## **Supplementary Information**

## Quantitative programming and use of Parylene-C fluorescence as an on-chip reference

Diego G. Dupouy, Ata Tuna Ciftlik, Joan Teixidor, and Martin A. M. Gijs

## Supplementary Figures, Tables and Text

Supplementary Figure 1 (Figure S1)	Model of the characterization of iPBLF under UV illumination
Supplementary Table 1 (Table S1)	Parameters extracted from the model for UV/blue observation
Supplementary Table 2 (Table S2)	Parameters extracted from the model for blue/green observation
Supplementary Figure 2 (Figure S2)	3D plot of the fluorescence intensity in the microfluidic channel and the on-chip reference



**Figure S1** Model fitting of the iPBLF under UV illumination for 2, 5 and 10 µm thickness of Parylene-C. The experimental curves  $I_{exc/em}$  were shown already in the main text (Figure 3) and were obtained by applying Eq. 1 and Eq. 2 to the raw data. (A) UV/blue and (B) blue/green observation channels were fitted according to the formula  $I(x) = ax * e^{-b*x} + y_0$ , representing an initial linear growth of the fluorescence followed by a single exponential decay, though the latter is hardly visible in (B). The parameters of the fitted curves  $I_{UV/blue}$  and  $I_{blue/green}$  were extracted from the model and shown in Table S1 and S2, respectively.

thickness	a	b	<b>y</b> <sub>0</sub>	*RMSE
2 µm	1.38E-3	6.35E-4	1.71	1.07E-2
5 µm	3.42E-3	6.74E-4	1.43	5.64E-3
10 µm	1.96E-3	5.44E-4	1.47	7.83E-3

Table S1 Fitting parameters for the UV/blue channel for 2, 5 and 10 µm thickness of Parylene-C.

Note: \*RMSE: Root Mean Square Error

Table S2	Fitting parameters	for the blue/green	channel for 2, 5 and	10 um th	ickness of Parvlene-C.

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Parameters	а	b	yo	*RMSE	
2 µm	4.81E-3	1.48E-4	-0.11	7.13E-2	
5 µm	5.72E-3	1.20E-4	-1.16	3.33E-1	
10 µm	3.52E-3	3.81E-5	-0.23	1.32E-1	

Note: \*RMSE: Root Mean Square Error



Figure S2 3D plot of the fluorescent signal intensity of the complete chip area, including the microfluidic channel and the on-chip reference dot. The bleaching effect of the blue light on the fluorescent molecules flowing in the channel is observed when comparing a flow rate of (A) 20 nL/s and (B) 4 nL/s. The well-known parabolic-like profile is formed as a consequence of the higher velocity of the liquid in the centre of the microfluidic channel with respect to the regions near the borders, whereas the reference remains invariant from the flow conditions.