

Electronic Supplemental Information for:

Spatial Modulation Spectroscopy for Imaging and Quantitative Analysis of Single Dye-Doped Organic Nanoparticles Inside Cells

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TEM Imaging of LPNPs

TEM images of LPNPs were recorded using a Titan 80-300 (FEI, USA) with operating voltage of 300 kV and a Gatan 4x4k bottom-mount CCD camera. An aqueous solution of croconaine-doped LPNPs was localized on a carbon coated copper grid (300 mesh) and the samples were stained with 2% (w/v) phosphotungstic acid. A typical TEM image of the LPNPs is presented in panel (A) of Figure S1. A histogram of the particle sizes determined from the TEM images is presented in panel (B). The average diameter of the particles determined from this analysis was 58 ± 20 nm (error equals Standard deviation).

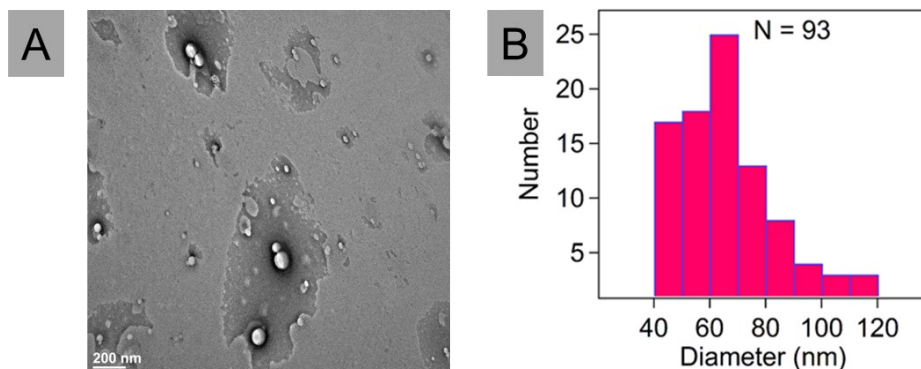


Figure S1: (A) Representative TEM image of the LPNPs and (B) size distribution histogram obtained from the TEM images.

The sample used for the TEM experiments above had a weight ratio of lecithin:DSPE-PEG(2000):dye:PLGA of 0.0125:0.2:0.5:1, compared the 0.0125:0.2:2:1 for the sample used in the optical imaging experiments. The increased amount of dye for the optical experiments will cause the particles to swell. The exact amount of swelling is difficult to determine, as the solvent composition may change as more dye is incorporated into the particles. Based on the mass of croconaine incorporated into the LPNPs, we estimate an average diameter of 90 ± 30 nm for the particles in the optical experiments.

Calibration Experiments with Gold Nanoparticles

The extinction cross-section determined by analysis of the SMS images depends on the laser spot size at the sample w_0 , and the modulation distance δ . The laser spot size is a fitting parameter, and is well determined in our experiments. However, the modulation distance must be independently measured. This was achieved with a ruled reticle (Edmund Scientific). In order to check that the system is properly calibrated, experiments were performed on a gold nanoparticle sample with a known size distribution (36 ± 5 nm). Figure S2 shows SMS images and analysis, a representative TEM image of the sample, and a histogram of the particle sizes determined from the SMS measurements using Mie theory. The average size from the optical measurements is 38 ± 7 nm, which is in good agreement with the TEM measurements. This demonstrates that the system is properly calibrated.

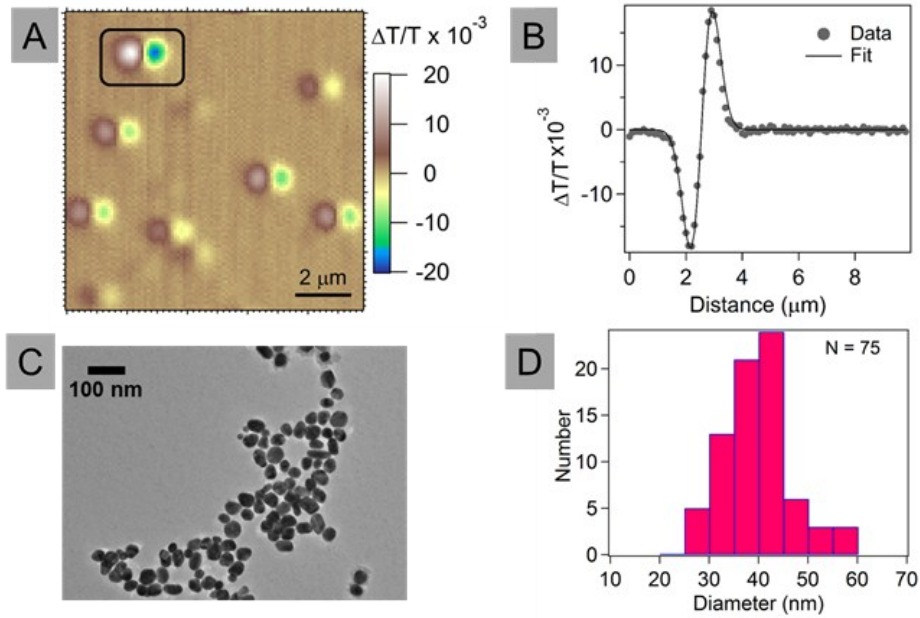


Figure S2: (A) Contour plot of the $1f$ SMS signal for a 36 ± 5 nm gold nanoparticle sample (size determined from TEM), measured at 532 nm. (B) Line profile for the particle inside the rounded rectangle in (A) along with a fit to the data. Data were recorded with a lock-in time constant of 10 ms and pixel dwell time of 30 ms. Power of the probe laser was 100 μW . (C) TEM image of the sample and (D) a histogram of the size distribution determined from the SMS experiments.

SMS Images of Dye-Doped Polymer Beads

Preliminary experiments on organic nanoparticles were performed using commercial dye-doped polymer beads. The beads were obtained from Polysciences Inc and were approximately 1 micron in diameter. SMS experiments were performed at 532 nm and 637 nm and are presented in Figure S3. The SMS signals from the beads was very consistent from bead to bead, both in absolute magnitude and in the relative signal at 532 nm compared to 637 nm. The large size of these beads means that there is a significant contribution from scattering to the SMS signal, which means we cannot determine the number of dye molecules per bead in a straightforward way.

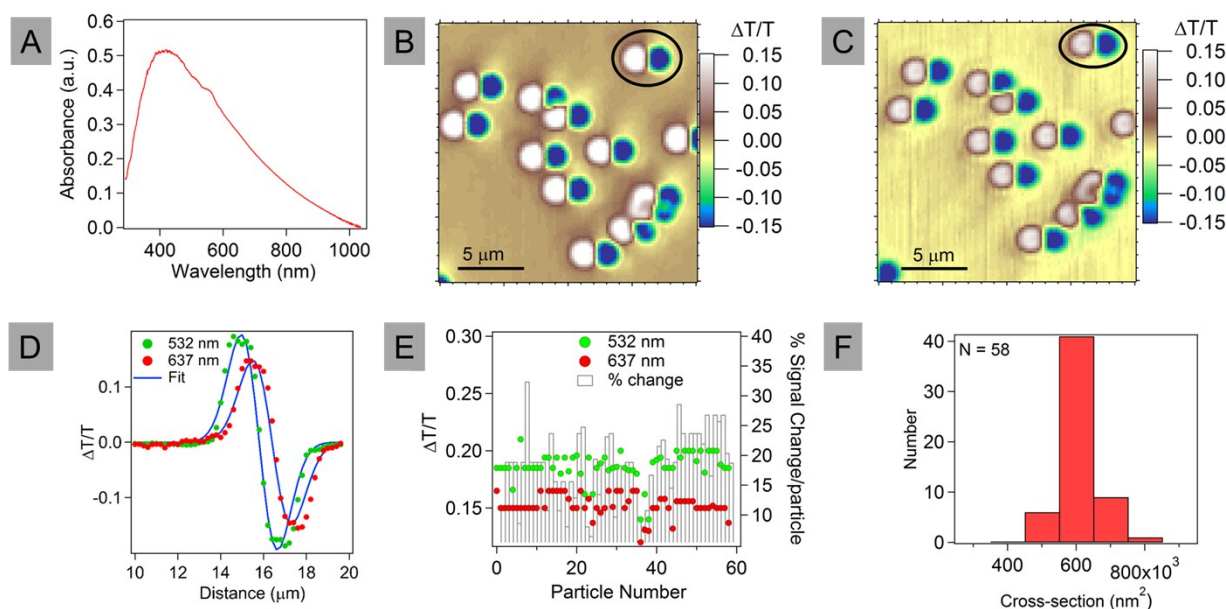


Figure S3: Analysis of a commercial dye-doped polymer nanoparticle sample (Fluoresbrite® YO, Polyscience Inc.). (A) UV-vis spectra of the fluoresbrite beads. (B) Contour plot of the $1/f$ SMS signal for the beads at 532 nm and (C) plot at 637 nm. (D) Line profile and fit for the particle marked in panels (B) and (C). Images were taken over a 20 x 20 μm area with a laser power of 100 μW . (E) Relative signal at 532 nm and 637 nm for 58 beads. (F) Histogram of the extinction cross-section per particle at 532 nm.

Fluorescence Microscopy of LPNPs in Cell Endosomes

The intracellular location of the LPNPs was studied using fluorescently labeled LPNPs that were loaded with croconaine and the green emitting fluorescent dye, DiI. EMT-6 cells were incubated with these nanoparticles for 24 hours and co-stained with DAPI for 10 minutes. A typical epifluorescence composite image is shown in Figure S4, exhibiting a punctate green staining pattern characteristic of endosomal localization. A movie of these punctate compartments shows diagnostic endosome shimmying over 5 minutes at 10x speed. See: <Cell_Movie_5min_10xspeed>

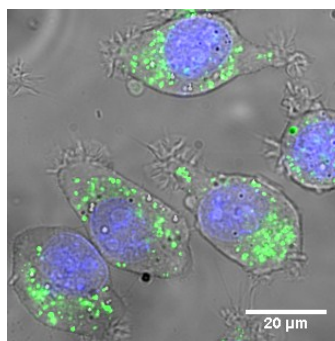


Figure S4: Composite image of fluorescently labeled LPNPs (green) within living EMT-6 cells. Blue is nucleus stained with DAPI.

Absorption Spectra of the SRfluor680 dye and SRfluor680/Croconaine Doped Lipid-Polymer Nanoparticles

Absorption spectra of the SRfluor680 Phenyl dye and the SRfluor680/croconaine co-doped lipid-polymer nanoparticles are presented in Figure S5 below. The wavelengths corresponding to the absorption maxima are labeled in the spectra. The concentration of SRfluor680 Phenyl dye in DMSO was 3.2 μM , and the concentration of the LPNPs in buffer was 0.25 mg/mL. The co-doped particles display considerably more absorption at 637 nm than the croconaine only LPNPs (see Figure 4 of the main text).

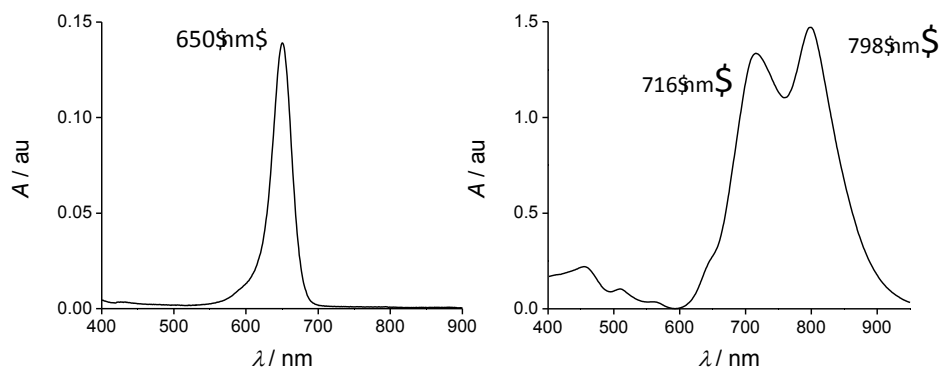


Figure S5: UV-visible absorption spectra of the SRfluor680 Phenyl in DMSO, and the hybrid lipid-polymer nanoparticles doped with croconaine and SRfluor680 Phenyl in buffer solution (pH 7.4).

Additional SMS Images of Croconaine Doped LPNPs in EMT-6 Cells

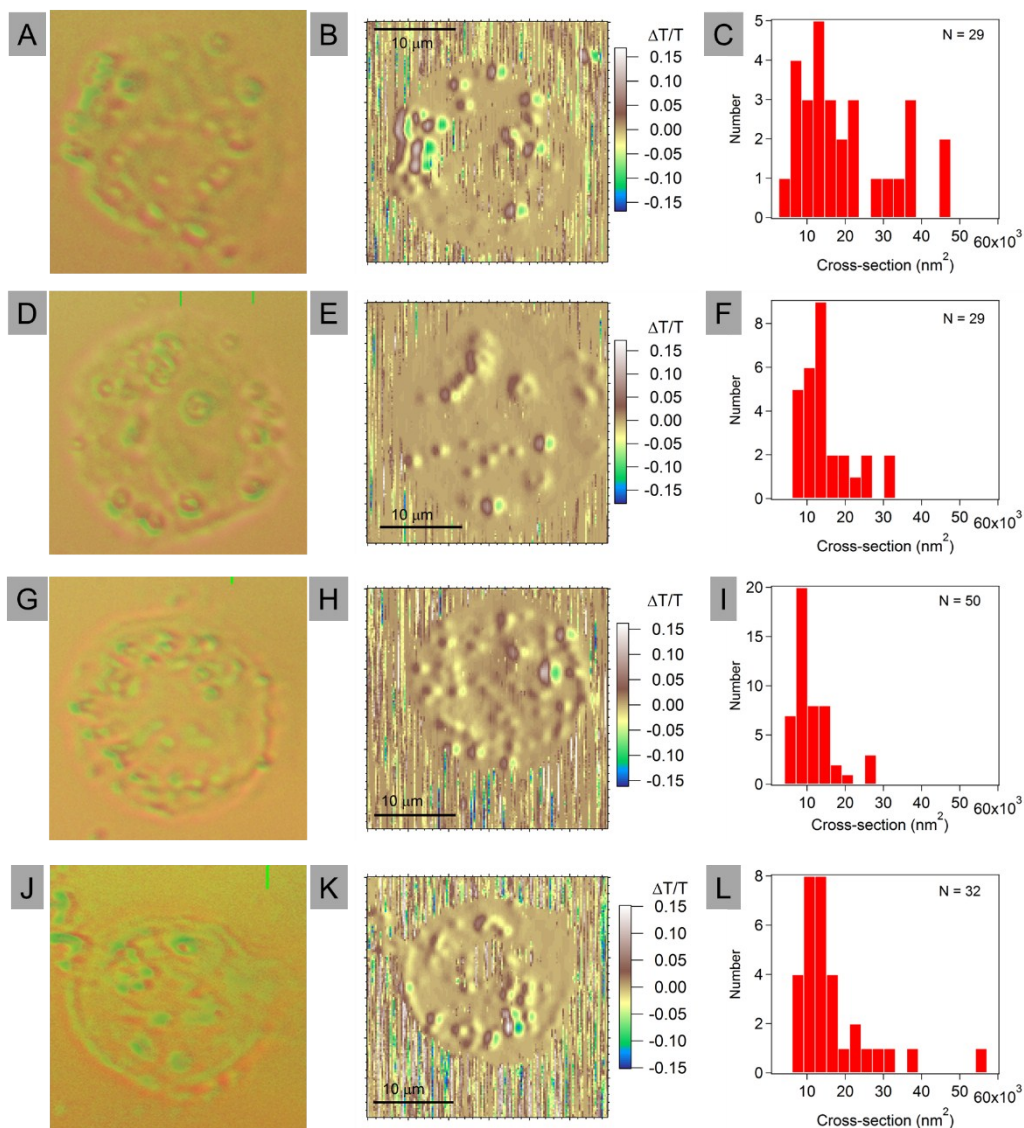


Figure S6: SMS signal comparisons for several EMT-6 cells incubated with croconaine-doped LPNPs. The left hand-side panels show the bright field images. The middle panels are contour plots of the $1f$ SMS signal, and histograms of the cross section distribution in each cell are presented on the right hand-side.