An Array of Planar Apertures for Near-Field Fluorescence Correlation Spectroscopy

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Abstract:
We have fabricated planarized apertures in a thin metallic film for near-field fluorescence correlation spectroscopy. This fabrication utilizes electron beam lithography to define the apertures, reactive ion etching to transfer the pillars in the fused silica substrate, and chemical mechanical polishing to planarize the 50 nm diameter apertures. The resulting device has 1.2 nm RMS roughness and an illumination area 14X smaller than a diffraction-limited area (0.11 vs. 0.0076 µm$^2$) on both supported lipid bilayers and live cell membranes.

Summary of Research:
The organization and dynamics of cellular membranes is fundamentally important for a variety of cellular processes. The plasma membrane contains numerous receptors and signaling molecules utilized by the cell to sense the surrounding environment and transmit signals into the cellular interior. However, the nanoscale reorganization of membrane-bound molecules is largely not understood due to the experimental difficulties associated with dynamic nanoscale phenomenon [1].

Fluorescence microscopy provides high temporal and chromatic resolution, but is conventionally limited to the diffraction limit of ~ 200 nm resolution. Near-field excitation is one of the methods for improving on the diffraction barrier by using nanoscale metallic structures to concentrate the fluorescence excitation light at the dimensions of the fabrication [2,3]. To achieve confinement of excitation light to 60 nm diameter area of the membrane, we fabricated 50 nm diameter planarized glass-filled apertures in Al$_{95}$Si$_{5}$Cu$_{1}$ for transmission near-field illumination (Figure 1) [2].

Figure 1: Scanning electron micrograph of 50 ± 4 nm diameter planarized apertures for near-field optical microscopy (PANOMs). The four fused silica apertures are indicated by white arrows and are surrounded by the Al alloy film, which has noticeable metallic grain structure. (Reprinted with permission from Cell Press [2])
Fabrication of the planarized apertures utilized electron beam lithography to create 90 nm diameter pillars of NEB-31A3 on a 500 µm thick fused silica wafer. A reactive ion etch with CHF₃ and O₂ was performed to etch the fused silica and create nanoscale glass pillars. The glass pillars were etched with buffered oxide etch to yield pillars as small as 20 nm diameter. The wafer was then sputter coated with 200 nm of Al₉₅Cᵤ₄Sᵢ₁ and planarized with chemical mechanical polishing, with a resulting RMS roughness of 1.2 nm.

Apertures were incorporated into a two-objective optical microscope (Figure 2) to examine membranes with fluorescence correlation spectroscopy (FCS). FCS analyzes the fluorescence intensity vs. time for individual fluorophores diffusing through the illumination spot. The characteristic dwell time for fluorophores in the illumination depends both on the diffusion rate of the fluorophores and the area of the illumination.

The small illumination area created by these apertures is shown by the fast dwell time for membrane-bound fluorophores via FCS (Figure 3). The half-time of diffusion for membrane-bound G₄₅-Bodipy in both POPC supported lipid bilayers and living RBL cells is 14x faster than in a calibrated far-field setup. Measuring the far-field illumination to be 190 nm diameter and the illumination area the near-field illumination was determined to be 60 nm diameter.

In future studies, we plan to utilize apertures of varying sizes for two-color cross-correlation analyses. This technique will permit us to examine microsecond dynamics of membrane-bound molecules and co-diffusion at 60 nm length scales.

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References:

