Microsphere Lithography and a Demonstration of Fluorescence Quenching and Reduced Photobleaching

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Abstract

Using microsphere lithography, a bottom-up fabrication method, we create large areas of gold Fischer pattern. Additionally, using a Polydimethylsiloxane (PDMS) substrate coated with a thin layer of gold, we demonstrate fluorescence quenching and enhancement when the PDMS-Au sample is in contact with the fluorescent dye Nile Blue. Additionally, reduced photobleaching of the Nile Blue is shown. Observations are made by raster scanning the sample in an inverted confocal microscope with laser excitation.

Introduction

There is currently a strong trend towards nanometer scales driven in part by the needs of electronics, data storage and biology. Nanotechnology requires not only unique fabrication techniques, but also an understanding of physical processes on such a small scale. In this paper, we attempt to use a bottom-up nanofabrication technique to explore the behavior of fluorescent molecules in contact with our nanostructure.

Current nanofabrication processes include electron-beam lithography\cite{1}, UV-photolithography\cite{2}, and scanning probe lithography\cite{3}, as well as other methods including nanosphere lithography (NSL)\cite{4} similar to that described within this paper. Unfortunately, none of the available methods are ideal for all applications; there is a delicate balance between the scalability, speed and cost of the techniques.

NSL is an inexpensive, high throughput nanofabrication process that is applicable to a wide variety of materials. It can produce a two-dimensional array of periodic structures to study the size-dependent optical, magnetic, electrochemical and catalytic properties of materials. In the proper conditions, nanospheres in solution that are placed on a substrate will self assemble into a two-dimensional crystal lattice. A thin layer of metal is then deposited onto the nanospheres, as well as the uncovered substrate, by any of a variety of methods. By removing the spheres after metal deposition, we obtain a "Fischer pattern", which is the periodic array of triangle-like structures created by the space between adjacent spheres.\cite{5} A computer generated Fischer pattern is shown in Figure 1.

![Figure 1: A computer generated Fischer pattern (black structure).](image_url)
when the fluorescent molecule is not saturated, it can easily be understood that by increasing the non-radiative decay rate enough, it will become less likely for the excited state to decay in such a way that the molecule will photobleach.[6]

This concept was first proposed in 1946 by Edward Purcell, specifically that nuclear magnetic resonance (NMR) relaxation times could be altered by the molecules environment.[7] Detailed calculations of the lifetime of a fluorescent molecule at different distances from a metal surface have been done by Kuhn. He has shown that as the distance between the fluorescent molecule and the metal is decreased, the ratio of the lifetime at the given distance and the lifetime infinitely far from the metal will oscillate, but at close enough distances (<~5nm) the lifetime will decrease and at the limit go to zero.[8] Specifically this means that the fluorescent molecule is quenched and is therefore quite resistant to photobleaching.

Microsphere lithography (MSL) was used for this project for convenience. The large, 3µm, polystyrene spheres used, as well as the resulting gold Fischer pattern, were both easily visible in a traditional wide field light microscope. The additional advantage of the large spheres is that they are more easily removed from the substrate.

Methods

Fabrication of Fischer Pattern. A solution of 3µm diameter polystyrene beads in water was deposited on the center of a methanol cleaned glass coverslip. The coverslips were placed in a plastic box with a small amount of magnesium chloride to slow the drying time from less than 1 hour in air to approximately 5 hours, resulting in a more uniform monolayer of beads. After drying, the samples were placed in a thermal vacuum evaporator (~ 1.9 × 10^-7 torr). Gold was evaporated in a Molybdenum boat (2.5V, 130A) for 10 minutes resulting in ~30nm of gold with ~2.5nm RMS surface roughness (as determined via AFM). Beads were removed using either Tapecon tape or medium tack dicing tape. Samples were then sonicated methanol followed by acetone to remove and dissolve, respectively, all remaining beads. Samples were viewed in a light microscope (100x, NA 0.9 air objective) to check the Fischer pattern and confirm that no beads remained.

Fluorescence Quenching with a Gold Surface. Polydimethylsiloxane (PDMS) was prepared on a glass slide and baked for 6 hours at 70°C. The PDMS was then cut into squares and removed from the slide, resulting in a smooth flexible substrate. A mask was placed over the PDMS and 18nm of gold was sputtered onto the surface. The gold side of the PDMS was then placed onto a spin coated sample of Nile Blue (40µL of 10^-4M Nile Blue, spun at 3000rpm). The sample was raster scanned in an inverted confocal microscope, illuminated with a Coherent RADIUS 635 (λ = 635nm), using a 100x NA 1.4 oil immersion objective.

Computer Simulation. Using Octave, a Fischer pattern template was made. The circular regions were assigned a value of 1 while the regions between the circles were assigned a value of 2, demonstrating the fluorescence of the fluorescent sample following photobleaching while in contact with the metal Fischer pattern. The numbers were chosen based on data collected from the PDMS-Au sample described above. Random intensity variations were then added to simulate the dye layer. The resulting pattern was convolved with the intensity of the exact solution of a tightly focused linearly polarized Gaussian beam and the output was scaled to values consistent with experimental results. The purpose of this was to see how visible the Fischer pattern would be given the size of the spheres (3µm) and a diffraction limited focal spot (~300nm).

Results

Light microscope images of characteristic regions of the Fischer pattern are shown in Figure 2. Regions as large as 25µm × 25µm of Fischer pattern
were observed. The main problem experienced in the formation of the Fischer pattern was the distribution of particle sizes limiting the area of a uniform monolayer of beads.

By placing the PDMS-Au sample in contact with the fluorescent dye Nile Blue, we observed fluorescence quenching, enhancement and reduced photobleaching. Figure 3(a) shows a confocal scan of a region covered in the PDMS-Au sample. The dark areas are quenched while the lighter areas show no effects and the brightest areas show enhancement. The pattern in the image is due to not all regions of dye being in contact with gold. Photobleaching was done by slowly raster scanning the sample through a strong laser excitation. Finally, the PDMS-Au sample was removed and the region was again imaged.

The results, shown in Figure 3(b), clearly show reduced photobleaching in the region that was quenched. The effect of quenching is shown to reduce the fluorescence by a factor of 12. The regions that were not influenced by the metal and those that showed enhancement did not have any increased resistance to photobleaching. The bright circles in all of the images are regions with a larger fluorescent dye density.

The computer simulation revealed that the reduced photobleaching due to quenching when in contact with a gold Fischer pattern is resolvable using our confocal microscope (Figure 4).

**Discussion**

It is well known that the fluorescent properties of a fluorescent molecule are influenced by the molecule’s environment. As a molecule is brought close to a metal surface (<~ 5nm) there will be fluorescence quenching. Several methods were attempted to place the metal and dye surfaces in contact, but finally only the PDMS-Au sample successfully demonstrated this effect.

In our first attempt, we found that simply evaporating gold onto a glass coverslip and physically holding it in contact with a fluorescent sample was insufficient to achieve quenching. Other methods, including forming an epoxy-Au stamp (fabricated similar to the method presented by Fischer and Zingsheim [9]), could not be placed in close enough to the dye molecules. The PDMS, on the other hand, was very easy to place in contact with the fluorescent dye; little ef-
fort beyond simply placing the samples together was required.

Further work will be required to demonstrate fluorescence quenching with a gold Fischer pattern. The remaining challenge is to create a good Fischer pattern on a PDMS substrate. Due to the flexibility of the PDMS, the gold layer is easily cracked. We did try and fabricate a gold Fischer pattern on a PDMS substrate but found that little Fischer pattern, and in fact little gold, remained on the PDMS after washing and soni-cating in water and then in methanol.

For future work, we recommend solidifying the PDMS on a mica substrate so that it will be smoother and to also add a support (such as a glass coverslip) to the backside of the PDMS substrate. The additional smoothness will be an improvement given that PDMS surface non-uniformities were easily observed in a light microscope. The added structural support may help prevent the gold from breaking off of the PDMS.

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References


