## Optically Fabricated Gradient Nanochannel Array to Access Translocation Dynamics of T4-phage DNA Through Nanoconfinement

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## **Supporting Information**

This PDF file includes:

**Supplementary Text** 

Fig. S1 to S5

Captions for Movie S1 to S3

Other Supplementary Materials for this manuscript include the following:

Movie S1 to S3

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## 1. Shape of TPP voxel depends on laser power and exposure time

Voxel shape of the two-photon direct laser writing platform is determined by moving the focal spot right above the substrate. Without supporting, the nanostructures fall down to their long axis after carefully removing the uncross-linked photoresist (i.e., developing). Cross-section of the TPP focal spot can, therefore, be observed using SEM (Fig. S2a). Our results demonstrate that shape and size of the effective photoresist crosslinking region varies with changing laser power and exposure time (Fig. S2). Since it is technologically challenging to adjust TPP laser power during fabrication, we propose to include the exposure time as tunable parameters.

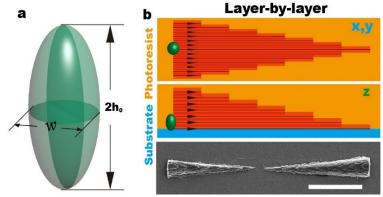


Figure S1 Nanostructure with gradually transitioning dimension produced by conventional TPP technique. a. Schematic showing the ellipsoid of TPP region. b. Conventional TPP technique fabricates micro- and nano-scale 3D structures by directing focal spots inside the photo-sensitive polymeric material via layer-by-layer staking, which makes stair-like structures unavoidable. Scale bars denote 5 μm.

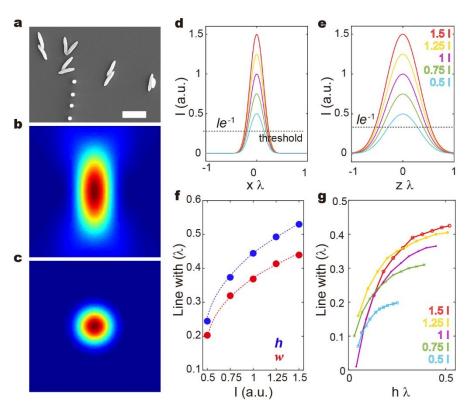


Figure S2 Dimension of the nanostructure (e.g., the line width) depends on the laser power and size of the TPP focal spot. a. SEM image of the nano-pillars, reflecting shape of TPP voxel. b,c. Simulated intensity distribution of TPP focal spot in (a) XZ (b) XY plane (see Fig. 1 for direction). d. The dependence of lateral line width on TPP intensity and threshold. e. The dependence of axial line width on TPP intensity I. f. The change of channel height width with different laser intensities. It is demonstrated that depending on the TPP exposure dose (i.e., intensity and time) and photoresist, the cross-linked region decreases with increasing distance from the center of the focal spot (i.e., the increasing height, h).

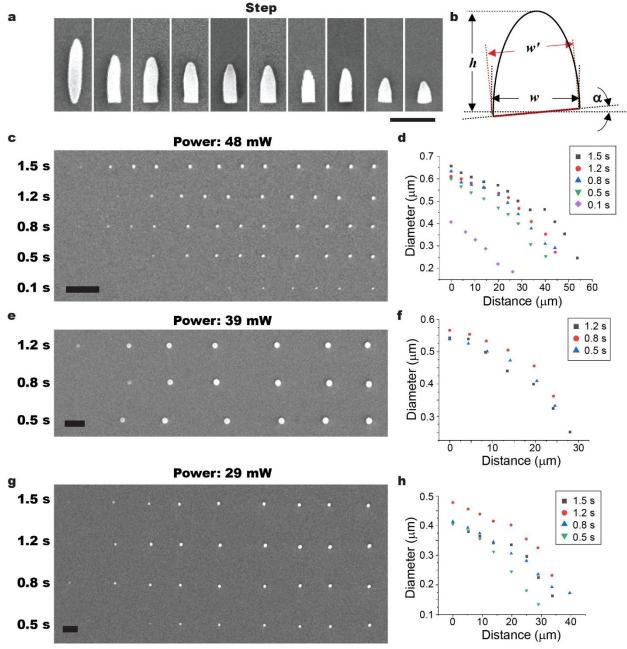


Figure S3 Shape and size of the TPP voxel depend on the laser power and exposure time. a. Lateral movement of the focal spot along the inclined direction of a substrate leads to decreased exposure volume in the photoresist, and thus the production of nanostructures with gradually decreasing dimension (see also Fig. 1b). 5EM images of the pillars produced on a tilted substrate using 48.3 mW laser power and 1.5 s exposure time. c. When the inclination angle  $\alpha$  is small, tilted nanostage brings no obvious changes in the projection area. d-i. SEM images (Fig. d, f, h) and dimension (Fig. e, g) of the nano-sized pillars made by directing the TPP focal spot on a tilted substrate with varying laser power (i.e., 48 mW, 39 mW and 29 mW) and exposure time (i.e., 0.1 s to 1.5 s). Scale bars denote 2  $\mu$ m in all figures.

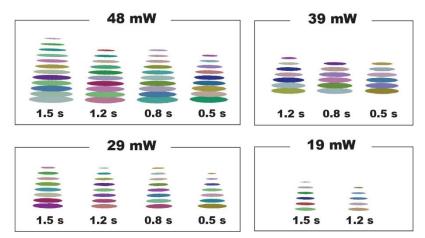
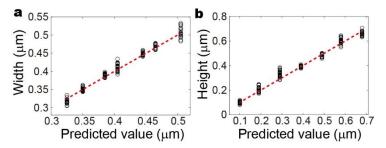
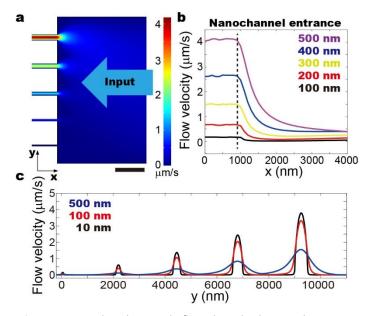


Figure S4 Cross-section of the nano-pillars reflect shape and size of TPP focal spot. At different TPP laser power, exposure time and substrate inclination angle of a stacks of nano-pillar cross-section reflect the photoresist cross-linking region in TPP focal spot (see Fig. 1b).



**Figure S5** Comparison between the TPP-produced nanostructure with the predicted value. **a.** Relationship between the predicted value (red dashed line) and width of the nanolines measured by SEM. **b.** Relationship between the predicted value (red dashed line) and height of the cone-like nanostructure measured by AFM.



**Figure S6** Numerical simulation on the flow velocity distribution at the entrance of the nanochannel array. **a.** Flow velocity varies greatly in nanochannels with different dimension, and shows non-uniformly distributed flow at the entrance of the nanochannel. **b.** Flow velocity increases greatly at the nanochannel entrance along the nanochannels (x direction). The difference in flow velocity is proportional to the nanochannel dimension. **c.** Distribution of flow velocity at the nanochannel entrance along y direction. Scale bar denotes 2  $\mu$ m.

- **Movie S1:** Deformation and partial stretching of T4-phage DNA at the entrance of nanochannel.
- **Movie S2:** Translocation of T4-phage DNA through 400 nm nanochannel driven by electric field.

**Movie S3:** Translocation of T4-phage DNA through 400 nm nanochannel driven by applying pressure at the inlet and causing shear flow.