Nanofluidic Devices for Single Molecule Spectroscopy

CNF Project # 762-99
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Abstract:
Micrometer and sub-micrometer fluidic devices are used in a variety of applications for analytical biochemistry. Previous work describes the benefits of performing single molecule spectroscopy in small engineered focal volumes with regard to uniform illumination intensity and controllable analyte flow [1]. The goal of this project is to further reduce the engineered focal volume with sub-100 nm fluidic channel dimensions. Reduction of the focal volume will reduce the probability of multiple molecule occupancy and could lead to spectroscopy with higher accuracy. Biophysical interaction at the channel-fluid interface becomes increasingly dominant at smaller dimensions and therefore warrants comparison of the practical benefits of each design [2].

Introduction:
A two-step lithographic process was used to create these nanofluidic devices. Electron beam lithography was used to pattern the nanofluidic channel region and an anisotropic reactive ion etch of the fused silica formed channels of dimension 100 nm by 45 nm, as shown in Figures 1 and 2. A photolithography step defined the reservoirs and analyte supply channels to the nanofluidic channel region. Channels were sealed in a direct bonding process using a fused silica cover wafer, to reduce autofluorescence and allow standard coverslip-corrected microscope objectives to be used.

To provide a comparison with nanofluidic devices, sub-micrometer fluidic channels were created with a similar process involving a single photolithography step. Sub-micrometer channels were patterned with projection photolithography and then created with an anistropic reactive ion etch resulting in channel dimensions of 500 nm by 250 nm, as shown in Figure 3. Direct bonding with a fused silica cover wafer is used again [3].

Fluidic channels have been used in experiments involving fluorescent dye labeled DNA and proteins. In particular, a relatively-new molecular engineered probe, the molecular beacon [4], is under investigation for use with fluidic channels in single molecule spectroscopy.

Summary:
Sub-micrometer (500 nm x 250 nm) and nanometer (100 nm x 25 nm) scale fluidic channels were fabricated in fused silica to conduct single molecule spectroscopy. A nanometer-scale engineered focal volume permits micromolar range analyte sample concentrations, while increased biophysical interactions at the channel-fluid interface warrant comparative study with sub-micrometer channels.

Investigation was conducted using molecular beacons in fluidic channels to provide more robust single molecule spectroscopy, with possibilities for the analysis of heterogeneous analyte solutions. The highly sequence-specific nature of molecular beacons, combined with rapid fluidic channel analysis, presents new possible opportunities for early detection of disease.

References:
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- Rapid analysis of analytes confined within the fluidic channel.
- Materials compatible with several types of biology and conducive to study with conventional microscope optics.
- Straightforward fabrication process requiring 2-step lithography or less.

Figure 1, top left: Optical micrograph of electron beam lithography fabricated nanofluidic channels.

Figure 2, bottom left: Atomic force microscope image of a single nanofluidic channel profile (approximately 100 nm wide x 45 nm deep).

Figure 3, above: Optical micrograph of an array of sub-micrometer fluidic channels. Spectroscopy is performed in the narrow region (500 nm wide x 250 nm deep) of each channel near the numbers.