Nanometer traceability of localization microscopy

Craig R. Copeland, Ronald G. Dixson, Andrew C. Madison, B. Robert Ilic, Samuel M. Stavis*

National Institute of Standards and Technology, Gaithersburg, Maryland 20899

Localization microscopy, including pointillist imaging and particle or edge tracking, enables resolution beyond the optical diffraction limit in diverse applications ranging from cell biology to microsystem technology.[1] Random effects, such as from photon shot noise, yield localization uncertainties that can extend below one nanometer. However, systematic effects, such as from the common but inadvisable assumption of nominal magnification, can become orders of magnitude larger across an imaging field.[2] Such an extreme discrepancy between precision and accuracy can result in severe overconfidence in position data. Moreover, any claim to traceability is not yet rigorous, further limiting the reliability of position data. The root cause of this critical problem is the absence of traceable standards and microscope calibrations that are fit for this purpose.

To solve this problem, we are developing aperture arrays to specify reference positions across an imaging field. A central goal of our work is to accurately and efficiently control and quantify the reference positions. Previously,[2] we tested the accuracy of electron-beam lithography to place apertures in rectilinear arrays. Two lithography systems each used two interferometers, which are implicitly traceable to the laser wavelength of 632.8 nm, to measure and calibrate stage positions within the lithography process. By localizing apertures and comparing arrays from the two systems, we estimated a difference of mean distance between apertures of one part in five thousand, or approximately one nanometer, and a standard deviation of the distances between apertures of a few nanometers from random effects during processing.

In this study, we validate the accuracy of aperture placement for the first time, by applying critical-dimension atomic-force microscopy[3] to traceably measure the distances between apertures. In an initial test, we analyze the locus of sidewall positions around the bottoms of a representative pair of apertures (Fig. 1a). By either centroid analysis or elliptical modeling of the sidewall positions, the distance between the apertures is within 1 nm of the nominal value of 5000 nm. Sampling of multiple pairs of apertures in the two lateral directions yields a mean distance between apertures of 5001 nm (Fig. 1b), with a standard error of less than 1 nm. These new results are excellent, validating our placement accuracy by electron-beam lithography, and establishing the explicit traceability of aperture arrays to the international system of units (SI).

With traceable aperture arrays in hand, we can calibrate our optical microscope,[2] enabling the traceable localization of new aperture arrays that we efficiently pattern with a focused ion beam. Without interferometric measurements and calibrations of stage position, however, our focused-ion-beam system produces arrays with systematic errors of aperture placement across the patterning field (Fig. 2a-b), depending on process parameters (Fig. 2c-d). Such data present a new opportunity to correct the placement of a focused ion beam, which is in use for commercial production of aperture arrays with high efficiency but uncertain specification of reference positions, leading to the possibility of unreliable standards and erroneous calibrations.

---

[*] samuel.stavis@nist.gov

[1] Mathai *et al.*, *Applied Physics Review*s, 2016

[2] Copeland *et al.*, *Light: Science & Applications*, 2019

[3] Dixson *et al.*, *Journal of Micro/Nanolithography, MEMS, and MOEMS*, 2012

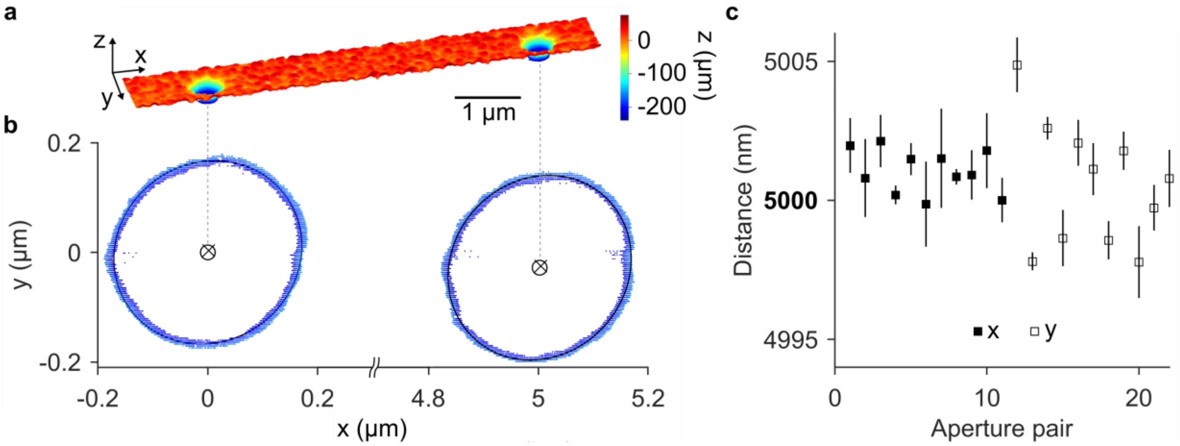

Fig. 1. Validating aperture arrays from electron-beam lithography and establishing traceability. (**a**) Critical-dimension atomic-force micrograph showing a representative pair of apertures. (**b**) Plots showing two-dimensional reductions of sidewall positions around the aperture bottoms. The color code is the same in (a) and (b). The central circles are the centroids from elliptical models of the sidewall positions. The central crosses are the centroids from direct analysis of the sidewall positions. (**c**) Plot showing mean distances between multiple pairs of apertures. Vertical bars are approximately equal to standard errors. The nominal distance between apertures is 5000 nm.

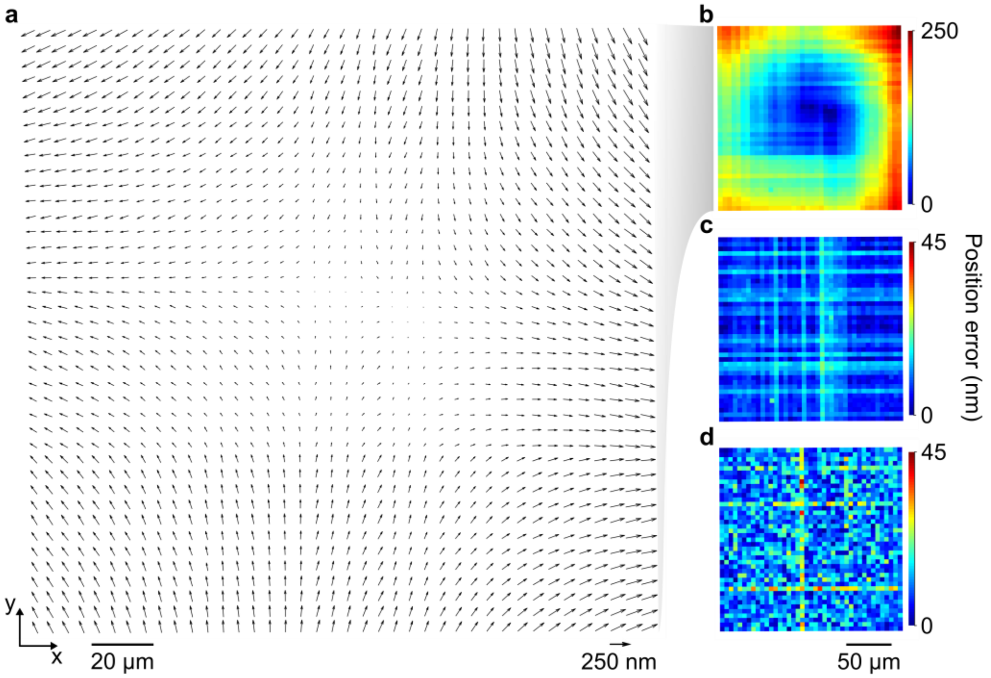

Fig. 2. Testing aperture arrays from focused-ion-beam milling. (**a**) Vector plot showing position errors, not to scale, for each aperture from focused-ion-beam milling, indicating compression in y and expansion in x of the patterning field. (**b**) Plot showing magnitudes of position errors in (a). (**c-d**) Plots showing position errors near the spatial frequency of the array of 5 µm. Plot (c) shows systematic errors from discretization of the patterning field. Plot (d) shows lesser systematic effects but greater random effects from removing discretization and randomizing placement of the focused ion beam. These data present an opportunity to characterize and optimize the patterning process.