Electronic Supplementary Material (ESI) for Analyst.
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Supporting Information

Facile Method to Stain the Bacterial Cell Surface for Super-Resolution Microscopy

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Abstract: Supporting information contains additional images of fluorescent bacterial cells and cells in combination with fluorescent nanomaterials. Fluorescent nanomaterial characterization is also included.

Fluorescent Nanomaterial Preparation and Additional Fluorescence Images

To provide broader context for this work, we include here additional image analysis and images of bacterial cells stained with amine-reactive Alexa Fluor 488 either alone or in combination with quantum dots. Quantum dot material characterization data is also provided.

Estimating the localization precision achieved by STORM. The equation below, taken from Thompson et al. 2002, was used to estimate the localization precision achieved by STORM.[1]

$$\left\langle (\Delta x)^2 \right\rangle = \frac{s^2 + a^2/12}{N} + \frac{8\pi s^4 b^2}{a^2 N^2}$$

By estimating N=500 photons, b=10 photons, a=100 nm, and s=FWHM/2.35=270/2.35=115 nm, the value of Δx was found to be 14.3 nm. According to this method, the FWHM resolution of STORM imaging performed herein is 14.3*2.35 = 34 nm.

A more conservative estimate was also calculated using the equation below, taken from Mortensen et al. 2010.[2]

$$Variance = \frac{\sigma_a^{\,2}}{N}\left(\frac{16}{9} + \frac{8\pi\sigma_a^{\,2} b^2}{N a^2}\right)$$

where $\sigma_a^{\,2} = \sigma^2 + a^2/12$

Estimating N=500 photons, b=10 photons, a=100 nm, and σ=FWHM/2.35=270/2.35=115 nm, the resulting Δx value was found to be 15.8 nm. Accordingly, the FWHM resolution of STORM is 14.3*2.35 = 37 nm.

Images of Cells and Nanomaterial-Cell Interactions

Figure 1 demonstrates the resolving power of STORM by comparing wide-field (top) and STORM (bottom) images of *Shewanella oneidensis* MR-1 stained and imaged as described in the methods section.

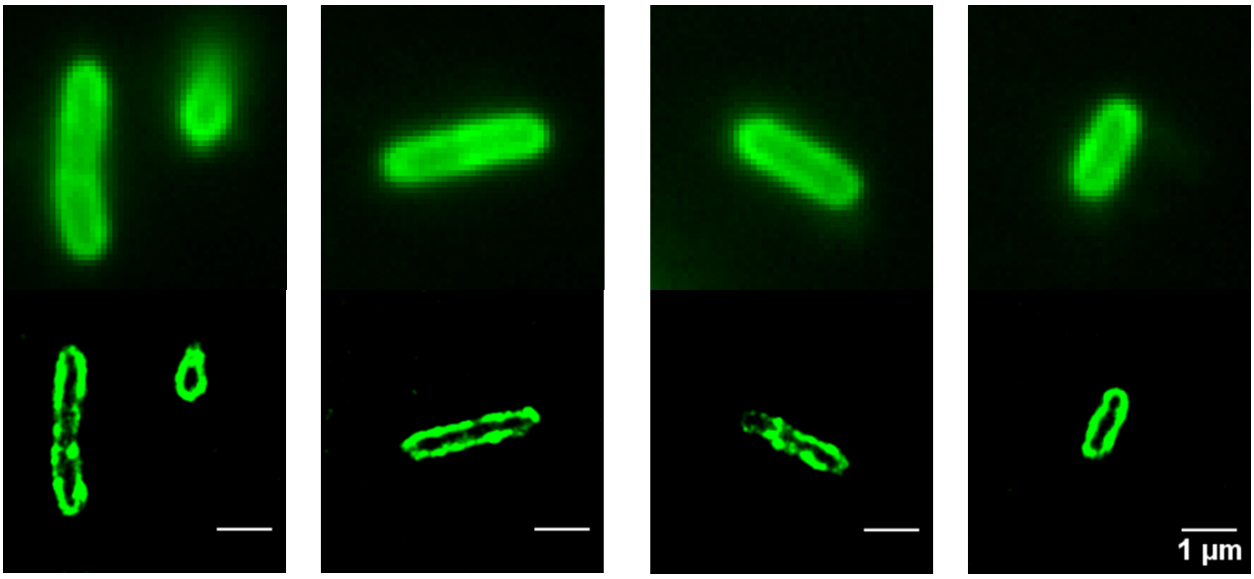


Figure 1: Wide-field (top) and STORM (bottom) images of *Shewanella oneidensis* MR-1 stained with amine-reactive AF-488.

Figure 2 presents an additional SIM image of *Shewanella oneidensis* MR-1 exposed to 250 nM amino-poly(ethylene glycol)-functionalized CdSe/ZnS core-shell quantum dots. Quantum dots, shown in orange, are observed to associate with the cell wall, again showing no penetration into the intracellular space.

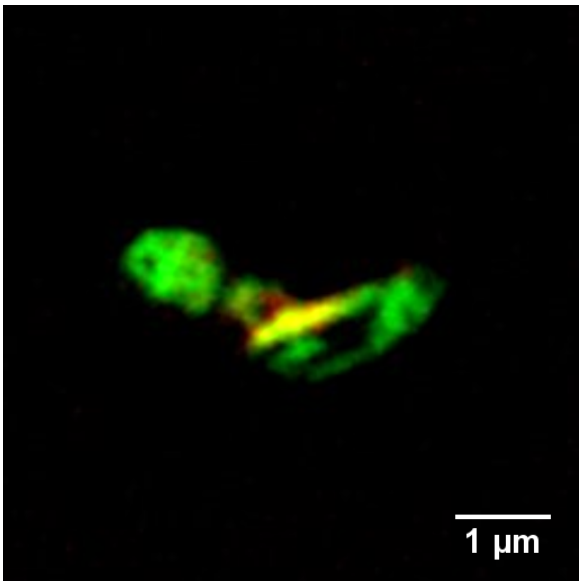
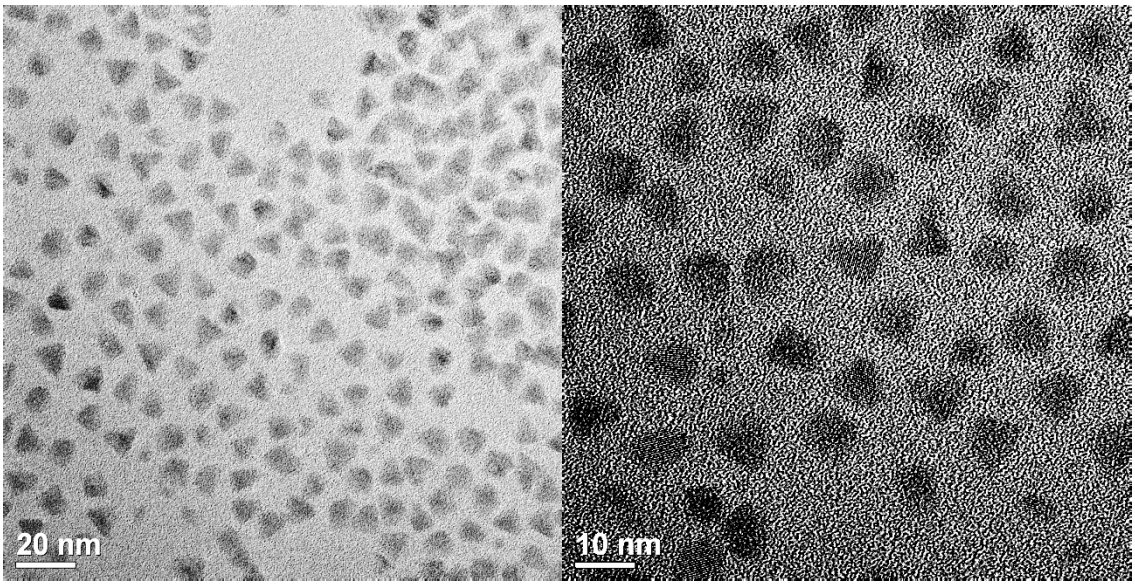


Figure 2: SIM image of *Shewanella oneidensis* MR-1 stained with amine-reactive AF-488 (green), exposed to 250 nM amino-poly(ethylene glycol)-functionalized CdSe/ZnS core-shell quantum dots (orange).

Commercial CdSe/ZnS Quantum Dot Size Characterization

Amino-poly(ethylene glycol) functionalized CdSe(core)/ZnS (shell) nanoparticles were analyzed by transmission electron microscopy (TEM). Particles were diluted 10-fold in ethanol, dried onto a 300 mesh pure carbon grid (Ted Pella), and viewed with a Philips FEG CM200 Ultra Twin TEM at 200 kV accelerating voltage. Particle size in longest dimension is 9.3 nm ± 1 nm (n=83).



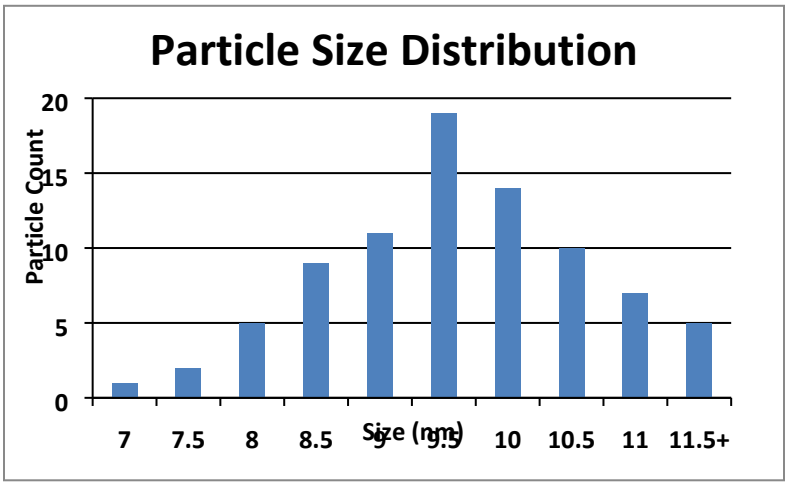


Fig. 4 Representative images at 250,000x and 460,000x magnification with size distribution (n=83).

Reference

(1) Thompson, R. E.; Larson, D. R.; Webb, W. W. *Biophys. J.* **2002**, *82*, 2775–2783.

(2) Mortensen, K. I.; Churchman, L. S.; Spudich, J. A.; Flyvbjerg, H. *Nat. Methods* **2010**, *7*, 377–381.