Electron Beam Induced

High Resolution Biofunctionalised Nanopatterns

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Biofunctionalised nano scale arrays are an important tool in the study of several biological processes, especially to monitor interactions between single molecules. A flexible technique to biofunctionalise surfaces is described by Schlapak et al [1]. Carbon containing nanopatterns (100 x 100 nm^2) were created on a glass/ITO (17 nm) substrate coated with PEG silane with the help of a focused electron beam in a Scanning Electron Microscope (SEM). Upon incubating this sample in a solution of an antibody-bound fluorescent dye (IgG-Cy3) followed by rinsing, the electron beam-patterned islands were observed to fluoresce with a high contrast (> 1000) with respect to the surrounding PEG silane layer. This is a versatile method as the antibody can be used with a variety of proteins, enabling easy tuning of the local biomolecule density. Further, since the binding conforms to the shape of the nanopatterns, the resolution of such a functionalization approach could in principle approach a few nanometers [2].

We aim to study in more detail the processes taking place with the ultimate goal of achieving ultra-high resolution (sub-10 nm) biofunctionalised patterns. The sample was patterned in a Nova Nano 650 Dual Beam system at 5 keV and 24 pA current. The pattern consists of an array of squares (1μm x 1μm) written with increasing dose from 50 C/m^2 to 1800 C/m^2. After incubation in a solution of IgG bound to Alexa488 fluorescent dye, the sample was examined in a fluorescence microscope. Surprisingly, we did not observe fluorescence from the irradiated areas, but from the edges of the structures (see Figure 1), with higher dose areas yielding greater intensity as in [1]. However, regions of the sample that were not patterned but imaged shortly in the SEM, and therefore received a low electron dose, are seen to fluoresce completely (Figure 2). This suggests that the binding is possibly electron dose dependent and results from chemical modification of the PEG silane layer and/or electron beam induced deposition (EBID) from hydrocarbons in the SEM chamber due to the secondary electrons generated in the process. Using these results, high resolution nanostructures were patterned and their dimensions determined by fluorescence as well as SEM imaging (Figure 3).

[1] R. Schlapak, J. Danzberger, T. Haselgrubler, P. Hinterdorfer, F. Schaffler, S. Howorka, *Nano Letters* **12** (2012) 1983-1989

[2] W. F. van Dorp, B. van Someren, C. W. Hagen, P. Kruit, *Nano Letters* **5** (2005) 1303-1307

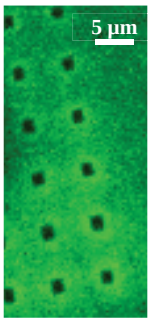

Figure 1: Fluorescence image demonstrating fluorescence from the sides of the nano patterns (black squares)

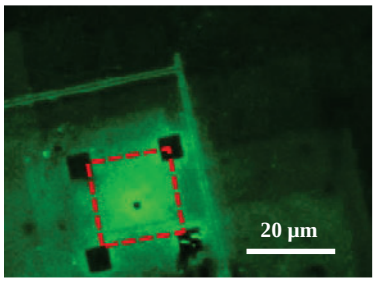

Figure 2: Observation of fluorescence from areas imaged with a low electron dose (shown with dotted lines)

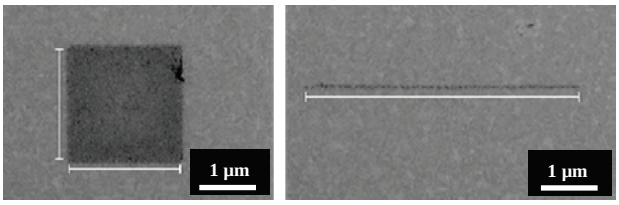

Figure 3: SEM image of a biofunctionalised square (L) and line (R) after patterning followed by incubation in a solution containing IgG-Alexa 488 tagged with 5 nm gold particles. The bars roughly indicate the regions actually irradiated by the electron beam.