Fabrication of zero-mode waveguides with a high-resolution FIB nanowriter

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Zero-mode waveguides (ZMWs) are optical nanostructures fabricated in a thin metallic film capable of confining the excitation volume to the range of zeptoliters. [1] This small volume of confinement allows single-molecule fluorescence experiments to be performed at concentrations of fluorescently labeled biomolecules that are physiologically relevant. The nanoapertures can be fabricated using electron-beam lithography, deep-UV lithography, nanoimprint lithography and focused-ion beam technology. [2,3] In this presentation we will detail our efforts aiming at fabricating such devices using a high-resolution FIB nanowriter[4] capable of prototyping arrays of such devices with pre-defined geometries, high precision and reproducibility. We have specifically addressed the question of sputtered material re-deposition and of local electrical charge removal in developing a methodology that will be thoroughly presented and discussed. Indeed, particle re-deposition has been found to pollute the floor of neighboring ZMWs when milling the nanoholes in the metal layer covering the surface of fused-silica substrate, impairing their optical performance and the capacity to immobilize biomolecules. Arrays of 400 nanoholes were milled in 100 nm thick metallic layers (Figure 1). Nanoapertures dimensions were measured and the corresponding volumes deduced. With a solution of fluorescently labeled DNA at μM concentration, a background of fluorescence was measured that was not uniform over the array (Figure 2). Fluorescence signals corresponding to single molecules bound inside the ZMWs were analyzed and show signal to noise ratios of 8 (Figure 3). Levels of background were analyzed and were shown to correlate with the protocol used for the FIB operation (ZMWs milled last presented no metallic re-deposition). This type of analysis was used to improve FIB milling protocols.

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[1] Zhu P. and Craighead H.G. Zero-mode waveguides for single-molecule analysis. *Annual Review of Biophysics*. 41:269-93. 2012.

[2] Wenger J, Lenne P-F, Popov E, Rigneault H, Dintinger J, Ebbesen TW. Single molecule fluorescence in rectangular nano-apertures. *Opt. Express* 13:7035–44, 2005.

[3] Wenger J, Gérard D, Lenne P-F, Rigneault H, Dintinger J, et al. Dual-color fluorescence crosscorrelation spectroscopy in a single nanoaperture: towards rapid multicomponent screening at high concentrations. *Opt. Express* 14:12206–16, 2006.

[4] Gierak J., Hawkes P., Jede R., *Nanofabrication with Focused Ion Beams* in the *Nanofabrication Handbook* Edited by Stefano Cabrini and Satoshi Kawata, CRC Press 2012

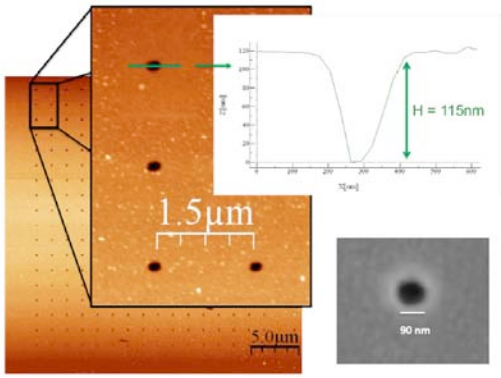

Figure 1: fluorescence background distribution. AFM and SEM (bottom right) images for inspection of ZMWs. AFM profile for inspection of a nanowell.

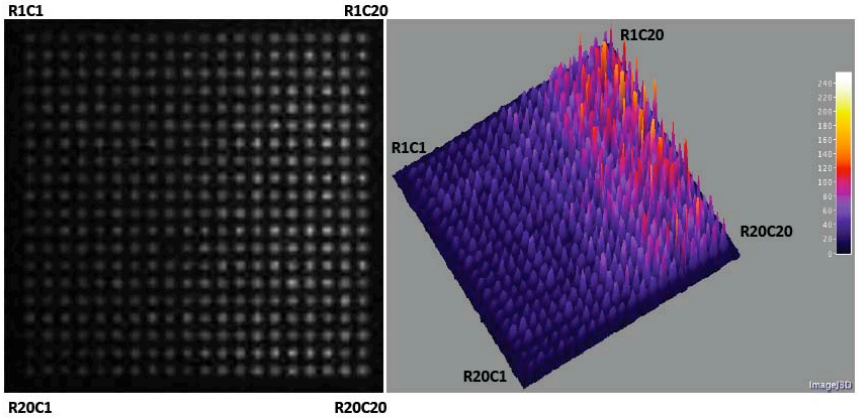

Figure 2: fluorescence background distribution. (left) Fluorescence image for 400 nanoholes in contact with a solution of fluorescently labeled DNA (5 µM). Arrays were imaged for 40 sec at 25 fps with a 642-nm laser excitation at room temperature. For each frame, the minimum intensity signal was extracted and integrated to generate the left image. Coordinates of rows and columns identify each nanohole. (right) 3D analysis of fluorescence levels for each nanoholes.

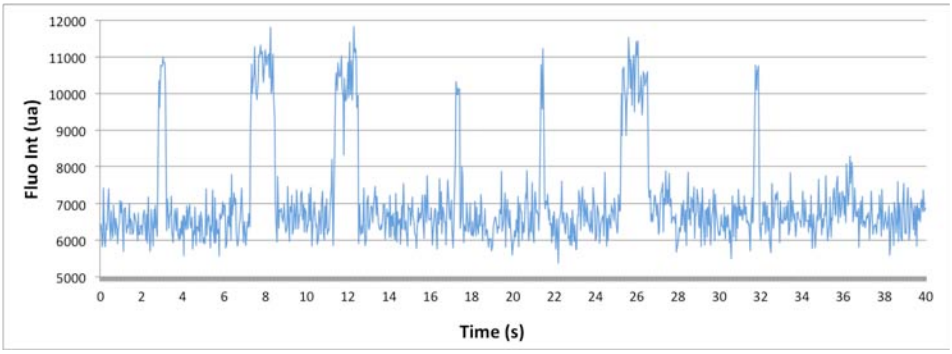

Figure 3: fluorophore detection inside ZMW. Representative trace of Cy5-labeled DNA oligonucleotides bound inside a single ZMW. Experiment was performed with a 642-nm laser excitation at room temperature and images recorded at 25 fps for 40 sec. Signal to noise ratio was calculated by the mean of the on state intensity divided by the SD of the background intensity.