Measurement Set-up for Lab-on-a-chip Fluorimetric Detection

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***Abstract*—Fluorimetric detection is widely utilized in analytical applications including pathogen detection, clinical diagnosis and DNA analysis. This method combined with lab-on-a-chip (LOC) technique provides new class of miniature and ultrasensitive bioanalytical tools and therefore new solutions are being widely developed. We present new measurement set-up for lab-on-a-chip fluorimetric detection which consists of silicon-glass LOC microreactor, detection unit with CCD-sensor, semiconductor laser module and dedicated software. Unique image-processing algorithm provides real-time fluorescence signal analysis and databasing.**

***Keywords*—software-based image conditioning, Laser Induced Fluorescence detection, lab-on-a-chip**

I. Introduction

Intensive progress in area of microsystems for (bio)chemical analysis (lab-on-chips) enable development of low-cost and ultra-sensitive miniature analytical devices. One of the most popular detection methods in these devices is fluorimetric measurement with Laser Induced Fluorescence (LIF) [1]. LIF is one of the most sensitive detection methods utilized for qualitative and quantitative analysis of nucleic acids and proteins in gel electrophoresis, microarrays, and fluorescence spectroscopy [2, 3]. Fluorescence occurs when a fluorescence–sensitive molecule (fluorophore) absorbs light photons of wavelength λ_A, and rapidly emits light photons of wavelength λ_E (usually $\lambda_A < \lambda_E$).

The miniaturized, portable measurement set-up for LOC fluorimetric detection which allows students to familiarize with lab-on-a-chip technique, optical LIF detection, electronic and software equipment has been developed. The measurement set-up provides measurement of the fluorescence signal of fluorophore dye which can be used in clinical diagnosis.

II. Laboratory Lab-on-a-chip Set-up

Measurement set-up for lab-on-chip fluorimetric detection was constructed by Division of Microengineering and Photovoltaics (Wrocław University of Technology, Poland). The set-up utilizes silicon-glass analytical lab-chips and simple but sensitive detection unit with CCD-sensor and semiconductor module laser for LIF. The measurement set-up (Figure 1) consists of:

- silicon-glass microfluidic chip,
- chip-holder,
- semiconductor laser – wavelength 635 nm,
- CCD camera,
- high-pass optical filter > 650 nm,
- zoom lens,
- PC with dedicated software,
- power supply
- samples of fluorescence dye – methylene blue.

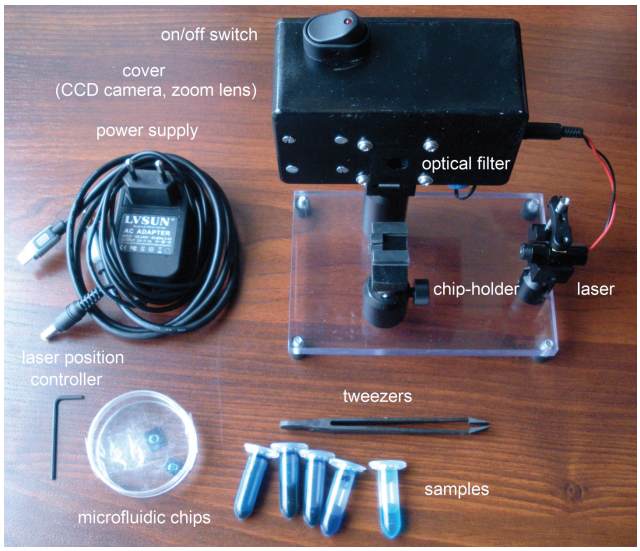

Fig. 1. The measurement set-up

The portable set-up for lab-on-a-chip fluorimetric measurement allows detection of an optical signal from LOC, processing of the optical signal for useful electric signal and its transmission to the computer. Dedicated software provides conversion of the signal and visualization of processed data. Block scheme of the measurement set-up is shown in Figure 2. Laser light beam is coupled into the microreactor chamber of the chip, containing sample with fluorophore dye. The fluorophore is excited by the laser light λ_A and emits fluorescence light λ_E. The CCD camera with zoom lens is recording the image of microreactor. The optical filter cuts off the laser light signal and transmits fluorescence light, so only fluorescence signal of the dye is detected by the camera. The

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analog video signal is converted to digital video signal by video grabber and sent to PC by USB interface. Recorded image is presented and processed by dedicated software.

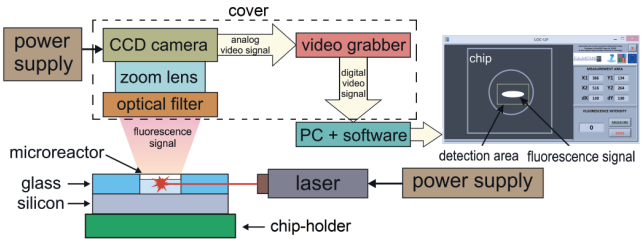

Fig. 2. Scheme of the measurement set-up with dedicated software

III. LAB-ON-A-CHIP CONSTRUCTION

Three silicon-glass chips with various microchamber volumes have been developed. Outer dimension of chips are 10 × 10 × 1.5 mm^3. Each LOC consists of two layers: a monocrystalline silicon body and borosilicate glass microchamber. The layers are permanently sealed utilizing anodic bonding technique. Fabrication procedure (Figure 3) includes masking of a glass layer, double-side deep isotropic wet etching in hydrogen fluoride (HF) and mechanical smoothing the side walls of the microreactor chamber. Following this, anodic bonding of glass layer and silicon body is performed in air at 450°C, 1000 V.

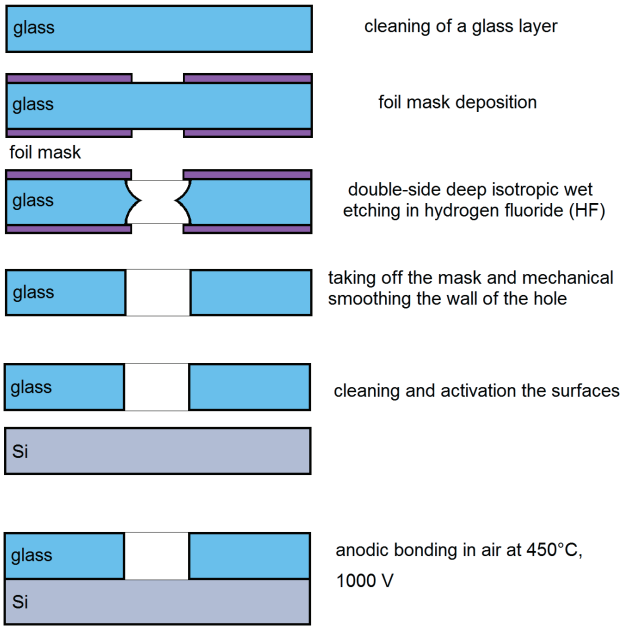

Fig. 3. Fabrication process of the chip

Microchamber volumes of fabricated chips are: 10, 15 and 20 µl, respectively (Figure 4). Due to high chemical resistivity of silicon-glass construction, LOCs may be cleaned with organic solvents, as well as acids and bases, and are fully reusable.

The glass layer is not only the body of the microreactor, but also optical waveguide for laser light beam, which is coupled perpendicular to optical detector. Such a solution has been developed by Division and Photovoltaics group and successfully applied in many LOC instruments with fluorescence detection [4, 5].

Fig. 4. Silicon-glass lab-on-a-chips at a glance; volume of the microractors (from left to right): 10, 15 and 20 µl

IV. OPTICAL DETECTION SYSTEM

Ultrasensitive UV/VIS detectors with active cooling, are commonly used in fluorimetric detection and analysis, but are rarely applied in LOC-based solutions due to high volume and mass, sophisticated operation and high cost. We presented simple but sensitive LIF detection system (Figure 5), consisting of diode laser module (635 nm), high-pass optical filter (Thorlabs), 16 mm zoom lens and non-cooled CCD camera (Sony) [6]. Selection of suitable dye and a corresponding laser wavelength determines the type of analysis.

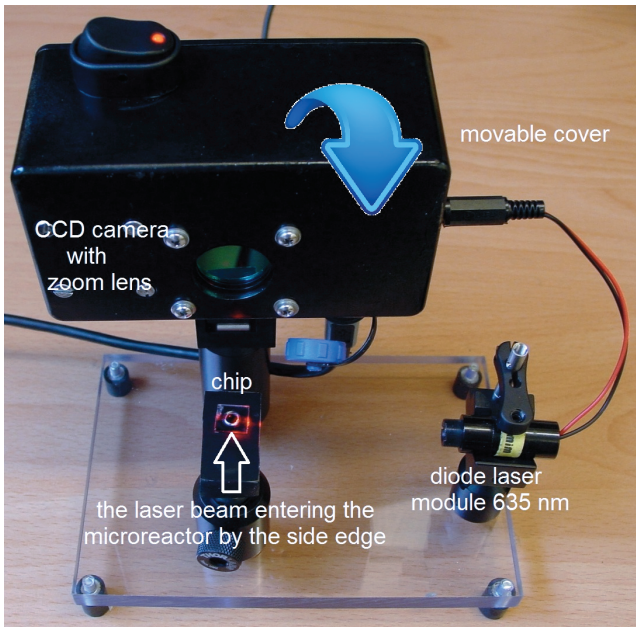

Fig. 5. LIF optical detection system

V. SOFTWARE

The Software is developed in C++ programming language using Qt framework for graphical user interface and OpenCV libraries for video data processing. Input data of the Software are bitmap images and/or AVI videos taken from the lab-on-a-chip set-up by CCD camera and output are series of measured fluorescence value. The software calculates the fluorescence intensity based on processing of image or video file.

User interface allows selection of image area using bounds on the picture (Figure 6) to avoid information errors (e. g. light blinks on the chip edge). Bounds are stored in form of rectangle top left and bottom right corner coordinates in configuration file. Threshold is a lightness value of most dark pixel which has to be taken into account during calculation process.

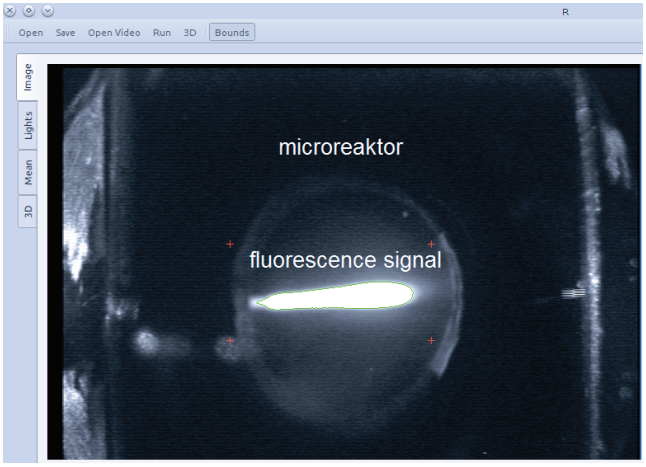

Fig. 6. Microchip chamber seen by CCD camera: selecting the area of fluorescence in software by green outline by user

All pixels in bounded area are processed row by row in loop. If the intensity of the pixel exceeds user-selected threshold value then counter is increased by 1 and the sum value is increased by lightness value of the pixel. In case that intensity of the pixel is below the threshold value and adjoins at least one pixel with intensity above the threshold then it is marked to show user the edge of light spot on image. The result is displayed as a graph frame in function of light points (Figure 7).

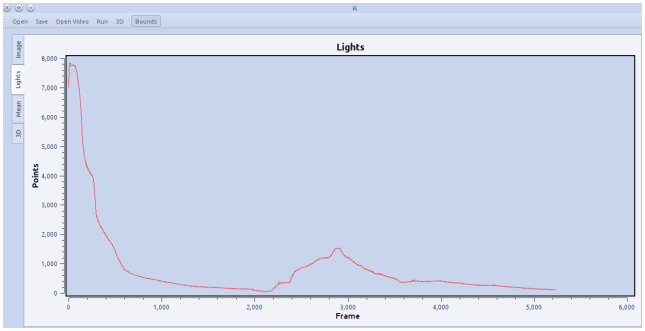

Fig. 7. Processed fluorescence signal: graph frame in function of light points – from 0 to 1000 signal from laser source, from 2000 to 4000 fluorscence signal

Sum of the intensity of all pixel with lightness value more than threshold value, which were accumulated while light points counting, are divided by counter. Result value is a mean value of lightness in the light spot (Figure 8).

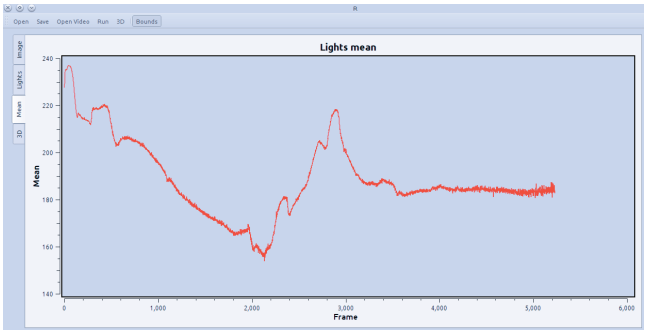

Fig. 8. Graph frame in function of mean value of lightness

In 3D view bounded area is represented as surface where height represents lightness of the pixel. Part which is red represents pixel that are lighter than threshold (Figure 9).

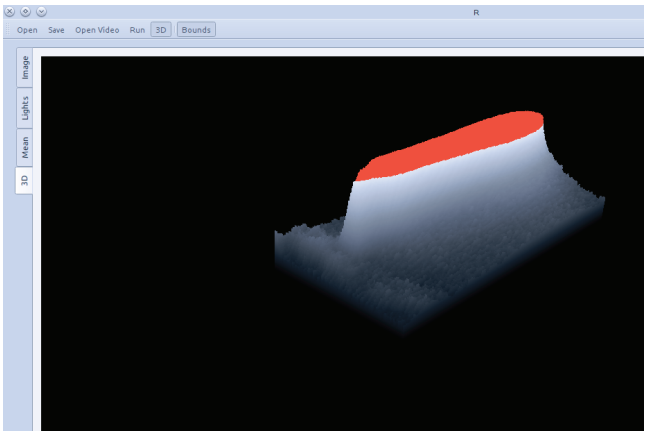

Fig. 9. 3D view of microreactor area – fluorescence area is marked in red

In case of video processing the file is cut into separate frames that are processed one by one. Resulting light count and mean lightness value for all frames are stored in arrays and are shown in plots. Results of video processing can be stored in plain text file for further processing (e. g. in MATLAB).

VI. CONCLUSION

Laboratory set-up for lab-on-a-chip fluorimetric measurement with software-based image conditioning has been designed and developed. The device is used by students of Lviv Polytechnic National University to familiarize with construction and parameters of modern microsystems, electronic modules used for processing the signals, as well as detection algorithm used in the dedicated software.

The software enables all the basic functions used in expensive, commercial, stationary devices for analytical analysis. The inexpensive, portable device for many types of analytical analysis is ideal as laboratory set-up for students of electronic and informatics technology as well as biotechnology.

ACKNOWLEDGMENT

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