Supporting Information

Rapid prototyping of electrochromatography chips for improved two-photon excited fluorescence detection

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Abstract

Supporting information contains more detailed information regarding the instrumentation, data acquisition and analysis and LPL-chip production as well as two tables and one figure.
Table S-1 summarizes the fluorescence lifetimes obtained by one-photon excitation and two-photon excitation as well as literature data of on-the-fly HPLC experiments.
Figure S-1 shows exemplary chromatograms obtained for borosilicate glass chips with excitation at 266 nm and 532 nm.
Figure S-2 shows the obtained plate heights depending from the flow rate in a wet-etched FS chip with monolith inside using TPE fluorescence detection.
Table S-2 summarizes retention times, efficiencies and resolutions of two selected flow rates from Figure S-2.

Experimental Section

Instrumentation

The laser beam is focused by backfilling an objective onto the desired detection point within the microfluidic channel. Emission light collected by the same objective passes a dichroic mirror and is guided either to a photon counting photomultiplier tube (PMA 165-N-M, PicoQuant) or to a single-photon avalanche diode (SPAD, PDM series, Micro Photon Devices (MPD), Bolzano, Italy) or to both simultaneously via a beam splitter. TCSPC was performed using a PicoHarp 300 module (PicoQuant) synchronized to the laser frequency. The laser intensity was monitored by an internal photodiode. When TPE was used, an optical density filter (ND 2.0, AHF Analysentechnik, Tübingen, Germany) was implemented in front of the diode. For excitation at 532 nm a 40x LUCPlanFLN objective (NA = 0.6, Olympus) or a 60x UPlanSApo objective (NA = 1.2 (water), Olympus) were applied. Dichroic mirrors Z532RDC and ZT532SPRDC (AHF Analysentechnik) as well as a BG3 filter (280 - 460 nm, Schott, Mainz, Germany) were implemented for fluorescence detection. For measurements at 266 nm excitation a 40x fused silica objective (NA = 0.8, Partec, Münster, Germany) or a 40x LMU-40x-UVB (NA = 0.5, Thorlabs, Dachau/Munich, Germany) were used. For detection, two Z266RDC dichroic mirrors (AHF Analysentechnik), a 150 µm diameter pinhole and either a 266 nm longpass filter (AHF Analysentechnik) or a DUG11 bandpass filter (295 – 385 nm, Schott) as well as a bandpass filter (420 – 480 nm, Olympus) were deployed. In order to maximize the detected fluorescence intensity tube lens- and pinhole-position as well as the shifted focus position, due to chromatic aberration, were optimized before measurements.

Data acquisition and analysis

A TCSPC module (PicoHarp 300, PicoQuant) processed the detected photons and the collected data were analyzed using the SymPhoTime software (Version 5.3.2.2, PicoQuant). Chromatograms were constructed by plotting the number of counted photons versus time. Applying a smoothing interval of 10 ms, the corresponding data acquisition rate was 100 Hz. The fluorescence lifetimes were determined by constructing a lifetime histogram of the region of interest, which was set interactively in the chromatogram, where the recorded intensity had at least reached 5% of the maximal peak height. Additional histograms were constructed selecting regions of background signal with the same timespan as the peak histograms. The background histograms were then substracted from the peak histograms and the resulting

corrected histograms were mono-exponential tail-fitted with OriginPro 8G SR4 (v8.0951, Origin Lab, Northampton, USA), yielding the fluorescence lifetimes of the analyte peaks. Retention times, chromatographic efficiencies and resolutions were determined using the software Clarity (version 3.0.4.444, DataApex, Prague, Czech Republic). Limits of detection were extrapolated by linear fitting with OriginPro 8G SR4.

LPL-chip production

Access holes were powder blasted at designated positions in glass and fused silica slides (height: 1.0 or 1.1 mm) to create robust lids of different materials. For covalent binding of bottom and lid to the polymer, glass and fused silica slides were surface modified according to protocols by Revzin *et al.*[1] and Brzoska *et al.*[2] Therefore, the cleaned and dried slides were treated with 5 mM 3-(trichlorosilyl)propyl methacrylate in n-heptane and chloroform (3:1, v/v) for 2 min, followed by rinsing with n-heptane and deionized water.

The microfluidic chips were prepared by spreading 45 µL (area: 52 x 26 mm) to 60 µL (area: 76 x 26 mm) of a mixture of 99% (w/w) poly(ethylene glycol) diacrylate and 1% (w/w) 2,2-dimethoxy-2-phenylacetophenone evenly onto a bottom slide. Afterwards, the lid was lowered carefully onto the prepolymer mixture avoiding air bubbles. A light-impermeable mask (offset print, 3600 dpi, DTP-System-Studio, Leipzig, Germany) was applied onto the lid and the assembly was placed in a MJB4 laboratory mask aligner (SÜSS MicroTec AG, Munich, Germany) equipped with a mercury arc lamp (15 mW/cm^2 at 365 nm) and illuminated for 1.4 s. Following this, uncured prepolymer was removed from the created channels and the chips were flushed with ethanol. Finally the chips were re-exposed to UV light for 1.4 s. The used chip layout resembles a simple cross with 6 mm long injection channels and a 30 mm separation channel. The line width of the photo mask (150 µm) transferred into 200 µm channel width in the crafted chip.

Table S-1. Fluorescence lifetimes in ns and standard deviations of four ChEC separations (exemplary shown in Figure 1 and Figure 2).

analyte	fluorescence lifetime τ (ns)		
	OPE	TPE	Literature
naphthalene	22.2 ± 1.9	-	27.47 [3]
fluorene	5.28 ± 0.01	5.35 ± 0.08	5.89 [3]
anthracene	-	4.06 ± 0.02	4.3, 4.22 [4,3]
fluoranthene	29.5 ± 4.0	20.1 ± 1.5	30.0 [4]
pyrene	-	18.0 ± 0.7	28.0, 35.82 [4,3]
benzo[*a*]anthracene	18.3 ± 0.4	14.5 ± 0.2	18.8, 20.0 [4,3]
benzo[*k*]fluoranthene	8.30 ± 0.04	8.16 ± 0.08	9.18, 8 [5,3]
benzo[*a*]pyrene	17.3 ± 0.4	15.0 ± 0.1	15.4 [4]

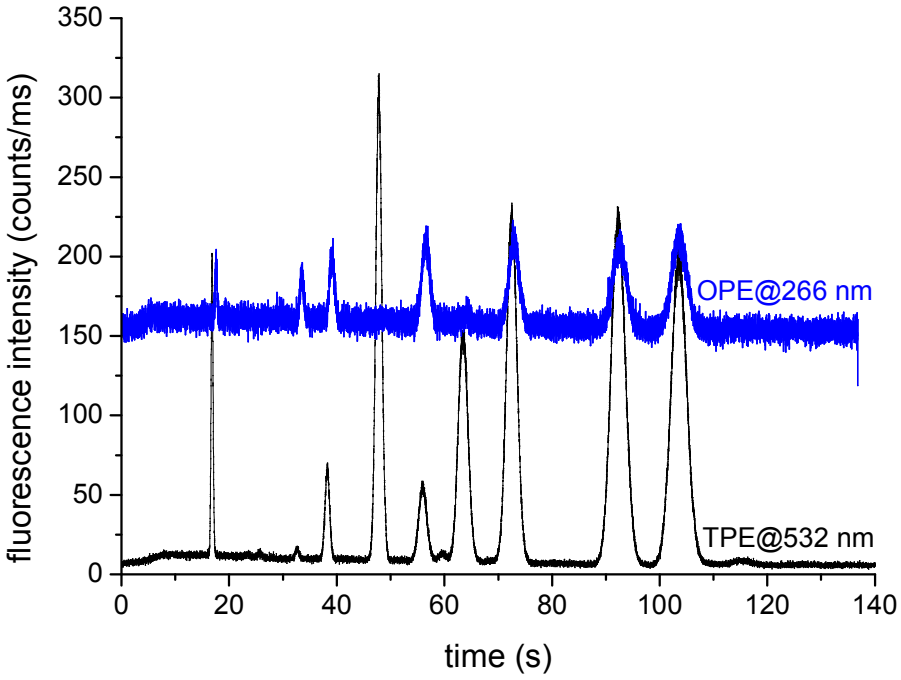

Figure S-1. Chromatograms of ChEC separations of 0.6 mM 7-amino-4-methylcoumarin, 0.2 mM (OPE)/2.4 mM (TPE) fluorene, 0.6 mM anthracene, 1.0 mM fluoranthene, 1.0 mM pyrene, 0.9 mM benzo[*a*]anthracene and 0.2 mM benzo[*a*]pyrene. chip: Micronit borosilicate glass, 3 cm monolith, eluent: MeCN/5 mM NH_4OAc pH 8.0 (4:1, v/v); λ_{ex}: 266 nm, 26.5 µW; λ_{em}: 295 – 385 nm and 420 – 480 nm; 40x Partec objective. λ_{ex}: 532 nm, 300 mW; λ_{em}: 270 – 465 nm, > 700 nm; 40x Olympus LUCPlanFLN objective.

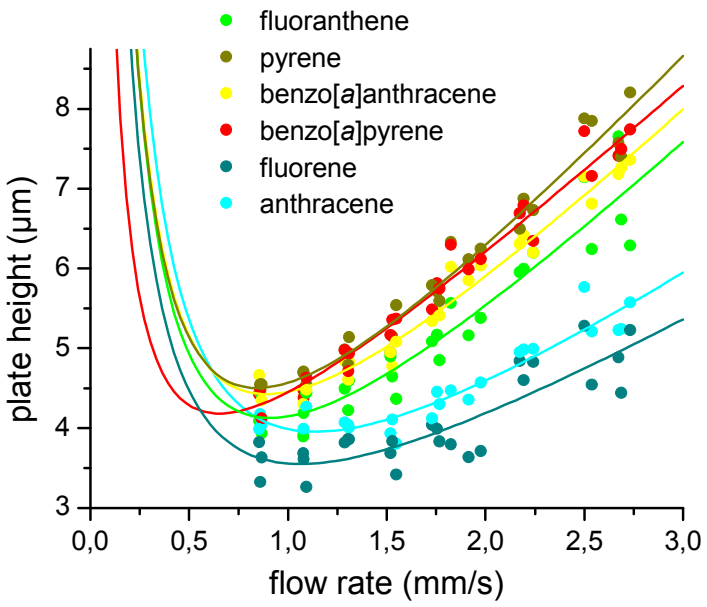

Figure S-2. Plate height vs. flow rate of the ChEC separations and calculated van Deemter curves; 0.6 mM 7-amino-4-methylcoumarin, 2.4 mM fluorene, 0.6 mM anthracene, 1.0 mM fluoranthene, 1.0 mM pyrene, 0.9 mM benzo[*a*]anthracene and 0.2 mM benzo[*a*]pyrene. chip: iX-factory fused silica, 3 cm monolith, eluent: MeCN/5 mM NH_4OAc pH 8.0 (4:1, v/v); λ_{ex}: 532 nm, 300 mW; λ_{em}: 270 – 465 nm, > 700 nm; 40x Olympus LUCPlanFLN objective.

Table S-2. Retention times, efficiencies and resolutions of two field strengths selected from Figure S-2.

field strength / analyte	208 V/cm			608 V/cm		
	retention time t_R (s)	efficiency (10^5 m^{-1})	resolution R	retention time t_R (s)	efficiency (10^5 m^{-1})	resolution R
fluorene	87.0 ± 1.9	2.79 ± 0.20	5.2 ± 0.1	30.1 ± 0.2	2.07 ± 0.17	4.7 ± 0.1
anthracene	110.1 ± 2.1	2.46 ± 0.06		38.5 ± 0.3	1.87 ± 0.07	
fluoranthene	129.9 ± 2.6	2.41 ± 0.15	3.5 ± 0.1	45.8 ± 0.5	1.47 ± 0.15	3.0 ± 0.1
pyrene	147.5 ± 2.9	2.22 ± 0.03	2.6 ± 0.0	52.1 ± 0.6	1.30 ± 0.07	2.1 ± 0.0
benzo[*a*]anthracene	170.8 ± 3.7	2.23 ± 0.08	3.0 ± 0.1	61.0 ± 0.9	1.38 ± 0.02	2.5 ± 0.1
benzo[*a*]pyrene	245.5 ± 4.3	2.31 ± 0.10	7.4 ± 0.1	88.7 ± 1.5	1.33 ± 0.03	5.9 ± 0.1

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