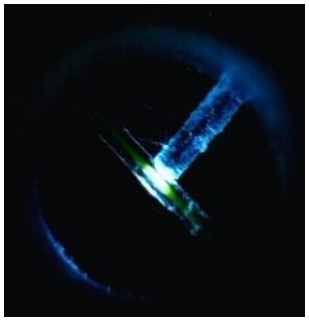
*Graphical Abstract

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

Markéta Vaculovičová[1,3,4], Mahbub Akther[2], Pleun Maaskant[2], Dermot Brabazon[1,5], and Mirek Macka[3*]

Graphical Abstract (please choose)



A new generation of integrated miniaturized fibre-coupled solid-state light sources based on microfabricated light emitting diode micro-array (μ-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.

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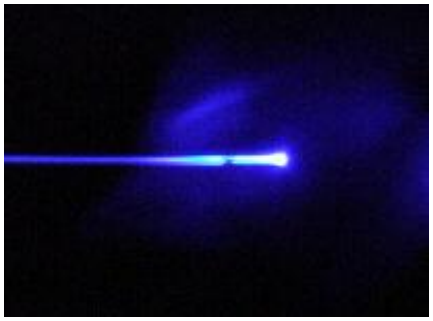

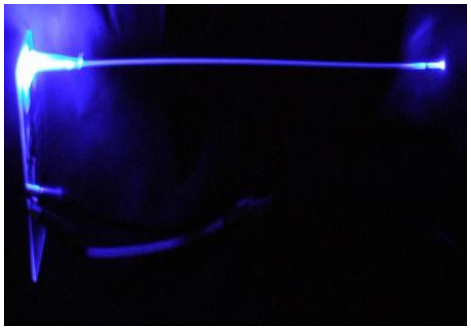

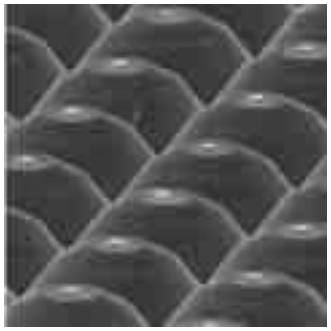

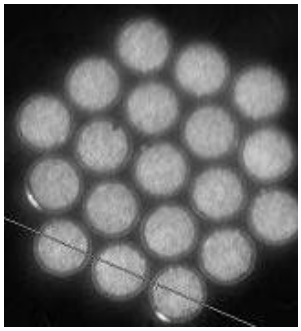

Fibre coupled micro-LED array light source with integrated band-pass filter for fluorescence detection in miniaturised analytical systems

Markéta Vaculovičová[1,3,4], Mahbub Akther[2], Pleun Maaskant[2], Dermot Brabazon[1,5], and Mirek Macka[3*]

Highlights

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

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

Markéta Vaculovičová[1,3,4], Mahbub Akther[2], Pleun Maaskant[2], Dermot Brabazon[1,5], and Mirek Macka[3*]

1) Irish Separation Science Cluster, National Centre for Sensor Research and School of Chemical Sciences, Dublin City University, Dublin 9, Ireland.

2) Tyndall National Institute, Dyke parade, University College Cork, Cork, Ireland.

3) Australian Centre for Research on Separation Science and School of Chemistry, University of Tasmania, Private Bag 75, Hobart 7001, Australia.

4) Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic

5) Advanced Processing Research Centre, Dublin City University, Dublin 9, Ireland.

*Correspondence:

Prof. Mirek Macka mirek.macka@utas.edu.au

Key words:

Micro-light emitting diode array; light source; optical fibre; capillary electrophoresis; fluorescence detection; separations.

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 (µ-LED) array with a custom band-pass optical interference filter (IF) deposited at the back of the LED substrate. The GaN µ-LED array consisted of 270 individual µ-LED elements with peak emission at 470nm, each about 14µm in diameter and operated as a single unit. Light was extracted through the transparent substrate material, and coupled to an optical fibre (400µm in diameter, numerical aperture NA = 0.37), to form an integrated µ-LED-IF-OF light source component. This packaged µ-LED-IF-OF light source emitted approximately 225µW 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_2/HfO_2 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 1×10^{-8} mol/L obtained under optimised conditions. The µ-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

rates, while their main deficiency is the lack of powerful enough emitters in the deep-UV (below 300nm) spectral region [26-28].

In the area of on-capillary detection including CE, LEDs have been used in miniaturised low-cost detection systems, both photometric [29-40] and LED induced fluorimetric (LED-IF) [41-45], with advantages especially for portable CE instruments [46]. A number of LED-IF detection designs for microfluidic chip-based CE [47-50] systems have been reported as well.

As LEDs, which otherwise would be more popular as miniaturised light sources for portable devices, are semi-monochromatic and naturally possess bandwidth of approx. 20-50 nm, when used as excitation sources in LED-IF for optimal performance and low baseline noise they have to be combined with low-pass filters [45, 51, 52].

Miniaturization of the individual optical components (light source, optical filters, lenses, mirrors etc.) and their assembly into a functioning optical system is the limiting factor when creating a miniaturized CE-LIF design either LIF or LED-IF. As LEDs have wide spatial light distribution, focusing optics is usually required for optimal sensitivity of LED-IF detection [45, 53, 54]. Optical fibres directly coupled to LEDs (pigtailed LEDs) [47, 55, 56] are a very popular alternative in creating a spatially directed LED light source. Fibre-coupled LED sources with integrated interference filter could be an attractive integrated micropackaged fibre-coupled light source component for miniaturised optical detection systems.

The μ-LED arrays [57] provide a quasi-collimated light emission and therefore can have a good coupling efficiency to optical fibres. When integrated and micropackaged with an optical fibre, an interference filter can be inserted between the μ-LED array and the fibre, by depositing this filter on the back surface of the

substrate emitting LED. Such LED-based integrated and micropackaged optical fibre light sources emitting from the fibre spectrally filtered light, could become a new option in custom designed optical fibre-coupled light sources for fluorescence detection in on-capillary and microfluidic chip separation systems. The authors to their best knowledge are not aware of any other similar work on integrated micropackaged fibre-coupled µ-LED array light sources.

In this work, for the first time an integrated and micropackaged µ-LED array with deposited SiO_2/HfO_2 interference filter and coupled to an optical fibre (µ-LED-IF-OF) was designed, fabricated, characterised and demonstrated using CE as an excitation light source for capillary separation techniques with fluorimetric detection.

Experimental

Materials. For the microfabrication of the LED micro-arrays, GaN substrate material was purchased from LUMILOG (Sophia Antipolis, France). On the top of this substrate, epitaxial InGaN layers were deposited using MOVPE (metal organic vapour phase epitaxy) at the University of Cambridge (UK). Device processing and deposition of the integrated filter onto the back side of the LED wafer material were carried out in the cleanroom facilities of the Tyndall National Institute in Cork (Ireland). The optical fibre was purchased from Thorlabs (Ely, UK). The fibre had a core diameter of 400 µm diameter and a numerical aperture of 0.37 (part no. BFH37-400). A perforated silicon platform was used to integrate the µLED chip with the optical fibre. This MEMS component was also fabricated in the cleanroom facilities of the Tyndall National Institute in Cork (Ireland).

The optical transmission spectrum of the glass slides was measured using a white light source and an Ocean Optics USB 2000 spectrometer (Ocean Optics, Dunedin, FL, USA).

Chemicals. Hafnium oxide (HfO_2) was purchased from PI-KEM (Tamworth, UK). Fluorescein and sodium phosphate were purchased from Sigma Aldrich (Dublin, Ireland), ammonium acetate, acetic acid, and 8-aminopyrene-1,3,6-trisulfonic acid (APTS) were obtained from Sigma Aldrich (St Louis, MO, USA). A solution of 1×10^{-7} 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 $1mm^2$ 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

deposited on the back to provide a good thermal contact with the sub-mount. The integrated interference filter was designed with Essential Macleod commercial software. The HfO_2/SiO_2 layers were deposited using a Leybold Lab600 type electron- beam evaporation system. 7.5 pairs of HfO_2/SiO_2 stack were deposited on the back side of the LED wafer and simultaneously on some 0.1 mm thick rectangular glass slides. The optical transmission spectrum of the glass slides was spectrophotometrically analysed using a white light source. For this measurement, the glass slides were placed in the light beam, perpendicular to the light beam. The measurements were normalized to the transmission of an uncoated glass slide. Once the LED micro-array device was fabricated and the filter deposited at the back, it was packaged with an optical fibre: it was mounted on silicon submounts with perforated holes to host the fibre. The device was wire bonded to the *p* and *n* pads and two external wires were soldered for connection to the power supply. The fibre was cleaved at the device end in order to maximize the light coupling. Subsequently, the fibre was inserted through a 500μm diameter hole in the silicon submount and the other end was placed across a detector to measure the maximum light output. Once the maximum coupling was achieved the fibre (through the hole) was affixed by a transparent epoxy and cured.

CE experiments. CE experiments were carried out using an in-house built CE system. High voltage power supply (Unimicro Technologies, USA) was used to run the separation. Separation took place in a fused silica capillary (375 μm O.D., 75 μm I.D.) with 40 cm total length and 35 cm effective length (to detection window). Prior to the first use, the capillary was flushed with 0.1 M NaOH for 10 minutes followed by flushing with 0.1 HCl for 10minutes and background electrolyte for 15 minutes. For CE separation of APTS derivatised maltooligosaccharide ladder, the background

electrolyte composed of 50mmo/L acetate buffer (pH 4.75) mixed with commercial DNA analysis gel from Agilent Technologies 1:1 (v:v) was prepared as described in detail elsewhere [12]. The sample was injected hydrodynamically for 15s at a level difference of 7cm and the separation was carried out at -10kV.

Results and Discussion

µ-LED array design

The schematic of the µ-LED array integrated with band-pass filter and optical fibre is shown in Fig. 1A. An important feature of the design of the individual µ-LEDs is a micro-reflector allowing the µ-LED array to emit quasi-collimated light [57]. This is shown in the red framed inset of Fig.1A with a single µ-LED and modelled light reflection emitted by the LED chip. The dimension of the LED chip is approximately 1mm^2, which is quite large compared to a standard surface emitting GaN based LED. The cluster size inside the chip area is approx. 450µm in diameter, consisting of 270 individual µ-LED elements. The silicon sub mounts are prepared separately to provide effective cooling of the µ-LED chip. The fabrication involves a self-aligning process with 5 different photolithography levels as described in detail in the experimental section. A scanning electron microphotograph of the µ-LED array and a photograph of the whole chip are shown in Fig. 1B and Fig. 1C, respectively.

Integrated interference filter

It has been shown that for LED-IF i.e. when using an LED as an excitation light source for fluorescence detection, the light emission characteristics of the LED can be improved by suppressing the emission at higher wavelengths, where the analyte emission intensity is measured, by inserting an excitation low-pass (or suitable band-pass) filter in front of the LED [45, 51]. Generally speaking, an interference filter is a

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 ~1.97 and good transparency in the blue part of the spectrum, whilesilicon oxide has a low refractive index (~1.47), yielding the desired high refractive index contrast needed for the interference filters. 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 HfO_2 and SiO_2 layers each of being ¼ 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 HfO_2/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

10mA, a silicon heat sink at the back of the micro fabricated LED µ-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.

µ-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 µ-LED elements (from the total 270 individual µ-LED elements arranged in an array of ca. 450 µm in diameter, each about 14 µm in diameter and optical power of ca. 15 µW) have to be considered based on the geometrical design: for the utilised 400 µm diameter OF, ca. 219 individual µ-LED elements fit into this area, corresponding to a maximum optical power of ca. 3.3 mW. The experimentally measured 225 µW from the µ-LED-IF-OF device correspond to

less than 10% fraction of the calculated maximum, with losses due to the spectral filtering by the integrated IF but likely also due to suboptimal light coupling efficiency in this research device. Although this was not the aim of this proof-of-concept exploration, it is likely the optical power of these μ-LED-IF-OF devices can be increased substantially, potentially to ca. 3 mW for this device (based on a 400 μm diameter OF).

Flow-through On-capillary detection

An in-house made fibre optic LED-IF detector as described previously [37] was used in this work as shown in the scheme in Fig. 5A. Microphotographs of the optical fibre guiding the light into the centre of an empty capillary and of a capillary filled with fluorescein solution are shown in Fig. 5B and 5C respectively.

To maximize the sensitivity the optimal pickup fibre diameter was selected based on detection limits of fluorescein. A flow-through method was used to determine LODs using manual syringe to flush the liquid through the capillary.

LODs for the non-filtered device were approx. 8x worse than LOD obtained for filtered in the case of all three driving currents as well as pick-up OF diameters. In Fig. 5D, the LOD was obtained for fluorescein using a 75 mA driving current. No significant difference between LODs obtained for OFs with diameter of 600 μm and 300 μm was observed using the filtered device. LODs of fluorescein obtained under optimal conditions (driving current and pick-up OF diameter) as well as optical powers of μ-LED devices are summarized in Tab.1. A comparison with commercially available pigtailed LED was performed. The pig-tailed commercial LED (LEDP-HB01-B_PF1000-050(SMA), Doric Lenses, Canada) used for comparison had an emission maximum at 470 nm and was equipped with 1000 μm diameter excitation

OF. The measurement showed a significantly lower LOD (ca.100x) due to the substantially higher optical output provided by the commercial LED which was coupled to an OF with 1000 μm diameter.

Capillary electrophoresis

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

Conclusions

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

Acknowledgement

MR and MM gratefully acknowledge the Marie Curie Excellence Grants grant and fellowship (MEXT-CT-2004-014361), Science Foundation Ireland (SFI) National Access Programme (NAP 171) for financial support of this work, and the Australian Research Council Future Fellowship (FT120100559). The authors would also like to acknowledge Andrasz Guttman (Horváth Laboratory of Bioseparation Sciences, Institute of Analytical Chemistry, University of Innsbruck, Austria) for providing the APTS derivatised oligosaccharide sample.

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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 [μm]	LOD Fluorescein [mol/L]
Without IF	75	0.400	600	9×10^{-8}
With IF	75	0.225	600	1×10^{-8}
Pig-tailed commercial LED Exc. OF 1000 μm	500	8	600	8×10^{-10}

Figure captions

Fig. 1: A) Scheme of μ-LED with modelled light reflection, Inset: scheme of μ-LED device with integrated IF and coupled to OF, B) SEM photograph of the μ-LED array, C) photograph of the μ-LED chip.

Fig. 2: Dependence of transmittance on number of deposited HfO_2/SiO_2 layers.

Fig. 3: A) Photograph of the μ-LED array integrated with band-pass filter and optical fibre device, B) Photograph of device in use.

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

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

Fig. 6: Electropherogram of APTS-labelled maltooligosaccharide ladder (50 mg/ml) analysed by CE-μ-LED-IF-OF, Conditions: BGE: 50 mM acetate buffer (pH

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.

Figures:

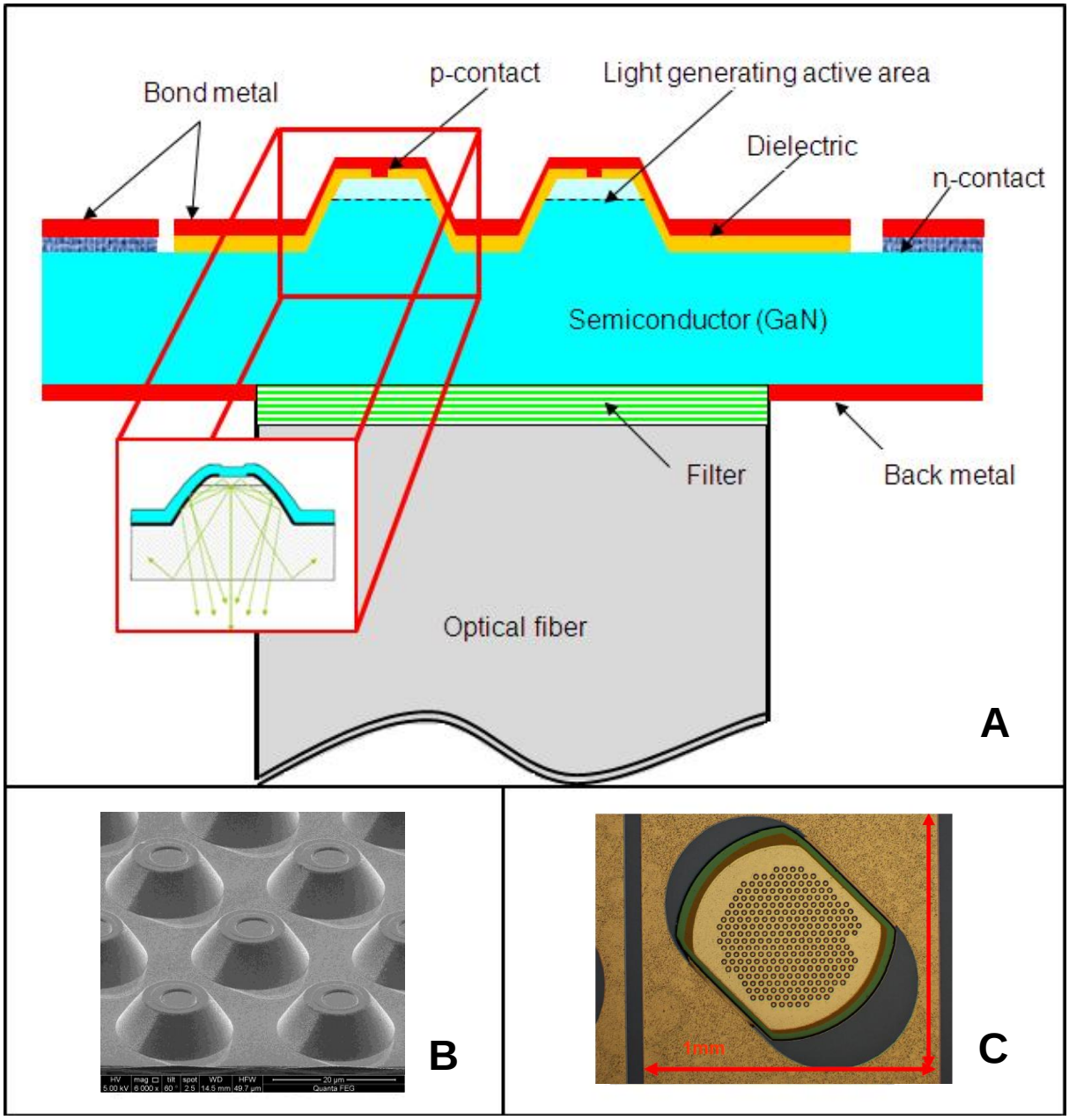

Fig. 1

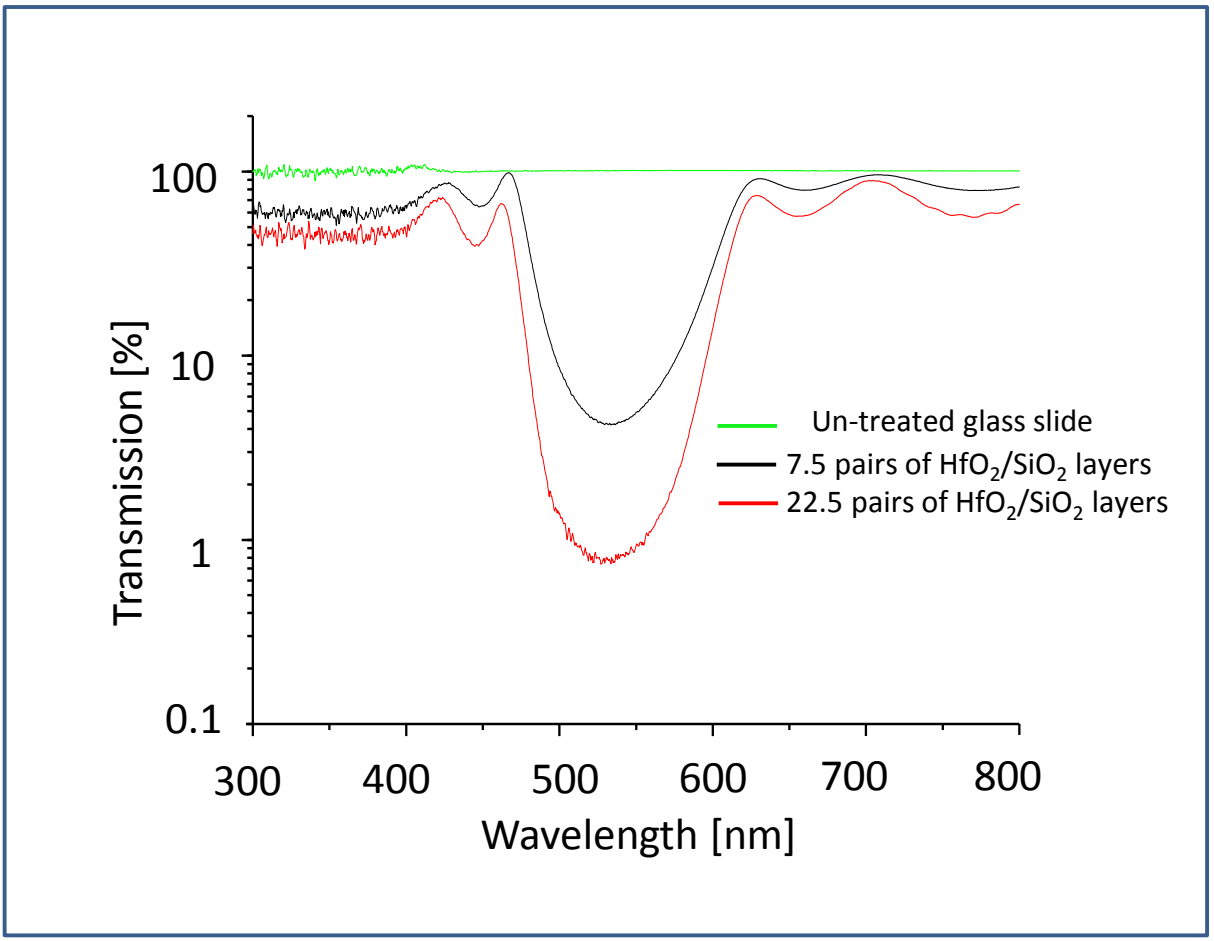
100
10
1
0.1
Transmission [%]
300
400
500
600
700
800
Wavelength [nm]
Un-treated glass slide
7.5 pairs of HfO2/SiO2 layers
22.5 pairs of HfO2/SiO2 layers

Fig. 2

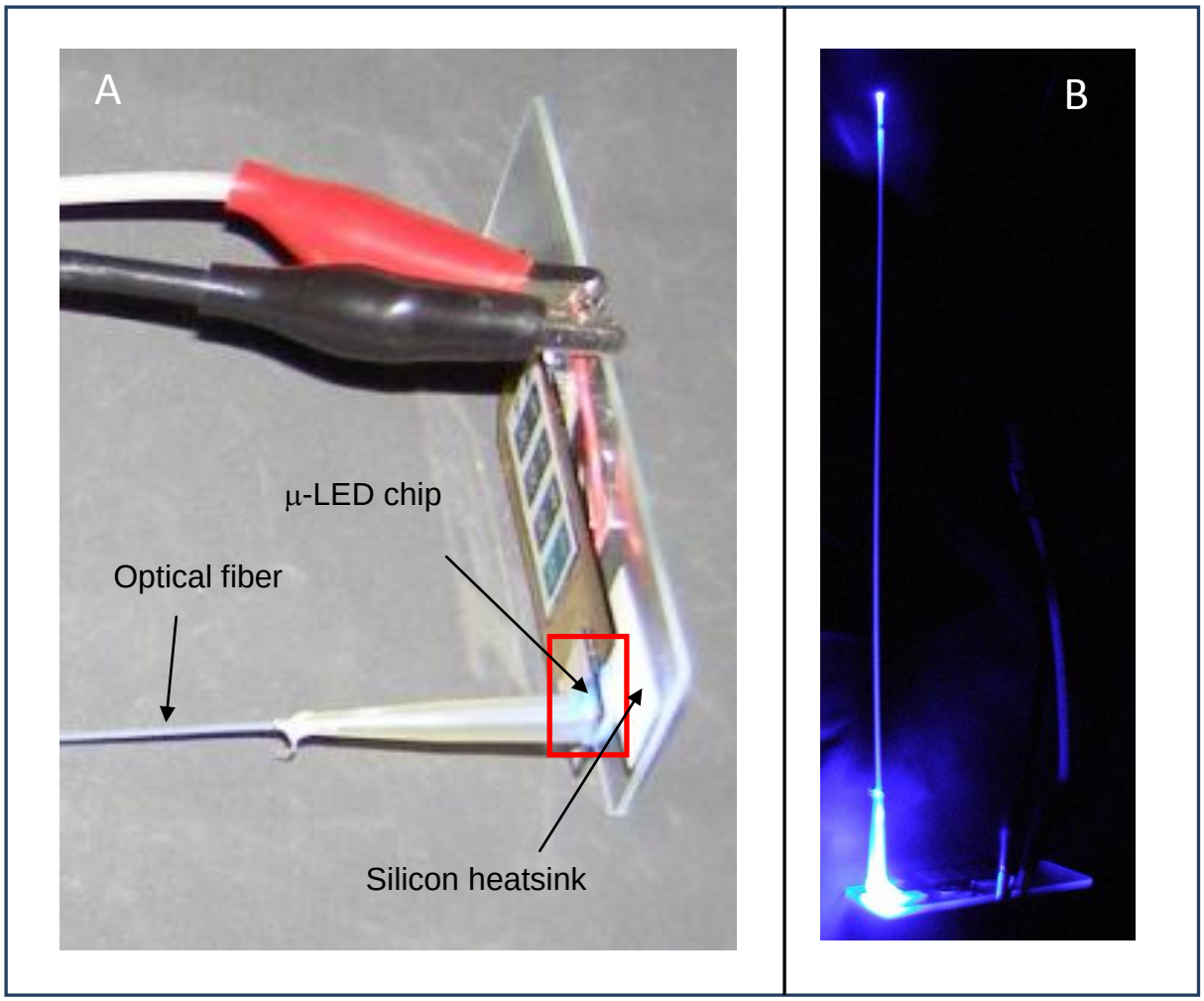

Fig. 3

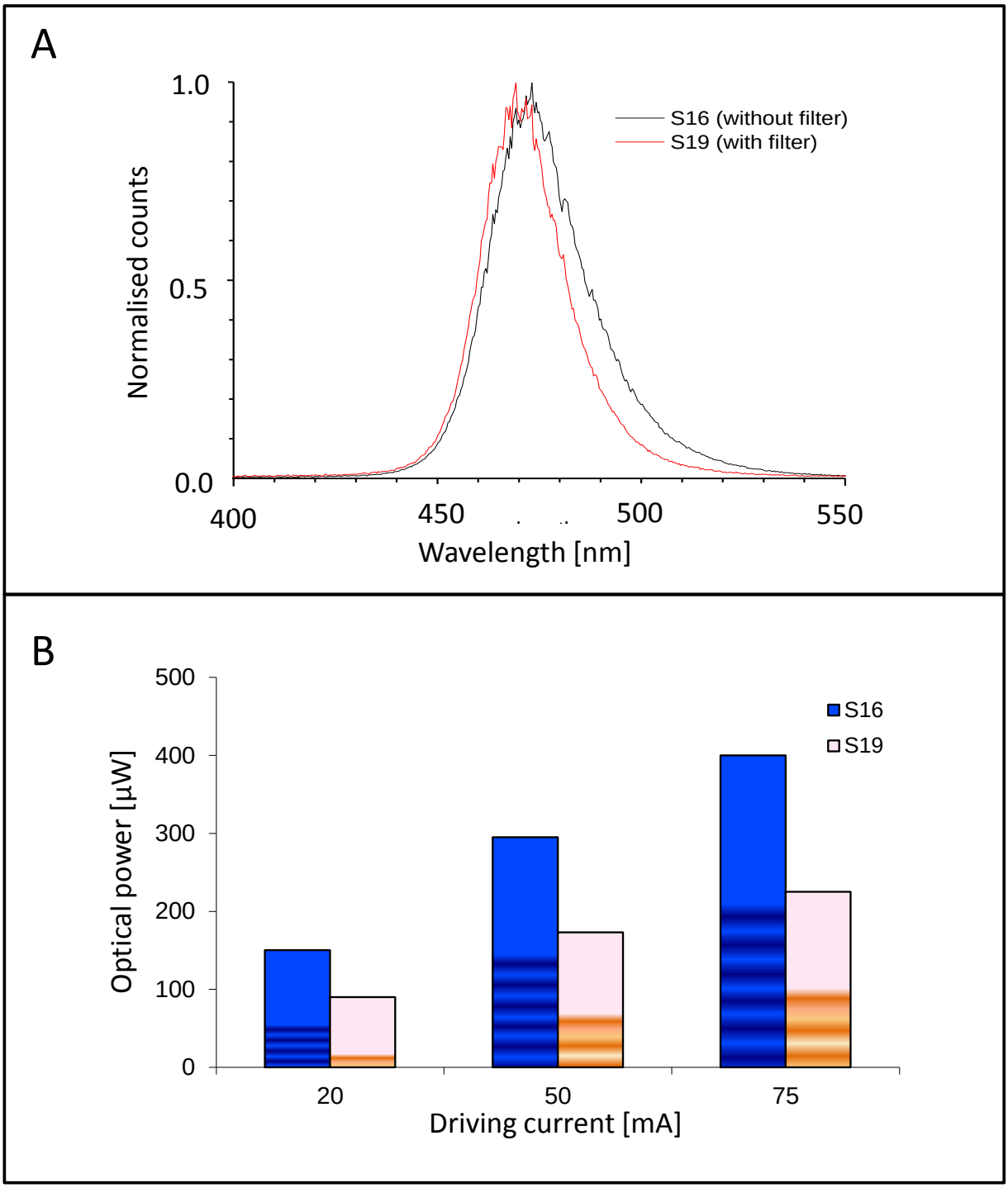
A
1.0
0.5
0.0
Normalised counts
400
450
500
550
Wavelength [nm]
S16 (without filter)
S19 (with filter)
B
500
400
300
200
100
0
Optical power [µW]
20
50
75
Driving current [mA]
S16
S19

Fig. 4

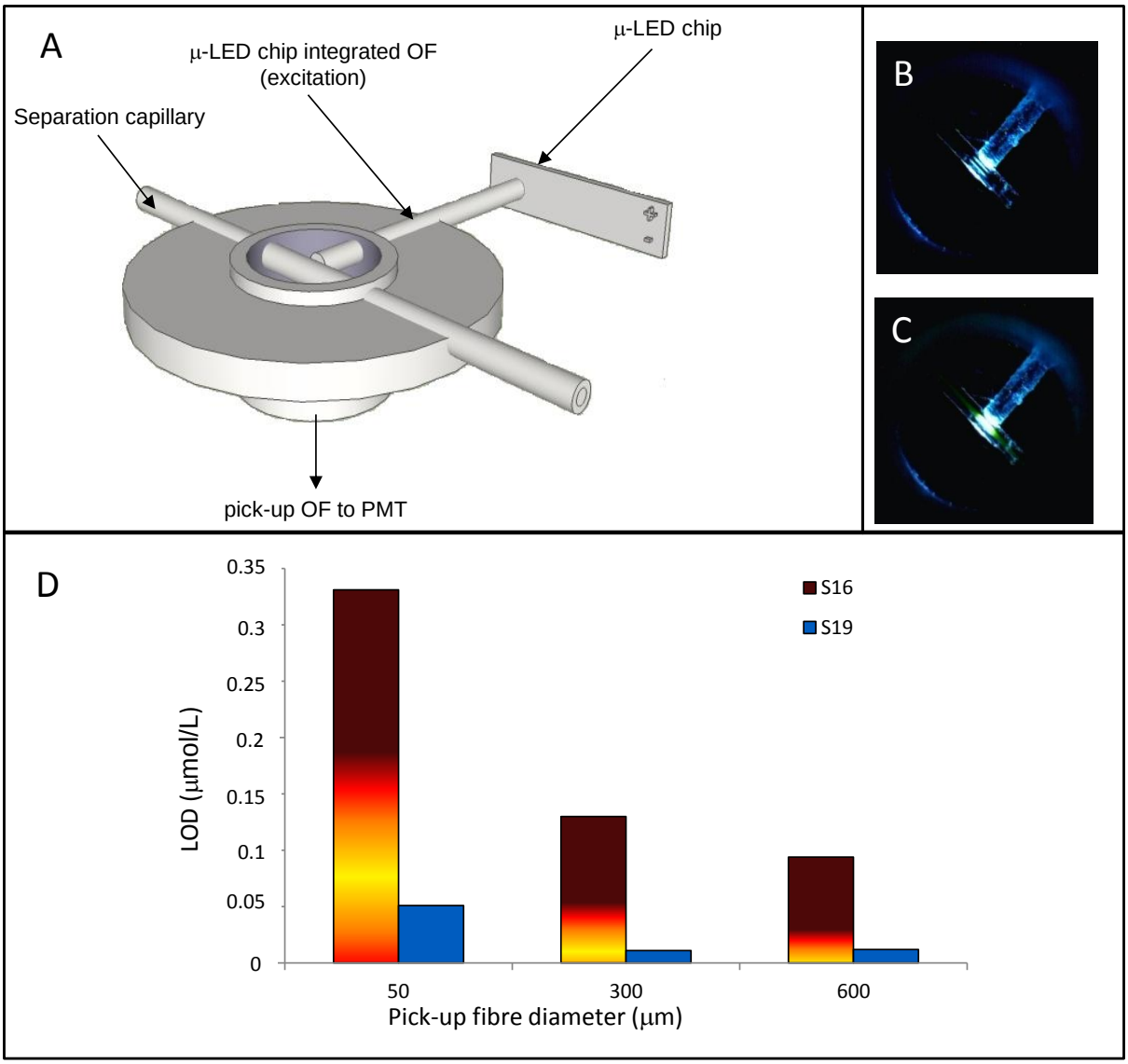
A
μ-LED chip
μ-LED chip integrated OF
(excitation)
Separation capillary
pick-up OF to PMT
B
C
D
S16
S19
LOD (μmol/L)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
50
300
600
Pick-up fibre diameter (μm)

Fig. 5

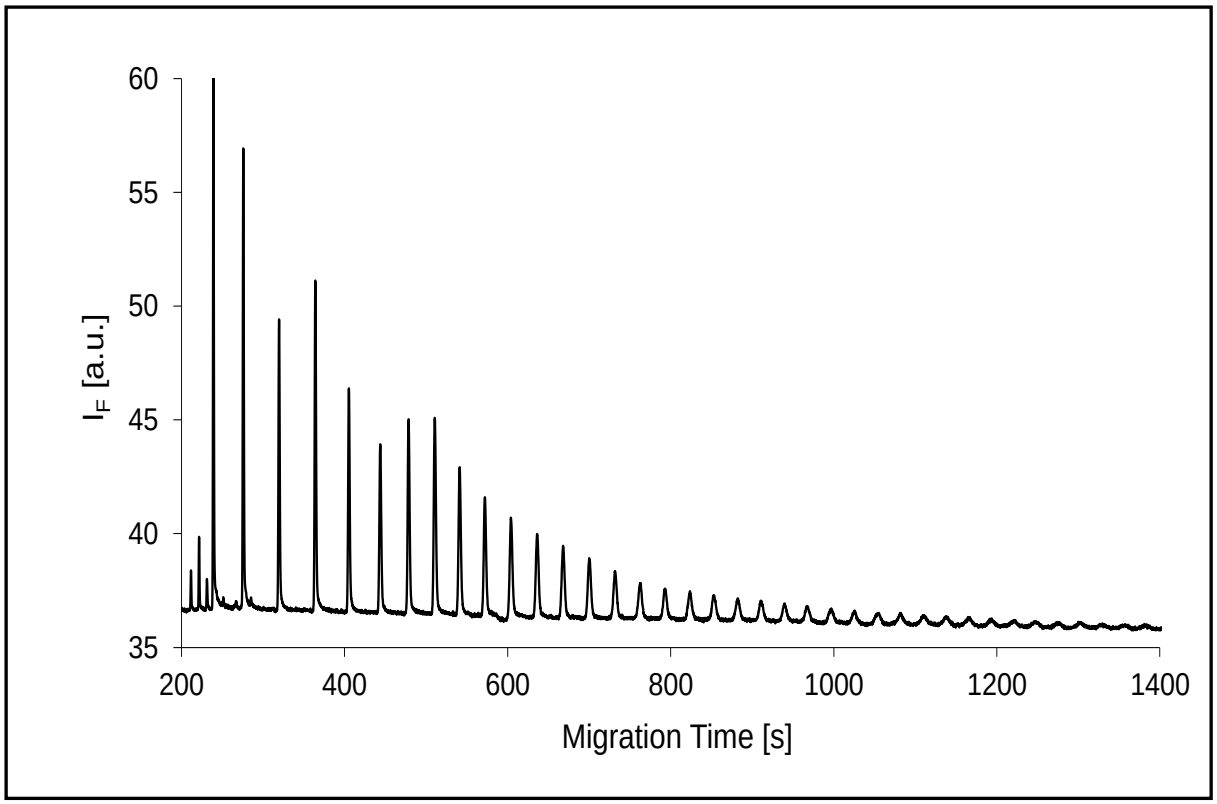
60
55
50
45
40
35
I_F [a.u.]
200
400
600
800
1000
1200
1400
Migration Time [s]

Fig. 6