundary of the camera view. The result of this segmentation is presented on the separate tab in the application window.

• Extracting points in which the NCV value exceeds the value defined by a clinician according to case-dependent rules. The assumed level depends on the tissue and the disease, being called the critical value. A proper selection of this value may be confirmed by the classical biopsy examination.

• When using the programme a user can observe all four stages on the tab control placed in the main window of the application. In the first tab the original photodynamic image is presented where the NCV value and the pixel position can be tracked.

![Application main window](image)

**Fig. 1. Application main window**

Rys. 1. Okno główne aplikacji

The second tab presents the grayscale representation of the image. In this picture the value of the grayscale levels of separate pixels may be read. This representation is used only for the background removal. On the basis of this image a user can adjust the threshold value. The third and fourth tabs contain the original image with its background removed and the map of excessive the NCV values, drawn on the original image respectively. On this map five colours from dark blue to red mark the increase of the NCV value by 20% of the assumed
critical value. If this threshold is set properly (according to the tissue and the disease), the colour map refers to the pathological region (Fig. 3, Fig. 4).

Fig. 2. Photodynamical image of the tongue
Rys. 2. Obraz fotodynamiczny języka

Fig. 3. Excessive NCV map
Rys. 3. Mapa obszaru o przekroczonym NCV
3. Results

An application for fluorescence (photodynamic) images analysis was prepared. It was written using .NET framework environment and C# programming language. This choice was made to ensure the portability of the application [6, 7]. It can be run without any dedicated computational or image processing application.

Over twenty clinical cases have been analysed. The results of our analysis are shown in the following table.

<table>
<thead>
<tr>
<th>Test results</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of images</td>
<td>50</td>
</tr>
<tr>
<td>True positives</td>
<td>43</td>
</tr>
<tr>
<td>True negatives</td>
<td>3</td>
</tr>
<tr>
<td>False positives</td>
<td>0</td>
</tr>
<tr>
<td>False negatives</td>
<td>4</td>
</tr>
</tbody>
</table>

These images reflect different diseases e.g. oral lesions like oral leukoplakia, oral cancer or bronchus cancer. For each case the processed image has been compared with the biopsy results. In every case examined the biopsy confirmed the presence of cancer in the selected ROI.
4. Conclusions

In our study we have shown a new approach to lesion location applied to the autofluorescence diagnostics. The analysis of red to green ratio allows changes with the highest fluorescence intensity and histological stage to be detected. The biopsy specimen always should be taken from places with the highest the NCV. Results of our study will be confirmed on a bigger group of patients.

BIBLIOGRAPHY

Omówienie

Biopsja jest głównym kryterium w początkowym stadium w diagnostyce raka. Skuteczność zależy od poprawnego wyboru miejsca akwizycji tkanki. Artykuł prezentuje proces akwizycji i analizy danych, której celem jest półautomatyczne wyznaczenie potencjalnego obszaru biopsji.

Opisywana metoda bazuje na zjawisku fluorescencji i autofluorescencji. Podczas badania, wykonywanego techniką endoskopową, z wykorzystaniem systemu Xilinx Onco Life, uzyskuje się dwa obrazy na których można zaobserwować różnice pomiędzy tkanką patologiczną a zdrową.

Opis zmian patologicznych odbywa się z wykorzystaniem współczynnika Numerical Color Value (NCV) wg równania (1). Poprawne wartości współczynnika NCV pozwalają na segmentację zmian patologicznych. Zaprojektowane oprogramowanie pozwala wykonać poszczególne kroki przetwarzania obrazów w sposób automatyczny.

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