

Nanostructural characterization is of critical importance for understanding the complex relationship between chemical composition, structure, property, and function for advanced materials design. Many modern imaging methods such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM), provide sub-nanometer resolutions, but each technique has limitations including high vacuum requirements, poor electron contrast, or difficulty in probing sub-surface features. In the past decade, single-molecule super-resolution microscopy has become a standard tool for visualizing nanostructures in the life sciences under native conditions. In this technique, a small fraction of spatially-separated fluorophores are activated from a non-emissive to an emissive state. Images of these bright molecules are then fit to their point-spread function, which determines their positions with tens of nanometer precision, after which the molecules are then de-activated to their non-emissive state. By repeating this process, a super-resolution image is reconstructed from the positions of all identified emitters over many thousands of frames. While this method has been routinely used in the biological community, super-resolution imaging in polymer science is much less reported. A key barrier to widespread adoption is the lack of suitable fluorophore chemistry in organic systems which are common in polymers. A photoswitchable fluorophore that can be easily covalently incorporated into polymers can promote super-resolution microscopy in this community, particularly for investigating nanoscale polymer dynamics in real space and real time, which is difficult through other characterization methods.

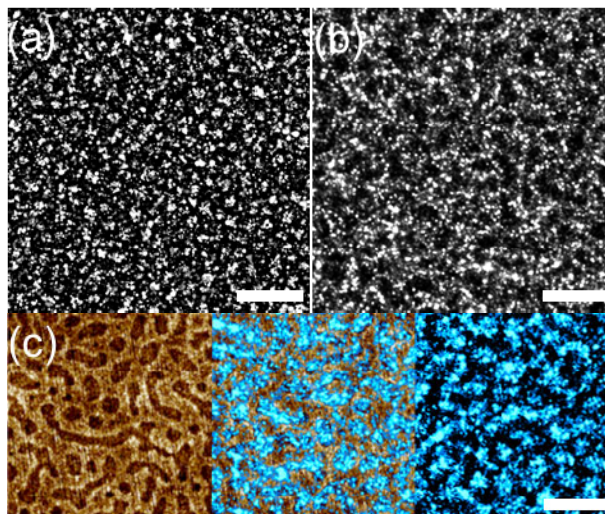


Figure 1. Super-resolution images of 28 nm thin films of PS/PMMA blends with mass ratios of (a) 1:3 and (b) 3:1. PS and PMMA homopolymers are $M_n=40,500$ g/mol, PDI: 1.3 and $M_n=120,000$ g/mol. c) Images of a 1:2 PS/PMMA blend. Left is an AFM phase image, right is a super-resolution image and they are partially overlaid in the middle. Scale bars are 1 μm .

In Year 2 of our ACS PRF support, we have continued our work in developing a photoswitchable fluorophore for super-resolution microscopy that can be directly copolymerized into polymer chains. This greatly streamlines labeling strategies into a single step. We demonstrated this scheme by polymerizing with radical monomers such as styrene and methyl methacrylate, which are labeled with our fluorophore with no further post-functionalization reactions or purifications needed. Unlike many aqueous fluorophore systems that require special buffers to promote transient dark states, our molecule switches between the two thermodynamically stable states and is controlled by relative intensities of ultraviolet and visible illumination, enabling super-resolution imaging through a photoactivated localization microscopy (PALM) scheme. We apply this technique to image the nanostructures of

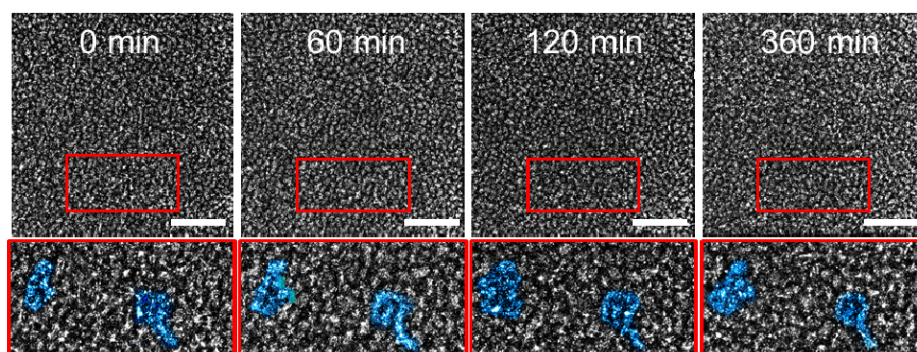


Figure 2. Time lapse super-resolution images of PS/PMMA blends thin film (1/2; w/w) under anisole vapor annealing ($p/p_0=0.95$) for 0, 60, 120, and 360 min. All scale bars are 4 μm . The corresponding zoomed-in regions below are magnified two-fold from the red areas in the above images, with highlighted features in blue visualizing specific features that evolve or remain fixed during annealing. The film thickness is approximately 28 nm.

fluorophore-labeled polymer blends and validate these images through correlation with AFM of the same region (Fig. 1). As each fluorophore can be switched several times, multiple time-lapse images can be acquired for observing the dynamic nanostructural evolution, which we demonstrate using polymer blends swollen with solvent vapor (Fig. 2). This work has been published as a cover article in *ACS Macro Letters*.¹

Further work has shown that optical microscopy can determine the position and orientation of every fluorophore in our experiment. This orientation information can detect alignment induced by mechanical strain, that can thus be mapped throughout a sample at the nanoscale. By circumventing the diffraction limit through PALM, this technique can pinpoint regions of deformed material as small as 20 nm and resolve them from neighboring unperturbed areas. Fluorophore orientations were determined by fitting model point-spread functions (PSFs) derived from vectorial diffraction calculations to the experimental widefield images. In practice, the single fluorophore orientations could be determined to within $\sim 10^\circ$ precision.

We then used this capability to determine the areas of mechanical deformation in a polymer film patterned by thermal nanoimprint lithography. We expected that the deformations from the imprint process would appear as locally-nonuniform fluorophore orientation distributions. A rigid mold with periodic square protrusions of 200 nm pitch and equal protruding and non-protruding area was heated and pressed into a PMMA (950 kg/mol) film doped with fluorophore, producing square wells that we imaged using PALM. Each fluorophore localization event was assigned an azimuthal orientation determined by PSF fitting. The resulting information was then combined into a spatial map of fluorophore orientation and alignment throughout the nanostructure. At every point within the structure, we determined an azimuthal orientation distribution by summing nearby events, and then determined a two-dimensional order parameter throughout the square wells. The fluorophore orientations were distributed predominantly uniformly throughout the well, but there was a preference for alignment along one of the four walls.

We explained this alignment phenomenon by examining the fabrication process more closely. After imprinting, separation of the rigid mold from the polymer film is performed at room temperature, which is deep in the glassy regime for PMMA.

Because the separation always requires peeling that is initiated from one side, the features of the mold are removed at an angle which induces plastic deformation and damage along one side of the wells (Fig. 3a). The location of the alignment phenomenon is always consistent with the peeling direction. Our proposed mechanism is further supported by AFM, which shows protruding features at the north side of each well, corresponding to the aligned region identified by super-resolution orientation microscopy (Fig. 3b). The excellent agreement between the protruding regions determined by AFM and the regions of high alignment determined by optical microscopy suggest that super-resolution orientation microscopy can detect mechanical deformation. The deformed regions are only ~ 20 nm in width and thus far below the optical diffraction limit, but are distinguishable from neighboring undeformed regions at the east and west sides and bottom of each well that are less than 200 nm away. To our knowledge, this is the first demonstration of super-resolution orientation microscopy to reveal deformation and damage at the nanoscale. This work was published in *Materials Horizons* and highlighted in several media outlets.²

Funding from the ACS PRF was received within the first year of the PI's faculty position, enabling a fast start to his research program and relieving significant early financial pressure. The proposed project is a core interest area in the group, and this support has seeded several related lines of research. Through ACS PRF funding, students and postdocs have learned techniques in optics, polymer chemistry, and nanoscale characterization in order to conduct their work. They are now largely independent researchers and will undoubtedly be successful in further efforts in the group and beyond.

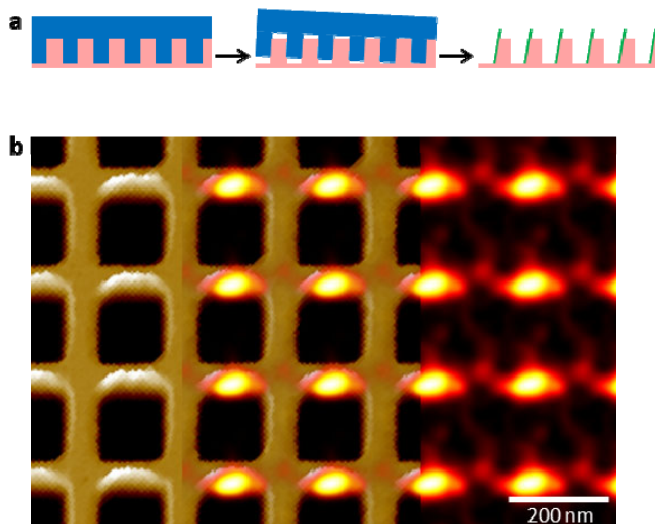


Figure 3. a) A rigid imprinting mold is removed from the PMMA film by peeling from one side, causing plastic deformation below T_g . This results in a damage zone indicated by green. **b)** This is confirmed by atomic force microscopy, where features protrude along the north wall of each well. In a composite of the AFM image (left) and orientational alignment from super-resolution (right), the excellent correlation between the protruding regions detected by AFM and the ordered (bright) regions determined *via* super-resolution suggests that orientation measurements can clearly identify nanoscale deformation.

(1) Qiang, Z.; Shebek, K. M.; Irie, M.; Wang, M. *ACS Macro Lett.* **2018**, *7*, 1432–1437.

(2) Wang, M.; Marr, J. M.; Davanco, M.; Gilman, J. W.; Liddle, J. A. *Mater. Horizons* **2019**, *6* (4), 817–825.