

## 1-Dimensional diffusion processes in two-photon fabricated PDMS nanochannel devices

Oliver Vanderpoorten\*, Quentin Peter, Pavan K. Challa, Ulrich Keyser, Jeremy Baumberg, Clemens F. Kaminski and Tuomas P. J. Knowles

\* Department of Chemical Engineering and Biotechnology,  
University of Cambridge

Philippa Fawcett Drive, Cambridge, CB3 0AS, UK

E-mail : [ov219@cam.ac.uk](mailto:ov219@cam.ac.uk)

**KEY WORDS:** Nanofluidics, Soft lithography, Single-molecule detection, Atomic force microscopy, Scanning electron microscopy, Two-photon lithography

The size of protein aggregates is of particular interest when studying biophysical mechanisms involved in protein misfolding diseases such as Alzheimers, Parkinson and Huntingtons disease. Nanofluidic devices have been demonstrated as useful tool to filter amyloid aggregates<sup>1</sup> and other biological samples e.g. exosomes, viruses and colloids according to their size.<sup>2</sup> Still, their availability is conventionally restricted to biological clean room laboratories with access to electron lithography or chemical etching facilities. To endorse this technology and show its applicability to study proteins in confined space, we combine conventional UV- mask lithography with 2-photon direct laser writing to produce nanofluidic master moulds in SU-8 for soft lithography.<sup>3</sup> The characterization of the masters is done with correlative AFM/SEM imaging and shows channel moulds in the range from microns down to 50 nm height and 230 nm lateral width. We verify functional nanochannels in the final PDMS device by super-resolution microscopy imaging (STORM) of diffusing single fluorescent molecules. Via tracks of single molecules (e.g. Rhodamine 6G, GFP, amyloid-beta, Tau) the 1-dimensional diffusion coefficient in confined space is evaluated and compared to diffusion coefficients in bulk. In comparison to other techniques this approach does not require surface binding of the protein of interest for detection. Using STORM reconstruction algorithms on the same data we obtain super-resolved fluorescence microscopy images which verify a nanochannel width of 300 nm (FWHM) on the final chip. To enhance lab-on-chip development we overcome the current microscale fabrication capabilities by merging mask whole-wafer UV-lithography with locally 2P-written nano-sized functional features and make nanofluidic chip prototyping in a biological laboratory available.

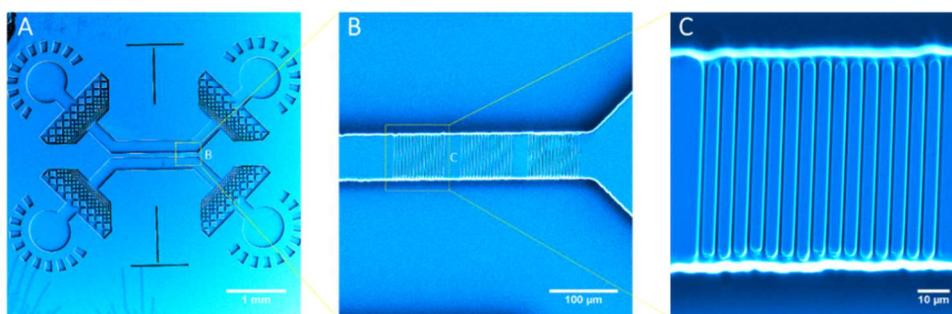


Figure 1: SEM-micrograph of PDMS nanochannels for single molecule diffusion studies

[1] Erik C. Yusko, et al., “Controlling protein translocation through nanopores with bio-inspired fluid walls”, *Nature Nanotechnology* 6, 253260 (2011)

[2] Wunsch, B. H. et al. „Nanoscale lateral displacement arrays for the separation of exosomes and colloids down to 20nm”. *Nat. Nanotechnol.* 11, 936–940, DOI: 0.1038/nnano.2016.134 (2016)

[3] Whitesides, G. M., “The origins and the future of microfluidics”, *Nature* 442, 368–373, DOI: 10.1038/nature05058 (2006). arXiv:1011.1669v3.