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Quantitative 3D Investigation of Nanoparticle Assemblies by Volumetric Segmentation of Electron Tomography Data Sets

Safiyye Kavak, Ajinkya Anil Kadu, Nathalie Claes, Ana Sánchez-Iglesias, Luis M. Liz-Marzán, Kees Joost Batenburg, and Sara Bals*



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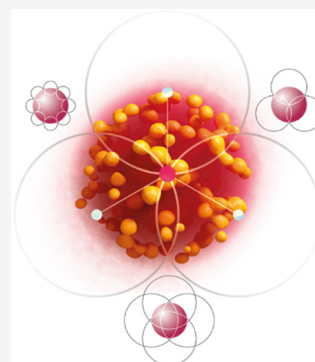


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ABSTRACT: Morphological characterization of nanoparticle assemblies and hybrid nanomaterials is critical in determining their structure-property relationships as well as in the development of structures with desired properties. Electron tomography has become a widely utilized technique for the three-dimensional characterization of nanoparticle assemblies. However, the extraction of quantitative morphological parameters from the reconstructed volume can be a complex and labor-intensive task. In this study, we aim to overcome this challenge by automating the volumetric segmentation process applied to three-dimensional reconstructions of nanoparticle assemblies. The key to enabling automated characterization is to assess the performance of different volumetric segmentation methods in accurately extracting predefined quantitative descriptors for morphological characterization. In our methodology, we compare the quantitative descriptors obtained through manual segmentation with those obtained through automated segmentation methods, to evaluate their accuracy and effectiveness. To show generality, our study focuses on the characterization of assemblies of CdSe/CdS quantum dots, gold nanospheres and CdSe/CdS encapsulated in polymeric micelles, and silica-coated gold nanorods decorated with both CdSe/CdS or PbS quantum dots. We use two unsupervised segmentation algorithms: the watershed transform and the spherical Hough transform. Our results demonstrate that the choice of automated segmentation method is crucial for accurately extracting the predefined quantitative descriptors. Specifically, the spherical Hough transform exhibits superior performance in accurately extracting quantitative descriptors, such as particle size and interparticle distance, thereby allowing for an objective, efficient, and reliable volumetric segmentation of complex nanoparticle assemblies.



1. INTRODUCTION

Nanoparticle assemblies with tunable three-dimensional (3D) structures and properties are of great interest in various fields.^{1–4} By altering the number, shape, size, and separation distance between individual nanoparticles, it is possible to assemble custom structures with specific properties for targeted use.⁵ Of particular interest are multifunctional nanostructures comprising particles with different compositions.⁶ However, a detailed understanding of the structure-property relationship often requires a thorough structural characterization that provides accurate information about the distribution and mutual separation between the different components. For example, when combining plasmonic nanoparticles with photoluminescent quantum dots, the overall optical response critically depends on their precise organization, which may result in photoluminescence quenching or enhancement, for shorter or longer separation distances.

Transmission electron microscopy (TEM) is a widely used technique for studying the shape, size distribution, composition, and structure of nanomaterials. However, conventional TEM images only provide a two-dimensional (2D) representation of a 3D object. One way to overcome this limitation is through electron tomography (ET), which involves acquiring a

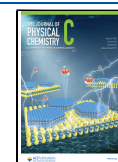
series of 2D projection images at different angles and using them to create a 3D reconstruction. This method has proven to be particularly useful for characterizing 3D assemblies of nanoparticles. Significant progress has been recently made toward optimizing the acquisition and reconstruction of ET tilt series.^{7–15} Still, reconstruction artifacts, such as misalignment, streak artifacts, or the missing wedge,¹⁶ hamper straightforward extraction of quantitative morphological parameters, such as, e.g., the number of particles, and the interparticle distances, from a reconstructed 3D tomography volume.

To extract quantitative morphological parameters from a 3D reconstructed volume, volumetric segmentation is essential. This process involves assigning regions of the data set to specific materials or compositions based on the gray levels of the voxels. Many approaches have been proposed to reach this goal, among which manual segmentation is most often used.

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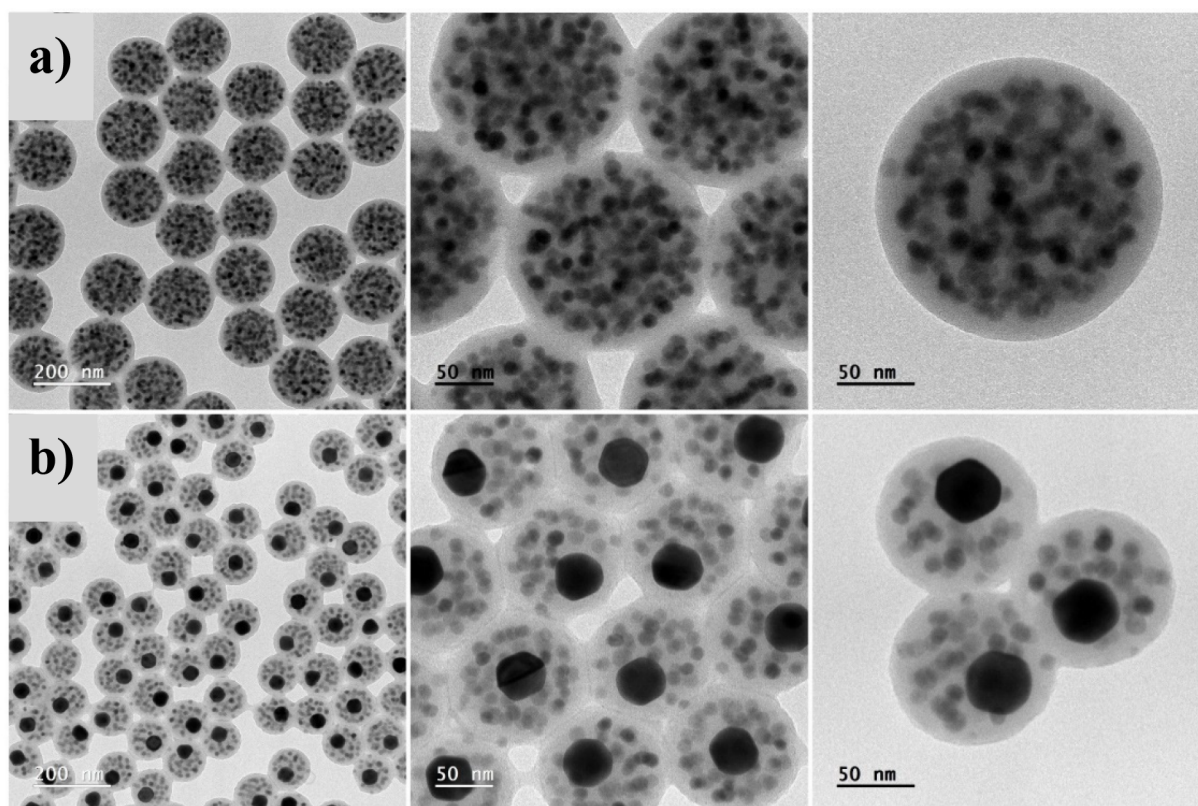


Figure 1. TEM images of clusters encapsulated in polymeric micelles: (a) CdSe/CdS; (b) AuNS@CdSe/CdS.

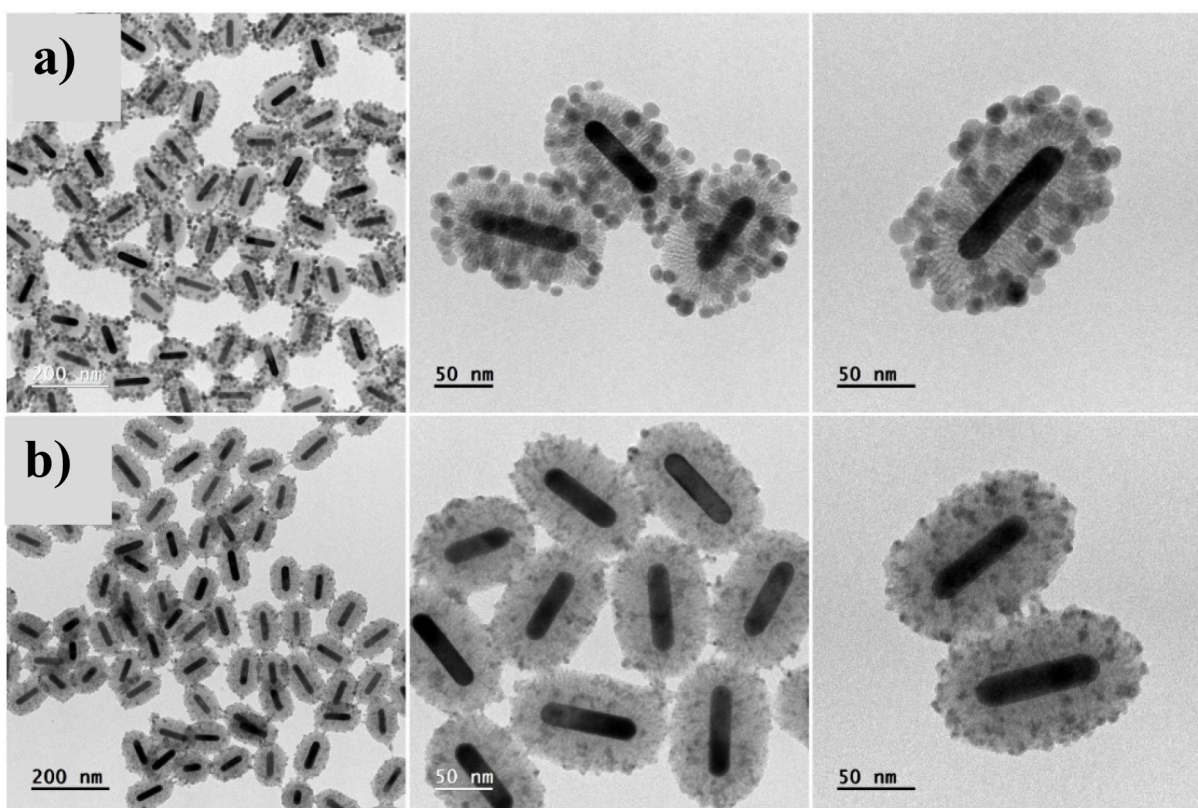


Figure 2. TEM images of AuNR@SiO₂@QDs: (a) CdSe/CdS; (b) PbS.

This process involves visually identifying the boundary between different gray levels in a 3D reconstructed volume.

Ideally, the procedure is repeated for each slice of the reconstructed volume in the three principal directions (x , y ,

and z). Manual segmentation of 3D reconstructions of nanoassemblies presents a challenge in terms of accuracy and efficiency. The subjective nature of manual segmentation and the difficulty in segmenting assemblies with a high number of small or closely spaced particles are the major drawbacks. To address these limitations, advanced reconstruction algorithms have been developed. One such example is the discrete algebraic reconstruction technique (DART),^{17–19} which assumes the sample consists of a small, discrete set of materials. While effective in certain cases, this method requires extensive parameter tuning and is susceptible to image formation artifacts. Another example is the sparse sphere reconstruction (SSR) method, which assumes that nanoparticles can be modeled as perfect spheres of known size.¹⁰ Although suitable for many nanoparticle assemblies, this algorithm cannot be generalized for assemblies made of anisotropic nanoparticles.

Considering the limitations of existing methods, the focus of the present study is on enabling automated volumetric segmentation for quantitative morphological feature extraction of 3D nanoparticle assemblies, using robust image processing operations and therefore not relying on the limiting assumptions of advanced reconstruction methods. Key to our approach is determining which automated volumetric segmentation methods are suitable for accurately extracting predefined quantitative descriptors for morphological characterization. This requires a rigorous validation of algorithmic performance with respect to a “gold standard” segmentation. In our methodology, we evaluate the accuracy of automated volumetric segmentation methods by comparing the quantitative descriptors obtained through manual segmentation with those obtained through automated segmentation.

Specifically, our experimental study is devoted to extracting quantitative parameters that describe 3D assemblies based on the assembly of cadmium selenide/cadmium sulfide (CdSe/CdS) quantum dots (QDs) and coassembly of spherical gold nanoparticles (AuNPs) and CdSe/CdS, both encapsulated in polymeric micelles of polystyrene-poly(acrylic acid) (PS-PAA), induced by hydrophobic interactions (Figure 1). We also investigated hybrid materials comprising silica-coated gold nanorods (AuNRs@SiO₂) decorated with QDs (CdSe/CdS and PbS) (Figure 2). These systems are of interest because the combination of semiconductor and plasmonic materials may enhance existing functionalities or may even lead to new synergy and applications in the fields of catalysis,^{20,21} plasmonics,^{22–25} sensors,²⁶ and cancer therapy.²⁷

Here, two different strategies were used, which can be applied toward tailoring the interaction between the plasmonic Au core and the satellite QDs through control over their respective positions and distances. Whereas in the first strategy hydrophobic NPs were encapsulated into block copolymer micelles upon the addition of water, the second involved silica coating of Au NRs and chemical binding of QDs onto the thiol-functionalized silica surface.

Our study compares the extraction of fixed quantitative descriptors, including the number of QDs, particle size, and interparticle distance, from 3D reconstructions through manual segmentation, with the results obtained using unsupervised image segmentation methods. We employ two alternative unsupervised algorithms for segmentation: the watershed transform²⁸ and the spherical Hough transform.²⁹

The watershed transform has been traditionally employed for volumetric segmentation,³⁰ and it has been successfully

demonstrated in quantifying spherical silica NPs from electron tomography reconstructions. However, this method may fail in certain scenarios due to its sensitivity to noise, over-segmentation, and difficulty in separating touching or overlapping objects.³¹ In contrast, recent research has shown that the spherical Hough transform can address some of these limitations, delivering more accurate and reliable results when extracting quantitative descriptors.³² We observe that the spherical Hough transform has found applications in biomedical imaging^{33,34} but has not been demonstrated in 3D electron tomography, to the best of our knowledge. While both the watershed transform and the spherical Hough transform can perform on par with manual segmentation techniques for the characterization of nanoparticle assemblies, a comparative analysis of the number of particles in the assembly, their average diameter, and interparticle distances reveals that the spherical Hough transform surpasses the watershed transform in terms of accuracy and efficiency. The Hough approach therefore offers a robust, objective, and efficient method for the volumetric segmentation of diverse nanoparticle assemblies.

2. MATERIALS AND METHODS

2.1. Materials and Synthesis Details. Samples are described in Table 1.

Table 1. Description of the Samples Used in This Study

Sample	Core	QDs	Encapsulated/Assembled
#1	-	CdSe/CdS	Polymeric micelles
#2	Au nanosphere	CdSe/CdS	Polymeric micelles
#3	Au nanorod	CdSe/CdS	SiO ₂ shell
#4	Au nanorod	PbS	SiO ₂ shell

2.1.1. Chemicals. Gold(III) chloride trihydrate (HAuCl₄·3H₂O, ≥99%), sodium borohydride (NaBH₄, 99%), L-ascorbic acid (AA, ≥99%), silver nitrate (AgNO₃, ≥99%), sodium oleate (NaOL, ≥99%), tetraethyl orthosilicate (TEOS, 98%), sodium hydroxide (NaOH, 97%), benzyldimethylhexadecylammonium chloride (BDAC), hexadecyltrimethylammonium bromide (CTAB, ≥99%), hexadecyltrimethylammonium chloride (CTAC, 25 wt %), tetrahydrofuran (THF, ≥99.9%), and 3-mercaptopropyl-trimethoxysilane (MPTS, 95%) were purchased from Sigma-Aldrich. Hydrochloric acid solution (HCl, 37 wt %) and ethanol (99.5%) were purchased from Scharlau. Thiol-terminated polystyrene (PS509-SH, MW: 53K) and poly(styrene-*b*-acrylic acid) (PS403-*b*-PAA62) were purchased from Polymer Source. Quantum dot nanocrystals stabilized with oleic acid (OA) in tetrahydrofuran, CdSe/CdS@OA (diameter: 11.4 ± 1.8 nm) and PbS@OA (diameter: 6.2 ± 0.6 nm), were purchased from Center for Applied Nanotechnology GmbH (CAN). All chemicals were used without further purification. Milli-Q water (resistivity 18.2 MΩ·cm at 25 °C) was used in all experiments. All glassware was cleaned with aqua regia, rinsed with Milli-Q water, and dried before use.

2.1.2. Synthesis of Gold Nanospheres.⁷ Gold nanospheres were synthesized via successive seed-mediated growth. First, gold seeds were prepared by fast reduction of HAuCl₄ with NaBH₄ in a CTAB solution. HAuCl₄ solution (0.025 mL, 50 mM) solution was added to a solution of CTAB (4.7 mL, 100 mM), and the mixture was stirred for 5 min at 27 °C until the Au³⁺:CTAB complex was solubilized. Subsequently, a freshly

prepared NaBH_4 solution (0.3 mL, 10 mM) was rapidly injected under vigorous stirring. The solution color changed from yellow to brownish yellow, and stirring was stopped after 2 min. The Au seed solution was aged at room temperature for 30 min prior to use, to allow the decomposition of excess sodium borohydride. The final concentration of metallic gold was 0.25 mM. The same seed solution was used to synthesize Au nanospheres and Au nanorods.

An aliquot of seed solution (0.6 mL) was added to a growth solution (100 mL) containing CTAC (100 mM), HAuCl_4 (0.18 mM), and AA (0.36 mM). The mixture was left undisturbed for 2 h at 25 °C. After synthesis, the colloidal dispersion containing 10 nm gold nanospheres was centrifuged (9000 rpm, 2 h) to remove excess CTAC and ascorbic acid and redispersed in an aqueous BDAC solution (10 mM) to a final Au concentration equal to 2.5 mM.

To grow 10 nm Au nanospheres up to 40 nm, a volume of Au seed solution (0.08 mL, 2.5 mM) was added under vigorous stirring to a growth solution (25 mL) containing BDAC (100 mM), HAuCl_4 (0.5 mM), and AA (1 mM). The mixture was left undisturbed for 30 min at 30 °C and then washed twice by centrifugation. The particles were finally dispersed in CTAB (1 mM) to a final Au concentration equal to 5 mM. The final diameter of Au spheres was 38 ± 1 nm.

2.1.3. Synthesis of Gold Nanorods.³⁵ Gold nanorods (AuNRs) were prepared through the well-known seeded growth method, based on the reduction of HAuCl_4 on CTAB-stabilized Au nanoparticle seeds in the presence of silver ions.

To prepare the growth solution, 1.8 g of CTAB and 0.25 g of NaOL were dissolved in 100 mL of warm Milli-Q water (~ 50 °C), in a 250 mL Erlenmeyer flask. Once NaOL was completely dissolved, the mixture was cooled down to 30 °C, and AgNO_3 (4.8 mL, 4 mM) was added under stirring. The mixture was kept at 30 °C for 15 min, after which HAuCl_4 was added (1 mL, 50 mM) under vigorous stirring. The mixture became colorless after 20 min at 30 °C, and HCl (0.3 mL, 37%) was introduced. After 15 min of stirring, AA (0.25 mL, 64 mM) was added, and the solution was vigorously stirred for 30 s. Finally, seed solution (0.16 mL, 0.25 mM) was injected into the growth solution under vigorous stirring for 5 min, and then, the solution was left undisturbed at 30 °C for 12 h. The solution was centrifuged twice (7000 rpm, 30 min) to remove excess reactants and dispersed in aqueous CTAB solution (1 mM). The final concentration of metallic Au was 5 mM. The final dimensions of AuNRs were 88 ± 3 nm (length) and 22 ± 1 nm (width).

2.1.4. Functionalization with Polystyrene, Clustering, and Encapsulation of Au and CdSe/CdS Nanoparticles.^{7,36} To replace the surfactant with a hydrophobic polymer, thiolated polystyrene (PS-SH) with a molecular weight of 53 kg/mol was used. The AuNS dispersion (2 mL, 5 mM) was added dropwise under sonication to a PS-SH solution (1 molecule of PS-SH per nm^2 of gold surface) in THF (20 mL). The solution was left for 15 min in an ultrasonic bath. To ensure ligand exchange, the resulting mixture was left undisturbed for 12 h and then centrifuged twice (7000 rpm, 30 min). The particles were finally dispersed in THF to a final Au concentration of 5 mM.

The clustering of PS-functionalized Au NPs (AuNPs@PS) and CdSe/CdS@OA quantum dots was carried out according to a previously reported method. In a typical self-assembly experiment, water (0.2 mL) was added dropwise to AuNP@PS

(0.25 mM, 10 mL) and CdSe/CdS@OA (0.02 mL, 10 mg/mL) in THF, under magnetic stirring. The dispersion was stirred for 5 min, and then, a solution of PS403-*b*-PAA62 in THF (1 mL, 6 mg/mL) was added dropwise under magnetic stirring. Subsequently, the water content was increased up to 35 wt %, followed by increasing the temperature up to 70 °C, which was maintained for 30 min. The dispersion of clusters was then centrifuged twice (4000 rpm, 30 min) and redispersed in water.

For clustering of CdSe/CdS@OA quantum dots, water (0.3 mL) was added dropwise to the QD dispersion (0.02 mL, 10 mg/mL) in THF, under magnetic stirring. The mixture was stirred for 10 min, and then, a solution of PS403-*b*-PAA62 in THF (0.2 mL, 6 mg/mL) was added dropwise under magnetic stirring. Subsequently, the water content was increased up to 35 wt %, followed by increasing the temperature up to 70 °C, which was maintained for 30 min. The clusters dispersion was centrifuged twice (18000 rpm, 30 min) and redispersed in water.

2.1.5. Mesoporous Silica Coating of AuNRs and Decoration with QDs.³⁷ Mesoporous silica shells were grown on AuNRs by adding three consecutive aliquots of TEOS solution (24 μL , 20 vol % in EtOH) at 60 min intervals, to a solution containing AuNRs@CTAB (8 mL, 1 mM) and NaOH (80 μL , 100 mM) in CTAB (1.5 mM). The reaction was allowed to continue while stirring for 2 days. The resulting silica-coated gold nanorods (AuNRs@ SiO_2) were washed twice with ethanol (5000 rpm, 30 min) and finally dispersed in EtOH to a final Au concentration of 1 mM. Then, MPTS (5 mL, 0.15% v/v) was added to the AuNRs@ SiO_2 (5 mL, 1 mM) dispersion, to modify the silica surface. The mixture was heated to 40 °C for 24 h, and then, thiol-functionalized AuNRs@ SiO_2 were washed three times with ethanol by centrifugation to remove excess MPTS and finally dispersed in THF to a final Au concentration of 0.5 mM.

CdSe/CdS or PbS QDs dispersed in THF (0.1 mL, 10 mg/mL) were mixed with the suspension of thiol-functionalized AuNRs@ SiO_2 nanoparticles in THF (5 mL, 0.5 mM) under mild stirring for 4 h. The QDs were self-assembled onto AuNRs@ SiO_2 nanoparticles due to the interaction of thiol groups and QDs. Finally, the hybrid AuNRs@ SiO_2 @QDs nanoparticles were centrifuged twice and redispersed in THF.

2.2. Methods. **2.2.1. TEM Imaging.** TEM images were acquired with a JEOL JEM-1400PLUS transmission electron microscope operating at an acceleration voltage of 120 kV.

2.2.2. Electron Tomography. The ET experiments for samples #1, #2, and #3 were performed using a Thermo Fisher Tecnai G2 microscope operating at 200 kV in high-angle annular dark-field (HAADF-STEM) mode. Tilt series for sample #1 were acquired from -72 to $+75^\circ$, for sample #2 from -75 to $+75^\circ$, and for sample #3 from -66 to $+69^\circ$. Sample #4 contained smaller quantum dots (QDs) compared to the other three samples. Therefore, a Thermo Fisher Scientific Tecnai Osiris operating at 200 kV with better spatial resolution was used to collect a tilt series from -75 to $+75^\circ$. A Fischione model 2020 single tilt tomography with a tilt increment of 3° was used for all series. From these tilt series, 3D volumes were reconstructed using the expectation maximization algorithm,^{38–40} which iteratively minimizes the maximum likelihood of the mismatch between the forward projection and the tilt series, assuming that the noise follows a Poisson distribution, which is appropriate for electron microscopy images. During each iteration, the algorithm first

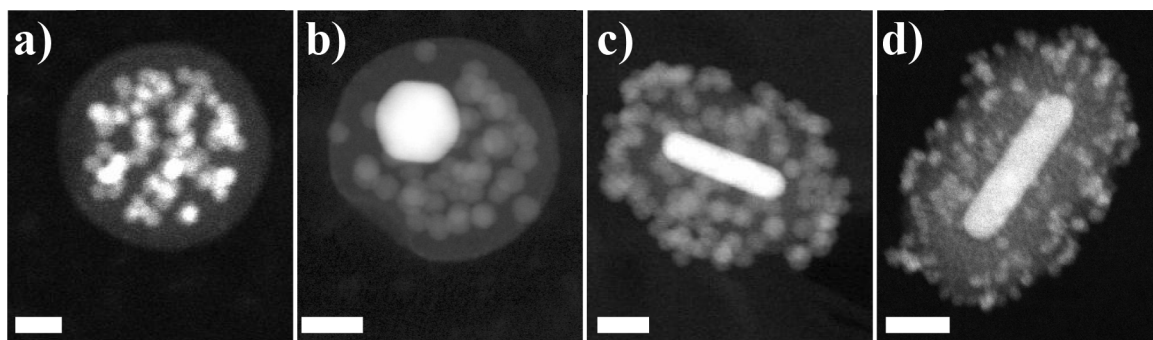


Figure 3. 2D HAADF-STEM images of different hybrid NP assemblies: (a) sample #1, CdSe/CdS QDs in polymeric micelles; (b) sample #2, AuNPs and CdSe/CdS coencapsulated in polymeric micelles; (c) sample #3, AuNR@SiO₂/CdSe/CdS; (d) sample #4, AuNR@SiO₂/PbS. All scale bars correspond to 30 nm.

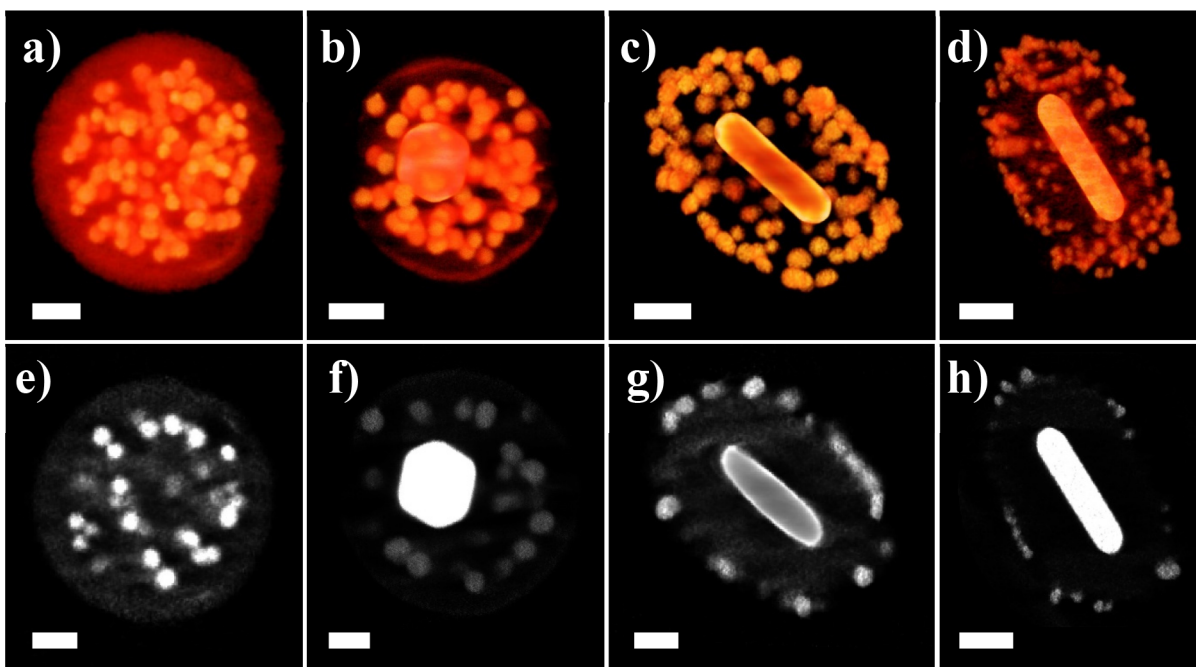


Figure 4. 3D visualizations of representative reconstructions (a–d: #1–#4), and orthoslices (e–h: #1–#4). All scale bars represent 30 nm.

estimates the hidden parameters of the model using the current estimates of the observed parameters and then updates the estimates of the observed parameters using the newly estimated hidden parameters. This process is repeated until convergence, resulting in a 3D volume with minimal noise and artifacts.

2.2.3. Watershed Transform. The watershed transform is a prevalent technique in the field of image processing for demarcating different regions or “basins” in an image. This method is based on the principles of a “watershed”, which is a region of land that drains water into a specific river or lake. In the context of image processing, the idea behind watershed segmentation is to treat the grayscale values in an image as a topographical map, where the darkest voxels represent the low points (valleys), and the lightest voxels represent the high points (peaks). The algorithm then “floods” the 3D image from the peaks, filling in the valleys and creating a set of connected regions. These regions can then be labeled and treated as individual particles in the image. Details about the method and its hyperparameters are given in the SI-section 2. In this study, the watershed transform was implemented using

Avizo3D visualization software, and the LabelAnalysis tool of Avizo3D was used to quantify morphological parameters, including center position and corresponding radius.

2.2.4. Spherical Hough Transform. The spherical Hough transform is an algorithm that detects spherical objects in the 3D image, characterized by a radius and a central position (as shown in SI-section 3).^{41,42} This approach has been primarily utilized in biomedical imaging for segmentation purposes, such as detecting multiform femoral heads and cell nuclei.^{43–45} In our study, the spherical Hough transform was employed to identify spherical particles in 3D anisotropic assemblies. The four-dimensional representation (i.e., center location and radius) allowed for easier manipulation of the spherical objects in three dimensions. The transform employs a voting mechanism to detect clusters of points that correspond to individual particles and construct a spherical model of the particles using these clusters (refer to the SI-section 3 for more information regarding the algorithm). The spherical Hough Transform offers several benefits over alternative methods, including robustness to noise and the ability to handle complex shapes. Its voting system allows for the accurate detection of

particles even in the presence of noise or distortion, thereby enabling the separation of attached and/or agglomerated particles without subjectivity. The implementation of this transform was performed in MATLAB, and the resulting code was validated on numerous synthetic structures.

3. RESULTS

In Figure 3, an overview of the different samples is presented through 2D high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) images. Sample #1, depicted in Figures 1a and 3a, comprises solely CdSe/CdS QDs embedded within a polymeric micelle shell. Sample #2, shown in Figures 1b and 3b, was prepared by coassembly of AuNPs and QDs,^{7,35–37} resulting in hybrid assemblies typically containing one Au nanosphere and numerous QDs. Two additional samples were examined, which were prepared by the second strategy, as described above. Figures 2a and 3c present the general appearance of the hybrid particles in sample #3, which consist of CdSe/CdS QDs assembled onto gold nanorods and surrounded by a silica shell (AuNRs@SiO₂). Sample #4 (Figures 2b and 3d) have a similar structure, but PbS QDs were used instead of CdSe/CdS.

The morphological characterization of all nanoparticle assemblies was performed through the application of ET. The technical details and the parameters used for the ET experiments can be found in the Materials and Methods section. The results of the ET reconstructions are depicted in Figure 4, where the visual representation of the 3D structures and representative orthoslices acquired through the 3D reconstructions are presented. Our observations indicate that the Au NPs are positioned at the central axis of the assemblies, with a uniform distribution of the QDs surrounding them. We can also confirm that the QDs in samples #3 and #4 are primarily located on the outer surface of the SiO₂ shell, whereas in samples #1 and #2, the QDs are dispersed throughout the polymeric micelle matrix. This different distribution of QDs was expected from the application of different synthesis methods, samples #1 and #2 being prepared from coassembly of NPs from a mixed dispersion and subsequently encapsulated by the block copolymer, whereas in samples #3 and #4, the QDs were added after coating of AuNRs with the SiO₂ shell and modifying its outer surface to facilitate QD binding.

The ultimate objective of our 3D analysis was the determination of quantitative descriptors for the nanoparticle assemblies, including the number of particles within the assembly, the interparticle distances between QDs, and the mean diameter of the individual QDs. To obtain these parameters, the center of mass position of each QD must be first determined. However, as the QDs in the assemblies exhibit varying shapes and sizes and frequently appear to be agglomerated in the reconstructions, algorithms such as DART and SSR are not suitable. Therefore, the focus of this study is to optimize the segmentation process applied to a reconstructed 3D data set, as it pertains to quantifying the morphological features of nanoparticle assemblies.

First, we performed careful manual segmentation for every orthoslice along the *x*, *y*, and *z* directions. The boundaries of the individual QDs and the Au nanoparticle were manually indicated and separated from the background for each assembly, as illustrated in Figure S1. This approach is time-consuming and can be subjective. To reduce user bias, manual segmentation was repeated several times. In this manner, we

consider the resulting quantitative parameters to be the accepted reference values, and they are listed in Table 2.

Table 2. Summary of the Most Important Descriptors for the NP Assemblies as Extracted by Means of Different Segmentation Approaches: Manual Segmentation, Use of the Watershed Transform, and a Spherical Hough Transform^a

		Manual seg.	Watershed transform	Spherical Hough transform
#1	number of QDs	136	127	136
	diameter (nm)	10.1 ± 0.2	10.6 ± 0.3	10.0 ± 0.1
	average distance (nm)	65.3 ± 0.2	67.7 ± 0.2	65.0 ± 0.2
	nearest neighbor distance (nm)	11.6 ± 0.3	12.0 ± 0.3	12.1 ± 0.2
#2	number of QDs	71	78	72
	diameter (nm)	10.9 ± 0.2	10.2 ± 0.2	11.0 ± 0.1
	average distance (nm)	53.7 ± 0.3	53.4 ± 0.3	53.9 ± 0.2
	nearest neighbor distance (nm)	14.2 ± 0.6	12.7 ± 0.7	14.6 ± 0.5
#3	number of QDs	130	131	128
	diameter (nm)	11.7 ± 0.2	11.5 ± 0.2	11.8 ± 0.1
	average distance (nm)	75.2 ± 0.2	74.8 ± 0.2	73.6 ± 0.2
	nearest neighbor distance (nm)	12.4 ± 0.2	12.6 ± 0.3	12.5 ± 0.2
#4	number of QDs	153	154	150
	diameter (nm)	6.7 ± 0.1	6.3 ± 0.1	6.5 ± 0.1
	average distance (nm)	66.5 ± 0.2	70.3 ± 0.2	70.4 ± 0.2
	nearest neighbor distance (nm)	8.2 ± 0.4	8.6 ± 0.3	8.4 ± 0.3

^aThe determined descriptors include: the number of QDs in an assembly, the diameter distribution of the individual QDs, the average interparticle distances, and the nearest neighbor distances, together with standard error values.

Next, we performed segmentation of our 3D reconstructions based on the use of a watershed transform³¹ (Figures S2–3), as summarized in Table 2 and Figure 5. The percentage error values for the calculated parameters are presented in Figure 6. It can be seen that the quantified results are in reasonable agreement with the manual segmentation results, which we accepted as reference values. The obtained diameters are furthermore consistent with the values provided by the vendor of the quantum dot nanocrystals (see section 2.1.1). On the other hand, larger deviations (see error bars) were found regarding the number of QDs per assembly for samples #1 and #2, average diameter for samples #1, #2, and #4, and nearest neighbor distance for samples #2 and #4. This unexpected result (in particular for QD diameter) is likely to result from an oversegmentation problem, which we discuss below.

As an alternative, we applied the spherical Hough transform, and a qualitative comparison to the manual segmentation and results obtained by the watershed transform are depicted in Figure 5. The quantified outcomes are illustrated in Table 2 and represented by the error percentage values in Figure 6. The analysis reveals that the quantified results obtained through the spherical Hough transform segmentation exhibit a high degree of concurrence with the reference data. The number of QDs demonstrates excellent overlap with the reference data, as demonstrated by the small error percentage

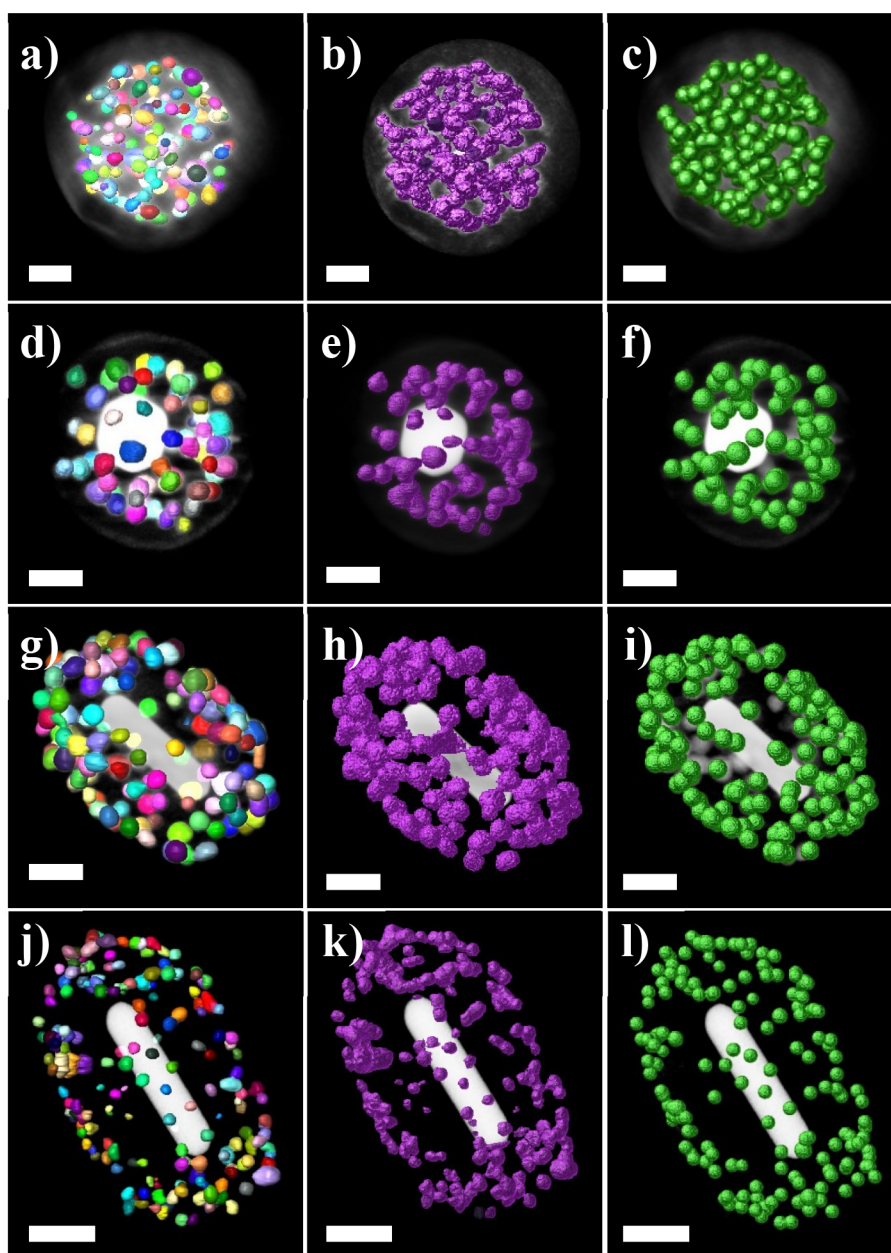


Figure 5. 3D visualizations obtained through manual segmentation (a,d,g,j), segmentation using the watershed transform (b,e,h,k), and segmentation using the spherical Hough transform (c,f,i,l), for samples #1, #2, #3, and #4, from the first to last rows, respectively. Scale bars represent 30 nm in each image.

values displayed in Figure 6. In comparison to the results obtained through the watershed transform, the spherical Hough transform exhibits significantly improved accuracy in determining the parameters of average diameter, average distance, and average nearest neighbor distance, as evidenced by the results in Figure 6.

4. DISCUSSION

The efficacy of each segmentation method was compared across four distinct nanoparticle assemblies. The applied methods comprised manual segmentation, the watershed transform, corresponding to a traditional approach for segmentation problems, and the spherical Hough transform, an alternative technique for segmentation.³² Our results indicate 136, 71, 130, and 152 particles were detected by manual segmentation for samples #1–#4, respectively. The

number of particles detected using the watershed transform was higher than the accepted reference values for samples #2, #3, and #4, an issue commonly referred to as oversegmentation (Figure S4), which arises from the division of an image of particles into an excessive number of regions or segments that do not correspond to distinct structures or particles. Conversely, the number of particles detected using the spherical Hough transform was in better agreement (lower percentage error values, Figure 6) with the accepted reference values, compared to the watershed transform, for all samples.

Subsequently, the diameters of the nanoparticles were computed using the three segmentation methods. The diameters computed through manual segmentation were 10.1, 10.9, 11.7, and 6.7 nm for samples #1–#4, respectively. The diameters calculated using the watershed transform differed from the accepted reference values by 2–7%. In

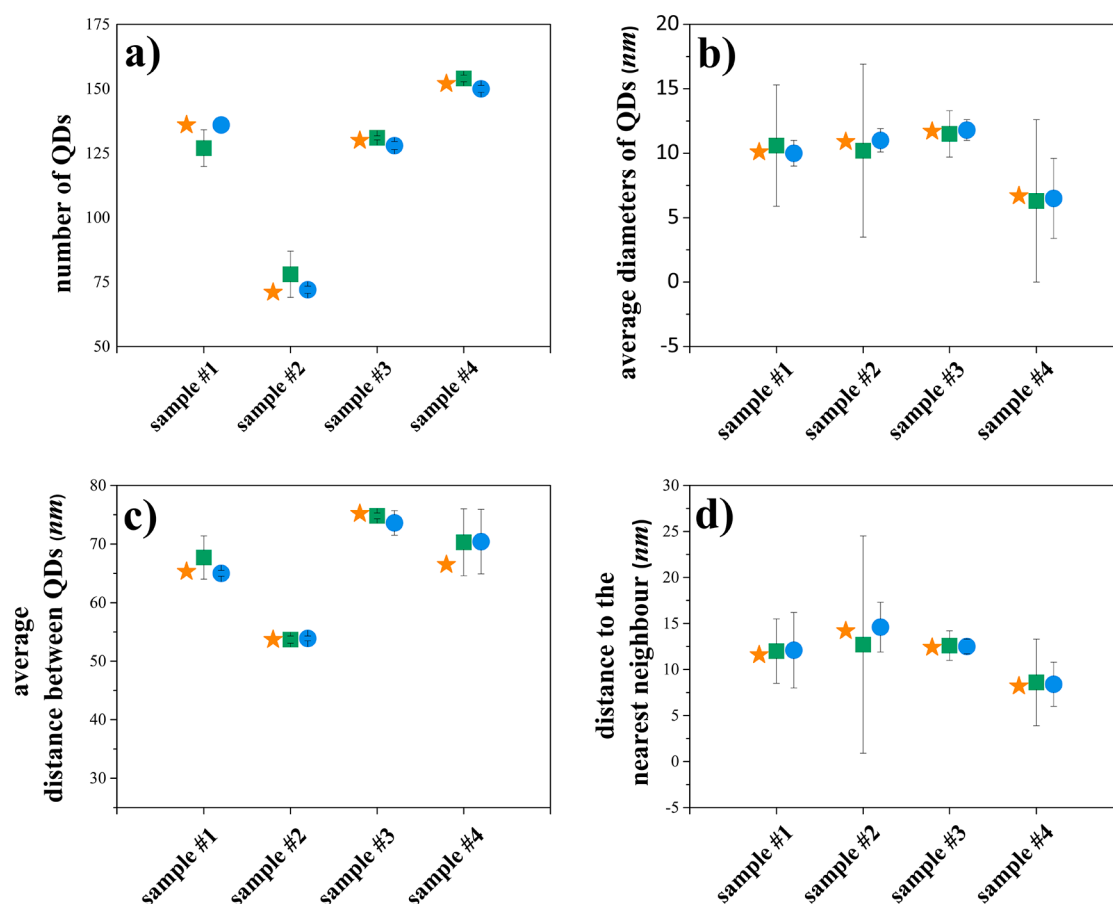


Figure 6. Percentage error values for: (a) number of QDs, (b) average diameters of QDs, (c) average distance between QDs, and (d) average distance of a QD to the nearest neighbor QD. Stars (★) indicate manual segmentation results, squares (■) indicate the watershed transform results, and circles (●) indicate the results from the spherical Hough function. Error bars were calculated from the difference (%) to the average of accepted reference values (manual segmentation).

contrast, the diameters calculated using the spherical Hough transform again exhibited better agreement with the accepted reference values, with differences ranging from 0.9 to 3.0%.

Additionally, the interparticle distances were calculated using all three segmentation methods. The average interparticle distances calculated through manual segmentation were 65.3, 53.7, 75.2, and 66.5 nm for samples #1–#4, respectively. The average distances computed using the watershed transform were different from the accepted reference values by 0.5–3.7% for samples #1–#3 and by 5.7% for sample #4. On the other hand, the average distances computed using the spherical Hough transform demonstrated improved agreement with the accepted reference values for all samples, with differences ranging from 0.4 to 2% for samples #1–#3. And for sample #4, the difference was similar to watershed transform #4 by 5.8% (Figure 6).

The final parameter evaluated was the average nearest neighbor distance, which quantifies the proximity of a nanoparticle to its closest neighboring particle. The average nearest neighbor distances obtained from manual segmentation were 11.6, 14.2, 12.4, and 8.2 nm for samples #1–#4, respectively. For sample #1, the average nearest neighbor distance calculated using the spherical Hough transform showed a slightly higher error of 4.3% compared to the watershed transform, which showed an error of 3.4%. However, for samples #2–#4, the spherical Hough transform demonstrated improved agreement with manual segmentation

results, by 0.8–2.8% difference, compared to the watershed transform which shows 1.6–10.6% difference, as evident from the data presented in Figure 6.

In conclusion, we have performed ET experiments on a collection of nanoparticle assemblies and evaluated two computational methods for automated volumetric segmentation—the watershed transform and the spherical Hough transform—alongside manual segmentation, the latter of which served as the accepted reference value. Our numerical results indicate that the spherical Hough transform is a superior approach for quantifying 3D nanoparticle assemblies, due to its ability to deliver objective, efficient, and accurate quantification results.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jpcc.3c02017>.

Details of manual segmentation, watershed transform, and spherical Hough transform(PDF)

■ AUTHOR INFORMATION

Corresponding Author

Sara Bals – EMAT, University of Antwerp, 2020 Antwerp, Belgium; orcid.org/0000-0002-4249-8017;
Email: sara.bals@uantwerpen.be

Authors

Safiyye Kavak – EMAT, University of Antwerp, 2020 Antwerp, Belgium; orcid.org/0000-0002-3910-5737

Ajinkya Anil Kadu – EMAT, University of Antwerp, 2020 Antwerp, Belgium; Computational Imaging Group, Centrum Wiskunde & Informatica (CWI), 1098 XG Amsterdam, The Netherlands

Nathalie Claes – EMAT, University of Antwerp, 2020 Antwerp, Belgium

Ana Sánchez-Iglesias – CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), 20014 Donostia – San Sebastián, Spain; orcid.org/0000-0003-1871-8742

Luis M. Liz-Marzán – CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), 20014 Donostia – San Sebastián, Spain; Centro de Investigación Biomédica en Red, Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), 20014 Donostia – San Sebastián, Spain; Ikerbasque, Basque Foundation for Science, 48009 Bilbao, Spain; Cinbio, Universidade de Vigo, 36310 Vigo, Spain; orcid.org/0000-0002-6647-1353

Kees Joost Batenburg – Computational Imaging Group, Centrum Wiskunde & Informatica (CWI), 1098 XG Amsterdam, The Netherlands; Leiden Institute of Advanced Computer Science (LIACS), Leiden University, 2333 CA Leiden, The Netherlands

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acs.jpcc.3c02017>

Author Contributions

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Notes

The authors declare no competing financial interest.

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