Zero-Mode-Waveguide (ZMW) for Surface-Cell Interaction Study

CNF Project # 599-96
Principal Investigator: Harold G. Craighead

Abstract:
Sub-wavelength metallic apertures have been used for optical single molecule studies. These devices are referred to as zero-mode waveguides (ZMWs). Because of their small observation volume, ZMWs allow single-molecule fluorescence correlation spectroscopy (FCS) at micro-molar concentration and microsecond temporal resolution [1]. Recently ZMWs have found further use in studies of diffusion of single lipids within a cell membrane [2]. Because ZMWs confines fluorescence excitation to zeptoliter volumes, they have become an effective tool in observation of cell functions with high spatial resolution. However, because the excitation volume is confined to the bottom ~ 50 nm of the holes, the question of how far cells probe into the waveguides still remains to be resolved [3]. In our project, we fabricated closely spaced waveguides allowing more sampling points in a given illumination area. These structures were used to study cell and surface interactions. We also compared cell adhesion to aluminum and SiO₂ surfaces by recording the fluorescence of Dil-labeled cell membranes that are excited inside the ZMW holes.

Summary:
Fused silica substrates are used in fabricating zero mode waveguides. A 100 nm thick aluminum film was patterned using electron-beam lithography. The substrate is subsequently diced into individual dies for experiment. For comparison, a few dies are further coated with a thin (3 nm) layer of SiO₂, by electron beam evaporation. Rat basophilic leukemia (RBL)-2H3 cells that contained Dil C16 suspended in solution were applied to these surfaces, incubated for 30 minutes, and fixed with formaldehyde. Fluorescent images are taken to compare the adhesion of cells to surfaces, and actively probe into the waveguides while the cells are still alive. The affinity difference of cells to SiO₂ and aluminum surfaces remains to be quantitatively determined. In addition, further experiments are planned to test the investigation of cells into ZMWs.

References:
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- ZMWs allow single molecule study at micro-molar concentrations.
- ZMWs can be used to monitor diffusion of single lipids in cell membranes.
- How far into the ZMW holes a cell membrane would investigate remains to be determined.

Figure 1: SEM image of ZMW aluminum surface, showing developed e-beam resist patterns.

Figure 2: SEM image showing ZMWs on aluminum surface after lifting off e-beam resist.

Figure 3: (a) Trans-illuminated ZMWs showing four different hole sizes, each at 1 µm spacing, and epi-fluorescence of RBL cells fixed to (b) SiO₂ surface, (c) Aluminum surface, showing qualitatively more cells adhering to SiO₂ surface than to Al surface.