

Electronic Supplementary information (ESI)

Synthesis of tracer nanoparticles: Two different microemulsions were prepared, respectively named “silver microemulsion” and “borohydride microemulsion”. The “oil phase” of both these microemulsions contained 1.5 g Triton X-100, 7.4 mL cyclohexane, 1.6 mL of 1-hexanol as the co-surfactant. The aqueous pool for the silver microemulsion consists of premade iron oxide nanoparticles (prepared by the co-precipitation of iron (II) and iron (III) salts) dispersed in 0.1 M silver nitrate and 1mM 4-mercaptobenzoic acid (total volume = 680 μ L) whereas for the borohydride microemulsion, 680 μ L of 0.1 M ice-cold sodium borohydride prepared in 0.04 M aqueous NaOH solution served as the aqueous pool. To the silver microemulsion, 77 μ L of tetraethylorthosilicate (TEOS) and 154 μ L of ammonium hydroxide (29.5% solution) were added. After 2 minutes, a 3 mL aliquot of borohydride microemulsion was added to the silver microemulsion. The color of the microemulsion immediately changed to dark brown/black indicating the formation of silver nanoparticles. Stirring was continued overnight (12-14 hrs) and subsequently 10 mL ethanol was added to induce precipitation of the microemulsion. The settled particles were purified (centrifugation @ 14000 rpm for 5 minutes) twice with ethanol and twice with deionized (DI) water and redispersed in 6.8 mL of DI water. This was used as a stock solution for ICP and other experiments. These as-prepared particles are highly concentrated as observed by their dark color, and were diluted at least 10-20 times for subsequent studies.

Examination of cell viability: Cell survival was measured using the MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. HT29 human colon adenocarcinoma cells growing in the exponential growth phase were dispensed into a 96-well flat bottom plate at a concentration of 10^4 cells/well in a 90 μ L volume. Tracer particles were washed by centrifuging and resuspended in sterile water using sonication. After allowing 24 hours for cell attachment, the tracer particle solutions were diluted appropriately i.e. 10 μ L added to 90 μ L media volume to give the final concentration, 6 wells per sample. Supernatants of the particles (prepared by centrifuging particles at 13.2×10^3 rpm for 10 mins) were used as

nm for iron oxide) nanoparticle diameters, concentration of silver and iron measured by ICP ($[\text{Ag}] = 5 \times 10^{-3} \text{ M}$, $[\text{Fe}] = 3.7 \times 10^{-3} \text{ M}$) the total number of nanoparticles/L were determined (silver = 1.05×10^{16} nanoparticles/L, or in terms of molarity = 0.17×10^{-7} or 17 nM in particles; iron oxide = 1.04×10^{17} nanoparticles/L, = $0.172 \times 10^{-6} \text{ M}$ or 172 nM).

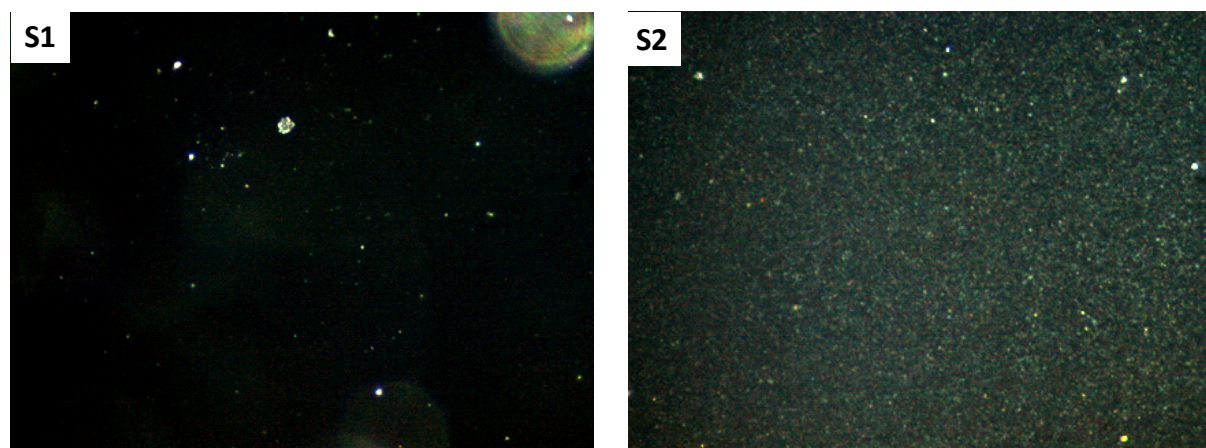
Calculation for silica particles:

Knowing the concentration of silver (30.1×10^{20} atoms of Ag/L) and knowing the atomic percent ratio by EDAX of silica to silver, (Si:Ag atomic % ratio = 4.8), we can get a rough estimate of the number of Si or silica units present ($=144.8 \times 10^{20}/\text{L}$), and ultimately the number of silica particles/L = 3.7×10^{16} .

Hence knowing the number of silver and iron oxide nanoparticles/L (calculated above), the total number of silver and iron oxide particles per silica particle are:

Ag particles/ Si particle = 0.3 and Fe oxide particles / Si oxide particle = 3.

Control images for darkfield light scattering:



Darkfield light scattering images recorded for plain silica particles (S1) and iron oxide coated silica particles (S2).

control treatment lacking particles. Tracer particles in the media were removed following 24hrs incubation with cells and fresh media was added in a volume of 200 μL . The cells were allowed another 3 days recovery period following which cell viability was determined by the addition of 20 μL MTT (5mg/ml dye in sterile PBS). The plate was incubated for an additional 5 hours at 37 $^{\circ}\text{C}$ and 5% CO_2 , allowing viable cells to convert the pale yellow MTT to an insoluble purple dye. The insoluble dye was pelleted by centrifugation, 300g for 5 minutes. The media was carefully removed and the pelleted dye was dissolved in 200 μL dimethyl sulfoxide. Absorbance values at 595 nm were collected. Cell viability following tracer particle exposure was calculated as a percentage compared to untreated control cells.

Calculations for silver, silica and iron oxide:

The data for crystal structures, unit cell dimensions and atoms per unit cell were obtained from the CRC Handbook of Chemistry and Physics.

The quantitation of silver and iron content in the tracer particles was carried out by inductively coupled plasma (ICP) measurements, performed on a Varian Vista ICP-AES spectrometer. Purified samples (1 mL) diluted to different concentrations were digested with aqua regia (1 mL nitric acid + 0.5 mL hydrochloric acid) overnight. The silver and iron concentrations (mg/L) were determined by comparing the atomic emission peaks with freshly prepared silver and iron standards of different concentrations. Taking into account the atomic weights of silver and iron, these concentrations were subsequently converted to moles/L. The concentrations obtained were: $[\text{Ag}] = 5 \times 10^{-3} \text{ M}$, $[\text{Fe}] = 3.7 \times 10^{-3} \text{ M}$. A 0.2 mL solution of the tracer particles was dried on aluminum stubs for EDAX measurements. The Si:Ag atomic % ratio was found to be = 4.8.

Calculations for silver and iron oxide:

Taking into account the following: crystal structures (silver: fcc, 4 atoms per unit cell; Fe_3O_4 : cubic inverse spinel, 8 formula units per unit cell), measured ($\sim 21 \text{ nm}$ for silver) or reported (10

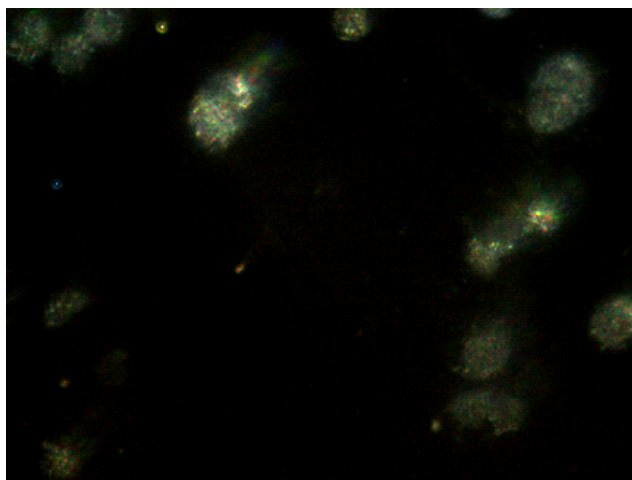


Figure S3: Darkfield light scattering of calcein-HT29 cells in the absence of tracer nanoparticles.