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MINIATURISING SUPERCRITICAL ANGLE FLUORESCENCE AND TOTAL INTERNAL REFLECTION FLUORESCENCE STRUCTURES FOR A MICROFLUIDIC SYSTEM USING IN DIAGNOSTIC APPLICATIONS

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ABSTRACT
This paper presents, for the first time, an update of a microfluidic system inspired from the work of Hill et al. [1] in which they reported a macroscopic (millimetre scale) disposal biochip produced for diagnostic applications. The employing principal of the chips in the previous work was based on the combination of supercritical angle fluorescence (SAF) and total internal reflection fluorescence (TIRF) for a fluorescent signal measurement (Fig. 1 & 2).

KEYWORDS: polymer, microfluidics, supercritical angle fluorescence, total internal reflection fluorescence.

INTRODUCTION
When a fluorophore sits at the interface between two different media, a large portion of the fluorescence is emitted into the medium with higher refractive index (the denser material) at the angle larger than the critical angle of the two media. The fluorescence observed in this phenomenon is hence called super-critical-angle fluorescence. One can use total internal reflection (TIR) phenomenon to direct this fluorescence to the observation points where a camera can be placed to capture the fluorescent signal. Inversely, excitation light can be directed from the light source using TIR to the interface where they hit and excite the fluorophores. By doing so, we solely excite the fluorophores which are located in the vicinity of the interface (within a distance less than 100 nm from the surface, Fig. 3), hence prevent the excitation of the fluorescent molecules in the bulk (far from the surface) which are the cause of signal noise. Therefore, SAF and TIRF are combined in this detection technique. We have successfully fabricated microarrays of SAF structures, presented in MicroTAS 2018 [2].

We in this work miniaturise the fluidic system, which has the combination of SAF and TIRF structures down to microscale with a robust and low-cost fabrication procedure. Going down from millimetre to micrometre scale enhances not only the numbers of reaction chambers and structures for multiple detections but also the capability of using in a portable device (due to lightweight) for point of care detection.

EXPERIMENTS AND RESULTS
The structures that we miniaturised, including two separated parts, i.e. the SAF part, and TIRF light directing part, (the annular mask). These two parts are then attached using a chip holder (made from aluminium by micro-milling) (Fig. 4).

For the TIRF light directing part, we use the cutting-edge maskless aligner (MLA150 from HEIDELBERG Instrument (Germany)) and e-beam deposition for creating the annular mask structures after finishing by a lift-off step (Fig. 5). We created the annular masks both on glass slides and on the disposable cyclic olefin copolymer (COC) chips for the sake of comparison. The results showed that we successfully fabricated the annular masks on both glass slide and COC polymer (Fig. 6). The lift-off time for the later is much longer (20 hours versus 5 mins), assumably due to the higher adhesion between COC polymer and the photoresist. For the SAF part, micro-milling is used [2].

This work has opened up a possibility for using a combination of SAF and TIRF in a microfluidic chip for point of care testing which may conduct either solid-phase polymer chain reaction or loop-mediated isothermal amplification.

REFERENCES

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Figure 1: Large amount of the radiation emitted into the higher refractive index medium [2]. See also Fig. 1 of ref. [1]

Figure 2: The CAD illustration of SAF and TIRF light directing annular ([Left] top view and [Right] side view).

Figure 3: Our theoretical modelling of the relative fluorescent intensity from the interface versus the incidence angle with different $z$ ($z$ is the distance of the fluorophore from the interface). The two media are water and glass, and the critical angle is 61 degree. $\lambda$ is the excitation wavelength

Figure 4: The CAD design of the chip holder.

Figure 5: The lift-off process flow for fabrication of the annular structures used as TIRF light directing. Our work is done in NanoLab of Denmark Technical University.

Figure 6: (a) The design of the mask. This design is then used with the maskless aligner for UV lithography. (b) Large view of the individual annular mask from the design. (c) The microscopic images of the fabricated annular on the glass slide surface after the lift-off step. The dimension of the annular agreed with the design. (d) The microscopic images of the fabricated annular on the COC polymer surface. The results show that we can obtain a clean (without a trace of metal), and quicker lift-off time for the case of using glass slide as the substrate compared to the COC polymer. (e) Digital picture of the chips and the glass slide having annular structures assembled in the chip holder.