Nanoscale Hyperspectral Microscopy Enables 17-Color Imaging and Benchmarks Endolysosomal Maturation in Living Systems

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Low-dimensional nanomaterials can be made sensitive to disease biomarkers and other bioanalytes. To build optical probes using these materials, we developed a new spectroscopic imaging technique that resolved up to 17 distinct near-infrared "colors" of carbon nanotubes with single nanotube spatial resolution in live mammalian cells, in murine tissues ex vivo, and in live zebrafish endothelium. We used this capability to precisely quantify the number of fluorescent nanotubes and their emission changes in a single live cell and within individual endosomes.

The approach facilitated the transient and quantitative imaging of endosomal maturation. Nanotubes monitored vesicular fusion by the coalescence of endosomal contents—nanoparticles could be counted in single endosomes during the fusion process. Concomitantly, nanotube emission was modulated by changes in the endosomal redox environment during endosomal maturation. The fluorescence changes could be reversed by inhibitors of the maturation process—thus providing a measure of drug action.