Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary material

Optimizing supercritical angle fluorescence structures in polymer microfluidic biochips for highly sensitive pathogen detection: a case study on *Escherichia coli*.

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1. Modeling of SAF optimal height

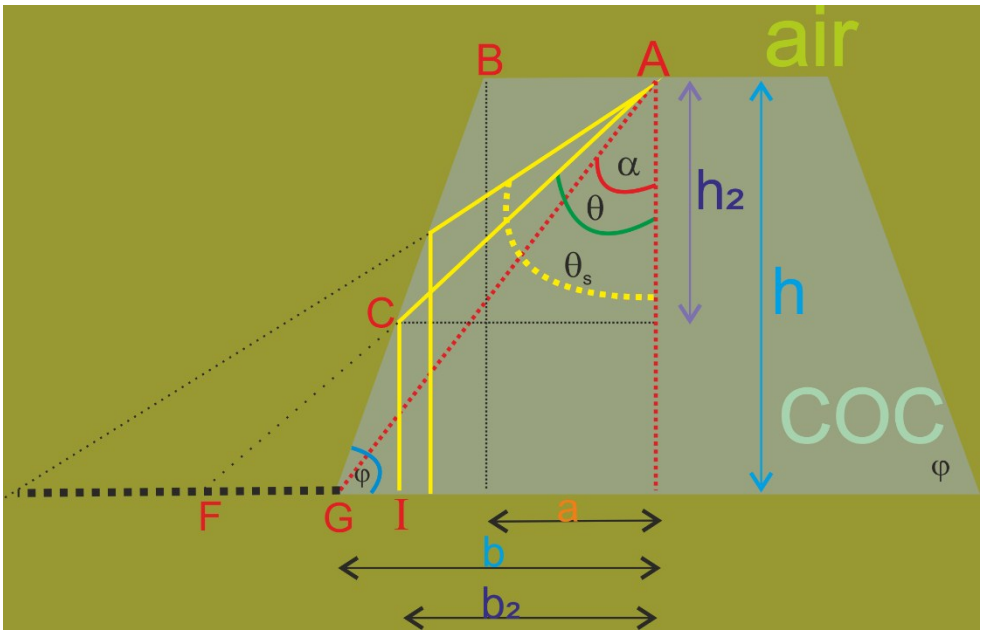


Figure S1: Schematic of a SAF structure in which half of the top and bottom diameters are *a* and *b* respectively. The fluorophore sits at the position A in the schematic. θ_s, θ and α are supercritical angle, critical angle and geometry angle, respectively. h is the height of the SAF structure.

In figure S1, air medium with a refractive index of 1 is represented with olive color. The COC medium with a refractive index of 1.53 is represented with grey color. Yellow lines represent the emitted lights from the fluorophore.

When a fluorophore sits at the interface of two different media, in this case, they are air and COC (in figure S1, the fluorophore is located at point A), the emitted light from the fluorophore goes into the denser medium (COC with refractive index 1.53) with the angle larger than the critical angle of the two medium. In this case, the critical angle $\theta = 40.8^{o}$. The emitted angle in this phenomenon is called supercritical angle, and the fluorescence is hence called supercritical angle fluorescence (SAF). The special structure, which we use to observe this SAF, is called SAF

structures. The SAF structure, as shown in fig. S1 has the truncated-cone shape aims to direct the SAF to the observation eyes or a camera by using total internal reflection phenomenon. The calculation bellows aims to derive the optimal height of the SAF structure so that we can observe the highest fluorescence intensity. In summary, the optimal height (h) of the SAF structure is obtained at the value of h_2 (denoted in figure S1) as the result of vanishing the area of the triangle GCI (area of GCI = 0, equivalently at the value of the line GI = 0).

From Fig. S1, we obtain the follows:

$$\begin{cases} GI = b - b_2 \\ b = \tan(\alpha)h \\ b_2 = \tan(\theta)h_2 \\ h = \tan(\varphi)(b - a) \\ h_2 = \tan(\varphi)(b_2 - a) \end{cases} \qquad \text{* MERGEFORMAT (1)}$$

By substituting h_2 to b_2 and h to b formula in eqn. 1, we obtain

$$GI = \frac{a\tan(\varphi)\tan(\theta)}{1-\tan(\varphi)\tan(\theta)} - \frac{a\tan(\varphi)\tan(\alpha)}{1-\tan(\varphi)\tan(\alpha)} \qquad \text{* MERGEFORMAT (2)}$$

From eqn. 2, by solving GI = 0, we obtain $\alpha = \theta$. From eqn.1, by substituting b_2 to h_2, we obtain the expression for h_2:

$$h_2 = \frac{a\tan\varphi}{\tan(\varphi)\tan(\theta) - 1} \qquad \text{* MERGEFORMAT (3)}$$

From eqn.1, we have $\tan(\varphi) = \frac{h}{b-a}$; $\tan(\alpha) = \frac{b}{h} = \tan(\theta)$ (since $\alpha = \theta$). Substituting these resulting eqns. to eqn. 3, we obtain $h = h_2$, this is the optimal h.

2. Measurement of refractive index of COC

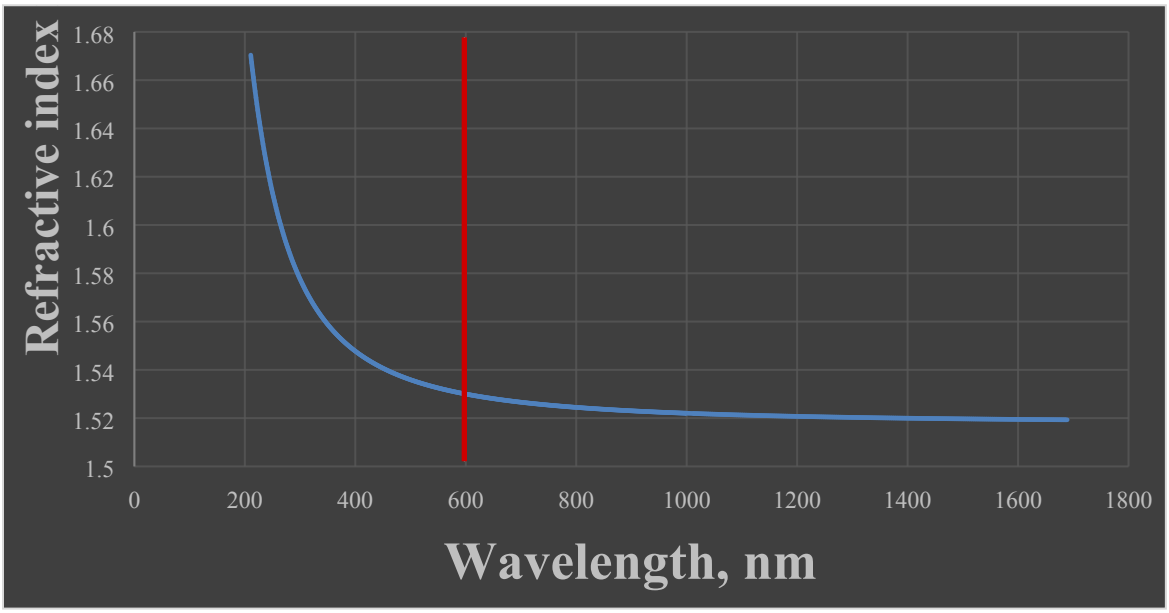


Figure S2: The dependence of COC refractive index on wavelength. Data was experimentally measured at DTU Nanotech using Ellipsometer M-2000V. In our experiments, we use cy3 fluorophores, λ = [532, 580] nm, n = 1.53.

3. Micro-milling shims

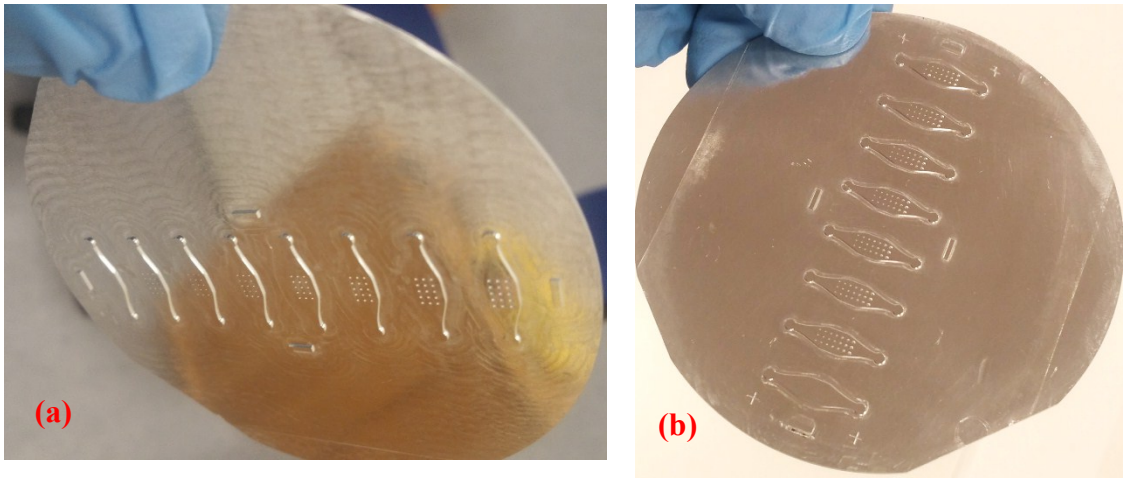


Figure S3: Aluminum shims made from a micro-milling machine. (a) 8-chamber shim, each chamber has one size of the SAF structures, varying from 0 µm to 300 µm height, corresponding to chamber number 1 to 8, respectively. (b) 8-chamber shim, in which each chamber has different of the SAF structures, varying from 0 µm to 300 µm height.

4. Digital picture of the polymer chips

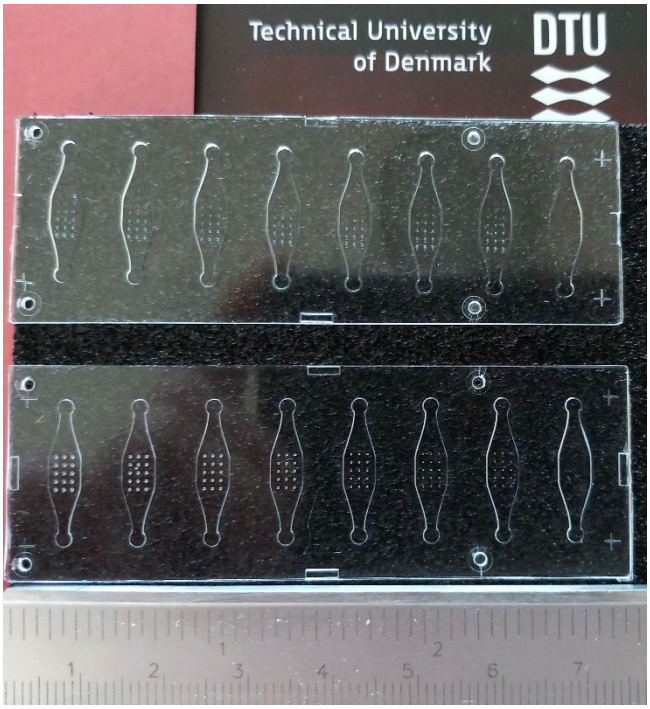


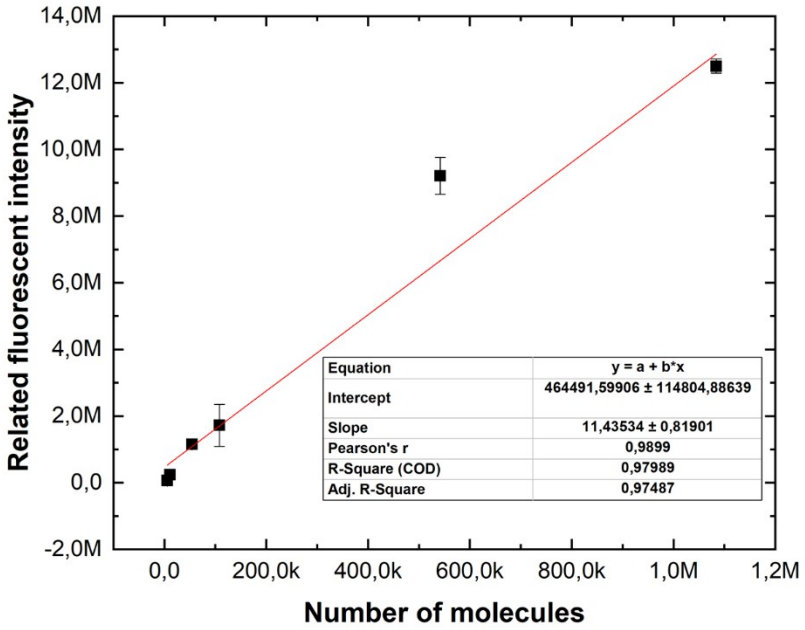
Figure S4: Digital pictures of the polymer injection moulding chips. (Top): the COC polymer chip having eight chambers in which each chamber contains SAF structures with different sizes. (Bottom): the COC polymer chip having eight chambers in which each chamber has SAF structures of only one size.

5. Fluorescent intensity and the number of molecules

The fluorescent intensity of a dilute solution is in a linear relationship with its solution concentration or number of molecules [1], [2]. This relation is a lightly modified version of Beer-Lambert relation [1]–[3] and can be expressed as:

$$F = 2.3K'\varepsilon bCP_o \qquad \backslash* \text{ MERGEFORMAT (4)}$$

in which, F is the fluorescent intensity; **K′** is the constant depends on the quantum efficiency of the fluorescence process and instrumental parameters; ε is the molar absorptivity of the fluorescing molecules; b is the path length; C is the concentration; P_o is the source power.



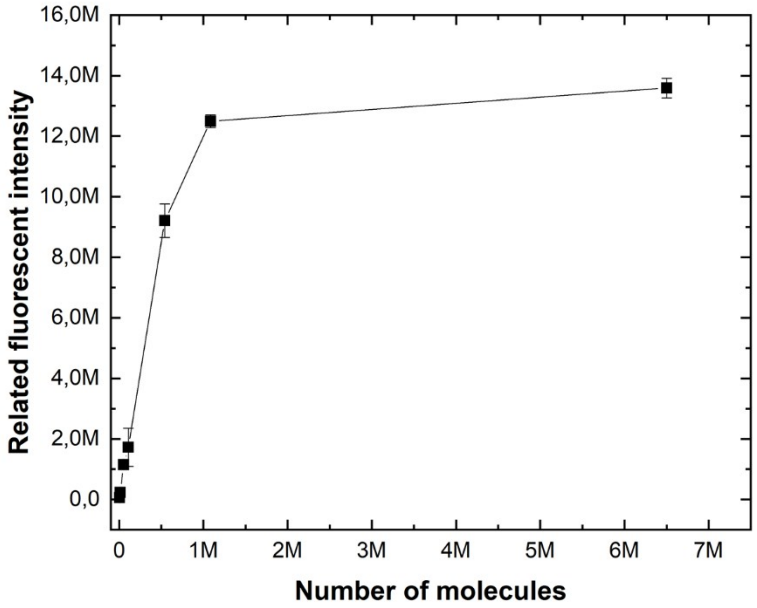


Figure S5: (a) The linear relationship between fluorescent intensity and the number of molecules at low concentration. (b) At high concentration, the non-linear relationship appears, same as shown in figure 5 of ref. [3].

6. References

[1] C. C. Chan, H. Lam, and X.-M. Zhang, *Practical approaches to method validation and essential instrument qualification*. Wiley Online Library, 2010.

[2] D. A. Skoog, F. J. Holler, and S. R. Crouch, *Principles of instrumental analysis*, 7th ed. Cengage learning, 2017.

[3] F. A. O. Joint, "Combined Compendium of Food Additive Specifications. Volume 4. Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications," vol. 4, p. 28, 2006.