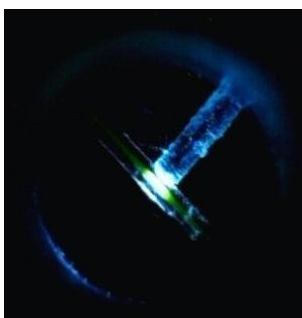


## Fibre coupled micro-LED array light source with integrated band-pass filter for fluorescence detection in miniaturised analytical systems

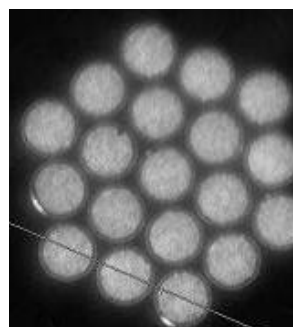
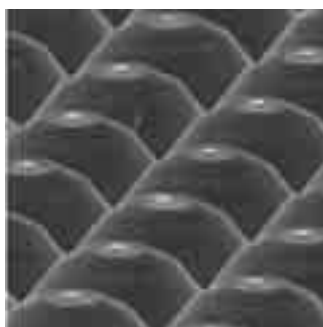
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**Graphical Abstract** (please choose)



A new generation of integrated miniaturized fibre-coupled solid-state light sources based on microfabricated light emitting diode micro-array ( $\mu$ -LED), micropackaged with a custom band-pass optical interference filter deposited at the end of an optical fibre, is presented and in this work and demonstrated as excitation light source for fluorescence detection in capillary electrophoresis.

ALTERNATIVE IMAGES:



## **Fibre coupled micro-LED array light source with integrated band-pass filter for fluorescence detection in miniaturised analytical systems**

Markéta Vaculovičová<sup>1,3,4</sup>, Mahbub Akther<sup>2</sup>, Pleun Maaskant<sup>2</sup>, Dermot Brabazon<sup>1,5</sup>, and Mirek Macka<sup>3\*</sup>

### **Highlights**

- A new integrated miniaturized fibre-coupled solid-state light source is presented.
- Based on a micropackaged microfabricated light emitting diode micro-array ( $\mu$ LED).
- Interference filter micropackaged with optical fibre and the  $\mu$ LED array.
- Demonstrated as excitation light source for fluorescence detection.

1 **Fibre coupled micro-light emitting diode array light source with**  
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4 **integrated band-pass filter for fluorescence detection in**  
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6 **miniaturised analytical systems**  
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51 **Key words:**  
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55 Micro-light emitting diode array; light source; optical fibre; capillary electrophoresis;  
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57 fluorescence detection; separations.  
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## Abstract

In this work, a new type of miniaturized fibre-coupled solid-state light source is demonstrated as an excitation source for fluorescence detection in capillary electrophoresis. It is based on a parabolically shaped micro- light emitting diode ( $\mu$ -LED) array with a custom band-pass optical interference filter (IF) deposited at the back of the LED substrate. The GaN  $\mu$ -LED array consisted of 270 individual  $\mu$ -LED elements with peak emission at 470nm, each about 14 $\mu$ m in diameter and operated as a single unit. Light was extracted through the transparent substrate material, and coupled to an optical fibre (400 $\mu$ m in diameter, numerical aperture NA = 0.37), to form an integrated  $\mu$ -LED-IF-OF light source component. This packaged  $\mu$ -LED-IF-OF light source emitted approximately 225 $\mu$ W of optical power at a bias current of 20mA. The bandpass IF filter was designed to reduce undesirable LED light emissions in the wavelength range above 490 nm . Devices with and without IF were compared in terms of optical power output, spectral characteristics as well as LOD values. While the IF consisted of only 7.5 pairs (15 layers) of SiO<sub>2</sub>/HfO<sub>2</sub> layers it resulted in an improvement of the baseline noise as well as the detection limit measured using fluorescein as test analyte, both by approximately one order of magnitude, with a LOD of  $1 \times 10^{-8}$  mol/L obtained under optimised conditions. The  $\mu$ -LED-IF-OF light source was then demonstrated for use in capillary electrophoresis with fluorimetric detection. Limits of detection obtained by this device were compared to those obtained with a commercial fibre coupled LED device.

## Introduction

Photometric and fluorimetric optical detection methods are frequently used in capillary based separation techniques including capillary electrophoresis (CE) and capillary liquid chromatography (cap-LC) [1-6]. While photometric detection is generally valuable and the most common detection in CE and cap-LC, the combination of the most sensitive detection method of laser-induced fluorescence (LIF) detection with CE provides a powerful separation platform with a wide range of advantages including speed, high resolution, efficiency, and sensitivity, as well as low sample and reagent consumption [7-9], for applications such as glycomics [10-12].

Traditionally used excitation sources for fluorimetric detection are incandescent or arc lamps (halogen or mercury) based on technologies going back over a century, and in the last decades on lasers and then increasingly on solid state light sources – diode lasers and LEDs [13-20]. Arc and incandescent lamps have an advantage in their broadband continuous emission; however due to their size, fragility, heat production, relatively low luminosity and optical output stability, they are not suitable for miniaturization purposes. Lasers are commonly used as excitation sources due to their high emission intensity, monochromaticity and advantageous spatial properties (collimated light, easy to focus), which allow the light to be focused to a very small area. Light-emitting diodes (LEDs) since their discovery in 1907 [21] and commercial technology developments from 1960s pushed down the wavelength scale from infrared and red to green, blue, violet and ultraviolet [22-25], and are nowadays considered as the light sources of the future. LEDs offer numerous advantages including quasi-continuous wavelength coverage, stable intensity, robustness including long lifetime, small size, low cost, and ability to be pulsed at fast

1 rates, while their main deficiency is the lack of powerful enough emitters in the deep-  
2 UV (below 300nm) spectral region [26-28].  
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5 In the area of on-capillary detection including CE, LEDs have been used in  
6  
7 miniaturised low-cost detection systems, both photometric [29-40] and LED induced  
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9 fluorimetric (LED-IF) [41-45], with advantages especially for portable CE instruments  
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11 [46]. A number of LED-IF detection designs for microfluidic chip-based CE [47-50]  
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13 systems have been reported as well.  
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17 As LEDs, which otherwise would be more popular as miniaturised light sources for  
18  
19 portable devices, are semi-monochromatic and naturally possess bandwidth of  
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21 approx. 20-50 nm, when used as excitation sources in LED-IF for optimal  
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23 performance and low baseline noise they have to be combined with low-pass filters  
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25 [45, 51, 52].  
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29 Miniaturization of the individual optical components (light source, optical filters,  
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31 lenses, mirrors etc.) and their assembly into a functioning optical system is the  
32  
33 limiting factor when creating a miniaturized CE-LIF design either LIF or LED-IF. As  
34  
35 LEDs have wide spatial light distribution, focusing optics is usually required for  
36  
37 optimal sensitivity of LED-IF detection [45, 53, 54]. Optical fibres directly coupled to  
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39 LEDs (pigtailed LEDs) [47, 55, 56] are a very popular alternative in creating a  
40  
41 spatially directed LED light source. Fibre-coupled LED sources with integrated  
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43 interference filter could be an attractive integrated micropackaged fibre-coupled light  
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45 source component for miniaturised optical detection systems.  
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53 The  $\mu$ -LED arrays [57] provide a quasi-collimated light emission and therefore can  
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55 have a good coupling efficiency to optical fibres. When integrated and  
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57 micropackaged with an optical fibre, an interference filter can be inserted between  
58  
59 the  $\mu$ -LED array and the fibre, by depositing this filter on the back surface of the  
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1 substrate emitting LED. Such LED-based integrated and micropackaged optical fibre  
2 light sources emitting from the fibre spectrally filtered light, could become a new  
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4 option in custom designed optical fibre-coupled light sources for fluorescence  
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6 detection in on-capillary and microfluidic chip separation systems. The authors to  
7  
8 their best knowledge are not aware of any other similar work on integrated  
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10 micropackaged fibre-coupled  $\mu$ -LED array light sources.  
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13  
14 In this work, for the first time an integrated and micropackaged  $\mu$ -LED array with  
15  
16 deposited  $\text{SiO}_2/\text{HfO}_2$  interference filter and coupled to an optical fibre ( $\mu$ -LED-IF-OF)  
17  
18 was designed, fabricated, characterised and demonstrated using CE as an excitation  
19  
20 light source for capillary separation techniques with fluorimetric detection.  
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## 25 **Experimental**

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28 **Materials.** For the microfabrication of the LED micro-arrays, GaN substrate material  
29  
30 was purchased from LUMILOG (Sophia Antipolis, France). On the top of this  
31  
32 substrate, epitaxial InGaN layers were deposited using MOVPE (metal organic  
33  
34 vapour phase epitaxy) at the University of Cambridge (UK). Device processing and  
35  
36 deposition of the integrated filter onto the back side of the LED wafer material were  
37  
38 carried out in the cleanroom facilities of the Tyndall National Institute in Cork  
39  
40 (Ireland). The optical fibre was purchased from Thorlabs (Ely, UK). The fibre had a  
41  
42 core diameter of 400  $\mu\text{m}$  diameter and a numerical aperture of 0.37 (part no. BFH37-  
43  
44 400). A perforated silicon platform was used to integrate the  $\mu$ LED chip with the  
45  
46 optical fibre. This MEMS component was also fabricated in the cleanroom facilities of  
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48 the Tyndall National Institute in Cork (Ireland).  
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1 The optical transmission spectrum of the glass slides was measured using a white  
2 light source and an Ocean Optics USB 2000 spectrometer (Ocean Optics, Dunedin,  
3 FL, USA).  
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6  
7 **Chemicals.** Hafnium oxide ( $\text{HfO}_2$ ) was purchased from PI-KEM (Tamworth, UK).  
8  
9 Fluorescein and sodium phosphate were purchased from Sigma Aldrich (Dublin,  
10 Ireland), ammonium acetate, acetic acid, and 8-aminopyrene-1,3,6-trisulfonic acid  
11 (APTS) were obtained from Sigma Aldrich (St Louis, MO, USA). A solution of  
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1×10<sup>-7</sup> mol/L fluorescein was prepared in sodium phosphate (20 mol/L, pH 9).

APTS derivatised maltooligosaccharide ladder standard [12] was provided by Prof. A. Gutman (Horváth Laboratory of Bioseparation Sciences, Institute of Analytical Chemistry, University of Innsbruck, Austria). Water was purified using a Millipore (Bedford, MA, USA) MilliQ water purification system.

**LED micro-array fabrication and characterisation.** LED chips with an area of 1mm<sup>2</sup> were fabricated on FS-GaN wafer material with a peak emission wavelength at 475nm. The fabrication started with the opening of the contact area for the *n*-GaN contacts. A shallow ICP etch was applied to expose the underlying *n*-GaN layer in places where the *n*-GaN contact was to be deposited. A multilayer Ti/Al/Ti/Au based *n*-contact was deposited subsequently on the *n*-contact area by lift-off. The next step was the lithography and subsequent GaN etching to form the parabolic mesa shapes. The mesa sidewalls were then covered with an insulating dielectric. The next level was the deposition of a *p*-contact metallisation on the mesa tops. A thick layer of Cr/Au was then evaporated over both *p* and *n* contact areas as the bondpad metal for wire bonding. The next step was to thin and polish the wafers from 500µm down to 120µm. Once the thinning and polishing were completed, the interference filter was deposited on the back. Around the light emitting window, a multilayer metal was



1 deposited on the back to provide a good thermal contact with the sub-mount. The  
2 integrated interference filter was designed with Essential Macleod commercial  
3 software. The  $\text{HfO}_2/\text{SiO}_2$  layers were deposited using a Leybold Lab600 type  
4 electron- beam evaporation system. 7.5 pairs of  $\text{HfO}_2/\text{SiO}_2$  stack were deposited on  
5 the back side of the LED wafer and simultaneously on some 0.1 mm thick  
6 rectangular glass slides. The optical transmission spectrum of the glass slides was  
7 spectrophotometrically analysed using a white light source. For this measurement,  
8 the glass slides were placed in the light beam, perpendicular to the light beam. The  
9 measurements were normalized to the transmission of an uncoated glass slide.  
10 Once the LED micro-array device was fabricated and the filter deposited at the back,  
11 it was packaged with an optical fibre: it was mounted on silicon submounts with  
12 perforated holes to host the fibre. The device was wire bonded to the  $p$  and  $n$  pads  
13 and two external wires were soldered for connection to the power supply. The fibre  
14 was cleaved at the device end in order to maximize the light coupling. Subsequently,  
15 the fibre was inserted through a 500 $\mu\text{m}$  diameter hole in the silicon submount and the  
16 other end was placed across a detector to measure the maximum light output. Once  
17 the maximum coupling was achieved the fibre (through the hole) was affixed by a  
18 transparent epoxy and cured.

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44 **CE experiments.** CE experiments were carried out using an in-house built CE  
45 system. High voltage power supply (Unimicro Technologies, USA) was used to run  
46 the separation. Separation took place in a fused silica capillary (375  $\mu\text{m}$  O.D., 75  $\mu\text{m}$   
47 I.D.) with 40 cm total length and 35 cm effective length (to detection window). Prior  
48 to the first use, the capillary was flushed with 0.1 M NaOH for 10 minutes followed by  
49 flushing with 0.1 HCl for 10minutes and background electrolyte for 15 minutes. For  
50 CE separation of APTS derivatised maltooligosaccharide ladder, the background  
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1 electrolyte composed of 50mM/L acetate buffer (pH 4.75) mixed with commercial  
2 DNA analysis gel from Agilent Technologies 1:1 (v:v) was prepared as described in  
3 detail elsewhere [12]. The sample was injected hydrodynamically for 15s at a level  
4 difference of 7cm and the separation was carried out at -10kV.  
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## 10 **Results and Discussion**

### 11 **$\mu$ -LED array design**

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14 The schematic of the  $\mu$ -LED array integrated with band-pass filter and optical fibre is  
15 shown in Fig. 1A. An important feature of the design of the individual  $\mu$ -LEDs is a  
16 micro-reflector allowing the  $\mu$ -LED array to emit quasi-collimated light [57]. This is  
17 shown in the red framed inset of Fig.1A with a single  $\mu$ -LED and modelled light  
18 reflection emitted by the LED chip. The dimension of the LED chip is approximately  
19  $1\text{mm}^2$ , which is quite large compared to a standard surface emitting GaN based LED.  
20  
21 The cluster size inside the chip area is approx.  $450\mu\text{m}$  in diameter, consisting of 270  
22 individual  $\mu$ -LED elements. The silicon sub mounts are prepared separately to  
23 provide effective cooling of the  $\mu$ -LED chip. The fabrication involves a self-aligning  
24 process with 5 different photolithography levels as described in detail in the  
25 experimental section. A scanning electron microphotograph of the  $\mu$ -LED array and  
26 a photograph of the whole chip are shown in Fig. 1B and Fig. 1C, respectively.  
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### 47 **Integrated interference filter**

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49 It has been shown that for LED-IF i.e. when using an LED as an excitation light  
50 source for fluorescence detection, the light emission characteristics of the LED can  
51 be improved by suppressing the emission at higher wavelengths, where the analyte  
52 emission intensity is measured, by inserting an excitation low-pass (or suitable band-  
53 pass) filter in front of the LED [45, 51]. Generally speaking, an interference filter is a  
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multilayer system consisting of alternate layers made of materials with high and low refractive index. Hafnium oxide is a suitable material with high refractive index  $\sim 1.97$  and good transparency in the blue part of the spectrum, while silicon oxide has a low refractive index ( $\sim 1.47$ ), yielding the desired high refractive index contrast needed for the interference filters.  $\text{HfO}_2$  is a material commonly used to manufacture UV filters due to its transparency down to about 250nm. State-of-the-art commercial filters (light transmittance over 90% in the “pass” region, very steep transition to the “no-pass” region with extremely low transmission down to ca.  $10^{-5}$ ) may contain hundreds of layers, and include metal layers as well as dielectric layers to form resonant cavities [31]. However, in this proof-of-concept work we chose to deposit only a relatively small number of layers namely 15 (7.5 pairs) of  $\text{HfO}_2$  and  $\text{SiO}_2$  layers each of being  $\frac{1}{4}$  wavelength optical thickness in order to cut-off the wavelengths above 490nm and to keep the level of experimental complexity at a reasonable level, while knowing that the filter characteristics will be inferior to those mentioned for the highest quality commercial filters, however being sufficient to demonstrate the concept.

In Fig 2, the black curve shows the transmission of one set of 7.5 pairs of  $\text{HfO}_2/\text{SiO}_2$  layers, and the red curve shows the transmission for three sets equalling 22.5 pairs (45 layers).

### **Fibre coupling and packaging**

Packaging process is a key element that assures the mechanical rigidity, as optical fibre coupling may be challenging especially in the here investigated research stage devices. As conventional materials used for OF fabrication (glass and silica) are very fragile and difficult to manipulate when the core jacket is removed, a polymer fibre was used in this work. To enable powering the device at electric currents higher than

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10mA, a silicon heat sink at the back of the micro fabricated LED  $\mu$ -array had to be employed. Once the device was fabricated and the filter deposited at the back of the device, it was packaged with an optical fibre, with details of this procedure presented in the experimental section. Photographs of finished ready to use packaged device or assembly are shown in Fig. 3A and 3B.

### **$\mu$ -LED-IF-OF device characterisation**

Two devices – with and without IF – were fabricated and characterized in terms of emission spectra and optical output and its dependency on bias current. The normalized emission spectra are compared in Fig. 4A, showing the emission maxima of the LED at 472nm, with a shift to a maximum at 470 nm. While this relatively small shift was expected for an IF of only 15 layers (7.5 pairs), importantly the relative emission spectrum intensity in the area at the LED emission maximum at 474 nm and above is considerably diminished for the device with the IF. Although this device provides lower light intensity, the light in the undesirable wavelength range (above 490nm) was considerably suppressed.

The optical output of both devices was measured for different bias currents (20, 50 and 75 mA) (Fig. 4B) and as expected the optical power was proportional to the bias current.

To consider the theoretical maximum optical power that could be coupled into the OF, the number of individual  $\mu$ -LED elements (from the total 270 individual  $\mu$ -LED elements arranged in an array of ca. 450  $\mu$ m in diameter, each about 14  $\mu$ m in diameter and optical power of ca. 15  $\mu$ W) have to be considered based on the geometrical design: for the utilised 400  $\mu$ m diameter OF, ca. 219 individual  $\mu$ -LED elements fit into this area, corresponding to a maximum optical power of ca. 3.3 mW. The experimentally measured 225  $\mu$ W from the  $\mu$ -LED-IF-OF device correspond to

1 less than 10% fraction of the calculated maximum, with losses due to the spectral  
2 filtering by the integrated IF but likely also due to suboptimal light coupling efficiency  
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4 in this research device. Although this was not the aim of this proof-of-concept  
5  
6 exploration, it is likely the optical power of these  $\mu$ -LED-IF-OF devices can be  
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8 increased substantially, potentially to ca. 3 mW for this device (based on a 400  $\mu$ m  
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10 diameter OF).  
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### 12 **Flow-through On-capillary detection**

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15 An in-house made fibre optic LED-IF detector as described previously [37] was used  
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17 in this work as shown in the scheme in Fig. 5A. Microphotographs of the optical fibre  
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19 guiding the light into the centre of an empty capillary and of a capillary filled with  
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21 fluorescein solution are shown in Fig. 5B and 5C respectively.  
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27 To maximize the sensitivity the optimal pickup fibre diameter was selected based on  
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29 detection limits of fluorescein. A flow-through method was used to determine LODs  
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31 using manual syringe to flush the liquid through the capillary.  
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36 LODs for the non-filtered device were approx. 8x worse than LOD obtained for  
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38 filtered in the case of all three driving currents as well as pick-up OF diameters. In  
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40 Fig. 5D, the LOD was obtained for fluorescein using a 75 mA driving current. No  
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42 significant difference between LODs obtained for OFs with diameter of 600  $\mu$ m and  
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44 300  $\mu$ m was observed using the filtered device. LODs of fluorescein obtained under  
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46 optimal conditions (driving current and pick-up OF diameter) as well as optical  
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48 powers of  $\mu$ -LED devices are summarized in Tab.1. A comparison with commercially  
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50 available pigtailed LED was performed. The pig-tailed commercial LED (LEDP-  
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52 HB01-B\_PF1000-050(SMA), Doric Lenses, Canada) used for comparison had an  
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54 emission maximum at 470 nm and was equipped with 1000  $\mu$ m diameter excitation  
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1 OF. The measurement showed a significantly lower LOD (ca.100x) due to the  
2 substantially higher optical output provided by the commercial LED which was  
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4 coupled to an OF with 1000  $\mu\text{m}$  diameter.  
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## 7 **Capillary electrophoresis**

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10 Even though the LODs for the devices presented here are higher compared to  
11 commercial pig-tailed LED, the applicability for CE detection was demonstrated by  
12 the separation of APTS-labelled maltooligosaccharide ladder under electrophoretic  
13 conditions as described in detail elsewhere [12] (Fig. 6). In comparison with chip-CE  
14 using a commercial Agilent Bioanalyzer platform with 475 nm LED-IF detection [10],  
15 under optimized condition, the signal to noise ratio obtained for the highest peak  
16 (marked with an asterics“ \* “ in Fig. 6.) of the maltooligosaccharide ladder of here  
17 studied device was only ca. 2x lower (S/N obtained for chip 1332, for CE with  $\mu\text{LED}$   
18 770).  
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## 32 **Conclusions**

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37 New integrated fibre optics light sources based on an LED micro-array integrated and  
38 micropackaged with an interference filter and optical fibre ( $\mu\text{-LED-IF-OF}$ ) have been  
39 designed, microfabricated and successfully tested. They may have a potential as a  
40 new option of integrated solid-state-optical filter-fibre light source with potentially wide  
41 applicability including as an excitation source for capillary and microfluidic separation  
42 techniques. Fabrication of next generation of  $\mu\text{-LED-OF}$  devices will require  
43 application of a higher number of IF layers to enhance the spectral properties of the  
44 interference filter, optimising the OF coupling efficiency, and maximising the  
45 radiometric power output of the individual micro-LEDs.  
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## References

- 1  
2  
3 [1] E. Szoko, T. Tabi, Analysis of biological samples by capillary electrophoresis with  
4  
5 laser induced fluorescence detection, *J. Pharm. Biomed. Anal.*, 53 (2010) 1180-  
6  
7 1192.  
8  
9
- 10 [2] H.M. Tseng, Y. Li, D.A. Barrett, Bioanalytical applications of capillary  
11  
12 electrophoresis with laser-induced native fluorescence detection, *Bioanalysis*, 2  
13  
14 (2010) 1641-1653.  
15  
16
- 17 [3] K. Swinney, D.J. Bornhop, Detection in capillary electrophoresis, *Electrophoresis*,  
18  
19 21 (2000) 1239-1250.  
20  
21
- 22 [4] J.P. Landers, *Handbook of Capillary and Microchip Electrophoresis and*  
23  
24 *Associated Microtechniques*, in: J.P. Landers (Ed.), CRC Press, 2008.  
25  
26
- 27 [5] P. Doble, M. Macka, P. Andersson, P.R. Haddad, Buffered chromate electrolytes  
28  
29 for separation and indirect absorbance detection of inorganic anions in capillary  
30  
31 electrophoresis, *Anal. Commun.*, 34 (1997) 351-353.  
32  
33
- 34 [6] C. Johns, M. Macka, P.R. Haddad, Indirect photomeric detection of anions in  
35  
36 capillary electrophoresis using dyes as probes and electrolytes buffered with an  
37  
38 isoelectric ampholyte, *Electrophoresis*, 21 (2000) 1312-1319.  
39  
40
- 41 [7] J. Grimshaw, Analysis of glycosaminoglycans and their oligosaccharide fragments  
42  
43 by capillary electrophoresis, *Electrophoresis*, 18 (1997) 2408-2414.  
44  
45
- 46 [8] W.J. Mao, C. Thanawiroon, R.J. Linhardt, Capillary electrophoresis for the  
47  
48 analysis of glycosaminoglycans and glycosaminoglycan-derived  
49  
50 oligosaccharides, *Biomed. Chromatogr.*, 16 (2002) 77-94.  
51  
52
- 53 [9] A. Pantazaki, M. Taverna, C. Vidal-Madjar, Recent advances in the capillary  
54  
55 electrophoresis of recombinant glycoproteins, *Anal. Chim. Acta*, 383 (1999)  
56  
57 137-156.  
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64  
65
- [10] K. Kakehi, Application of capillary electrophoresis to glycomics: High-speed screening of carbohydrate chains, *Glycobiology*, 14 (2004) 57.
- [11] Y. Mechref, M.V. Novotny, Glycomic analysis by capillary electrophoresis-mass spectrometry, *Mass Spectrom. Rev.*, 28 (2009) 207-222.
- [12] P. Smejkal, A. Szekrenyes, M. Ryvolova, F. Foret, A. Guttman, F. Bek, M. Macka, Chip-based CE for rapid separation of 8-aminopyrene-1,3,6-trisulfonic acid (APTS) derivatized glycans, *Electrophoresis*, 31 (2010) 3783-3786.
- [13] P.K. Dasgupta, I.Y. Eom, K.J. Morris, J.Z. Li, Light emitting diode-based detectors absorbance, fluorescence and spectroelectrochemical measurements in a planar flow-through cell, *Anal. Chim. Acta*, 500 (2003) 337-364.
- [14] S. Gotz, U. Karst, Recent developments in optical detection methods for microchip separations, *Anal. Bioanal. Chem.*, 387 (2007) 183-192.
- [15] D. Xiao, L. Yan, H.Y. Yuan, S.L. Zhao, X.P. Yang, M.M.F. Choi, CE with LED-based detection: An update, *Electrophoresis*, 30 (2009) 189-202.
- [16] X. Zhang, J.N. Stuart, J.V. Sweedler, Capillary electrophoresis with wavelength-resolved laser-induced fluorescence detection, *Anal. Bioanal. Chem.*, 373 (2002) 332-343.
- [17] X.H.C. Huang, M.A. Quesada, R.A. Mathies, Capillary array electrophoresis using laser-excited confocal fluorescence detection, *Anal. Chem.*, 64 (1992) 967-972.
- [18] D.Y. Chen, H.P. Swerdlow, H.R. Harke, J.Z. Zhang, N.J. Dovichi, Low-cost, high-sensitivity laser-induced fluorescence detection for DNA sequencing by capillary gel-electrophoresis, *Journal of Chromatography*, 559 (1991) 237-246.
- [19] M. Macka, T. Piasecki, P.K. Dasgupta, Light-emitting diodes for analytical chemistry, *Annual Review of Analytical Chemistry*, 2014, pp. 183-207.

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- [20] P.C. Bui D. A.; Hauser, *Anal. Chim. Acta*, in print (2014).
- [21] H.J. Round, A note on carborundum, *Electrical World*, 49 (1907) 309.
- [22] T. Gessmann, E.F. Schubert, High-efficiency AlGaInP light-emitting diodes for solid-state lighting applications, *J. Appl. Phys.*, 95 (2004) 2203-2216.
- [23] H.P. Maruska, Stevenso.Da, W.C. Rhines, preparation of mg-doped GaN diodes exhibiting violet electroluminescence, *Materials Research Bulletin*, 7 (1972) 777-781.
- [24] S. Nakamura, M. Senoh, T. Mukai, High-power InGaN/GaN double-heterostructure violet light-emitting-diodes, *Applied Physics Letters*, 62 (1993) 2390-2392.
- [25] S. Nakamura, M. Senoh, T. Mukai, P-GaN/N-InGaN/N-GaN double-heterostructure blue-light-emitting diodes, *Japanese Journal of Applied Physics Part 2-Letters*, 32 (1993) L8-L11.
- [26] B. Kuswandi, Nuriman, J. Huskens, W. Verboom, Optical sensing systems for microfluidic devices: A review, *Anal. Chim. Acta*, 601 (2007) 141-155.
- [27] A.K. Malik, W. Faubel, Photothermal and light emitting diodes as detectors for trace detection in capillary electrophoresis, *Chem. Soc. Rev.*, 29 (2000) 275-282.
- [28] D. Xiao, S.L. Zhao, H.Y. Yuan, X.P. Yang, CE detector based on light-emitting diodes, *Electrophoresis*, 28 (2007) 233-242.
- [29] M.C. Breadmore, R.D. Henderson, A.R. Fakhari, M. Macka, P.R. Haddad, Separation of Nile Blue-labelled fatty acids by CE with absorbance detection using a red light-emitting diode, *Electrophoresis*, 28 (2007) 1252-1258.

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62  
63  
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65
- [30] L. Krcmova, A. Stjernlof, S. Mehlen, P.C. Hauser, S. Abele, B. Paull, M. Macka, Deep-UV-LEDs in photometric detection: A 255 nm LED on-capillary detector in capillary electrophoresis, *Analyst*, 134 (2009) 2394-2396.
- [31] S. Schmid, M. Macka, P.C. Hauser, UV-absorbance detector for HPLC based on a light-emitting diode, *Analyst*, 133 (2008) 465-469.
- [32] M. Macka, P. Andersson, P.R. Haddad, Linearity evaluation in absorbance detection: The use of light-emitting diodes for on-capillary detection in capillary electrophoresis, *Electrophoresis*, 17 (1996) 1898-1905.
- [33] M. Macka, C. Johns, P. Doble, P.R. Haddad, Indirect photometric detection in CE using buffered electrolytes - Part II, practical rules, *Lc Gc N. Am.*, 19 (2001) 178-188.
- [34] C. Johns, M. Macka, P.R. Haddad, Enhancement of detection sensitivity for indirect photometric detection of anions and cations in capillary electrophoresis, *Electrophoresis*, 24 (2003) 2150-2167.
- [35] C. Johns, M. Macka, P.R. Haddad, Design and performance of a light-emitting diode detector compatible with a commercial capillary electrophoresis instrument, *Electrophoresis*, 25 (2004) 3145-3152.
- [36] N. Vachirapatama, M. Macka, P.R. Haddad, Separation and determination of vanadium in fertiliser by capillary electrophoresis with a light-emitting diode detector, *Anal. Bioanal. Chem.*, 374 (2002) 1082-1085.
- [37] M. Ryvolova, J. Preisler, F. Foret, P.C. Hauser, P. Krasensky, B. Paull, M. Macka, Combined Contactless Conductometric, Photometric, and Fluorimetric Single Point Detector for Capillary Separation Methods, *Anal. Chem.*, 82 (2010) 129-135.

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- [38] M. King, B. Paull, P.R. Haddad, M. Macka, Performance of a simple UV LED light source in the capillary electrophoresis of inorganic anions with indirect detection using a chromate background electrolyte, *Analyst*, 127 (2002) 1564-1567.
- [39] C. Johns, M. Macka, P.R. Haddad, Highly sensitive indirect photometric detection of cations by capillary electrophoresis with the cationic dye chrysoidine, *J. Chromatogr. A*, 997 (2003) 87-94.
- [40] A.R. Fakhari, M.C. Breadmore, M. Macka, P.R. Haddad, Non-aqueous capillary electrophoresis with red light emitting diode absorbance detection for the analysis of basic dyes, *Anal. Chim. Acta*, 580 (2006) 188-193.
- [41] W.W. Bi, S.R. Lei, X.P. Yang, Z.M. Xu, H.Y. Yuan, D. Xiao, M.M.F. Choi, Separation of tyrosine enantiomer derivatives by capillary electrophoresis with light-emitting diode-induced fluorescence detection, *Talanta*, 78 (2009) 1167-1172.
- [42] K.N. Ren, Q.L. Liang, X. Mu, G.A. Luo, Y.M. Wang, Miniaturized high throughput detection system for capillary array electrophoresis on chip with integrated light emitting diode array as addressed ring-shaped light source, *Lab on a Chip*, 9 (2009) 733-736.
- [43] J. Xu, Y. Xiong, S.H. Chen, Y.F. Guan, A lamp light-emitting diode-induced fluorescence detector for capillary electrophoresis, *Talanta*, 76 (2008) 369-372.
- [44] F.B. Yang, J.Z. Pan, T. Zhang, Q. Fang, A low-cost light-emitting diode induced fluorescence detector for capillary electrophoresis based on an orthogonal optical arrangement, *Talanta*, 78 (2009) 1155-1158.
- [45] E.P. de Jong, C.A. Lucy, Low-picomolar limits of detection using high-power light-emitting diodes for fluorescence, *Analyst*, 131 (2006) 664-669.

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- [46] M. Ryvolova, J. Preisler, D. Brabazon, M. Macka, Portable capillary-based (non-chip) capillary electrophoresis, *Trac-Trends Anal. Chem.*, 29 (2010) 339-353.
- [47] H.F. Li, Z.F. Chen, J.M. Lin, Optical Fiber Coupling of Light-Emitting Diode Induced Fluorescence Detection for Microchip Electrophoresis, *Chinese Journal of Analytical Chemistry*, 36 (2008) 1587-1591.
- [48] T. Zhang, Q. Fang, S.L. Wang, L.F. Qin, P. Wang, Z.Y. Wu, Z.L. Fang, Enhancement of signal-to-noise level by synchronized dual wavelength modulation for light emitting diode fluorimetry in a liquid-core-waveguide microfluidic capillary electrophoresis system, *Talanta*, 68 (2005) 19-24.
- [49] C.J. Evenhuis, R.M. Guijt, M. Macka, P.R. Haddad, Determination of inorganic ions using microfluidic devices, *Electrophoresis*, 25 (2004) 3602-3624.
- [50] T. Piasecki, M. Macka, B. Paull, D. Brabazon, Numerical model for light propagation and light intensity distribution inside coated fused silica capillaries, *Optics and Lasers in Engineering*, 49 (2011) 924-931.
- [51] E.P. de Jong, C.A. Lucy, Spectral filtering of light-emitting diodes for fluorescence detection, *Anal. Chim. Acta*, 546 (2005) 37-45.
- [52] C. Moser, T. Mayr, I. Klimant, Filter cubes with built-in ultrabright light-emitting diodes as exchangeable excitation light sources in fluorescence microscopy, *J. Microsc.-Oxf.*, 222 (2006) 135-140.
- [53] G.A.B. Heras, M.C. Breadmore, C. Johns, J.P. Hutchinson, E.F. Hilder, P. Lopez-Mahia, P.R. Haddad, Indirect photometric detection of anions in nonaqueous capillary electrophoresis employing Orange G as probe and a light-emitting diode-based detector, *Electrophoresis*, 29 (2008) 3032-3037.
- [54] G. Ryu, J.S. Huang, O. Hofmann, C.A. Walshe, J.Y.Y. Sze, G.D. McClean, A. Mosley, S.J. Rattle, J.C. deMello, A.J. deMello, D.D.C. Bradley, Highly sensitive

1 fluorescence detection system for microfluidic lab-on-a-chip, Lab on a Chip, 11  
2 (2011) 1664-1670.  
3

4 [55] L. Campagnola, H. Wang, M.J. Zyka, Fiber-coupled light-emitting diode for  
5 localized photo stimulation of neurons expressing channelrhodopsin-2, J.  
6 Neurosci. Methods, 169 (2008) 27-33.  
7  
8  
9

10 [56] S. Ek, B. Anderson, S. Svanberg, Compact fiber-optic fluorosensor employing  
11 light-emitting ultraviolet diodes as excitation sources, Spectroc. Acta Pt. B-  
12 Atom. Spectr., 63 (2008) 349-353.  
13  
14  
15  
16  
17  
18

19 [57] S. Tanriseven, P. Maaskant, B. Corbett, Broadband quantum dot micro-light-  
20 emitting diodes with parabolic sidewalls, Applied Physics Letters, 92 (2008).  
21  
22  
23  
24  
25  
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27  
28  
29  
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Tab. 1: Parameters of the fibre LED sources and resulting LOD values.

Conditions:

	Current [mA]	Optical power [mW]	Pick-up fibre diameter [ $\mu\text{m}$ ]	LOD Fluorescein [mol/L]
Without IF	75	0.400	600	$9 \times 10^{-8}$
With IF	75	0.225	600	$1 \times 10^{-8}$
Pig-tailed commercial LED  Exc. OF  1000 $\mu\text{m}$	500	8	600	$8 \times 10^{-10}$

## Figure captions

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7 **Fig. 1:** A) Scheme of  $\mu$ -LED with modelled light reflection, Inset: scheme of  $\mu$ -LED  
8 device with integrated IF and coupled to OF, B) SEM photograph of the  
9  $\mu$ -LED array, C) photograph of the  $\mu$ -LED chip.

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17 **Fig. 2:** Dependence of transmittance on number of deposited  $\text{HfO}_2/\text{SiO}_2$  layers.

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23 **Fig. 3:** A) Photograph of the  $\mu$ -LED array integrated with band-pass filter and optical  
24 fibre device, B) Photograph of device in use.

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31 **Fig. 4:** A) Emission spectra of filtered and non-filtered device, B) Optical power vs.  
32 driving current for filtered (S19) and non-filtered device (S16).

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40 **Fig. 5:** A) Scheme of CE- $\mu$ -LED-IF-OF setup, B) Photograph of OF focused to the  
41 empty capillary C) Photograph of OF focused to the capillary flushed with  
42 fluorescein, D) Limits of detection of fluorescein obtained using different  
43 pick-up optical fibres (driving current 75 mA).

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53 **Fig. 6:** Electropherogram of APTS-labelled maltooligosaccharide ladder (50 mg/ml)  
54 analysed by CE- $\mu$ -LED-IF-OF, Conditions: BGE: 50 mM acetate buffer (pH  
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4.75) mixed with commercial DNA analysis gel from Agilent Technologies

1:1 (v:v), injection: hydrodynamic (15 s, 7 cm), separation voltage: -10 kV.

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Figures:

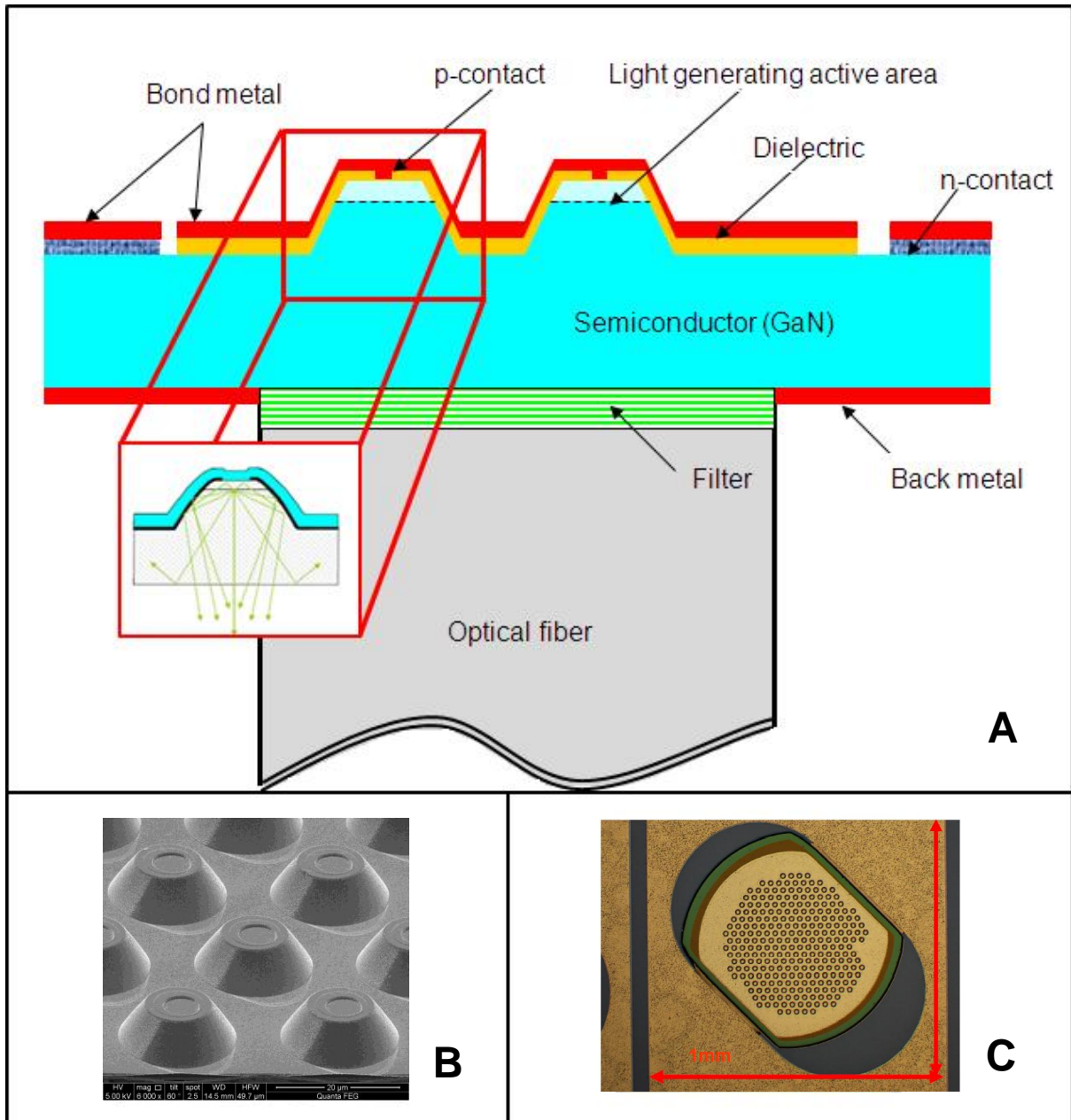


Fig. 1

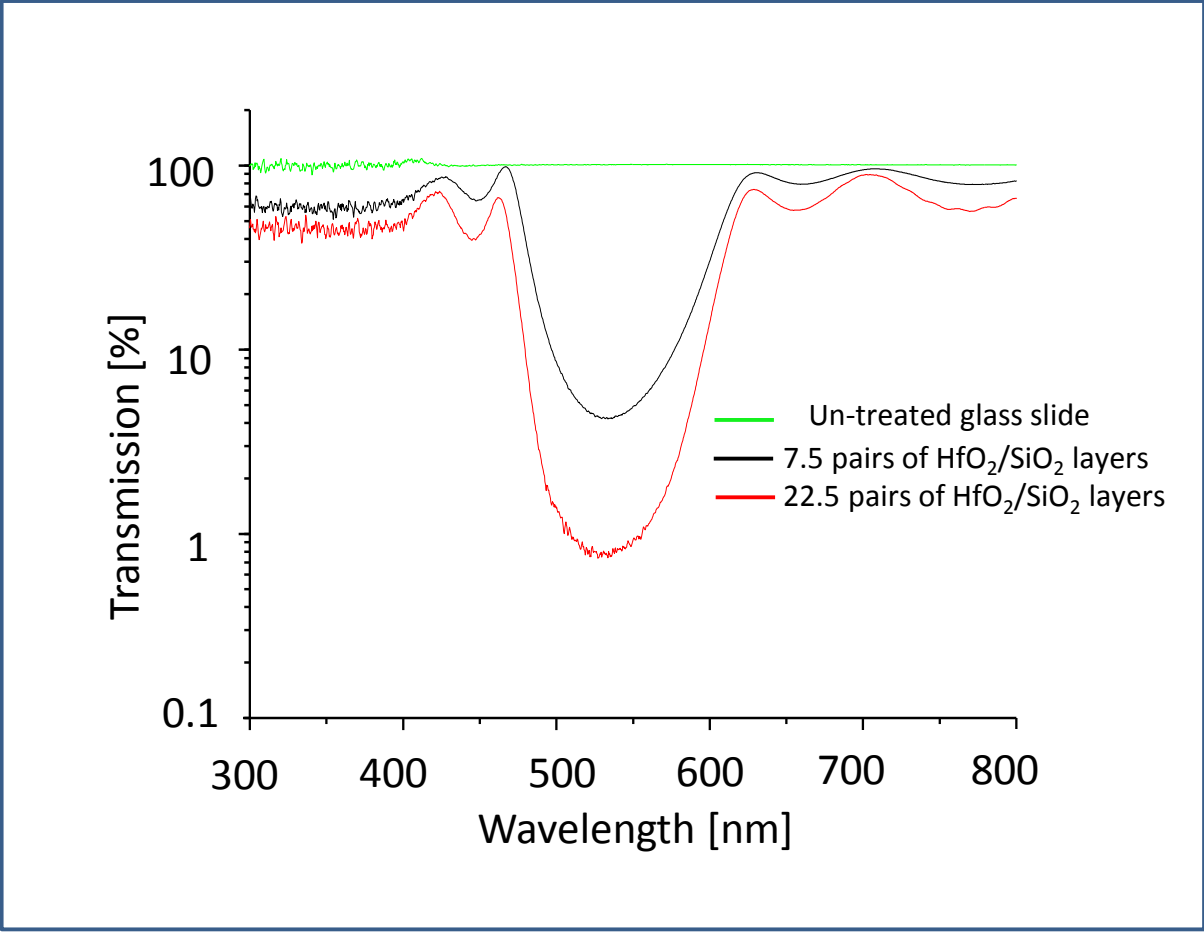
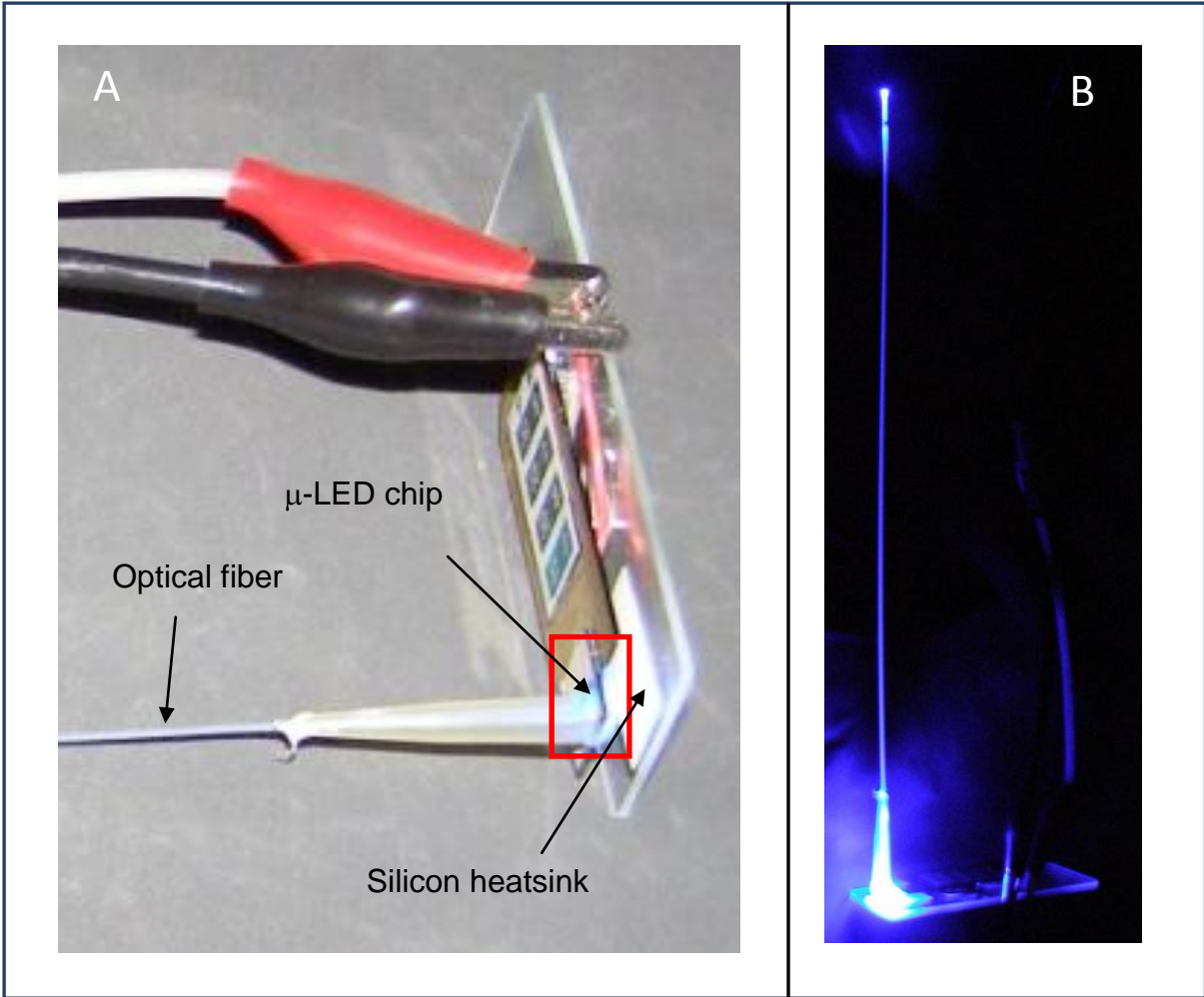


Fig. 2



**Fig. 3**

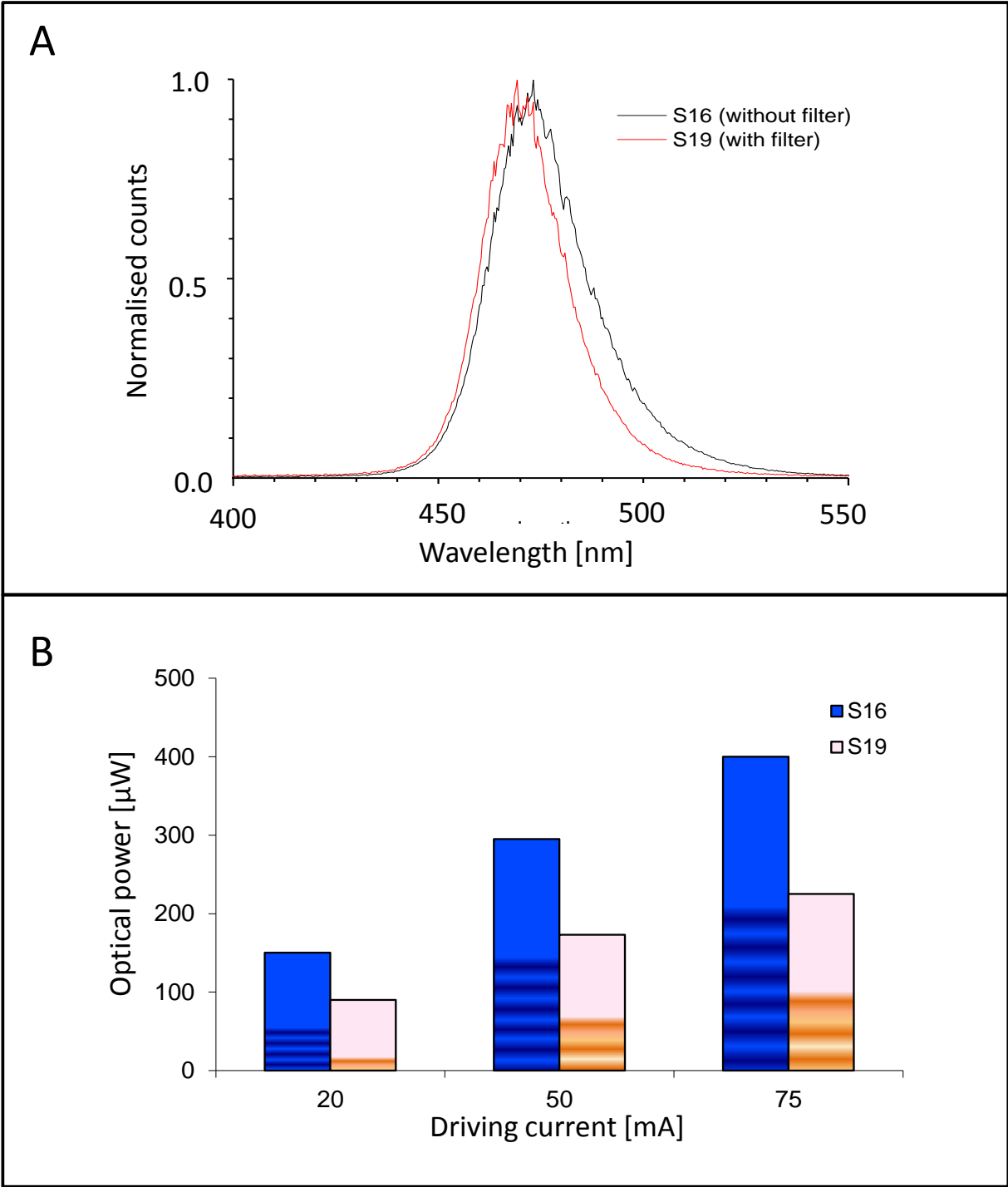
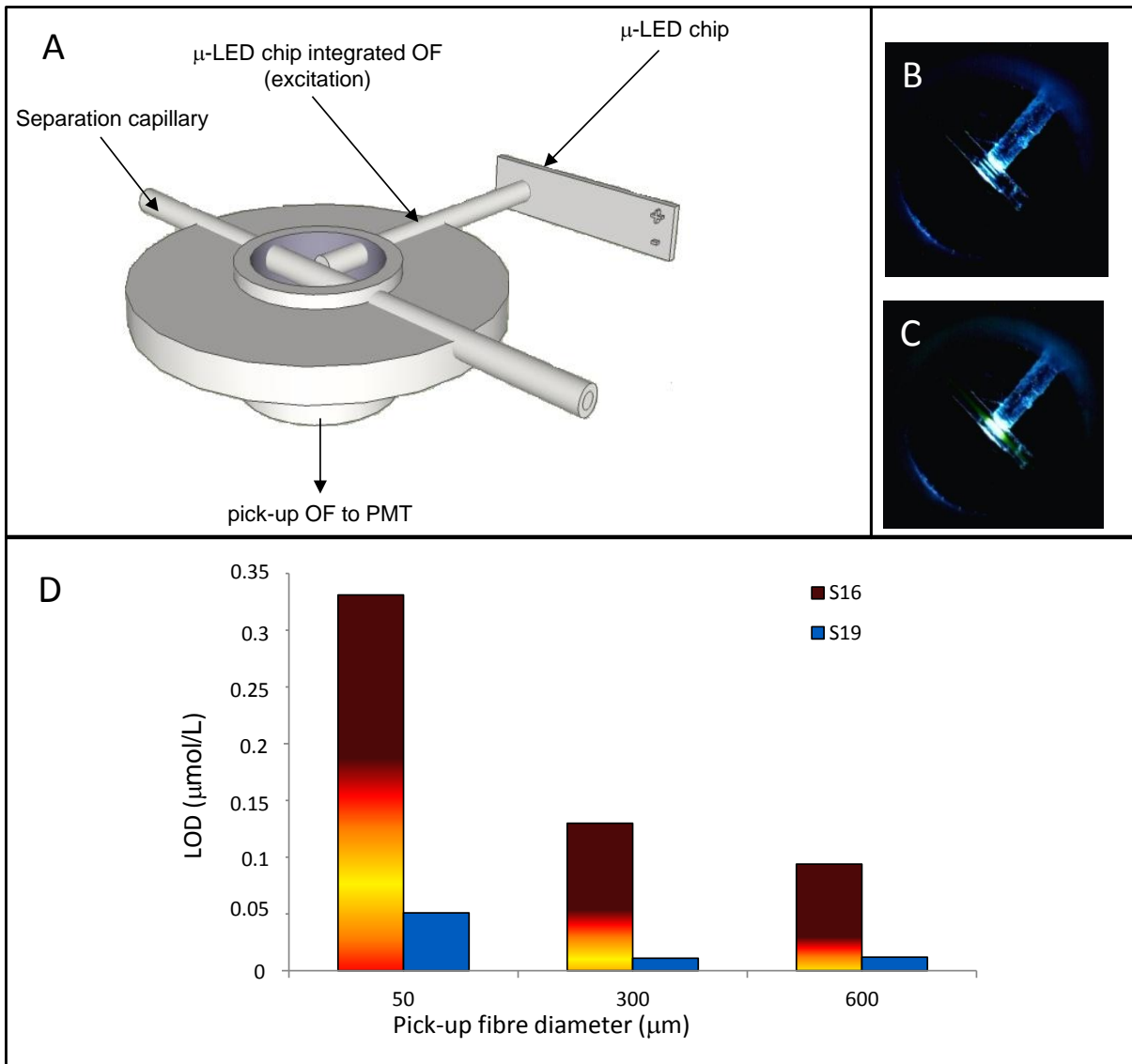
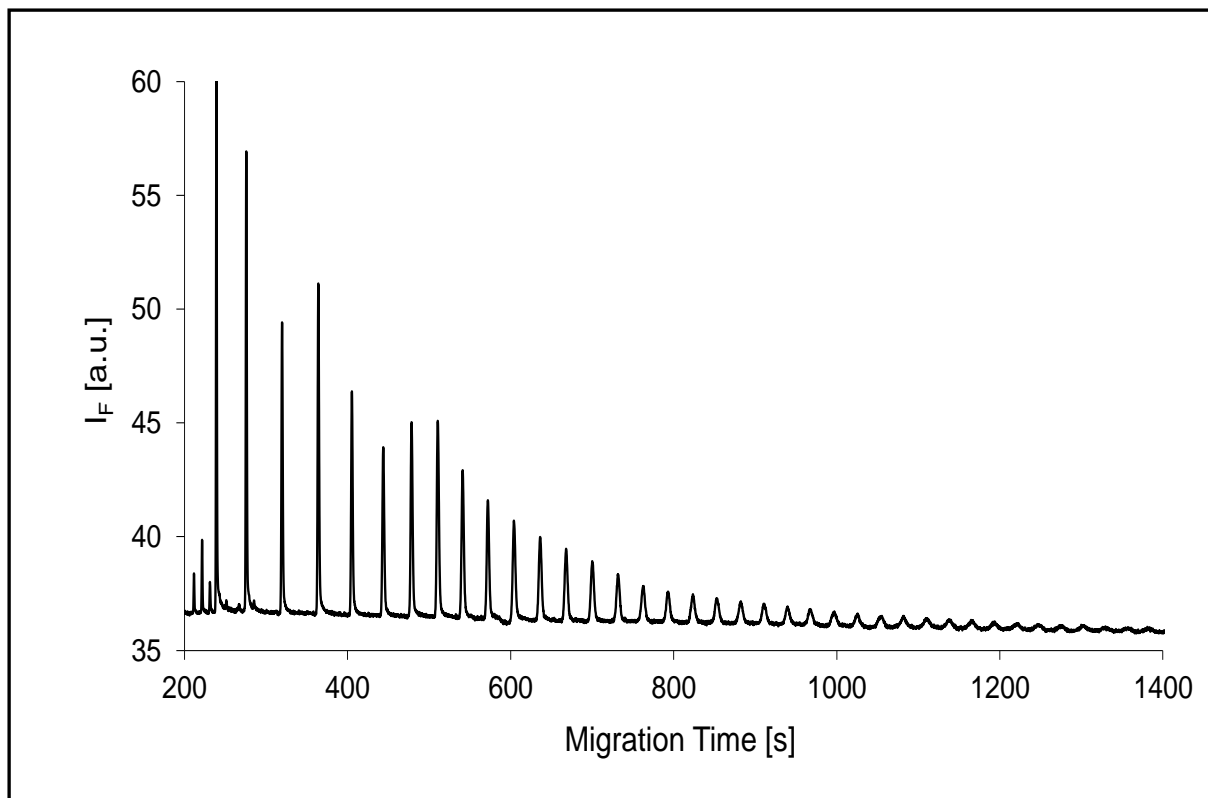


Fig. 4



**Fig. 5**



**Fig. 6**