Super-Resolution Single-Molecule Microscopy: a Characterization Tool for Materials

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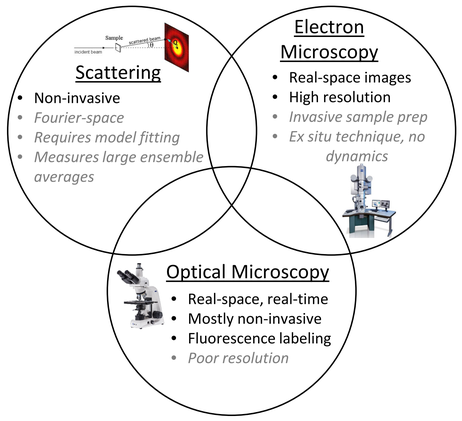
NIST National Institute of Standards and Technology U.S. Department of Commerce

MOTIVATION

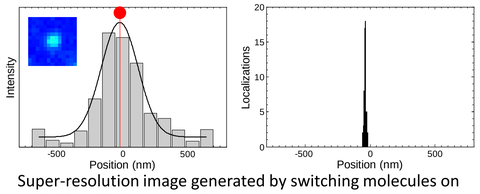
Polymer Structural Characterization



Need a structural characterization technique that combines the strengths and overcomes the limitations of existing methods.

SINGLE-MOLECULE MICROSCOPY

Positions of molecules are estimated to greater precision than the size of their PSFs.



Super-resolution image generated by switching molecules on and off and localizing each PSF.

Point-Spread Function (PSF):

$$I \propto \exp\left(-\frac{r^2}{2\sigma^2}\right) \qquad \sigma = 0.22\frac{\lambda_{em}}{NA}$$

Factors Affecting Resolution

1. PSF accuracy
2. Number of photons (signal-to-noise)

$$\sigma_x \propto \frac{\Delta}{\sqrt{N}}$$

3. Number of molecules

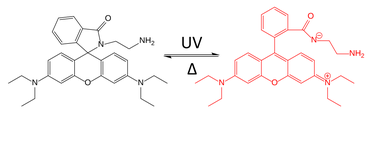
Nyquist-Shannon theorem: At least one event every $x/2$ to resolve a feature of size x.

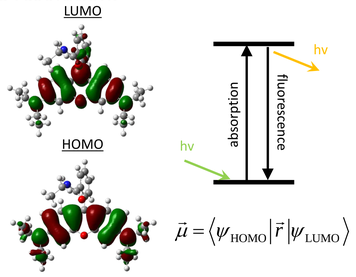
$$\frac{500\ \text{amu}}{(10\ \text{nm})^3} = 0.00083\ \text{g/mL} = 0.08\ \text{wt\% dye}$$

LIMITS OF SUPER-RESOLUTION

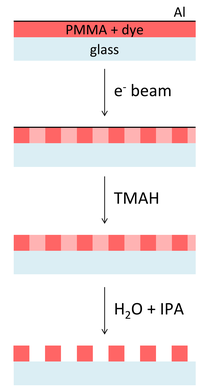
SR microscopy is used in biology, but very few examples have been shown in polymers and soft materials. No systematic study of the ultimate resolution.

Photoactivatable fluorophore based on Rhodamine B switches from dark to bright state when illuminated with UV light, and fluoresces under green light. Absorption and emission dipoles are aligned with planar ring structure.

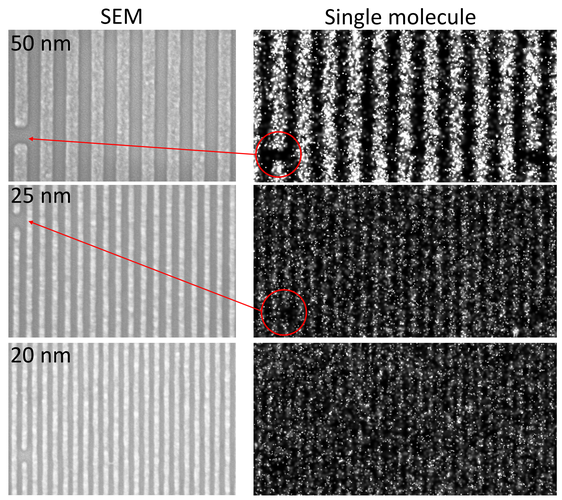


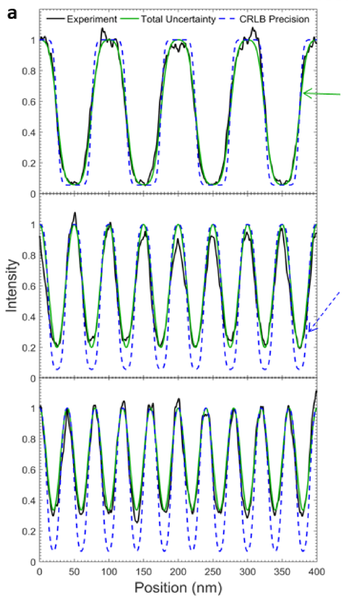


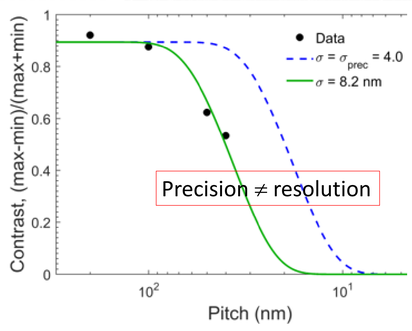
$$\vec{\mu} = \langle \psi_{\text{HOMO}} | \vec{r} | \psi_{\text{LUMO}} \rangle$$

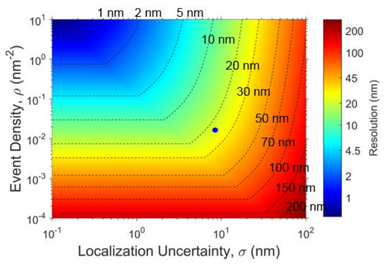


E-beam lithography patterns polymer film doped with photoactivatable dye.



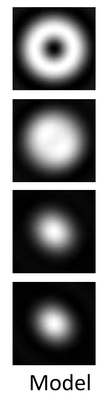




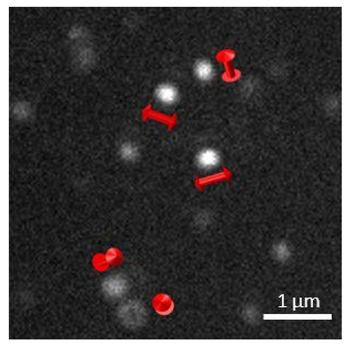


PSF REVEALS ORIENTATION

PSFs of single molecules are not simple Gaussians and encode their orientations.

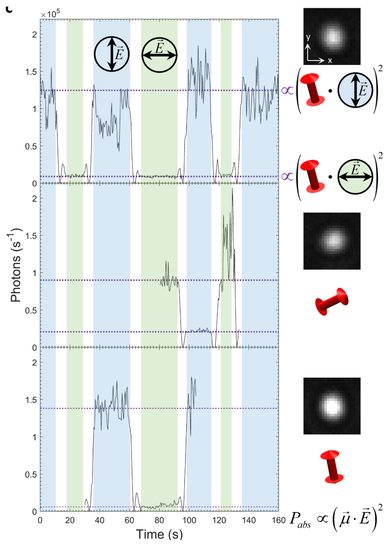


Model

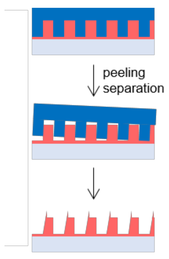


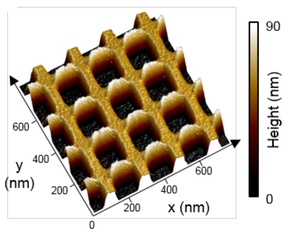
Experiment

Orientations determined from PSFs are consistent with orientations from switching the polarization of illumination.

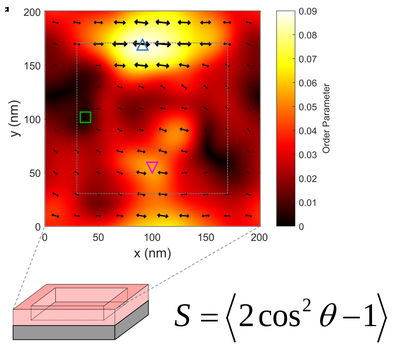


ORIENTATIONS REVEAL DEFORMATION

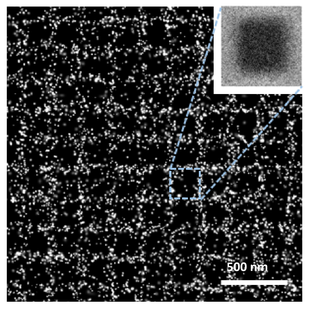




Sum of 1,300 wells

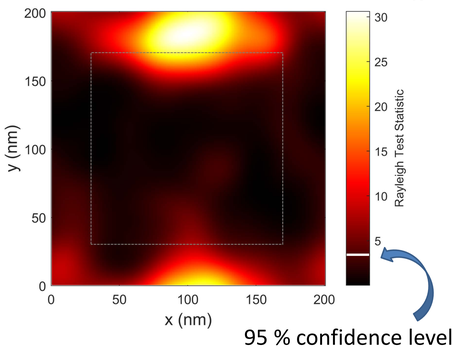


$$S = \langle 2\cos^2\theta - 1 \rangle$$



Integration over array gives high effective labeling density without perturbing material

Rayleigh test statistic = nS^2 – follows χ^2



95 % confidence level