

Abstract Submitted
for the MAR16 Meeting of
The American Physical Society

Limits of single-molecule super-resolution microscopy in thin polymer films MUZHOU WANG, MARCELO DAVANCO, JAMES M. MARR, J. ALEXANDER LIDDLE, JEFFREY W. GILMAN, National Institute of Standards and Technology — Structural characterization by super-resolution microscopy has become increasingly widespread, particularly in the biological community. The technique is powerful because it can produce real-space images with resolutions of tens of nanometers, while sample preparation is relatively non-invasive. Previous studies have applied these techniques to important scientific problems in the life sciences, but relatively little work has explored the attainable limit of resolution using samples of known structure. In this work, we apply photo-activated localization microscopy (PALM) to polymer films that have been nanopatterned using electron-beam lithography. Trace amounts of a rhodamine spiroamide dye are dispersed into nanostructured poly(methyl methacrylate), and UV-induced switching of the fluorophores enables nanoscale localization of single molecules to generate a final composite super-resolution image. Features as small as 50 nm are clearly resolvable. To determine the ultimate resolution limit, we investigate sources of error in the system, particularly from systematic mislocalizations due to the effect of fluorophore orientation on the single-molecule point-spread function.

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Date submitted: 06 Nov 2015

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