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Supporting information Bridging the Gap: 3D Real-Space Characterization of Colloidal Assemblies via FIB-SEM Tomography

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Stage1



Stage 2



Stage 3



Figure S1: **Stepwise overview of the gradient tracking procedure.** Stage 1: blurring the image, removal of the background and calculation of the gradients in 3D. Stage 2: image generation of expected particle shape and calculation of 3D gradients. Stage 3: 3D convolution of the gradients in the original image and the expected particle shape in 3D, and identification of the local maxima.



Figure S2: Transmission electron tomography result for AuNR assembly with d = 340 nm, which is too large for a reliable reconstruction. a) 0° tilt image, acquired in HAADF-STEM mode. b) XY, XZ and YZ views of the 3D reconstruction. The tilt series was acquired from -70 to +70 °with a tilt step of 2°. The full tomogram can be view in Movie S12.



Figure S3: The FFT filtering procedure to remove curtaining stripes. a) Unfiltered image of colloidal crystal consisting of silica spheres (d = 531 nm). b) FFT of the image in a. c) Filtered FFT. d) The filtered image without curtaining stripes, after preforming an inverse FFT of c.



Figure S4: Misidentification of AuNRs near the Pt layer, due to poor contrast. The rod tracking code is not capable of reliably identifying the orientation and position of the nanoparticles close to the outer Pt layer, as indicated by the arrow.