Supporting Information

1. Experimental

The position markers were fabricated by electron-beam lithography. To improve the nanoparticle distribution when depositing aqueous Ag nanoparticles onto the window, we changed the hydrophobic surface (Si$_3$N$_4$) to hydrophilic by evaporating a thin film of silicon monoxide (SiO, 8 nm thickness) onto the window. Thermal evaporation of SiO was performed with a home-built thermal evaporator (at 1x10$^{-6}$ torr). A special baffle-boat (R. D. Mathis) for SiO powders (Ted Pella) was used to avoid streaming of SiO particulates. AFM measurements show that the as-prepared SiO film is pin-hole free and uniform (roughness of SiO ~ 0.5 nm, rms). The SiO-coated substrate was then spin-coated with a MMA-MAA copolymer (methyl methacrylate-co-methacrylic acid polymer, ~150 nm thickness) using a 6 wt% solution in ethyl lactate (spin conditions: 3500 RPM for 70 seconds), followed by baking in an oven at 130 °C for 1 hr. The substrate was further coated with PMMA (polymethylmethacrylate, Mw~950,000, ~200 nm thickness) using a 3 wt% solution in chlorobenzene (4000 RPM for 70 seconds), and then annealed at 140 °C for 1 hr. This double-layer technique allows easy liftoff of the film and a clean surface for Ag nanoparticle deposition.

Prior to e-beam writing, the substrate was further coated with a thin layer of Cr (8 nm) to improve the electrical conductance. The position markers were fabricated by a SEM (Hitachi 2700) integrated with a nanometer pattern generation system (NPGS, version 8) and operated at 30 kV. A typical position marker pattern consists of bars and nanodots as well as a U-shaped orientational keying structure. An electron beam (beam size ~10 nm, current 10 pA) was scanned at a controlled rate over the substrate (exposure dose ~1400 µC/cm$^2$, dot-to-dot distance ~10 nm, line-to-line distance ~10 nm). After e-beam writing, the substrate was soaked in a Cr etchant solution (1:3 diluted with water) for 60 seconds (manually agitated) to remove the Cr film, followed by water rinse and air drying. Pattern development was accomplished by soaking the substrate in a mixed solution of MIBK (methyl isobutyl ketone) and iso-propanol (1: 3) for 40 seconds and rinsing with iso-propanol and water.

The PMMA pattern was translated into metal markers by thermal evaporation of a 30-nm thick Cr or Ag film onto the substrate. The substrate was soaked in acetone at r.t. for ~15 minute to lift-off the polymer/metal film. The substrate was further coated with a layer of positively charged poly(diallyldimethylammonium chloride) (PDDA, low molecular weight, Aldrich) by soaking in a 0.2 wt% PDDA aqueous solution for several hours. The substrate was then rinsed with water and dried. The roughness of the final substrate is ~ 0.5 nm (rms).

Ag nanoparticles were prepared by citrate reduction. The as-prepared nanoparticles are predominantly quasi-spherical (average dia.~50 nm) along with ~10% being Ag nanorods.

The SH activity map was acquired with a home-built nonlinear optical microscopy system consisting of an inverted microscope (Nikon TE2000) and a tunable femtosecond pulsed Ti:Sapphire laser source (Spectra Physics Mai-Tai Broadband). A galvanometer, imaged at the back focal plane of a 60x 1.4 NA oil-immersion objective by a scan lens and tube lens that also served to expand the beam to overfill the objective back aperture, raster scanned the beam over the sample. An xyz piezo scan stage (PI-561) provided fine sample translation and focusing. A dichroic shortpass mirror, bandpass filter, and two-photon blocking filter isolated the epi-scattered SH signal that was projected onto a 1000x1000 pixel electron-multiplying CCD (Andor DV-885). A 4x expander was used for a total magnification of 240x and a pixel size of 33 nm. Images are an average of 20 scans acquired with an integration time of 5s each.
2. Data Analysis and Image Processing

SH and TEM image data processing was done with the freely available and open source ImageJ software (version 1.35, http://rsb.info.nih.gov/ij/). Image registration and centroid localization was performed with Matlab (Mathworks) and its Image Processing Toolbox.

A bright field image and SH map of the marked area of the sample were acquired with white light (i.e. halogen) condenser illumination and the femtosecond near-IR laser pulse excitation, respectively. The images typically covered 35x35 µm square areas (on the order of the entire marker bar pattern). ImageJ was used to crop a specific area of interest, typically 5x5 to 10x10 µm square, that corresponded to the TEM image of the same area. Individual Ag nanoparticles and SH signals were then identified in both images. This allowed direct particle to SH emission correlation.

To transcend simply identifying particles and allow precise colocalization of emission and morphology, SH images were mathematically registered to TEM image counterparts. The registration-correlation-localization algorithm is given in supporting Fig. S1. The procedure is based on a simple point-to-point matching correlation and transformation. Briefly, centroids of hexagonal-arrayed spherical markers were determined from the TEM image. Next, localization of the marker SH emission was accomplished by either fitting a 2D Gaussian to the SH signal or when that failed due to low signal-to-noise ratio, determining the centroid. The Matlab “cpselect” tool was used to determine starting points for the localization. Optimization of the analysis parameters (box size, box center, convergence criteria) was done by hand. The set of corresponding marker points was then used to solve for a linear conformal transformation matrix $T$ relating the SH to the TEM coordinate space.

$$I_{\text{TEM}}(x', y') = T I_{\text{SHG}}(x, y)$$

(1)

$$T = \begin{pmatrix}
    s \cos(\theta) & -s \sin(\theta) \\
    s \sin(\theta) & s \cos(\theta) \\
    t_x & t_y
\end{pmatrix}$$

(2)

The transformation $T$ allows for scaling ($s$), translation ($t_x$, $t_y$), and rotation ($\theta$) and so avoids the complications of polynomial fitting or local image warping that would not fit the entire image. Consequently, no severe imaging aberrations must be present in the TEM and SH images. This condition is true for the small 10x10 µm areas that were carefully analyze in this work. The process was iteratively repeated until a satisfactory transform was found that successfully and simultaneously correlated all TEM and SH markers. This could be automated by minimizing goodness-of-fit parameters.

When the registration transform was determined, localization of the other SH emission features were then performed as above. These centroid coordinates were then transformed into the TEM image space and plotted. Additionally, the entire SH image was transformed and overlayed with the TEM image in ImageJ. The error of the image registration and correlation is estimated to be 25 nm and is limited by the size and structural irregularity of the 100 nm markers.
Figure S1. SH Image Registration Algorithm using Matlab functions.
Figure S2. TEM image prior to image correlation (corresponding to the same area shown in Figure 4 in main text)
Figure S3. SH image prior to image correlation (corresponding to the same area shown in Figure 4 in main text)
Figure S4. SH-active, local structure in a multiparticle aggregate, (a) TEM image, (b) SH optical map, and (c) zoom-in image of aggregate #1. The cavity formed by three adjacent Ag nanoparticles (see (c), indicated by an arrow) was determined to be the SH emission centroid.