Aperture Arrays for Subnanometer Calibration of Optical Microscopes

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***Abstract*—We fabricate and test subresolution aperture arrays as calibration devices for optical localization microscopy. An array pitch with a relative uncertainty of approximately three parts in ten thousand enables localization with subnanometer accuracy.**

Keywords—aperture; subnanometer; localization; microscopy

I. Introduction

Optical microscopy methods of imaging and localizing subresolution emitters are having impact in diverse studies ranging from microelectromechanical to biological systems [1–4]. Such measurements can have subnanometer precision, even for single-molecule emitters [5], but if calibrations ensuring accuracy at corresponding length scales are absent, then this can be false precision. There has been commercial interest in developing subresolution aperture arrays as calibration devices for optical microscopes [6], as well as recent application of such devices to localization microscopy in three dimensions [7]. However, aperture arrays enabling measurements that are traceable to the SI at subnanometer scales are not yet in common use. Here, we fabricate and test such nanostructures, introducing a practical approach to optical localization that is accurate at subnanometer scales.

II. Methods and Materials

A. Device Fabrication

We fabricate aperture arrays in a platinum film on a silica substrate. A film thickness of approximately 80 nm results in low background noise from light transmission, and a substrate thickness of approximately 0.17 mm enables the future calibration of objective lenses with oil immersion for detection of single fluorophores. We use electron-beam lithography and ion milling to pattern sub-resolution apertures on a pitch of 10 μm, defining the critical dimension of our calibration device. Our lithography system positions the electron beam with a nominal accuracy of 2 nm, which we take as an initial estimate of the standard deviation of aperture placement in one lateral dimension. The uncertainty of the array pitch is greater than this value by a factor of $\sqrt{2}$, giving a relative uncertainty of the array pitch of approximately 3×10^{-4}. We will revisit this analysis in a future study. The extent of the aperture array exceeds the field of our optical microscope.

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B. Optical Microscopy

A light emitting diode trans-illuminates the aperture array at a wavelength of approximately 630 nm. An objective lens with corrections for chromatic and flatfield aberrations, a nominal magnification of 50×, and a numerical aperture of 0.55 collects light transmitted through the apertures. A tube lens projects the image of the aperture array onto a complementary metal-oxide-semiconductor (CMOS) camera with 2048 × 2048 pixels with a nominal size of 6.5 μm. The camera records optical micrographs at a video rate of 10 Hz. Fig. 1 shows a representative optical micrograph of a small region of the aperture array. Each subresolution aperture appears as the point spread function of the imaging system. We use weighted least-squares estimation to fit each point spread function to a symmetric Gaussian model. Our localization analysis accounts for the gain and noise of each pixel of the CMOS camera over its full field. We will describe the details of our localization analysis in a future study. In each micrograph, the localization precision for each aperture is less than 1 nm.

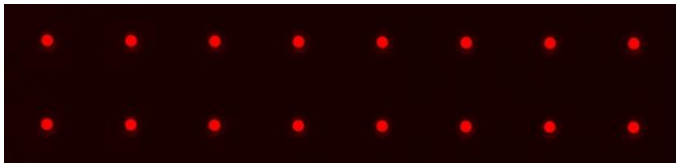

Fig. 1. Optical micrograph showing an array of superresolution apertures in a platinum film on a silica substrate. The array pitch is 10 μm with a relative uncertainty of 3×10^{-4}. Aperture localization enables microscope calibration.

III. Results and Discussion

A. Magnification Calibration

Calculation of distances between apertures across a micrograph enables mapping of the image pixel size. Fig. 2 shows such a map, with linear interpolation between apertures. A radial pattern is evident, possibly from lens manufacture. The image pixel size varies from 127.10 nm to 127.43 nm. In contrast, the nominal value of the image pixel size is 130 nm, resulting in a range of errors of 2 % or more. Many previous studies have used relatively rudimentary methods to calibrate image pixel size, potentially undermining the accuracy of otherwise precise localization measurements.

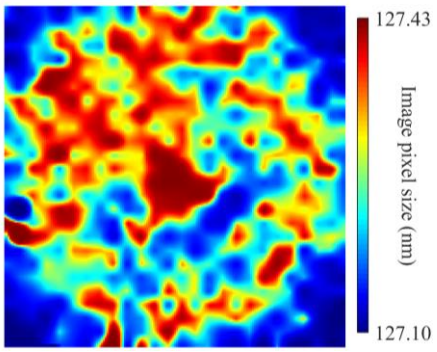

Fig. 2. Spatial map showing variation of image pixel size, possibly from lens manufacture, over the full field of the imaging system. The nominal value of image pixel size is 130 nm, corresponding to errors of 2 % or more.

B. Noise Analysis

We assume that the aperture array is mechanically stable, allowing analytical elimination of any lateral motion that is common between apertures. This isolates the apparent lateral motion of each aperture due to photon shot noise, which is the physical limit of uncertainty in the localization of subresolution emitters [8, 9]. Temporal averaging of photon shot noise reduces the Allan deviation for single apertures through the subnanometer scale and into the picometer scale. Fig. 3 shows a representative analysis for a single lateral dimension. The black line is the mean value and the gray bound denotes the standard deviation of the Allan deviation of 50 apertures. The slope of approximately -0.5 is consistent with the inverse square root of photon count, which increases linearly with averaging interval. Uncertainty in the size of image pixels from the relative uncertainty of the array pitch sets an inaccuracy floor of approximately 3×10^{-2} nm, which we approach in an averaging interval of less than 1 min. Much of this precision would be false precision in the absence of the preceding magnification calibration.

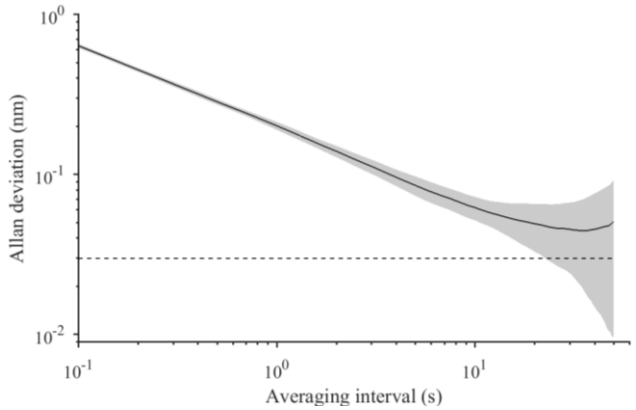

Fig. 3. Temporal averaging of photon shot noise reduces the mean value of Allan deviation of aperture location toward an inaccuracy floor of 3×10^{-2} nm. The gray bound denotes the standard deviation of 50 apertures.

IV. Conclusions

We are concerned that the increasing interest in achieving localization precision at subnanometer scales has advanced ahead of the corresponding metrology foundation for ensuring localization accuracy. Here, we have introduced a practical approach to achieve both accuracy and precision in optical localization microscopy extending below the subnanometer scale. In a future study, we will revisit the various topics that we have noted here, and we will apply our new measurement capability to quantify any motion of fluorescent nanoparticles adsorbed to imaging substrates. While many previous studies have assumed that this arrangement results in nominally static fiducials, there are open questions in the literature on this topic [9, 10] that we can now answer more definitively.

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