Zero-Mode Waveguides for Single-Molecular Imaging

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Abstract and Introduction:

Zero-mode waveguides (ZMW) are arrays of sub-wavelength apertures in a metal film that allow for the observation of single-molecule interactions at micromolar concentrations through the excitation of fluorescently tagged molecules. When light is shown through a zero-mode waveguide, photons with longer wavelengths are unable to propagate through the waveguide and only evanescent modes will exist. In a fluorescent microscope, the evanescent waves will exponentially decay at the glass/water interface, leading to a detection volume on the scale of zeptoliters (10^{-21} L) [1]. Zero-mode waveguides have many biological applications, such as allowing real-time deoxyribonucleic acid (DNA) sequencing [2], and observing protein-protein interactions [3]. To date, fabrication of ZMWs requires expensive and/or low-throughput nanolithography such as focused ion beam milling, e-beam lithography and dry etching, e-beam lithography and lift-off, deep UV lithography and lift-off, and nanoimprint and lift-off.

The aim of this project was to utilize an alternative method of fabrication involving conventional photolithography, lift-off, and electrodeposition to achieve affordable and efficient single-molecular imaging that could be adopted in most cleanroom facilities.

Experimental Procedure:

Conventional photolithography (AutoStep200) and negative photoresist was used to create a square pattern of waveguides surrounded by four square trench patterns. Lithography doses were adjusted, ranging from 0.15s to 0.22 s, and tested to determine the best parameters. Following photolithography, samples were baked in a large oven for 30 min for image reversal properties of the negative resist to take effect. Baking temperatures of 102ºC, 107ºC, and 112ºC were tested to determine the temperature that would yield optimum results. After baking, all samples were flood-exposed (40s, MA-6/BA-6 mask/bond aligner) and developed in solution. Development time was adjusted once again to determine the optimum time for each exposure dose.

After the photolithography patterning, 100Å of chrome (Cr) and 1000Å of gold (Au) were deposited on the surface using electron-beam evaporation physical-vapor deposition (EnerJet Evaporator). Lift-off of photoresist was performed using a heated solution of PRS-2000 (Baker).

In order to shrink the size of waveguides produced after lift-off, samples were further electroplated with gold. Waveguide diameters were measured using a scanning electron microscope (SEM) and variation in diameter locally and globally were calculated to quantify uniformity and consistency of fabrication procedures.

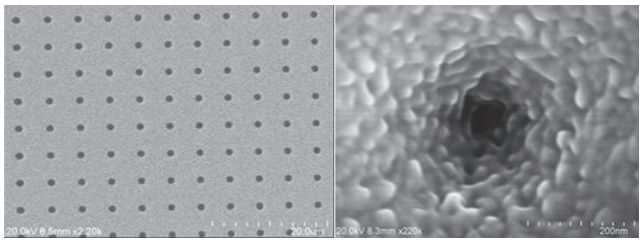

Figure 1: Image of waveguides after lift-off (left). Image of final waveguide after plating (right).

Results and Discussion:

Waveguides 50 nm to 100 nm in diameter were successfully fabricated, and molecular diffusion testing showed single-molecule detection at a concentration of 1 μM. The optimum conditions for fabrication included lithography doses from 0.16s to 0.19s, a baking temperature of 102ºC, and lithography dose dependent development times from 27s to 36s. To determine the uniformity and controllability of the fabrication process, the global and local uniformity in diameter size was calculated for 3×3-inch samples. Local uniformity refers to waveguides within the same cell whereas global uniformity refers to diameter measurements throughout the entire sample.

Under optimum conditions, the best local uniformity achieved was in a cell in the center of the sample, with a mean diameter of 54.4 nm, standard deviation 9.6 nm, and a range of ± 20.2 nm. However, the samples were not as uniform globally as they were locally. While many cells achieved high uniformity, cells around the outer edges of the sample generally had a lower uniformity, indicating a problem in the fabrication process. This may be due to lack to uniformity in photoresist thickness, which then creates irregularities in pillar size and waveguide diameter. Furthermore, random fluid movements during development may cause sections around the edge to be less uniform.

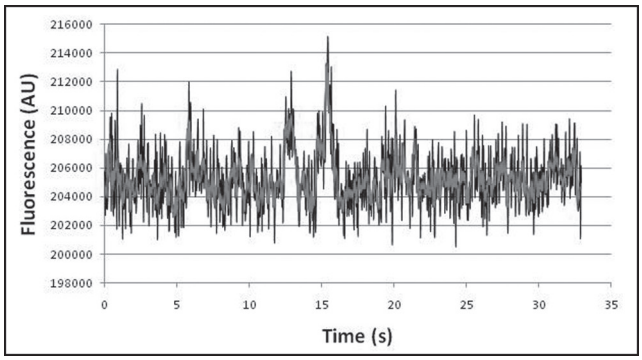

Figure 2: Single-molecule detection of 1 μM TMR-streptavidin in a 70 nm waveguide. Peaks of higher fluorescence indicate excitation of single molecules in the waveguide.

Using a waveguide 70 nm in diameter, diffusion experiments were conducted to test the effectiveness in single molecule detection. Figure 2 shows that single molecule detection was achieved in a solution 1 μM in concentration with a signal to noise ratio of about two.

Background noise was still fairly significant and could be further reduced to improve detection. Background fluorescence noise may be caused by auto-fluorescence of contaminants or surface reflection of Au or Cr. This may be reduced through better cleaning methods prior to Au electroplating.

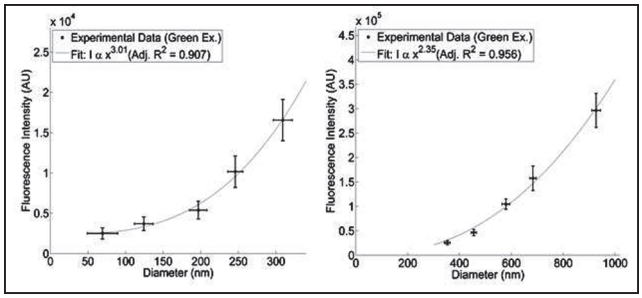

Figure 3: Fluorescence intensity at waveguides of various diameters ranging from 50-1000 nm. Experiments are conducted with green light.

Figure 3 shows the relationship between fluorescence intensity and waveguide diameter for diameters ranging from 50 nm to 1000 nm. Waveguides below and above 350 nm in diameter showed 3.01 and 2.35 power dependences of fluorescence intensity versus diameter, respectively.

In small waveguides, fluorescence intensity is a function of excitation intensity in the waveguide and the fluorescence out-coupling efficiency. In larger waveguides, light is able to propagate through the aperture, and fluorescence intensity is mainly determined by the area of the aperture, which is theoretically second-power dependent on the radius or diameter.

Conclusions:

Waveguides of 50-100 nm were fabricated and tested to yield single-molecule detection at a concentration of 1 μM. The signal to noise ratio of detection was approximately 2:1 and can be further improved. Fluorescence intensity measurements taken using waveguides of different diameters showed an approximately third power dependence in smaller waveguides and an approximately second power dependence for larger waveguides. While local uniformity in diameter is fairly good, future studies should aim to improve global uniformity.

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References:

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