Volume Nano-Structured Optics for 3D Superresolution Imaging

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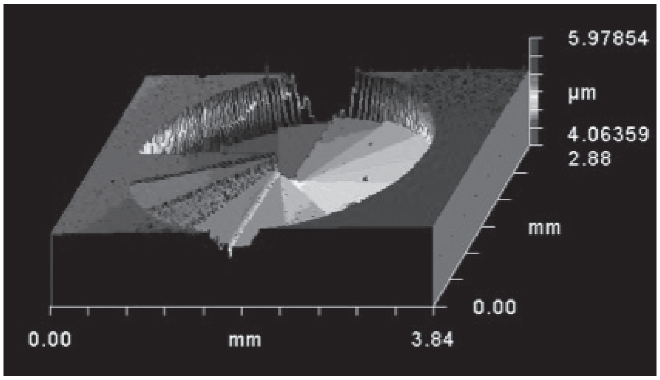

Figure 1: 2D optical profilometry of a phase mask fabricated at the CNL. This is a computer generated model of the mask based off height map measurements. (See cover for full color version.)

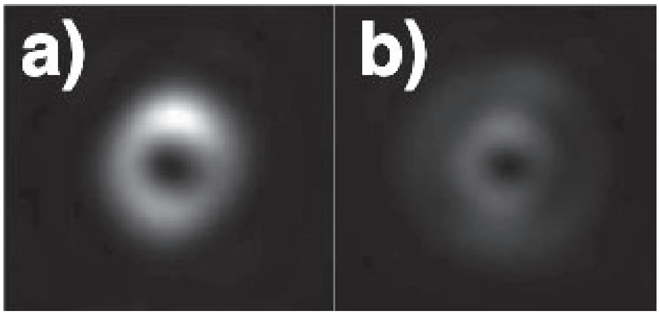

Figure 2: PSF of the phase mask a) in focus, and b) out of focus.

Abstract and Introduction:

Superresolution microscopy is an imaging technique capable of resolution beyond the diffraction limit [1]. One way to overcome the diffraction limit is by imaging a sparse group of fluorescent molecules so that none or only a few of the neighboring molecules emit photons simultaneously. By repeating this process, an image can then be generated by precisely localizing all the molecules seen up to a given instant [2-4].

Superresolution is now limited by the ability to localize a single molecule. In this paper, we present a phase mask that can be inserted in the Fourier plane of a microscope between the object and the camera. The phase mask modifies the transfer function of the imaging system in such a way that information about a molecule's position can be extracted more effectively than with a clear aperture. The phase mask was etched into quartz using four binary masks to achieve sixteen distinct heights. An accelerated image template-matching algorithm is described to retrieve the molecules' location from the captured images.

Fabrication:

The choice material for the phase mask was quartz because it is transparent and sturdy. We started by cleaning a 1″ × 1″ quartz slide in nanostrip bath. Then a 40 nm thick layer of chrome was thermo-evaporated onto one side of the slide. Circular apertures were inserted into the chrome using photolithography and chemical etching. The chrome side was now considered the bottom for the following reactive ion etching (RIE) steps, to physically separate the two different processes.

The optical path length, and therefore the phase of light, is changed depending on the thickness of the quartz slide at any particular point. We selected certain depths with a technique known as binary masks: using N masks, we could select a location on our phase mask to a certain depth with N bits of precision. Each mask patterned an area in the phase mask to be depressed by a certain depth by photolithography. The following RIE step extruded vertically downward any exposed quartz. Thus N masks yield up to 2^N distinct heights, but requires N-1 precise alignments, a nontrivial task.

Increasing the number of distinct heights increases the diffraction efficiency. For example, 16 levels via four phase masks can potentially achieve 99% diffraction efficiency [5]. Figure 1 shows a 16-level phase mask fabricated at the Colorado Nanofabrication Laboratory (CNL) causing a phase shift from 0 to 2π.

Algorithm:

An accelerated image template-matching algorithm is described to retrieve a molecule's location from the captured images. The parameters for 3D superresolution imaging are X, Y, and, Z, where X and Y are the transverse coordinates of the captured image, and Z is depth along the optical axis. The centroids of the sparse blurs in the captured image set the X and Y coordinates. The Z coordinate is quickly found after normalizing for image size and intensity, in the following way. Rather than searching for the maximally matching template, the algorithm finds the highest matching weighted average of all the templates. If the Z-resolution of the templates is high enough, the algorithm should report a weighting vector with nonzero entries for only two neighboring templates. This is implemented by solving the following optimization problem:

$$\begin{array}{ll} \text{minimize} & \left\| V_{imp}(Vw - v_0) \right\| \\ \text{subject to} & \mathbf{1}^{\mathrm{T}} w = 1 \\ & w \geq 0, \end{array}$$

where V is a library matrix in which every column is one of the template images reshaped into a vector, w is the free variable representing the weights of the templates, v_0 is the measured image reshaped into a vector, and V_{imp} is a diagonal matrix allowing the pixels located nearer the center to be considered more important to match.

The best choice of objective function is not yet determined; both the $l_{1\text{-}}$ and the $l_{2\text{-}}$ norms seem promising in preliminary simulations but other convex functions may work too. The nonzero entries in the vector w correspond to certain distances with different weights. To solve the problem, we used CVX, a package for specifying and solving convex programs [6].

Another advantage this approach has over brute force template-matching is that sub template Z-resolution can be achieved.

Results and Conclusions:

The phase mask was successfully fabricated and characterized by optical analysis and white light profilometry, showing that the measured 2D profile was close to the intended shape. In a photon-limited environment, such as molecular imaging, it is critical that the phase mask have high power transmission efficiency. The measured power transmission efficiency of the fabricated phase mask at the designed frequency was 92.0 ± 0.5%. The point spread function (PSF) was confirmed to have a ring shape, as the various phase shifts should all destructively interfere at the center, shown in Figure 2. We have confirmed that our algorithm can match a template with simulated noise successfully.

Future Work:

As future work, the optimization algorithm could be finalized, such as determining the objective function and the V_{imp} term. An antireflection coating could enhance the power transmission efficiency even further, increasing the signal-to-noise ratio. In addition, superresolution experiments could be performed, and the data could be processed.

Acknowledgments:

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