UV-MEDIATED LIGAND IMMOBILIZATION FOR MULTIPLEXED ANALYSIS IN EXTENDED NANO-SPACE CHANNELS
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ABSTRACT
A general method for immobilization of capture ligands in a single channel of extended nanospace depth is reported. For multiplexed analysis in a single channel, several ligands must be immobilized in the same channel. Since the bonding procedure (by heat/ HF) for glass and fused silica chips would destroy the ligands, we designed and synthesized a UV-activated linker for covalent ligand attachment inside the channels after fabrication. A modified mask aligner was used to align OHP or Cr masks to the channels to facilitate immobilization of the DNA capture oligos to pre-defined areas.

KEYWORDS: Ligand immobilization, DNA, UV, Extended nanospace, Nanochannel

INTRODUCTION
For analysis of minute sample volumes, such as secretions from or contents of single cells, the analysis chamber could be scaled to better suit the sample volume. By using very shallow channels of extended nanospace depth (10-1000 nm) the diffusion time is decreased to allow a higher fraction of the analyte to interact and be captured by the immobilized ligands, thus increasing sensitivity.

For multiplexed analysis in a single channel, several ligands must be immobilized and spatially separated inside the same channel. Since the bonding procedure for glass and fused silica microchips destroy the ligands (by heat or HF), ligand immobilization must be performed after fabrication.

Reports of UV-mediated ligand immobilization in microchannels have been relatively sparse, however, there are previous reports of protein and DNA immobilization in fused silica capillaries using surface immobilized UV-activated benzophenons on an APTES surface [1] or a novel hydrogel which is cleaved under UV-irradiation, creating aldehyde groups for protein coupling [2].

We synthesized a new linker for UV-mediated covalent attachment of multiple ligands inside channels of extended nanospace depth and modified a mask aligner to facilitate precise alignment of OHP or Cr masks used for patterning inside the channels.
EXPERIMENTAL

We fabricated a fused silica chip with 460 nm deep nanochannels flanked by microchannels, to facilitate liquid handling, using e-beam lithography, inductively coupled plasma (ICP), and thermal bonding (Fig 1), as previously described [3]. Channel depth was verified with AFM.

![Fig 1. A 70 x 30 mm chip containing 20 nanochannels (40 μm wide and 460 nm deep) in sets of twos (indicated by black arrows) flanked by microchannels (500 μm wide and 20 μm deep). The microchannels were fabricated with wet etch, and the nanochannels with e-beam lithography, ICP and thermal bonding to a 170 μm thick cover plate with 8 pre-drilled holes for chip-to-world interfacing. Right picture: a microchannel (the horizontal black lines are the channel walls) connecting two nanochannels (vertical grey lines).](image)

A new UV-linker consisting of a biomolecule repelling PEG-linker and a benzophenone moiety (Fig 2) was designed and synthesized using Fmoc solid phase synthesis and coupled to APTES modified nanochannels using HBTU/ Houb/ DIEA in DMF, to create a surface of biomolecule repelling PEG spacers with benzophenone “heads” for UV-mediated DNA or protein immobilization.

![Fig 2. The photolinker contains three units: a) a carboxyl groups which can be activated and coupled to amine groups inside the nanochannel. b) A PEG linker to reduce non-specific binding of analytes to the channel walls, and c) a benzophenone moiety which can be activated with 365 nm UV to covalently attach to -CH groups in capture ligands.](image)

We verified the principle of immobilization of several capture ligands in a single nanochannel by introducing two DNA capture oligos at 10 mg / ml in series and immobilizing them by exposure to 365 nm UV for 120 s at 200 mW / cm² through an OHP mask with a 250 μm slit. The channels were blocked with 5 x SSC + 0.1 % SDS + 1% BSA, and incubated for 120 min with a mix of 15 μM fluorescein and texas red labeled complementary oligos. The channels were washed and photographed under fluorescein and texas red excitation (Fig 3 c,d).
RESULTS AND DISCUSSION
As can be seen in Fig 3, the capture oligos bind only their complementary, fluorophore labeled oligos and not the non-specific oligo, clearly demonstrating that the several oligos can be patterned inside the same nanochannel with retained specificity.

Fig 3. Multiplexed DNA detection in extended nanospace. a) Schematic image of two nanochannels, each containing capture oligos A and B. The channels were incubated with a fluorophore labeled oligo mix. b) Transmission image of the nanochannels (40 μm wide and 460 nm deep). Channels are not visible. c) Under fluorescein excitation. The fluorescein labeled oligo binds to its complementary oligo B, but not to oligo A. d) Under texas red excitation. The texas red labeled oligo binds to its complementary oligo A, but not to oligo B.

CONCLUSIONS
We have patterned several functional ligands in channels of extended nanospace depth for the first time for multiplexed analysis of minute sample volumes.

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