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Field-emission light sources for lab-on-a-chip microdevices

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Abstract. Microfluidic devices called lab-on-a-chips utilize two kinds of characterization of a biosample, which are based on spectrofluorimetric and spectrophotometric methodologies. Lab-on-a-chips are equipped with an optical instrumentation and a software system that allow detecting of the optical signals and their processing into spectral characteristics.

In the paper, technology of lab-on-a-chip (cytometer microdevice) and miniature silicon-glass field-emission light source (FELS) is described. Electrons emitted from a silicon cathode covered with carbon nanotubes excite light emission from nanocrystalline rare earth doped yttrium oxide phosphor (anode). The emitted light can be precisely tailored to the main absorption line of a fluorescent marker. The presented system has been successfully used for testing of the cow embryos as well as fluorescent-marked porcine oocytes.

Key words: lab-on-a-chip, field emission, light source, microcytometer.

1. Introduction

Lab-on-a-chips (biochips) are miniature devices developed for carrying out chemical/biochemical analyses in micro scale. The biochips are fabricated in silicon, glass or polymer substrates/layers. They contain microchannels, mixers, pumps, valves, and microreactors. The main advantages of these microdevices are: small amount of used chemicals, high sensitivity, and high velocity of analysis, low cost, low dimensions and high portability. Optical methods are usually utilized in lab-on-a-chip systems for detection of reaction/analysis results [1]. The most popular is a spectrofluorometric method.

The spectrofluorometric analysis is based on detection of the fluorescent light (on a particular wavelength) emitted from the fluorophor (biochemical fluorescent particles), which are bonded to a biosample (e.g. single cell or protein) and serves as a fluorescent tag. In contrast with the spectrofluorometric analysis, spectrophotometry relies on analysis of the transmission characteristic obtained for a biosample, which is illuminated by visible or infrared radiation.

The main objective of this work was elaboration of a lab-on-a-chip system which consists of a microcytometer, a miniature light source and detection circuits (Fig. 1). For a few years, we have been working on the instrumentation for the investigations of biosamples. Recently we have developed technology of biochips for real-time PCR (Polymerase Chain Reaction) of DNA (Deoxyribonucleic Acid) samples that can be monitoring by fluorescence measurements [2]. Absorption characteristics can be also very useful for the investigations of quality of living animals' cells [3].

Biochips containing optical fibers or planar waveguides integrated with their microfluidics structure are built (Fig. 2a). In this paper a different concept of integration is proposed (Fig. 2b). We recommended a modified version of the field-emission light source, which was presented in detail in our previous work [4].

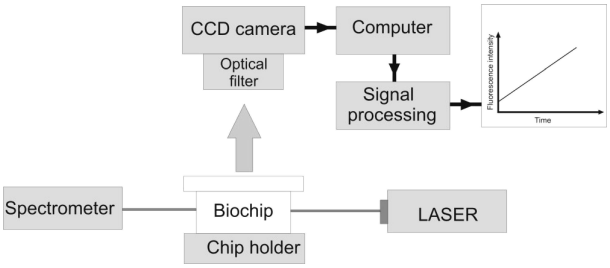

Fig. 1. Schematic of the lab-on-a-chip system

Such light source is fabricated as a silicon-glass structure with a silicon cathode, which surface is modified by deposition of carbon nanotubes (CNT). The anode is equipped with an appropriate nanocrystalline low-voltage phosphor based on yttrium oxide matrice doped with rare earth ions (or micrograined silver activated zinc sulfide phosphor for comparison). The new silicon-glass light source or halogen lamp is connected to a micromachined cytometer biochip. The transmitted light can be absorbed by biosample and analyzed by a microspectrometer (Fig. 2a). If a source light, adequate for the investigated fluorescent-marked biosample, excites fluorescence the optical signal is digitally processed as a CCD camera screenshots (Fig. 2b).

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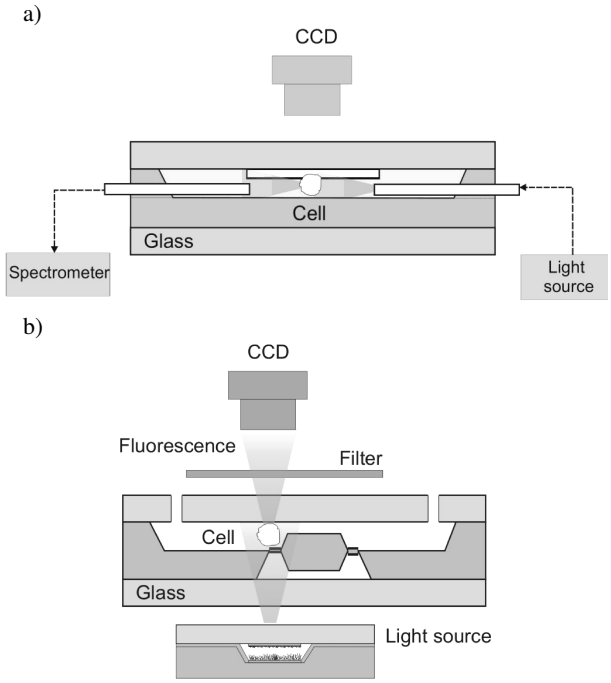

Fig. 2. Microcytometer for: a) spectrophotometric measurements, b) spectrofluorometric measurements with a new field-emission light source

2. Experiments

2.1. Microcytometer. Microcytometer is a glass/silicon/glass microfluidic biochip that makes possible the precise manipulation of a single animal cell and obtaining of the fluorimetric/photometric spectra. Silicon part of the microchip is fabricated in n-type (1–5 Ω·cm), double-side polished, (100) oriented silicon wafer. It is composed of a microfluidic channel with two via-holes and two V-grooves (Fig. 3).

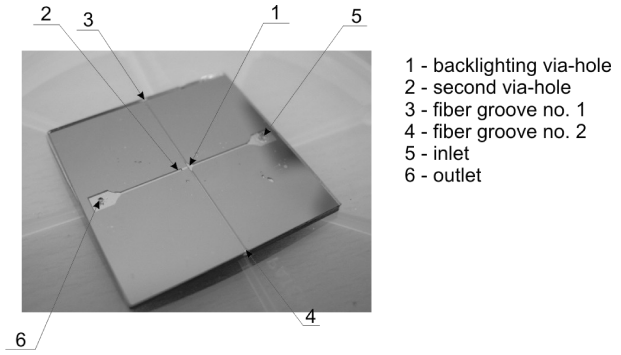

Fig. 3. Glass-silicon-glass microcytometer

The first via-hole (1) is made for backside illumination of the investigated biological cell, and the second one (2) works as a relief valve that allows flowing of an excess fluid, in which the cell is transported. Microchannels, via-holes and V-grooves are formed by wet anisotropic etching of silicon from both sides of the wafer (10 mol KOH, 80°C). After that, the silicon part of microcytometer is bonded to the two glass sheets (upper and lower covers) in the air at 450°C under 1200 V. Afterwards, two glass fibers are placed in V-grooves, precisely positioned in the chip's microchannel using an optical microscope and fixed by UV-cured hard epoxy optical glue.

The investigated biological cell is semi-automatically stopped in the shallowest part of the microchannel above the via-hole (1), and between inline aligned two glass fibers (3 and 4), which are precisely embedded inside V-grooves etched in silicon chip (Fig. 3). For the VIS/NIR absorption/transmission tests, light is guided from external light source (halogen lamp) by glass fiber (3 or 4), absorbed by cell and detected by external microspectrometer with use of the second fiber. For the spectrofluorometric tests, light is guided from FELS or external light source (laser, LED with optical filter) through via-hole (1) for backside illumination of the investigated cell (marked by fluorescent tag). In this case optical signal is detected by CCD camera at the front side of the chip and digitally processed as a video frames.

2.2. Light source. Field-emission cathodes have been fabricated in 3 in., (100) oriented, n-type (1–5 Ω·cm), 380 μm thick silicon wafers. At first, shallow rectangular cavity with dimensions of $5\times5\times0.07$ mm^3 was formed on each cathode's substrate with dimensions of 15×15 mm^2 (Fig. 4). Formation of the cavity was carried out through 1 μm thick thermal silicon oxide mask, by wet anisotropic etching (10 M KOH, 80°C). After the second thermal oxidation process and photolithography rectangular 3×3 mm^2 windows in etched cavity were opened. Next, low resistive ($2\cdot10^{-4}$ Ω·cm), highly transparent ($> 80\%$), 100 nm thick indium-tin oxide (ITO) layer was deposited by use of the magnetron sputtering method. Then, ITO layer was photolithographically patterned to form a conductive electrode at the bottom of each silicon cavity. Next, silicon wafer was cut into cathode structures, and carbon nanotubes working as an emissive material were electrophoretically deposited. This process was carried out in 2-propanol (IPA) sol with dispersed carbon nanotubes, and with addition of magnesium nitrate salt ($Mg(NO_3)_2\cdot6H_2O$). After the deposition of carbon nanotubes, cathodes were aged at 100°C for one hour to obtain higher CNT adhesion.

The anode of FELS was made of borosilicate glass (Borofloat 3.3 – Schott, Germany). Transparent ITO layer was sputtered onto glass substrate and photolithographically patterned to form a contact electrode (3×7 mm^2). Next, appropriate phosphor is deposited on the specified anode area (3×3 mm^2). The phosphor layer, likewise carbon nanotubes, was deposited from IPA sol with the appropriate additives using an electrophoresis method.

Two kinds of phosphors have been used. The first phosphor type (red or green) was based on nanocrystalline yttrium oxide matrices doped with rare earth ions (Re:Y_2O_3, Re = Eu^{3+}/Tb^{3+}). The second phosphor (blue) contained commercially available micro-grained silver activated zinc sulfide (ZnS:Ag). Rear earth doped yttria phosphors were very interesting because of their efficient luminescence under UV or cathode ray excitation. It has been reported that under excitation of electron beam with the middle accelerating voltage the cathodoluminescent intensities of the Re:Y_2O_3 nanocrystalline phosphors are higher than those of commercial micro-size ones [5].

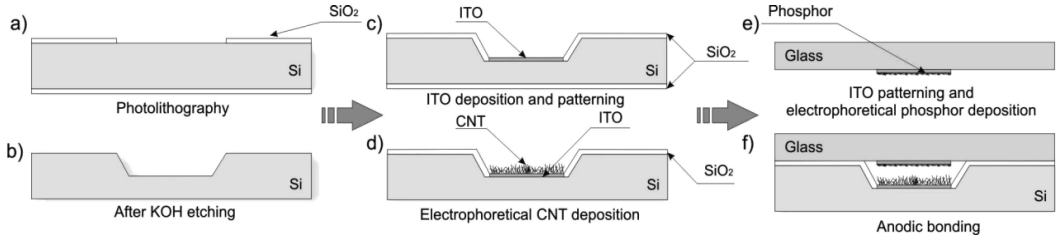

Fig. 4. Flow chart of the field-emission light source fabrication

In this study, Re:Y2O3 Re = Eu^{3+}, Tb^{3+} phosphors were synthesized in the combustion reaction between metal nitrates and urea $CO(NH_4)_2$ [5–7]. Europium oxide (99.999% Aldrich), terbium oxide (99.99% Aldrich) and yttrium oxide (99.999% Aldrich) were used as starting raw materials. After dissolving oxides in ultra pure hot nitric acid (HNO_3), adequate nitrates were obtained: $Eu(NO_3)_3{\cdot}5H_2O$, $Tb(NO_3)_2{\cdot}5H_2O$ and $Y(NO_3)_3{\cdot}6H_2O$. Depending on the phosphor, relevant nitrates water solutions were mixed according to the formula $(Y_{1-x}Re_x)_2O_3$, with addition of a suitable urea-to-metal nitrate ratio amount. Prepared solution was concentrated at 80°C and finally heated up to 600°C to initiate a combustion synthesis. After the synthesis, the obtained white residue was annealing at 1000°C for five hours.

The final product, in a form of the white nanopowder, was dispersed in IPA with magnesium nitrate additive, and electrophoretically deposited onto the anode glass substrate with ITO electrode. Phosphor nanocrystals were suspended in isopropyl alcohol with the addition of $Mg(NO_3)_2{\cdot}6\ H_2O$. The solution was ultrasonically mixed for 30 minutes, then glass substrate was immersed, negative polarization 60 V for 2 minutes was applied. Next, the glass substrates were dried at 100°C for 15 minutes and annealed at 450°C for 10 minutes. The annealing process was applied to obtain a strong adhesion between glass substrate and nanocrystallines of the phosphor by generation of MgO molecules.

Next, the silicon cathode and glass anode were prepared for vacuum sealing by the anodic bonding method. The procedure of substrates cleaning: rinsing in DI water and activation in hydrogen peroxide (40% H_2O_2) has been used without noticeable damages of the electrophoretically deposited layers (carbon nanotubes on cathode and phosphor on anode). The process of anodic bonding of silicon and glass parts of FELS was carried out in a vacuum chamber ($p = 10^{-3}$ hPa) at 450°C under 1200 V, after previous thermal treatment at 400°C for 30 minutes.

2.3. Biological tests. Preliminary tests of the microcytometer system have been made in order to verify its usefulness for characterization of biological properties of porcine oocytes and cow embryos. The system composed of glass-silicon-glass microcytometer, halogen lamp or field-emission light source, external microspectrometer or CCD camera.

First, porcine oocyte immersed in PBS buffer (phosphate buffered saline) was sucked into pipette and inserted into biochip inlet. Next, the cell was transported in microchannel and located above via-hole (1) and below CCD camera. System of data processing made possible obtainment of on-line photographs of the investigated cells.

The fluorimetric tests have been performed for porcine oocytes with use of the microspectrometer (Ocean Optics, USA) and the light source with addition of a band-pass filter. For this purpose Annexin-V FLUOS fluorescent kit (composition of two fluorophors: Annexin-V-Fluorescein and Propidium iodide (PI)) has been chosen. This kit is used for discrimination between necrotic and apoptotic cell and determination of the stage of the programmed cell death [3]. Firstly, porcine oocytes were washed in PBS. Afterwards, incubation of the cells with Annexin-V was carried out. Next, single oocyte was sucked into pipette and inserted into biochip inlet. The cell was transported in Annexin-V medium and positioned above first via-hole (1) between optical fibers.

The spectrophotometric tests have been made for cow embryos, which were available for measurements in one day after fertilization, and seven days after fertilization. Their biological properties were investigated by analysis of the transmission characteristics with use of the external halogen lamp and microspectrometer. Each embryo was washed in PBS buffer before performing a test. Next, embryo was sucked into pipette and transported in PBS into the microcytometer channel. The obtained spectra were analyzed to determine differences which could be related with biological properties of the measured cells.

3. Results and discussion

The vacuum sealing of FELS structure was the main technological problem to solve. The anodic bonding procedure for a silicon cathode with CNTs and a glass anode with nanocrystalline phosphor was elaborated. Leak-proof vacuum sealing through 1 μm thick silicon oxide has been realized.

Optical and electrical properties of the fabricated FELS have been measured. Light sources showed well defined and narrow spectral lines due to use of nanocrystalline Eu^{3+}:Y_2O_3 or Tb^{3+}:Y_2O_3 phosphors (Fig. 5). The X-ray diffraction spectra (HR XRD Philips, K_α, $\lambda = 1.540$Å) obtained for rear earth doped yttria powders showed up patterns indexed to Y_2O_3 cubic phase. The broadening of the diffraction peaks suggested a nanometer size of phosphor particles (smaller than 30 nm). Photoluminescent spectra were obtained by pulsed laser excitation system (Nd: YAG LASER SYSTEM LS2137/2M) with harmonic generator (FH/266 nm). They were described by well known emission lines of Eu^{3+} or Tb^{3+} ions in Y_2O_3 lattice.

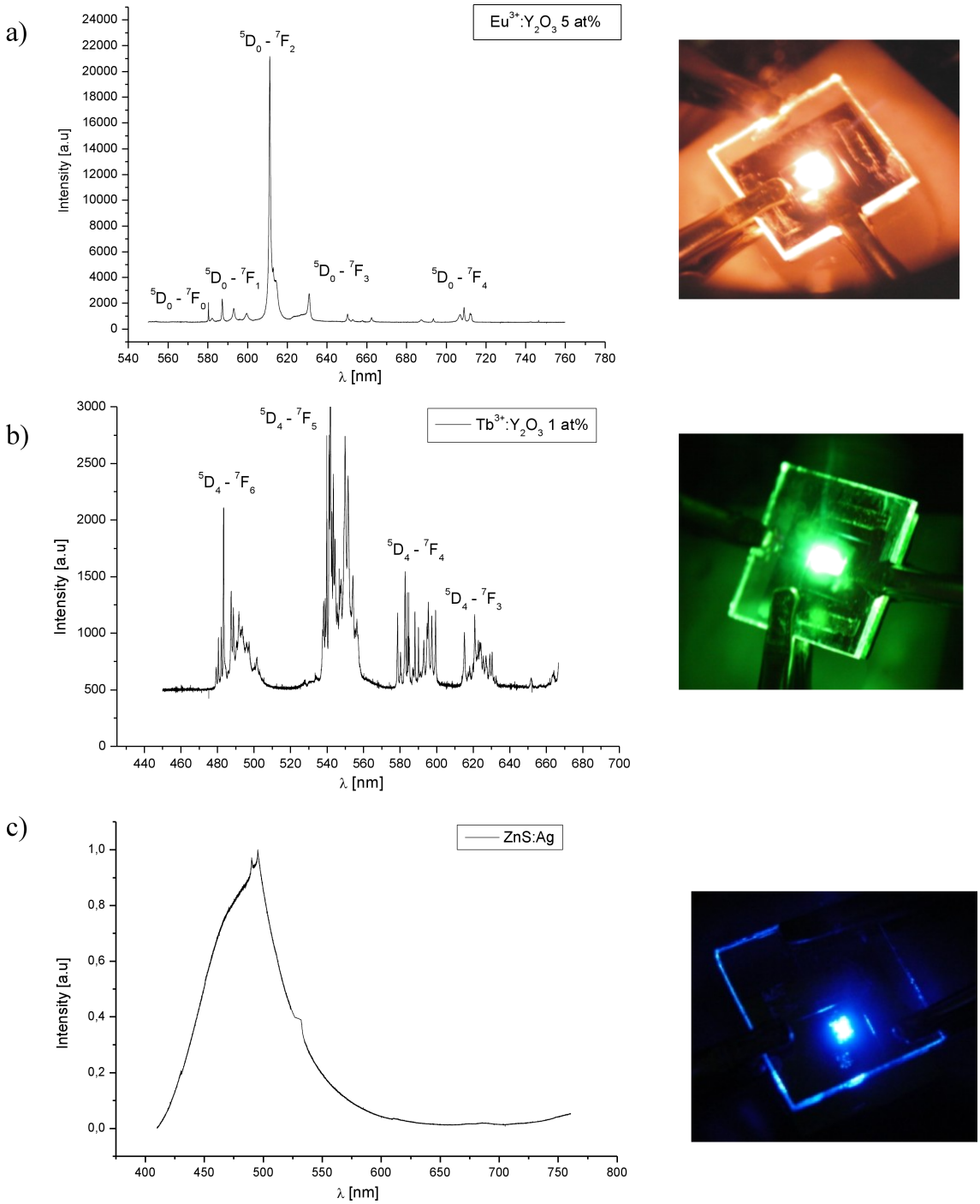

Fig. 5. Photoluminescent spectra of phosphors dedicated for fluorimetric measurements and working FELS: a) nanocrystalline Eu^{3+}:Y_2O_3 5 at% phosphor, b) nanocrystalline Tb^{3+}:Y_2O_3 1 at% phosphor, c) micro-grained ZnS:Ag phosphor (commercial)

Current-voltage measurements were also carried out for the fabricated FELS. Emission characteristics (with typical Fowler-Nordheim plots) showed that the threshold voltage was about 400 V. Current value up to tens of microamperes was obtained for voltage lower than 900 V (emission area was about 3×3 mm^2). The obtained current densities were enough for efficient excitation of synthesized nanophosphors.

The first tests of the fabricated glass-silicon-glass microcytometer showed its usefulness for the living cells optical studies. The microcytometer allows investigation of animal cells with dimensions from 70 μm to 150 μm. Preliminary spectrofluorometric studies were made for porcine oocytes. Each oocyte was marked by Annexin V-FLUOS kit and positioned inside the biochip channel. Illumination of positioned oocyte caused excitation of the fluorescence signal, which was detected by microspectrometer (Fig. 6a). The "good" oocytes may tend not to fluoresce, whereas an apoptotic or necrotic oocytes marked by fluorescent dye are going to fluoresce at charac-

teristic emission line (necrotic – red light from propidium iodide, apoptotic – green light from annexin-V-fluorescein). Presence of the fluorescence signal – green light – suggested programmed apoptotic-like death of the investigated oocyte.

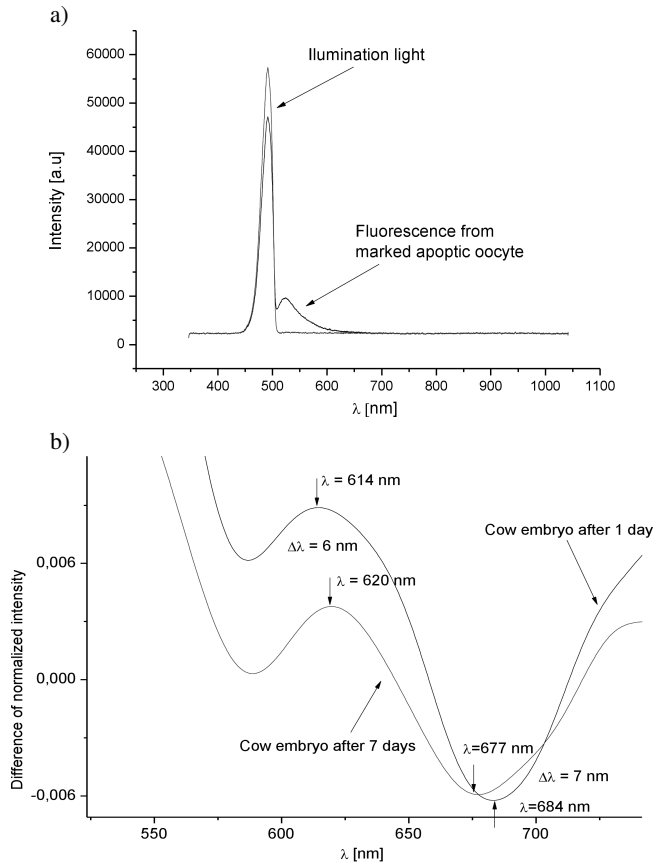

Fig. 6. Spectral characteristics of living animal cells: a) spectrofluorometric test of porcine oocyte, b) spectrophotometric test of cow embryos in one day and seven days after fertilization

Transmission/absorption measurements were carried out for cow embryos. The obtained spectra are presented in Fig. 6b. It is noticeable that lifetime of the embryo influences on the spectrum. The shift between the spectra is a very promising effect, which can be used for elaboration of the optical classification method of animal living cells.

Cow embryo at different stage of maturity can be described by characteristic points of absorption spectrum. A time-dependent decreasing of transmission intensity of embryo can be considered as another qualification factor.

4. Conclusions

A new miniature field-emission light source destined for the glass-silicon-glass microcytometer has been presented. The silicon cathode of the FELS modified by deposition of carbon nanotubes has been anodically bonded to glass anode substrate in vacuum. The nanocrystalline phosphor: Eu^{3+}:Y_2O_3, Tb^{3+}:Y_2O_3 or ZnS:Ag covers an important range of emitted light wavelengths used for excitation of fluorescent markers in biochemical analyses. Combustion synthesized nanocrystalline phosphors exhibit very narrow main emission lines, which are well tailored to main absorption lines of fluorescent markers.

A microcytometer for optical evaluation of the properties of biological living cell has been shown for the first time. This lab-on-a-chip was successfully used for nondestructive optical measurements. The device can be used for VIS/NIR spectroscopy and fixed-wavelength spectroscopy for specific wavelengths, which are adjusted to fluorescent markers. The optically induced fluorescence spectra of single porcine oocyte marked with Annexin-V fluorophor and absorption spectra of single cow embryo have been made.

It seems that the microcytometer integrated with the miniature light source can be competitive in comparison with the commonly applied systems with solid-state lasers, LED's and DYE-lasers. We believe that the presented solution will be developed in future to obtain a cheap diagnostic system for rapid measurements of quality of living cells.

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