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Novel Multichannel Fluorescence Detection for Lab-On-a-Chip Applications with Quantum Rods Fluorochromes

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Abstract

In this paper, for the first time results of works on novel miniature instrumentation for quantum rods (QR) fluorescence excitation and detection will be described. Presented solutions were developed to co-work with lab-on-a-chip (LOC) applications of QR fluorochromes in multichannel high sensitive fluorescence detection.

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Keywords: fluorescence, quantum dots, multichannel, lab-on-a-chip

1. Introduction

Quantum rods (QR) are a novel class of inorganic fluorochromes composed of nanometre-scale crystals made of a semiconductor material. Biological applications of quantum dots are widely described in literature [1, 2] as an alternative for conventional organic dyes in fluorescence resonance energy transfer analysis, cell tracing, flow cytometry or *in vivo* animal imagining. The tiny size of nanocrystals gives these materials unique physical properties which seem tailor-made for multicolour fluorescence analyse [2]. It has been also showed that quantum dots – based fluorochromes had no loss in intensity after 14 h, and were nearly 100 times as stable as, and also 20 times as bright as, rhodamine 6G [3].

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Described here QR are built up from a CdSe core-like spherical quantum dots, but CdS shell is grown as an elongated rod on top of the spherical core (Fig. 1). This can be realized by using ligands during the particle surface which block the crystal facets except for 001. This leads to an elongated growing of the CdS shell. The synthesis is a 2-step procedure. In the first step the CdSe cores are produced, isolated via centrifugation and redissolved. This step is performed at high temperatures above 350 °C to ensure a perfect monocrystalline wurtzite structure of the CdSe core which is needed for the elongated shell growth. In the second step, the CdS shell is grown on top of the core at high temperatures in the presence of above described ligands.

It has been found that QR particles combine an increased brightness with enhanced stability compared to the spherical quantum dots and organic dyes. Compared to the core/shell/shell particles which usually have quantum yields around 40% this would only increase the sensitivity by a factor of 2.

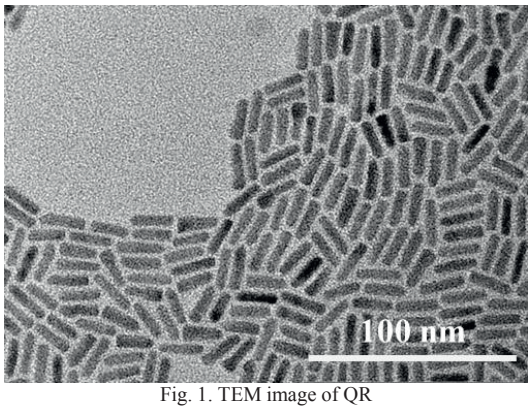

Fig. 1. TEM image of QR

Main feature of QR fluorochromes is possibility to excite fluorescence with wide-band wavelength region (below 500 nm) (Fig. 2a) and narrow-band fluorescence light emission (above 500 nm) (Fig. 2b) with emission centre wavelength and band width tailored by QR size and QR size distribution. Thus, single light source can be used to excite few QR fluorochromes simultaneously. In case of standard organic dye one light source is used to excite fluorescence from one dye. This feature causes that standard detection units with optical properties tailored to a single fluorochrome cannot be used in multichannel fluorescence detection in multiplex analyses.

a) b)

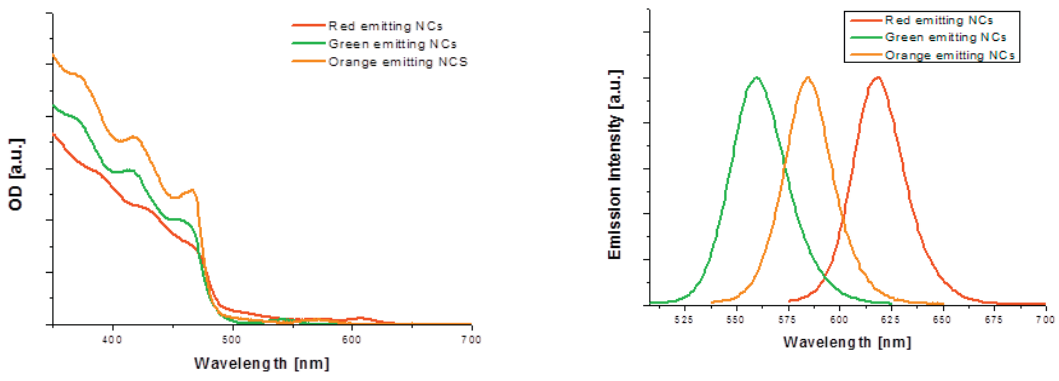

Fig. 2. Exemplary absorbance (a) and emission spectra (b) of QR

Here we present for the first time novel four-channel fluorescence detection units co-working with QR fluorochromes and LOC. Soluble in water QR fluorochromes were synthesised in order to obtain emission spectra characteristics tailored to four optical detection channels positioned above 500 nm (Fig. 3).

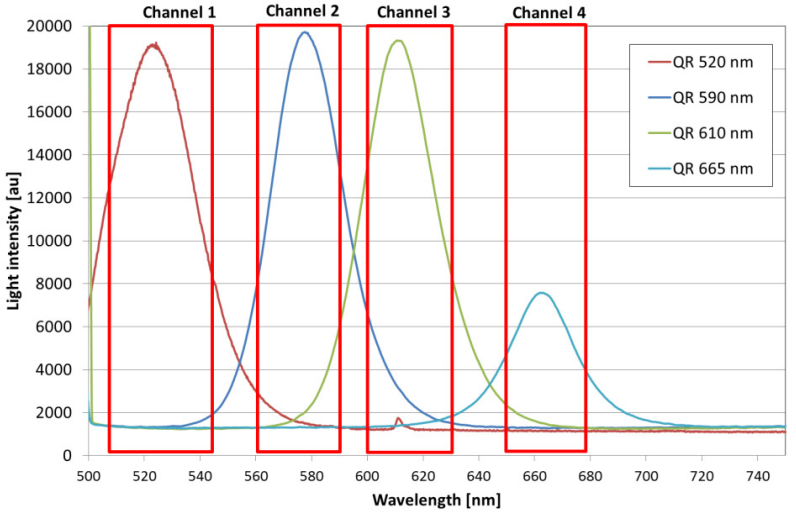

Fig. 3. Spectral characteristics of QR emission with marked fluorescence detection channels

2. Experiment

In the experiments silicon-glass chips with 10 µl chamber were used. The chamber was filled with QR solution and closed with 1 mm – thick PDMS top cover. Images of the chip during fluorescence measurements for each examined QR fluorochrome (fluorescence detection channel) are shown on fig. 4.

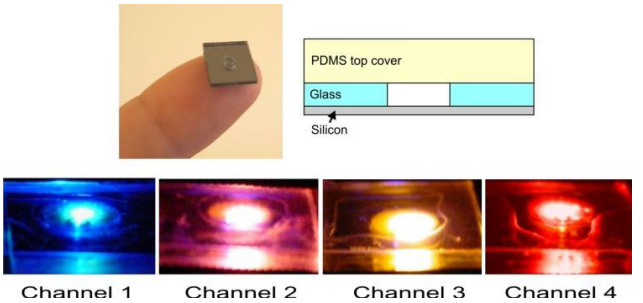

Fig. 4. View of the silicon-glass chip (including schematic cross-section) and images of the chip during fluorescence measurements in each examined optical detection channel

Two configurations of fluorescence read out units (Fig. 5, 6) were developed and tested. Both of them utilize BLED (480 nm) light source to excite QR fluorescence and epifluorescence configuration to collect the fluorescence. The excitation light beam has 3 mm diameter at 10 mm focal length. The excitation/light collection unit is connected to optoelectronic units by flexible 1-mm core polymer optical waveguide with SMA connectors. In both configuration, the main challenge is to distinguish fluorescence

signal during multichannel detection with very high sensitivity and minimum cross-talks between channels.

In the 1st configuration (Fig. 5), the miniature filter wheel and semiconductor photodiode integrated with transimpedance amplifier is used. The filter wheel has four 5 x 5 mm^2 interference filters with optical properties tailored to specific QR fluorochrome. The wheel was rotated both directions by the use of miniature stepper motor. During fluorescence readout, position of the filter in relation to the photodetector determines readout channel (up to 4 channel in presented here solution). Fluorescence intensity value is obtained immediately after electronic readout of analog signal from IC photodetector is finished. Steering and control unit of the 1st configuration has been developed under LabView platform.

In the 2nd configuration, collected light is guided to MOEMS spectrophotometer (Hamamatsu, Japan) (Fig. 6) where spectral analysis of the light is carried out. This configuration was tested with two integration times of the spectrophotometer readout – 1 and 10 seconds. Software-based analyse of the spectral characteristics enables determination of the fluorescence intensity in up to four selected wavelengths corresponding to readout channels.

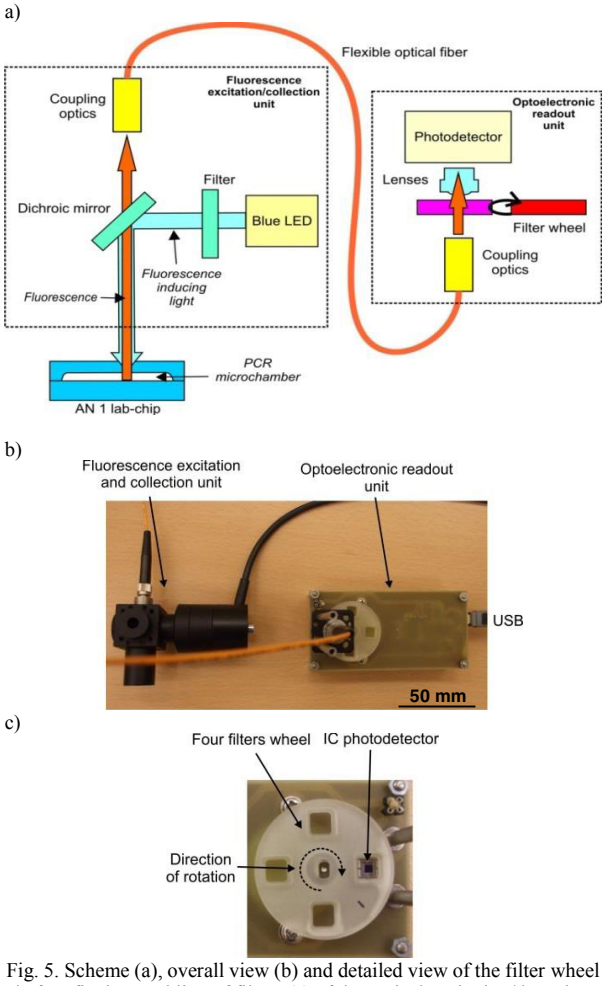

Fig. 5. Scheme (a), overall view (b) and detailed view of the filter wheel before final assembling of filters (c) of the optical excitation/detection unit with configuration 1

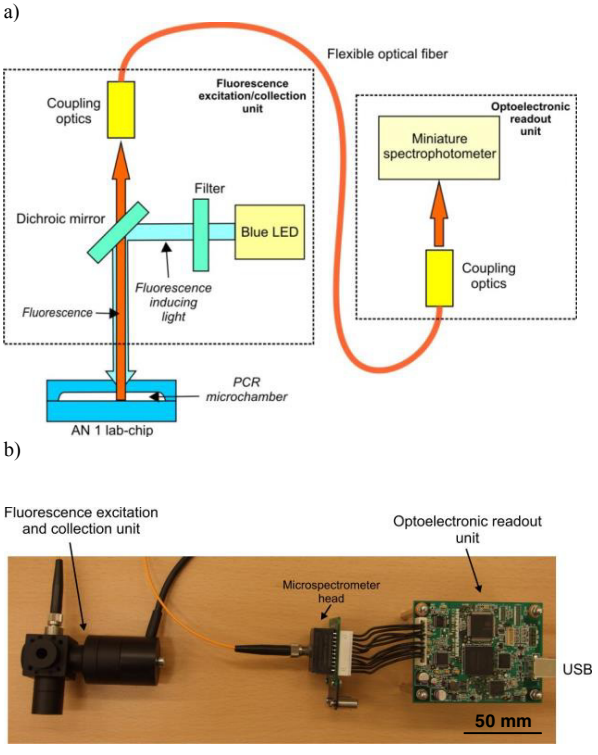

Fig. 6. Scheme (a) and view (b) of the optical excitation/detection unit with MOEMS spectrophotometer (configuration 2)

3. Results and summary

Fluorescence detection carried out with application of silicon-glass chip and developed units have shown very good limits of detection of QR fluorochromes in configuration 1 (below 0,3 nM) and satisfactory limits of detection in configuration 2 (2 nM) (Fig. 7) (Table 1).

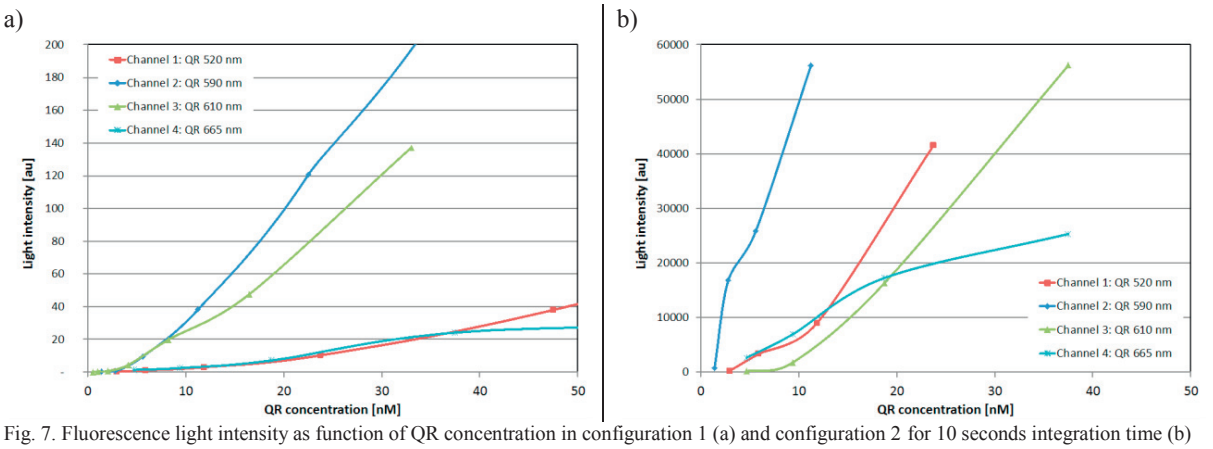

Fig. 7. Fluorescence light intensity as function of QR concentration in configuration 1 (a) and configuration 2 for 10 seconds integration time (b)

Table 1. Limits of detection of QR fluorochromes for 4 optical detection channels o described here optical detection configurations.

Channel	Configuration 1	Configuration 2 (1 / 10 sek integration)
1	0,15 nM	10 nM/2 nM
2	0,18 nM	10 nM/2 nM
3	0,22 nM	10 nM/2 nM
4	0,2 nM	10 nM/2 nM

In both configurations, multichannel fluorescence detection was obtained with proper distinguishing between optical channels and cross-talks between channels below 5% (Fig. 8). However due to simplicity and high sensitivity of the 1st configuration it has been chosen for further works.

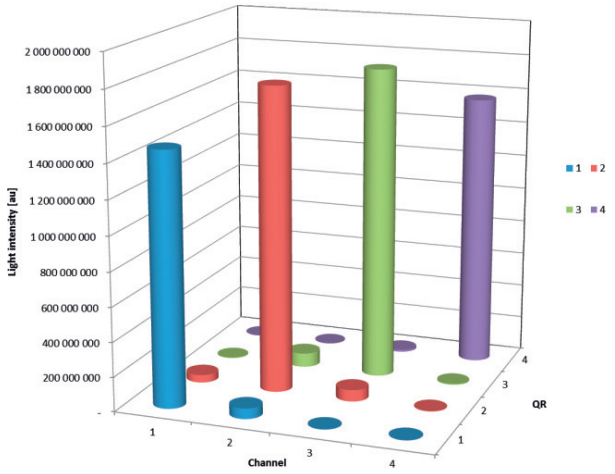

Fig. 8. Crass-talks graph between optical channels for 1st configuration of optical readout instrumentation

Further works will be focused on biological functionalization of QR fluorochromes towards its application in on-chip qPCR. Various conjugation protocols could be established to prepare QR-DNA probes.

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