

Two-Photon Polymerization Assisted Molding Process Fabricating 3D CYTOP Microfluidic Chips for Super-Resolution Live Imaging

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Live imaging of cellular dynamics in confined micro-spaces is crucial for understanding biological processes including cancer cell invasion, immune response, and neuronal development [1, 2]. However, conventional glass- or PDMS-based microfluidic chips that provide such micro/nano-environments suffer from image distortion due to refractive index mismatch with water-based culture media, making it difficult to capture high-resolution images. To address this problem, we have developed a technique fabricating three-dimensional (3D) microfluidic chips using fluoropolymer CYTOP, because its refractive index (~1.34) closely matches that of water (~1.33). Our fabrication approach combining two-photon polymerization (2PP) of SU-8 with molding processing enabled creating defect-free, flexible 3D structures with sub-micrometer precision.

In the fabrication procedure (Fig. 1), a 3D microstructure of SU8 was first fabricated by 2PP of SU8 using a femtosecond laser (wavelength: 515 nm, pulse width: 220 fs). The fabricated 3D structure was then used as a mold to be filled with liquid CYTOP, followed by thermal treatment for curing CYTOP. Finally, the SU8 microstructure remaining in CYTOP was selectively removed by chemical treatment, resulting in fabrication of the 3D microfluidic structure inside CYTOP.

The fabricated 3D CYTOP microfluidic chips realized super-resolution imaging of cancer cells in the microchannels and clearly visualized the rupture and repair of nuclear envelope during the migration. The ability of super-resolution imaging in confined micro-spaces expands opportunities for investigating many biological studies other than cancer cell migration study.

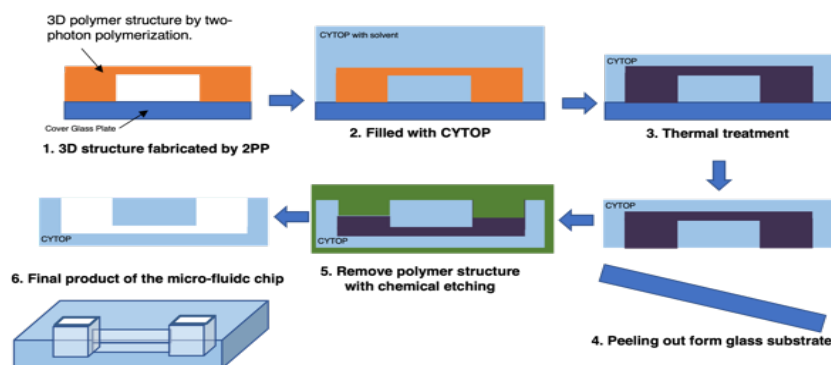


Fig. 1. Fabrication procedure of 3D CYTOP microfluidic chips by combination of two-photon polymerization with molding process.

References:

- [1] C. M. Denais, et al., *Science*, **352**, 353-358 (2016).
[2] F. Sima, et al., *Adv. Mater. Technol.* **5**, 2000484 (2020).