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Lab-on-a-chip fluorescence detection with image sensor and software-based image conditioning

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Abstract. The fluorometric detection in lab-on-a-chip devices for life-sciences is one of the main detection methods. Well know from "large-scale" analytical chemistry methodologies and instrumentation has been applied in lab-on-a-chip solutions. In most cases, optical instrumentation for fluorescence induction and detection is based on configuration and components borrowed from large laboratory instruments. As a result, optical instrumentation surrounding lab-on-a-chip is bulky, expensive and dedicated for operation only inside laboratory. In this paper a discussion on fluorescence detection in lab-on-a-chip is carried out. A novel image sensor – based detection instrumentation co-working with a "clever" software is described. Instrumentation is dedicated for operation in portable and low-cost devices for different life-science applications. Examples of applications of the novel method and instrumentation co-working with various lab-on-a-chips are presented on the base of author's works.

Key words: fluorescence, microfluidics, lab-on-a-chip, point-of-care.

1. Introduction

Lab-on-a-chip (LOC) integrates on single substrate (chip) one or more laboratory steps well known in an analytical chemistry, like sample handling, analysis and detection, but carried out on a few picoliters to hundreds of nanolioters range of sample volume. Compared with macroscale devices for analytical chemistry, LOC systems engender significant advantages in terms of speed, throughput, yield, selectivity and control. In spite of many advantages of LOC, some issues will arise while analytical systems are miniaturized. One of them is microscale detection in LOC. Effective detection within LOC is clearly defined by a close interrelationship of factors such as volume of detected sample, detector sensitivity, response times, detection limits and information content. Application of LOC leads to decrease of analyte available for detection. Reduction of analyzed volume means reduction in detection volume and making it more difficult to detect [1]. For example, with 10 μL volume of detection volume it is possible to reach concentration detection limit in order of 10^{-10} mol/L, whereas for 10 pL of detection volume, detection limit decreases to 10^{-4} mol/L [1]. Therefore, only high sensitivity and expensive sensing instrumentation have to be applied to ensure proper detection of various signals from nano- and picoliter volumes of analyte. What more, sensing problems arise while LOC is a part of point-of-care (POC) device, where portability, sensitivity, power requirements and price are major challenging factors.

One of the most commonly applied detection method in both macroscale analytical chemistry and LOC is based on fluorescence readout. LOC co-working with fluorescence detection systems are widely applied at laboratory level in many fields of life-sciences – for example in separation and detection of biological material by electrophoresis [2] or analyze of genetic material by polymerase chain reaction (PCR) with real-time analyze of amplification kinetics (real-time PCR) [3]. However, it is obvious that "conventional" laboratory fluorescence detection systems are too bulky and expensive to be applied in POC devices. Therefore, new methodologies and technical solutions must be used to fulfill requirements of LOC POC devices.

2. Detection of fluorescence in LOC

Two main issues have to be taken into account while fluorescence detection in LOC is considered: (1) construction and material of LOC and (2) fluorescence induction and detection methodology and instrumentation.

Construction of LOC is very often forced by the material of LOC. From point of view of LOC material, two main requirements have to be fulfilled: (1) optical transparency necessary for introduction of the fluorescence excitation light and collection of the fluorescence light from an area of interest within the chip, and (2) low autofluorescence of LOC's material to minimize optical background signal. The chips can be whole made as multilayer construction of light-transparent materials – like glass, PDMS, SU-8, COC or other polymers, or only a top cover of the chip is made of these materials whereas body of the chip is made of silicon or ceramic [4–11]. It is also possible to make LOC from non-transparent materials like low-temperature co-fired ceramics (LTCC) but an integration of optical waveguides enabling introduction of the fluorescence inducing light into a detection chamber and covered with glass or PDMS is necessary [12]. Technology of LOC involves many microengineering and microelectronic techniques enabling selective etching, deposition and bond-

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ing of few layers [13, 14]. While choosing LOC material, the autofluorescence effect of polymers have to be considered. The lowest autofluorescence signal is observed for borosilicate glass, PDMS or COC [15], the highest for PMMA or SU-8 [15, 16]. It is also confirmed that longer excitation wavelength causes lower autofluorescence signal. Therefore, in visible light region fluorescence detection, "red" light sources for fluorescence induction are preferred.

Commonly used compact apparatus for fluorescence induction and detection in LOC is an epifluorescence microscope or devices with configuration of the optical paths similar to applied in this microscope (Fig. 1).

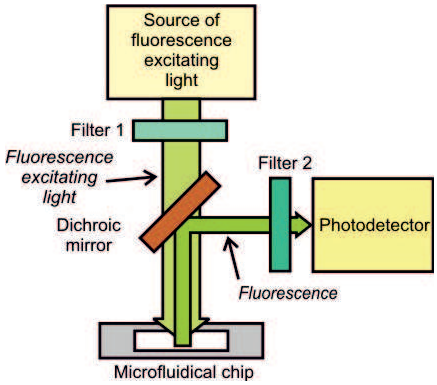

Fig. 1. Scheme of the typical optical path for fluorescence excitation and detection co-working with lab-on-a-chip

In these devices, two optical channels are distinguished: the fluorescence induction channel and the fluorescence detection channel. Fluorescence induction light, usually in a visible light range, comes from halogen, tungsten or xenon lamp, rather light emitting diode (LED) or a laser. The light is restricted to a narrow range of wavelengths that can effectively excitate the fluorochrome and be strongly excluded by the detection channel. The narrow wavelength range is ensured by one or more interference filter(s) and a dichroic mirror(s). In the detection channel, fluorescence light emitted by the fluorochrome is collected by the microscope objective with proper magnification, and guided to a photodetector by passing through filter(s) and dichroic mirror(s) to exclude the excitation light. Common detectors are cooled photomultiplier tubes (PMT) [5, 6, 11], semiconductor photodiodes [7, 9, 10], rather cooled charge coupled devices (CCD) as sensing matrix in video cameras [4] or lines in spectrophotometers [8].

In spite of sensitivity of the photodetector itself, an important issue in highly sensitive detection of fluorescence in LOC is conditioning of an electrical signal generated by the photodetector. A role of the conditioning electronics is to amplify electrical signal with simultaneous reduction of background noises to ensure high signal-to-noise ratio (SNR). Most of the conditioning electronics is realized by the use of analog circuits. The configuration of these circuits is sophisticated and to ensure high SNR only the highest quality elements can be used. Analog conditioning electronics prepares an electrical signal to be next digitalized and sent to a personal computer (Fig. 2).

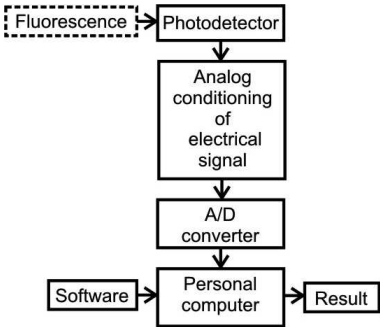

Fig. 2. Scheme of the typical path of analog fluorescence signal conditioning

A computer receives ready to display data, software does not perform advanced conditioning operations on delivered data. In most cases, a software play role as a user interface for setting parameters of the analysis and "visualization" of the fluorescence signal collected by an optical detection unit.

Although, the LOC fluorescence detection has been widely used for many years, the detection apparatus is based on solutions developed over the last 30 years. In most cases, these solutions are technically and dimensionally incompatible with LOC. It causes that "live" of LOC ends on a laboratory stage because detection instrumentation co-working with these chips is bulky, expensive, often built on laboratory scale and able to operate only in laboratory conditions. Therefore, rapid development of LOC must be followed by development of novel methodologies and technical solutions surrounding the chips and leading toward a successful application of LOC in the point-of-care devices.

3. Novel image sensor – based fluorescence detection

In the novel concept of optical instrumentation for fluorescence induction and detection, application of recent developments in microengineering techniques, optoelectronics and computer sciences are involved.

Recent progress in development of low-cost optoelectronic components and devices observed in the past two decades enables mass-scale fabrication of cheap and miniaturized semiconductor lasers as well as miniature image sensors. A laser can be used as the fluorescence excitation light source, whereas the image sensor can be a part of image–based fluorescence detector.

Currently, there is a lot of miniature semiconductor lasers working at visible light spectrum (for example around 408 nm, 532 nm and 635 nm) with optical power varying from 1 mW to hundreds of mW. These wavelength and power regions are enough to excite effectively fluorescence of many fluorochromes applied in life-sciences. Narrow-spectrum of the laser light eliminates application of emission filters. Low power consumption enables a long-term battery operation or power supplying by a computer USB port. It is also possible to apply light emitting diode (LED) or organic LED (OLED) but due its optical properties it requires application of a narrow-band filter and light beam forming optics. In the novel con-

cept, a collimated laser light (preferred) or LED light (as alternative) is introduced – by edge coupling to a light guiding side wall of the chip – directly into the detection area or microchamber of LOC (Fig. 3a).

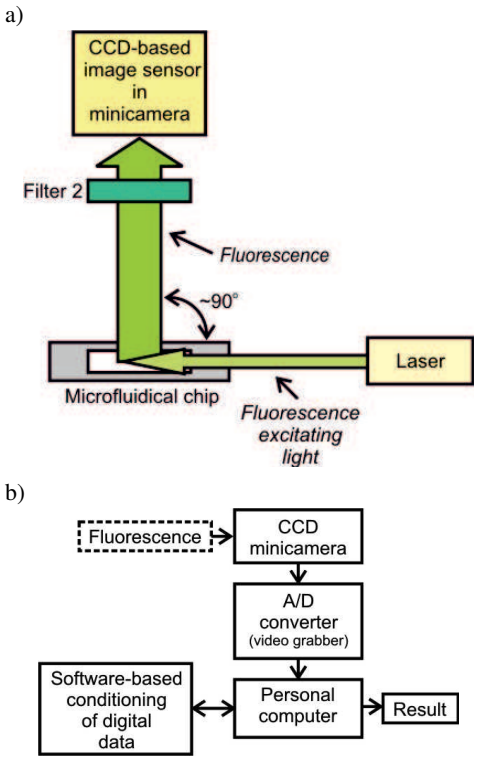

Fig. 3. The novel fluorescence excitation and detection method utilizing miniature optical components co-working with LOC: a) scheme of the method of laser light introduction and fluorescence collection, b) schematic presentation of novel, digital path of fluorescence signal conditioning

Laser light does not illuminate whole area of LOC but it does selectively only area of interest. Positive factor of proposed configuration is that low power lasers can be used – power density of laser in irradiated volume is high enough to induce fluorescence effectively; negative – fluorescence is induced only in a small sample volume determined by light distribution within fluid what decreases detection volume down to picoliters range and, as a consequence, high sensitivity detector have to be used.

The described here fluorescence detection instrumentation is based on two components: (1) image sensor – based unit and (2) specialized software for image data analysis. The chip is "observed" by an analog minicamera equipped with low-cost non-cooled CCD image sensor and a miniature objective with a long-pass interference filter. The minicamera is positioned perpendicularly in relation to the surface of the chip and laser beam (Fig. 3a). This configuration ensures geometrical separation of the laser excitation light and fluorescence signal without application of dichroic mirror as it is in "conventional" apparatus. It also significantly simplifies configuration and decreases number of components necessary to built induction and detection optical channels.

A non-conditioned "raw" analog output signal from the minicamera is digitalized by the one-channel low-cost video frame grabber connected to a personal computer. A computer stores images in a memory and analyzes them in real-time. Specialized software carries out analysis of the captured video images to give to an operator numerical values of fluorescence intensity (Fig. 4).

The images are in 8-bit greyscale which corresponds to 256 levels of fluorescence intensity. An important feature of a developed method is that images are analyzed in selected areas where the fluorescence image is present. Rest of the image which may contain artificial optical signals is not taken into account. Thus, digital conditioning of the fluorescence signal by the software-base image analysis, instead of analogue conditioning, is proposed here (Fig. 3b). Another important issue is storing of images in a computer memory (hard disk or other memory carrier) which enables re-analysis of the fluorescence images when it is necessary. This feature is very useful while "hot-point" fluorescence areas may occur in different places of the "observed" microchamber of LOC. This is unique feature of the novel instrumentation which is not available in typical devices with "non-imagining" photodetectors (PMT or photodiode) when an operator has only "one shoot" analyis.

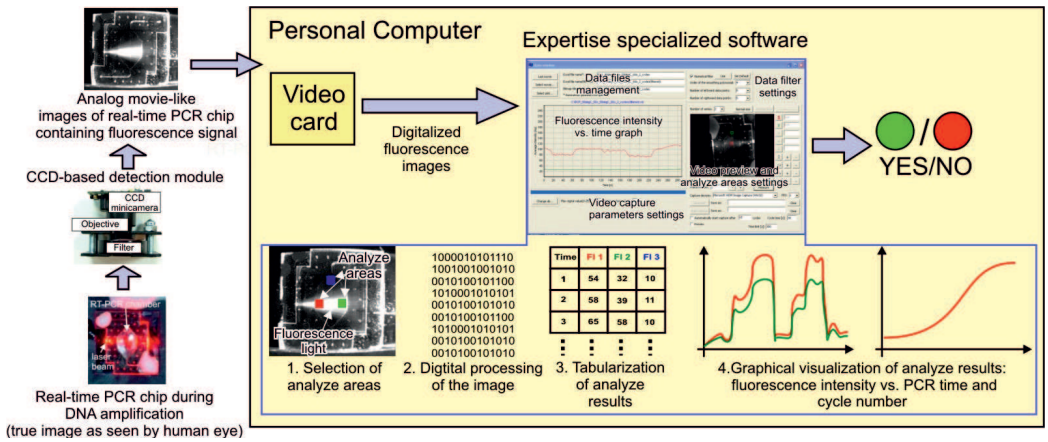

Fig. 4. Scheme of image processing towards fluorescence signal analyze

The comparison of main technical features of fluorescence induction and detection by "conventional" apparatus and presented here novel instrumentation are shown in Table 1.

Table 1

Feature	Instrumentation based on epifluorescence microscope	Novel instrumentation
Optical channels configuration	Dual channels with optical and geometrical separation by so called "filter/mirror cube"	Dual channels with optical and geometrical orthogonal separation
Components of fluorescence induction channel	1) High power and bulky lamp or laser, rather LED 2) Set of filter, dichroic mirror and light beam forming lenses	1) Semiconductor miniaturized low-power laser with collimating lens
Components of fluorescence induction channel	1) Cooled PMT, silicon photodiode, rather cooled CCD matrix or line 2) Set of filter, dichroic mirror and lenses	1) Miniature CCD image sensor-based minicamera 2) Miniobjective with filter
Signal conditioning	Analog, no software conditioning	Digital, advanced software-based analyze
Power requirements	High power light sources and cooling facilities, tenths to hundreds of Watts necessary for power supplying	Power consumption below 2.5 W all components can be supplied by USB port
Size, portability	Desktop size works as non-portable instruments	Hand-held size, dedicated for portable instruments

4. Examples of applications of novel fluorescence readout instrumentation

A novel fluorescence detection instrumentation co-working with various LOC has been successfully applied in some life-science applications, recently. Some examples are presented here: starting with portable real-time PCR DNA analyzer, than on-chip DNA gel electrophoresis setup, miniature microcytometer for optical characterization of biosamples, LOC for apoptosis detection in mouse embryos, finishing with a portable reader for cocaine detection in human's sweat.

4.1. DNA analyzer by real-time PCR – detection of food pathogens. Described here optical instrumentation became a part of device for food pathogens detection developed under European 6. Framework Programme OPTOLABCARD [17]. The goal of the project was to develop LOC-based compact instrumentation enabling detection of *Salmonella spp*. in human samples and *Campylobacter j.* in broiler chicken farms, by utilizing the real-time PCR technique [18, 19].

The disposable LOC (1×1 cm^2), made of negative photoresist SU-8 and glass, has integrated a heater and a temperature sensor [2]. LOC is placed in a plastic chip holder ($2.8 \times 2.8 \times 0.5$ cm^3) with integrated electrical contacts to the chip and some electronic circuits for temperature management of 2.5 μl in volume PCR microchamber of LOC. The chip holder has a miniature electrical connection to the specialized PCR temperature controller connected to computer. The holder with ready to use LOC is positioned in a docking station ($15 \times 5 \times 7$ cm^3) in the way ensuring the direct laser light introduction into PCR microchamber and fluorescence light collection [20]. The laser light (636 nm) does not illuminate whole PCR microchamber but it does only part of the chamber which corresponds to the laser light cone distribution. Therefore, fluorescence is induced and emitted from volume in the pL range. A view of the chip and hand-held docking station with a positioned chip holder just before start of PCR process is shown in Fig. 5.

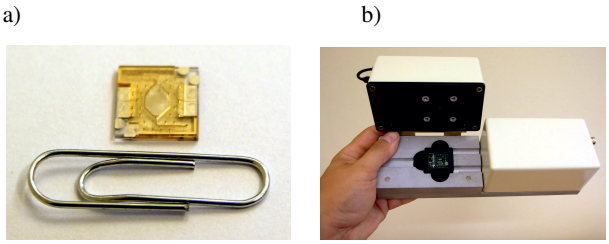

Fig. 5. Portable real-time PCR DNA analyzer: a) SU-8/glass LOC in comparison to a paper clip, b) view of docking station ready to work at Food Laboratory of Danish Technical University

The pre-validation tests of LOC – based system for detection of *Campylobacter j.* were carried out with 48 chicken fecal samples [19]. All the steps – from sample preparation to final result – were performed in the single chip with 2.5 μl volume of reagents. The ratio of PCR efficiencies between on-chip and on-tube was up to 300%. The sensitivity of on-chip PCR was determined as 0.7–7 ng/ml of template DNA. It is on the same level as on-tube PCR. The real-time PCR process took 30 min – at least 4 times shorter than PCR on-tube.

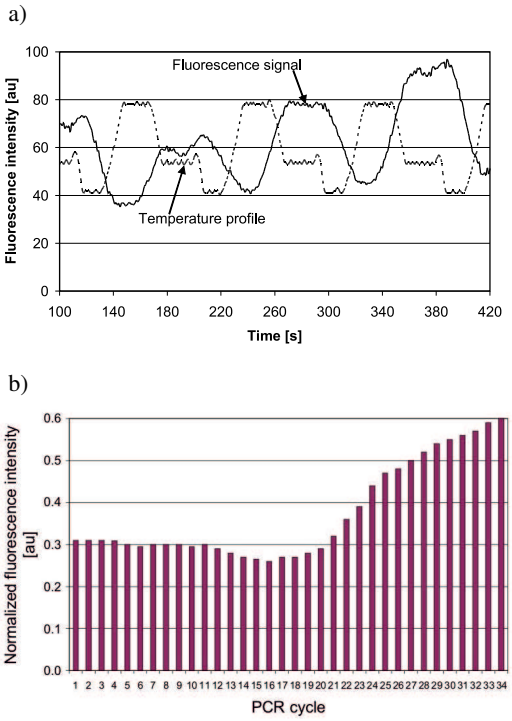

Fig. 6. Real-time PCR of *Campylobacter j.* DNA carried out in device developed under OPTOLABCRD project: a) fluorescence intensity change following PCR temperature profiling , b) S-curve – like graph describing kinetics of DNA amplification

An example of the fluorescence intensity change of TO-PRO 3 fluorochrome (BioScience, USA) and DNA complex following temperature profiling is shown in Fig. 6a. S-curve graph of real-time PCR process, describing DNA amplification kinetics, has been compiled on the base of average fluorescence intensity during extension step of each PCR cycle (Fig. 6b). The obtained S-curve is similar to the characteristic achieved by the use of the chip observed under epifluorescence microscope equipped with PMT as the reference.

Carried out pre-validation tests confirmed usefulness of the developed optical instrumentation, as well as the whole LOC-based system, for a real-time PCR detection of *Campylobacter j*. Further tests of similar LOC-based solutions carried out by under Polish projects gave us real-time PCR positive results with detection of *E. Coli* or complementary DNA of genetic material from woman breast cancer [21].

4.2. DNA analyze by on-chip gel electrophoresis separation. In spite of the real-time specific detection of DNA amplified during real-time PCR, the post-PCR DNA detection and identification by electrophoresis are also commonly used. A miniature system for gel electrophoresis of DNA with a fluorometric detection consists of LOC placed in a plastic chip holder, a fluorescence detection unit and high voltage electrophoresis driving a power supplier [22]. A chip holder enables introduction of fluids into the chip and electrical connections of fluid reservoirs to the high voltage power supplier. The construction of the holder ensures proper introduction of the red (636 nm) laser light into a detection area of the microchannel and the collection of the fluorescence light by the CCD minicamera-based detection unit (Fig. 7a).

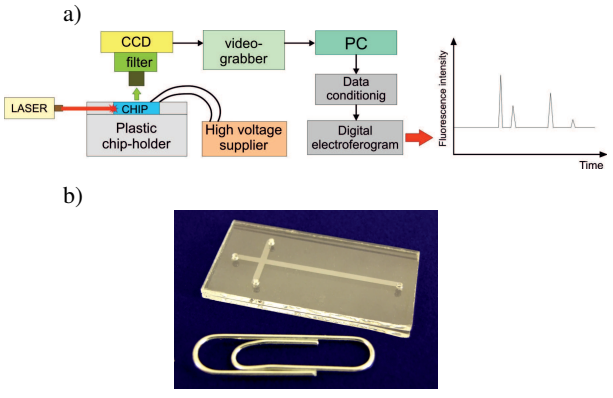

Fig. 7. DNA gel electrophoresis with on-chip real-time fluorometric detection: a) scheme of the measurement set-up, b) view of the all-glass LOC for gel electrophoresis in comparison to a paper clip

The microfluidical chip is as a whole made of borosilicate glass (Fig. 7b). One of the glass substrates is micromachined by wet etching to obtain T-shape cross-section of 560 μm wide and 30 μm deep microchannels. The second substrate has drilled holes for gel and fluids introduction. Both substrates together with glass reservoirs are bonded in one process by the use of high temperature direct bonding. After fabrication, the microchannels are filled with 10% polyakryloamid gel according to procedure proposed by von Heeren'a [23].

Gel electrophoresis (90 V/cm, 10–12 min) of 100 bp and 200 bp double-strain (ds) DNA labeled with Cy5 fluorochrome (USA) was carried out as a model separation. Fluorescence signal collected from 50 pL volume of irradiated flowing through sample was recorded and stored on hard disk. Data were in real-time processed into time-graphs of fluorescence intensity by the use of developed software. Proper separation of dsDNA and detection of fluorescence signal has been observed (Fig. 8). Separation time of injected sample was below 4 minutes in comparison to several minutes in standard gel electrophoresis system.

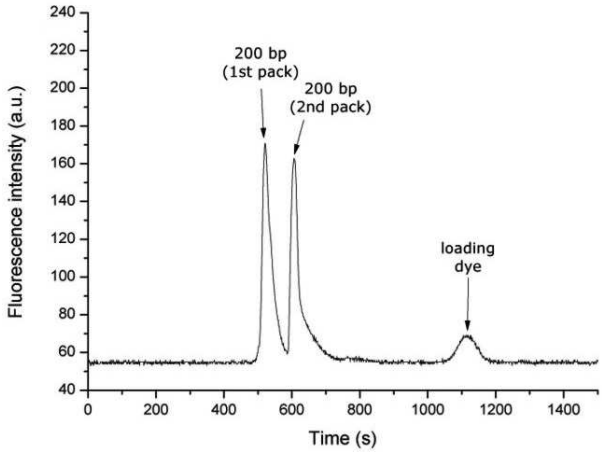

Fig. 8. Electroferogram of dsDNA separation in gel electrophoresis microfluidical chip co-working with CCD-based fluorescence detection unit, first large peak corresponds to 100 bp DNA, the second to 200 bp

It is expected, that developed LOC with fluorescence readout can be used for screening of food pathogens by a multiplex DNA amplification in PCR and on-chip separation [20], as well as investigations on single nucleotide polymorphism towards detection of DNA mutations by rapid tests.

4.3. Fluorometric characterization of biosamples in LOC. Among many sensing methods, optical methods – spectrophotometric and fluorometric – are main tools in characterization of biosamples. However, there are no reported solutions enabling simultaneous and independent on-chip measurements of light absorbance and fluorescence of biosample – liquid or cell. The proposed by us microcytometric-like instrument consists of multi-fiber LOC with flowing through measurement cell co-working with external miniaturized instrumentation for optical signals readout. The chip is a silicon structure with etched by deep reactive ion etching (DRIE) microchannels, anodically bonded top glass cover and four multimode glass fibers (125/100 μm) dedicated for VIS/NIR spectroscopy (Ocean Optics, USA). The fibers are arranged around a widened part of the fluidic microchannel, forming an optical cell with volume of 40 nL (Fig. 9a). Two fibers are perpendicular to the fluidic microchannel, while two other are at the angle of 40°. Thus, two path lengths (520 μm and 600 μm) for absorbance measurements are ensured (spectrophotomet-

ric measurements are described in [24]). During the fluorescence measurements, the excitation light – emitted by LED or laser – os coupled into a chosen fiber and transmitted to the measurement cell of LOC. Fluorescence light emitted by the fluorochrome labeled sample is collected by described here fluorescence detection system based on CCD image sensor co-working with miniobjective with $\times 20$ magnifications and interference filters: long-pass 500 nm for fluorescein or 650 nm for TO-PRO 3 fluorochromes (Fig. 9b). Collected images are conditioned in the same way as in real-time PCR application described earlier.

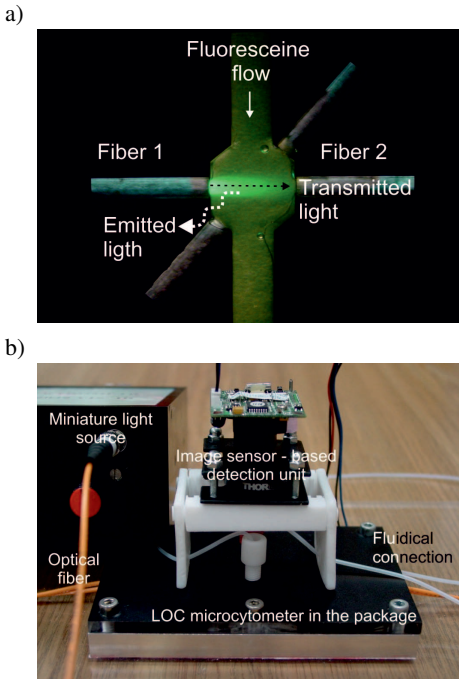

Fig. 9. Multi-fiber microcytometer: a) image of the optical cell with 4 integrated fibers, b) view of the test setup with CCD minicamera as fluorescence detector on the top of packaged microcytometer

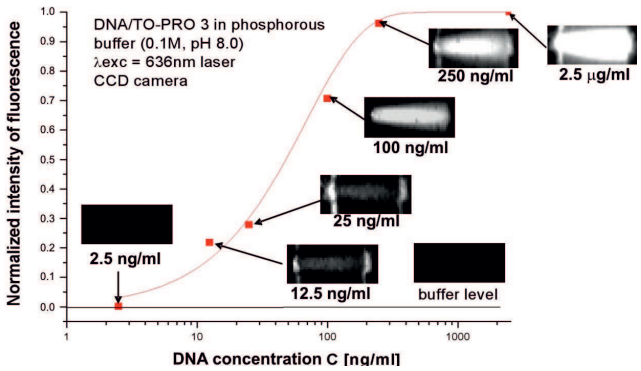

Fig. 10. Calibration curve of the microcytometer for CCD-based detection unit and DNA labeled with TO-PRO 3, images of the detection areas for different DNA concentration are also shown

Tests of the on-chip microcytometer were performed using standard fluorescent dyes and biochemical samples. The lowest detection limit of fluorescein fluorescence excited by LED with λexc = 490 nm was 70 nM. The salmon sperm DNA (Sigma Aldrich, USA), marked with 0.5 μM TO-PRO 3 fluorochrome was tested in the setup equipped with laser (λexc = 636 nm, 5 mW). The lowest detection limit of labeled DNA was 5 ng/ml (Fig. 10). In further experiments it has been confirmed that it is possible to measure simultaneously spectrophotometric and fluorometric properties of flowing through liquids, as well as immobilized inside measurement, cells living bovine embryos [24].

4.4. Microcytometer for apoptosis detection in mice embryos. Microcytometers are widely used for counting, characterization and separation of flowing through cells. However, the main limitation of existing microcytometers is diameter of examined cells, usually from a few to tenths of micrometers. On the other hand, there is a strong demand from life-science researchers to examine apoptosis (programmed cell death) of the largest cells – like small mammals or breeding animals with diameter in 50 μm to 300 μm range. One of the methods of the apoptosis detection is application of fluorescence readout after application of dedicated detecting kits with flourishing markers. A "colored" cell is observed under epifluorescence microscope. Fluorescence intensity and its distribution inside a cell inform on an apoptosis presence and on an actual stage. Recently, we have proposed LOC with fluorescence instrumentation enabling the fluorometric detection of apoptosis in mice embryos [25].

The developed LOC is micromachined in a silicon/glass structure which consists of one microfluidic channel with a cell trap which ensures an immobilization of embryo during an optical characterization (Fig. 11a). The cell trap has also two in-line grooves for positioning of optical fibers. During the measurement single oocyte/embryo is immobilized accurately onto the front of fibers. Fibers are connected to a fluorescence inducing LED light source (490 nm). Filtered fluorescence light is detected by the use of CCD minicamera mounted in an optical path of microscope (Fig. 11b). Images of investigated embryo are in real-time analyzed by developed software. After measurements the embryo is flushed-back to a sterile transporting container for further operations.

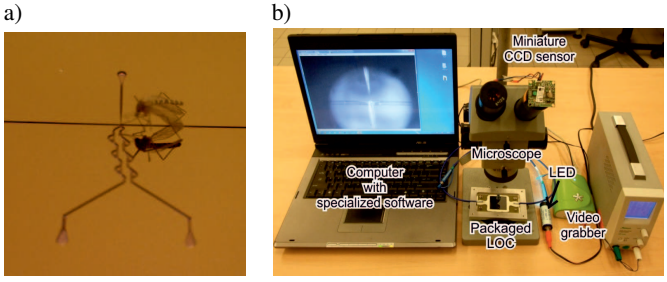

Fig. 11. LOC-based instrumentation for mice embryos detection: a) view of silicon part of LOC in comparison to a mosquito, b) view of the measurement setup

In the experiments, three groups of mice embryos were set. First group was a reference one without any treatment. Second group was treated by a fluorescence marker from Annexin-V apoptosis detection kit. Third group was treated by actino-

mycin D for inducing artificial apoptosis and then colored by Annexin-V kit. The differences of fluorescence intensity obtained for three tested groups were clearly observed (Fig. 12). LOC fluorometric measurements were in good correlation with mice embryo images obtained by the use of epifluorescence microscope.

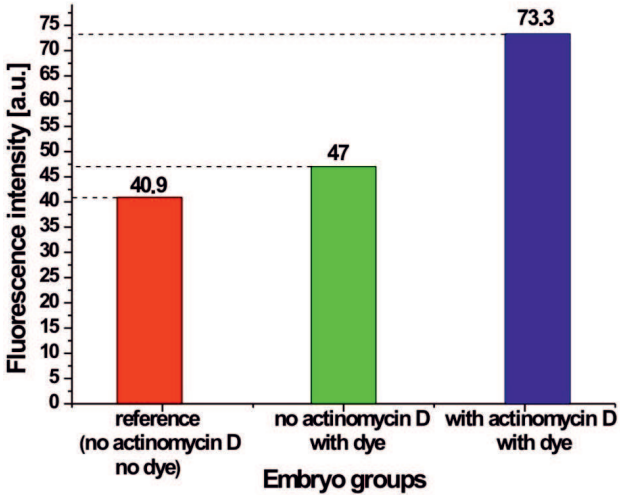

Fig. 12. Average fluorescence intensity for three groups of investigated in LOC mice embryos

Further tests confirmed that non-apoptotic embryos investigated by LOC kept theirs viability. It seems that the developed methodology, LOC and fluorometric instrumentation can be used for non-invasive assessment of cells viability, especially reproductive cells (oocytes, embryos) of breeding animals.

4.5. Portable cocaine detector in human's sweat. Drugs abuse of drivers is important problem of road safety across whole Europe. It has been reported recently, that in Poland, during prevention controls of drivers, about 1% was under influence of alcohol, whereas, more than 2.5% under drugs abuse [24]. These numbers can be even more dramatic when a new recommendation of drugs detection limits (from 300 ng/ml down to 30 ng/ml), prepared by European Commission, is to be implemented. To fulfill this new requirement new portable and rapid tests have to be developed.

The instrumentation for detection of cocaine or its metabolites in humans sweat is now being developed under EU 7. FP LABONFOIL project [17]. The cocaine test is forecasted to be used as a prevention test for professional drivers of heavy trucks or buses. The device consists of a disposable cartridge lab-on-a-paper for cocaine/metabolite detection and a hand-held optical reader connected to portable computer for detection result readout and analyze.

Configuration of measurement setup for preliminary tests of developed instrumentation is shown in Fig. 13a. It contains a detection strip placed on OLED playing as fluorescence excitation source and image sensor-based detection unit. OLED with spectral characteristic tailored to applied fluorochrome co-works with integrated 650 nm short-pass interference filter to cut off undesired wavelengths and efficiently excitate applied fluorochrome. In the latest version, OLED has been replaced by miniature semiconductor laser integrated with the hand-held reader and illuminating detection area of the lab-on-a-paper. Detection strip is immunochromatographic paper-based lab-on-a-paper with cocaine/metabolite capturing areas. Test and control lines of the strip are deposited in detection area with 5 mm spacing. Lines contain Dylight649 fluorochrome with maximal excitation wavelength of 649 nm and emission at 670 nm. Fluorescence images of the detection area are collected by hand-held detection (Fig. 13b) unit and send to computer equipped with video card and specialized software. Software analyzes areas of control/test lines, calculates fluorescence intensity and the ratio of fluorescence intensity of the test to control lines. This ratio is compared to calibration data. It enables determination of the cocaine concentration in the sample.

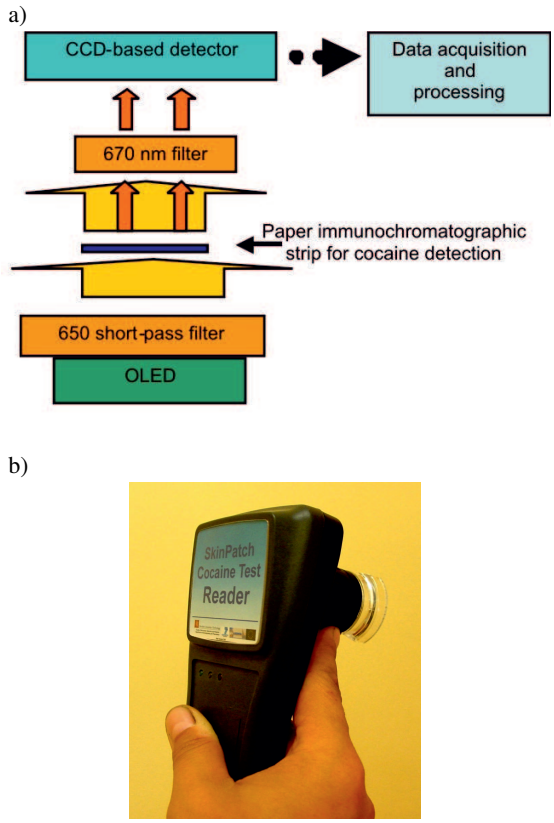

Fig. 13. Lab-on-a-paper – based test for detection of cocaine: a) scheme of the measurement setup for, b) view of the hand-held reader co-working with the test

The experiments were carried out with artificial samples containing from 0 ng/ml (negative test) to 60 ng/ml of cocaine/metabolite concentration with 15 ng/ml step. All samples with cocaine concentration above 30 ng/ml should be qualified as positive test result.

Views of strips as seen by the optical readout system are shown in Fig. 14a. According to assumption, the lower the fluorescence intensity of test line, the higher cocaine concentration in the sample. The lowest detection limit was around

5 ng/ml of cocaine what is over significantly better than for commercially available tests (300 ng/ml) and fulfils new detection requirements (Fig. 14b) [26]. Recent works on the hand-held reader equipped with a miniature laser instead of OLED confirmed high sensitivity of the detection unit [27, 28]. Reported the lowest detection limit of cocaine was better than 2 ng/ml which fulfills new requirements for the lowest detection limit.

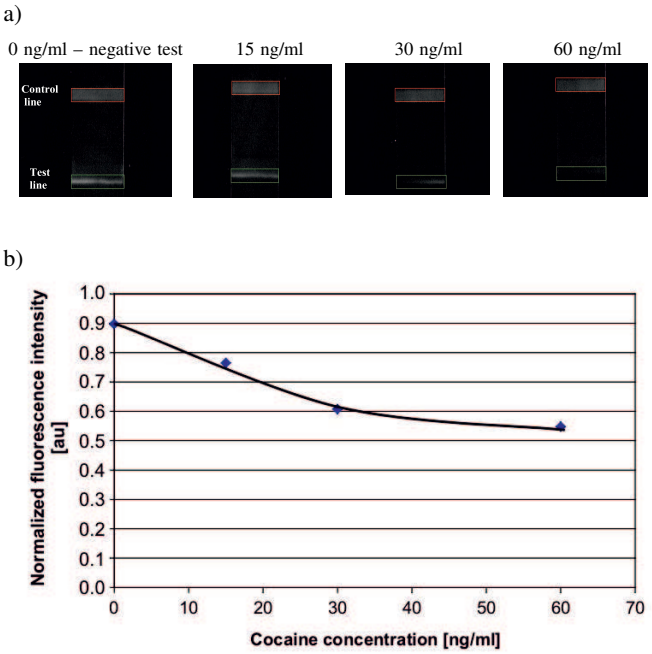

Fig. 14. Optical readout of the fluorescence signals: a) views of the test strips for various cocaine concentration, b) normalized fluorescence intensity as function of cocaine concentration

5. Summary and conclusions

The presented in the paper idea and technical realization of a novel fluorescence readout has been successfully applied with lab-on-a-chips for various applications in the filed of life-sciences. The main advantage of the presented here solution is a ratio of price of the optical components to the sensitivity of the whole detection unit. This has been obtained by application of low-cost components and "intelligent" software for conditioning of collected data. It seems that the novel image sensor-based fluorescence detection instrumentation enables development of point-of-care devices with advanced fluorescence detection and with the sensitivity level comparable to the standard laboratory equipment. Currently, there are works on further development of CCD image sensor – the based fluorescence detection method and instrumentation toward the multiwavelength detection.

It have to be pointed out that the described detection instrumentation have been developed by Polish researchers under frame of European and national projects. Most of the described LOC were also developed in co-operation between Polish R&D institutions, with a special technological input from Institute of Electron Technology in Warsaw. Tests of LOC and optical instrumentation were possible thanks to multidisciplinary co-operation of research teams from Polish universities and institutes. It leads to the conclusion that Polish researchers have potential to be successful and also they can be European and world leaders in some high-tech multidisciplinary niche fields.

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