SINGLE-MOLECULE FLUORESCENCE MEASUREMENT OF LOCAL POLYMER PROPERTIES

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The composite interphase is a vital region whose properties are notoriously difficult to measure. Fluorescent molecules can act as uniquely sensitive probes of their local environment, displaying changes in fluorescence lifetime, polarization anisotropy, and spectral shifts, all of which could provide useful information about this region. A prerequisite to making use of this information is the ability to determine the positions and orientations of single molecules accurately and precisely so that molecular behavior can be correlated with material structure. Here, we show the ability to determine the position and orientation of single molecules with an uncertainty of approximately 9 nm and a few degrees, respectively, allowing us to resolve features as small as 20 nm. Another challenge is to introduce probe fluorophores with a sufficient density to capture local material property variations in detail, but without perturbing the property of interest. We solve this problem by means of lithographically-fabricated test structures. These enable us to produce thousands of essentially identical replicas of a feature, the image data from which can be overlaid and integrated. In this way, a sparse fluorophore distribution can still yield a spatially-dense data set. Additional complications involve the interaction of fluorophore emission with variations in the local refractive index of the sample. We address these by fabricating and measuring nanoscale test structures that vary fluorophore environments in a precisely-controlled fashion. In this talk, I will describe our unique, wide-field, single-molecule fluorescence microscope, that allows us to measure the position, orientation, lifetime, and spectrum of fluorescent probes distributed within lithographically-fabricated analogs of real composite structures, and our progress in correlating single-molecule behavior with local material properties.

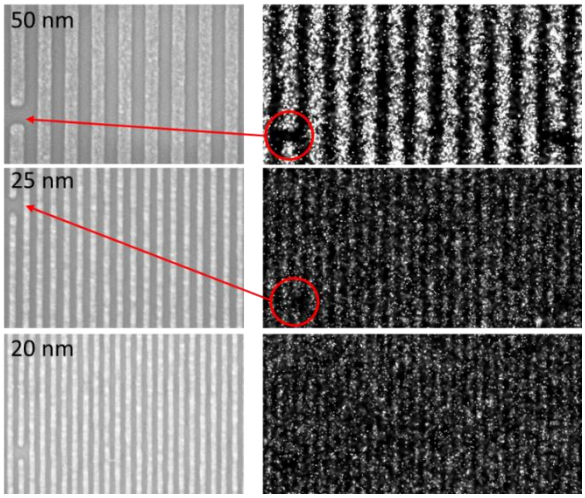

Figure 1. Left) Scanning electron micrographs of 100, 50 and 40 nm pitch lines and spaces. Defects are written into the lines to test the resolution of the system. Right) Single-molecule super-resolution images of the patterns. Lines and spaces are visible for all pitches. Point defects of size 50 nm and 25 nm are resolvable, but the 20 nm point defects in the third image shown in the same locations are barely visible

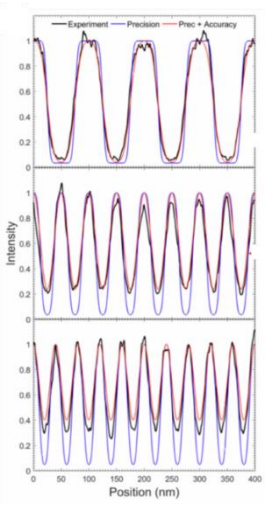

Figure 2. Image data integrated along the axes of the lines shown in Fig. 1. Although the areal density of fluorophores is low, the integrated images provide a high-density measure of the detected line profiles, and a quantitative measure of the system resolution