Software for Optical Detection Module for Microfluidic Devices

Danylo Lizanets[1], Oleh Matviykiv [2], Mykhailo Lobur[3]

1. CAD Department, Lviv Polytechnic National University, UKRAINE, Lviv, S. Bandery street 12, E-mail: danylo.lizanets@gmail.com

2. CAD Department, Lviv Polytechnic National University, UKRAINE, Lviv, S. Bandery street 12, E-mail: matviykiv@gmail.com

3. CAD Department, Lviv Polytechnic National University, UKRAINE, Lviv, S. Bandery street 12, E-mail: mlobur@polynet.lviv.ua

Abstract – **in this paper software for processing an output signal from microfluidic devices with optical detection is presented. Such microdevices (lab-chips) are utilized e.g. in rapid analysis of DNA, proteins and cells and mainly utilize fluorometric detection technique. Here we present dedicated software for smart signal processing and automatic recognition of fluorescence in silicon-glass and entirely glass lab-chips for DNA amplification and separation.**

Keywords – **software-based image conditioning, image processing, microfluidics**

I. Introduction

Microfluidic devices are widely used in biological and chemical research, as they provide rapid analysis, are portable, require less reagents, samples and are sensitive. The Lab-chips for biochemical analysis often utilize ultrasensitive optical detection method of biomolecules, fluorometry. In this technique, a beam of laser light is coupled into a microfluidic channel or reactor of the microdevice and induces flashes of fluorescent dye, labelled to biomolecule. The emitted light is next recorded by optical detector, e.g. miniature CCD matrix, and video signal is transmitted to the computer. In order to obtain result plot of fluorescence, digital data of recorded images must be processed to identify fluorescence (fluorescent flashes) at the noisy background of the image. As configuration of microdevices and optical modules varies, it is still a challenge to develop a smart algorithm for automatic recognition of fluorescent light.

In this paper we present software for automatic processing of recorded video signal from optical detection module of microfluidic devices. The program provides output data as mean fluorescence and histogram of fluorescence.

II. Developed solution

All the measurements were performed utilizing microfluidic glass and silicon glass lab-chips with fluorometric detection setup, developed by a MEMSLab team from the Wrocław University of Technology. Silicon-glass chip for PCR amplification of DNA with 10 µl reaction chamber has been used for static measurements of fluorescence intensity in a function of fluorescent dye concentration. Moreover, the simulations of the behavior of the liquid in the reaction chamber have been taken [1]. The Lab-chips for biochemical analysis are also made by glass [2]. A glass chip with straight 500 µm wide and 30 µm deep microfluidic channel for electrophoretic separation of DNA has been utilized in dynamic measurement of dye flow in a function of time [3]. Detection setup consisted of: lab-chip holder, module laser (λ = 635 nm) with adjustable mount, CCD matrix detector with low-pass optical filter (λ > 650 nm) and USB frame grabber for A/D conversion of video signal. In all the tests methylene blue DNA fluorescent dye has been utilized as a sample. Construction details of used here optical detection module for CCD-based identification of DNA in microdevices has been described elsewhere [4].

Application software has been developed by CAD Department from Polytechnic National University, using C++ programming language, Qt framework for graphical user interface, and OpenCV libraries for video data processing and device capturing.

Graphical user interface (Figure 1) consists of: working area for image manipulations and display, main toolbar for file management, time and threshold settings for user settings input, data panel – shows the results of processing.

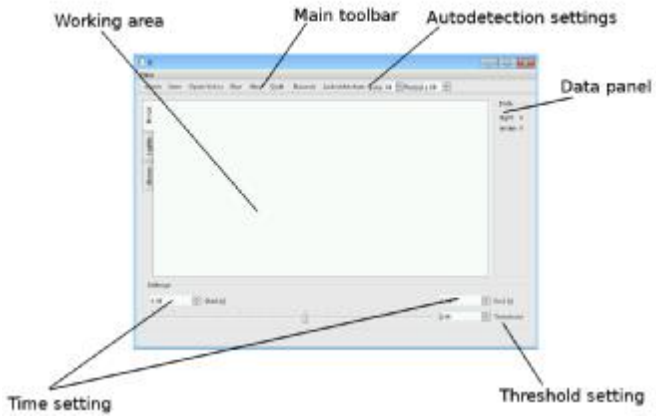

Fig. 1. Graphical user interface of the Software

Input data of the application are raster, monochrome, 8-bit per pixel images in BMP, PNG or JPG format and output is values of the measured fluorescent area and mean lightness value. The application calculates the fluorescence intensity based on processing of image files. User interface allows selection of image area using bounds on the picture to avoid information errors (e. g. light blinks on the chip edge). Bounds are stored in the form of rectangle top left and bottom right corner coordinates in the configuration file. Threshold is a lightness value of darkest pixel which has to be taken into account during the calculation process.

All pixels in a region of interest are processed in a few steps (Figure 4).

The first step is thresholding. Each pixel of matrix D, which represents an image, with coordinates i,j is thresholded

by a threshold value. It means all pixels with value greater than threshold become white (value 255) and all pixels with smaller value become black (value 0) (1).

$$D(i,j)=\begin{cases}255, D(i,j)>threshold\\ 0, otherwise\end{cases};(1)$$

The second step is contour search. During this step program searches for all the contours inside the region of interest on the image being processed using a contours search algorithm. Each contour is represented as an array of points which set its limits. The contour search algorithm is based on looking for edges of white and black areas on the image. Direction of contour points inside array (clockwise or counterclockwise) is based on rule that black and white areas must be on specific sides of contour edge. It gives an ability to separate external contours of fluorescent areas from the internal holes [5].

The third step is a calculation of fluorescence area and their mean lightness value. The fluorescence area is represented by area sum of the external contours. The mean value is calculated as a division of the sum of all pixel values inside the contours by their area.

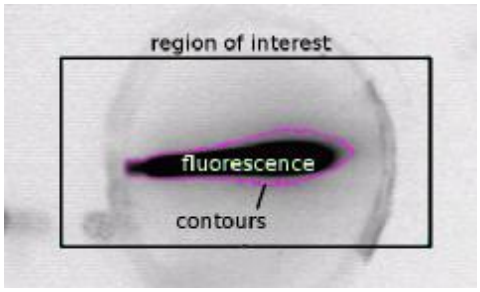

Fig. 2. Silicon-glass microchip reaction chamber (fabricated by MEMSLab) seen by CCD camera during processing (negative)

There is also calculated a histogram for all pixels inside a current region of interest (Figure 3).

The obtained results are displayed in the Data panel in numerical form.

Application can be used for processing and recording video directly from lab-on-chip setups using included image processing tools [6].

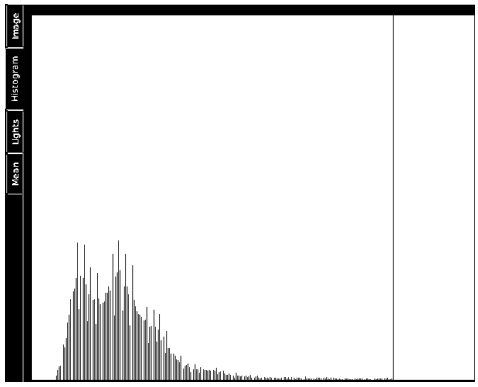

Fig. 3. Histogram for region of interest from Figure 2. x axis shows lightness values, y axis – the numbers of pixels with specific value

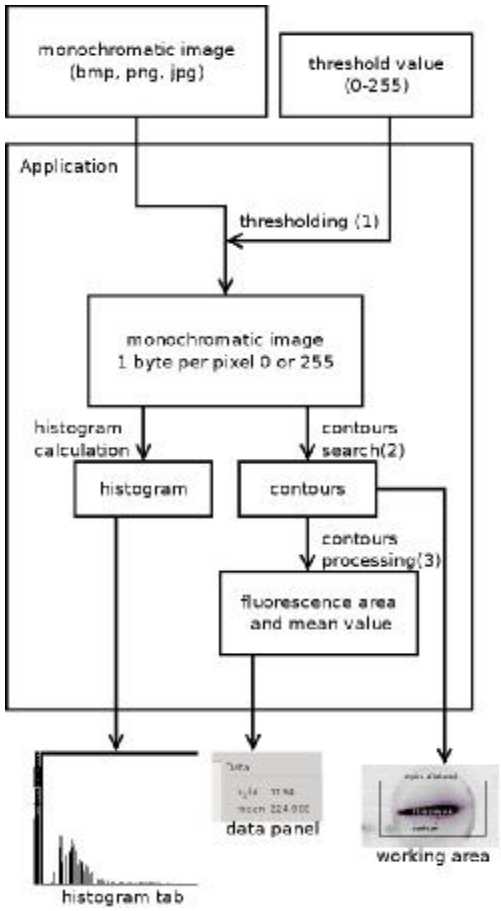

Fig. 4. Image processing algorithm

In case of video processing a video file will be cut into separate frames which are processed one by one. Resulting fluorescence area values and mean lightness values for all frames are stored in arrays and are shown in the plots (Figures 5, 6). Results of video processing can be stored in plain text format for further processing. The automatic detection mechanism is implemented in the case of processing video samples with changes of fluorescence.

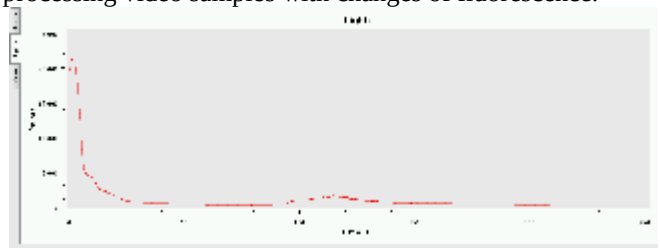

Fig. 5. Lightness values as a function of frame number

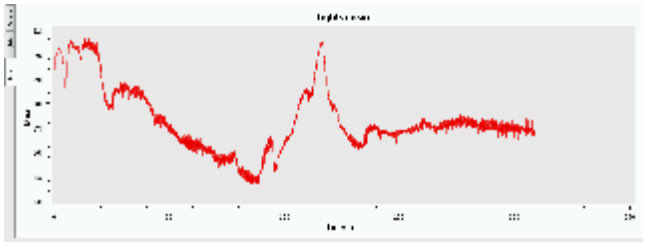

Fig. 6. Mean fluorescence values as a function of frame number

The main task for video processing is to find places on the each frame with the most or the least lightness level and calculate its fluorescence area or mean value. To find these places on an image there can be used thresholding by some maximum or minimum value then contour search algorithms and calculating the sum the found contours area. These operations take a lot of machine time and performance and it can be a problem to solve while processing the high resolution images in a long duration video file.

The approach used in software is to find quickly and mark the places on a video, where lightness level changes the most during playback and then process only this small part of each frame. It is hard to do so manually, but it can be performed automatically by software with smart algorithm.

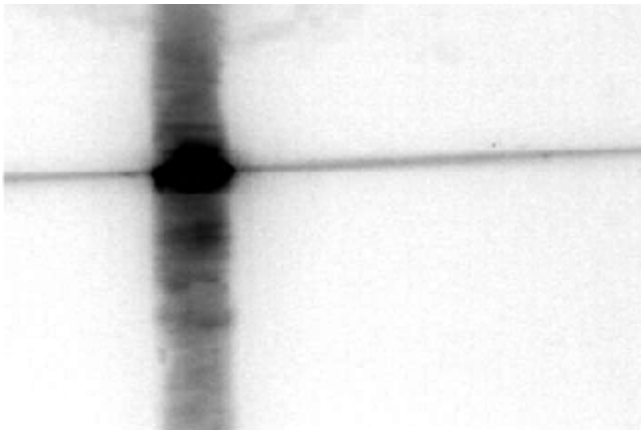

Fig. 7. Negative of sample video frame recorded during flow of fluorescent dye in glass microdevice; vertical line corresponds to gel-filled microchannel, horizontal line is the laser beam, darkest area at the crossing represents flash of fluorescence light

The algorithm is based on movement detection in a video frame. Dynamic tests of fluorescent dye flow in microchannels have been performed for the glass lab-chip. The process of microflow has been recorded by CCD detector, transmitted to PC as a movie, and next divided into separate frames. In movement detection each frame is subtracted from the previous one, pixel by pixel. Static pixels provide no change of processed signal and pixels which change their values produce positive absolute value, added to the signal. Although fluorescence spots on recorded video are not moving, they change lightness values of the pixels so it can be also detected by frame subtraction. For speed increasing there are taken not all frames but frames with regular interval. Each frame is subtracted by the previous in a sample and then result is thresholded by some value to be less sensitive to small changes of the lightness level (Figure 8).

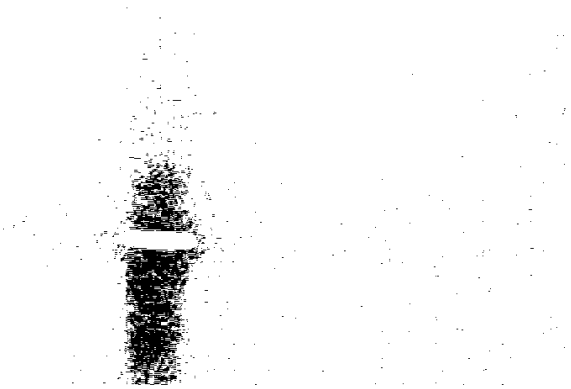

Fig. 7. Frames difference (negative)

All subtraction results are combined in one image by logic “AND” operation, as it allows gathering all needed information in one monochrome image (Figure 8).

Fig. 8. Result image (negative)

Then the result image is processed with the noise reduction algorithm to avoid small errors. At the end bounding box for all fluorescent areas are found on the image and stored as a bounds for further video processing [7] (Figure 9).

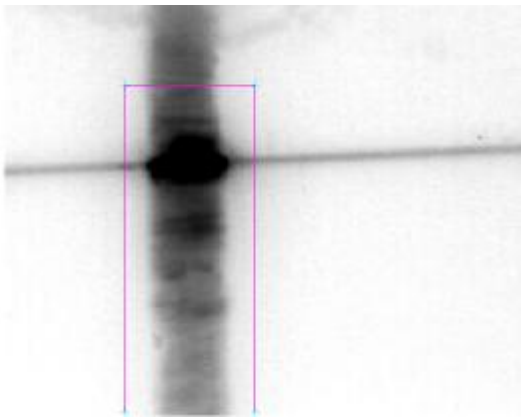

Fig. 9. Bounding box for a video processing (negative)

The application has also been utilized for detection of fluorescent signal in silicon-glass lab-chip with 10 µl reaction chamber. The chamber has been filled with water-based solution of methylene blue fluorescent dye and next illuminated by a beam of laser light. The measurement of fluorescence signal was performed for concentrations of the dye ranging from 0.01% to 0.1%. Recorded images were

recorded in the PC and processed by the application into plots of lightness spot and mean value (Fig. 10, 11). It has been confirmed, that the software provides automatic recognition of the fluorescent light in wide concentration range of the dye.

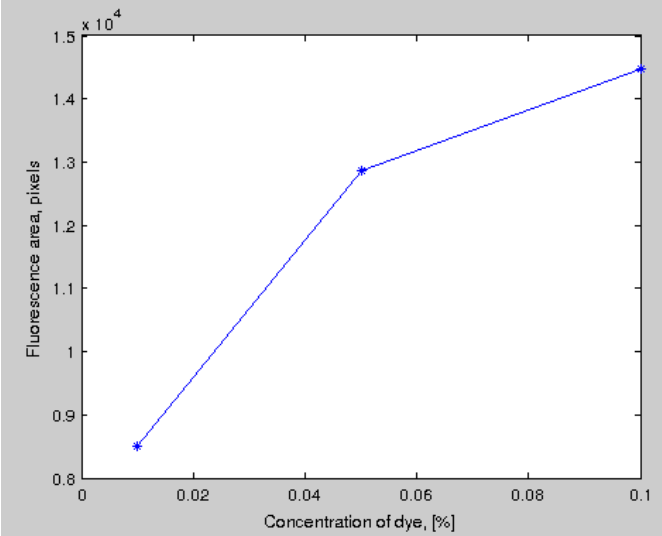

Fig. 10. Measured total fluorescence values as a function of methylene blue concentration

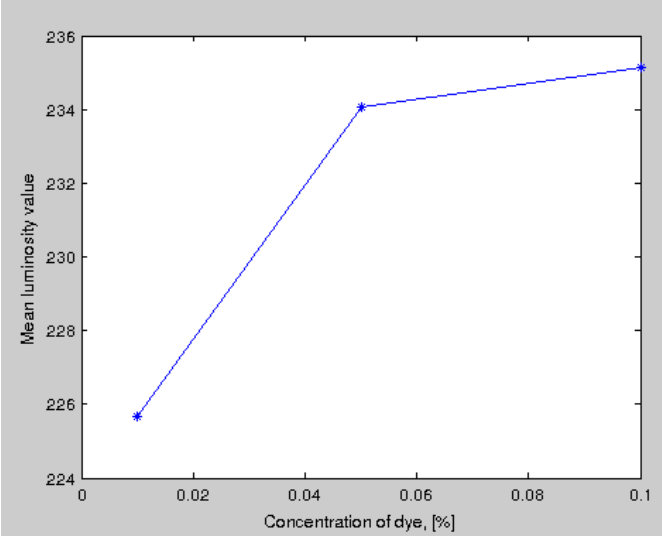

Fig. 11. Measured mean fluorescence values as a function of methylene blue concentration

III. Conclusions

Software which is presented in this article uses developed image processing and computer vision methods and algorithms to work with microfluidic systems with optical detection.

The software enables all the basic functions used in expensive, commercial, stationary devices for analytical analysis.

Experiments have confirmed the proper work of the developed software.

Acknowledgment

This research was supported by a Marie Curie International Research Staff Exchange Scheme Fellowship within the 7th European Community Framework Programme – Project EduMEMS no. 269295.

The authors would like to thank the MEMSLab team from the Wrocław University of Technology in Poland for manufacturing and delivery of glass and silicon-glass chips and fluorometric detection setup.

References

[1] *O. Matviykiv, I. Augustyniak,* Hybrid Petrov-Galiorkin Finite Element Method for Simulation Multiscale Micro&Nanofluidic Flow Effects, 12th International Conference on the Experience of Designing and Application of CAD Systems in Microelectronics (CADSM), Lviv, UKRAINE, FEB 19-23, 2013, pp. 449-450

[2] *I. Augustyniak, W. Kubicki,* Chip mikrofluidyczny do generowania nanokropel, Elektronik: konstrukcje, technologie, zastosowania, 51(6), 2010, pp. 21-23

[3] *W. Kubicki, R. Walczak, J. Dziuban* Injection, separation and fluorimetric detection of DNA in glass lab-on-a-chip for capillary gel electrophoresis, Optica Applicata, 41, 2011, pp. 409-416

[4] *W. Kubicki, R. Walczak, J. Dziuban* Miniature instrument for lab-on-a-chip capillary gel electrophoresis of DNA utilizing temperature control technique, Procedia Engineering, 25, 2011, pp. 1237-1240

[5] *Bradsky G. Kaehler A.* Learning OpenCV, O'Reilly, Sebastopol, 2008.

[6] *I. Augustyniak, W. Kubicki, J. Dziuban, D. Lizanets, O. Matviykiv, M. Lobur* Measurement Set-up for Lab-on-a-chip Fluorimetric Detection, MIXDES 2014, 19-21 June, Lublin, Poland, pp. 85-88

[7] *D. Lizanets, M. Lobur* Computer-Aided Approach to Increase the Performance of Analysis of the Optical Measurement MEMS Output, CADMD 2014, 10-11 October, Lviv, Ukraine