applied sciences

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Algorithm for Reconstruction of 3D Images of Nanorice Particles from Diffraction Patterns of Two Particles in Independent Random Orientations with an X-ray Laser

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Academic Editor: Kiyoshi Ueda

Received: 31 March 2017; Accepted: 8 June 2017; Published: 23 June 2017

Abstract: The method of angular correlations recovers quantities from diffraction patterns of randomly oriented particles, as expected to be measured with an X-ray free electron laser (XFEL), proportional to quadratic functions of the spherical harmonic expansion coefficients of the diffraction volume of a single particle. We have previously shown that it is possible to reconstruct a randomly oriented icosahedral or helical virus from the average over all measured diffraction patterns of such correlations. We point out in this paper that a structure of even simpler particles of 50 Å or so in diameter and consisting of heavier atomic elements (to enhance scattering) that has been used as a test case for reconstructions from XFEL diffraction patterns can also be solved by this technique. Even though there has been earlier work on similar objects (prolate spheroids), one advantage of the present technique is its potential to also work with diffraction patterns not only due to single particles as has been suggested on the basis on nonoverlapping delta functions of angular scattering. Accordingly, we calculated from the diffraction patterns the angular momentum expansions of the pair correlations and triple correlations for general particle images and reconstructed those images in the standard way. Although the images looked pretty much the same, it is not totally clear to us that the angular correlations are exactly the same as different numbers of particles due to the possibility of constructive or destructive interference between the scattered waves from different particles. It is of course known that, for a large number of particles contributing to a diffraction parttern, the correlations converge to that of a single particle. It could be that the lack of perfect agreement between the images reconstructed with one and two particles is due to uncancelling constructive and destructive conditions that are not found in the case of solution scattering.

Keywords: XFEL; nanorice; angular correlations

1. Introduction

An X-ray free electron laser (XFEL) produces X-rays of unprecedented brilliance of about 10 billion times what was previously possible. As such, it has given rise to the speculation that it may be possible to determine the structures of uncrystallized individual biomolecules [1]. Although the ultimate aim is to determine the structures of biomolecules, it would be helpful to demonstrate the feasibility of the approach to simpler objects initially. In this vein, there has been some work already on reconstructing prolate spheroids [2] of metallic particles. What such experiments demonstrate is the feasibility of reconstructing the structure of particles of random unknown orientations. The aim of the present paper is to show that reconstruction of the structure of such particles is possible even with two particles in independent random orientations contributing to a single diffraction pattern. If it

can be demonstrated for two randomly oriented nanoparticles, and we think that since the angular correlations seem to be identical, the number of identical particles may be increased without limit. In this respect, the method has some similarities with one proposed in the 1970s by Kam [3] to generalize the methods then current for small angle X-ray scattering (SAXS). In SAXS, an ensemble of many particles is suspended in solution in random orientations. A major problem with SAXS is that one attempts to recover the structure of a particle from a set of experimental data consisting of a single line plot $I(q)$. From the spherical harmonic expansion of a crude model of the particle, its shape is normally obtained by SAXS techniques. Kam's innovation was to point out that if an experiment could be performed that measures the diffraction patterns on a time scale shorter than the rotational diffusion time of the molecules, extra 3D information on the molecular structure could be obtained from the correlations in the angular variations of the measured intensities. There has also been work on determining the structure of Au nanoparticles to atomic resolution [4] by this technique. A major advantage of this method, if feasible, is the ability to determine the detailed structure of an ensemble of randomly oriented molecules. One would thereby be able to use the intensity enhancement caused by working with an ensemble of molecules rather than a single one, and yet not have to crystallize the molecules so that they are all in exactly the same orientation. Kirian et al. [5] have shown that even though there is nothing to be gained by having more particles per diffraction pattern since the noise goes up in the same proportion as the signal, one of the other results of their own analysis is that the noise can be reduced compared to the signal by averaging over many diffraction patterns, It should be pointed out that the need to avoid crystallization was the original aim of XFEL studies of biomolecules. If the experiment we propose is possible, it may be possible to avoid crystallization in structural studies and still avoid the very low scattered intensities that are inevitable in single particle studies even with an X-ray free electron laser (XFEL).

An experiment has recently been reported in which the structure of the mimivirus has been determined experimentally by a variant of the single particle methods described earlier [6]. At least for an icosahedral particle, as the mimivirus largely is, it has been previously shown by us [7] that it is possible to determine the structure of the particle by an analogous method to that described here from simulated diffraction patterns. In an experiment to recover time-resolved structural variations, starting from a knowledge of a nearby structure, using many of the same quantities, namely, the pair correlations, have also been shown to be capable of recovering time-resolved changes in a structure from the knowledge of a closely related structure of a single molecule in realistic simulations, including shot noise [8]. This possibility is unprecedented in structural work with XFEL diffraction patterns.

It is with the aim of further developing this idea that the work here is undertaken. Initially, following the basic theory presented here, the aim is only to reconstruct from an ensemble of randomly oriented particles of simple form, its low resolution 3D structure. Indeed, there has already been experimental work on "nanorice" particles of the type we describe here. While it may be true that such a simple structure may be obtained by SAXS alone, the demonstration of such a method even for a simple structure paves the way to its application to more complex structures that may not be accessible to SAXS. Furthermore, "machine learning" algorithms based on manifold embedding [9] have been used to perform an approximate sorting of the experimental diffraction patterns. Following this paper, we have begun to work with the authors of that paper on going all the way from measured diffraction patterns to structure. We describe here how the structure of "nanorice" particles on which experimental data is already available at the public web site cxidb.org can be reconstructed in principle. There has also been some work reported on the reconstruction of prolate and oblate spheroids by the method of cryptotomography [2]. However, this does require diffraction patterns from single particles to be identified beforehand from an experimental data set [10]. In the present paper, we describe an algorithm that works on either prolate or oblate spheroids and yet allows the possibility of working with diffraction patterns of multiple randomly oriented particles. To do this, we employ a method [11] of reconstructing the structure of a particle from XFEL diffraction patterns of random unknown orientations of the object.

This method has close similarities to a method proposed much earlier [3] for recovering the structure of particles randomly oriented, as in the method of small angle X-ray scattering (SAXS). Although, of course, in the 1970s, it was not possible to focus an X-ray beam to hit just a single particle, this has now become possible with the recent advent of the X-ray free electron laser (XFEL). It should be stressed that the method we describe in this paper is flexible enough to reconstruct a structure from "single-particle" experiments such as in the recent Single Particle Initiative (SPI) at the Linac Coherent Light Source (LCLS) in Stanford, CA, USA. What is used is the average over measured diffraction patterns of the angular correlations on each diffraction pattern. Since this uses diffraction data of perhaps millions of diffraction patterns, its expected shot noise would be significantly reduced by this averaging process [12]. Such methods are very different from those of traditional crystallography on crystals, as the orientation of each particle in generally unknown a priori and there are not even Bragg spots that enable their orientation to be determined via indexing [13].

We describe our method next. One of its advantages is that it has been shown to be equally applicable to ensembles of molecules of random orientations [8]. This could be an advantage experimentally, as it will allow a fuller use of available experimental data, and obviates the need for methods [10] that eliminate diffraction patterns from multiple particle hits from the analysis.

2. Method of Angular Correlations

The first step in using this method is to calculate angular cross correlations on each diffraction pattern in polar coordinates. Polar coordinates are natural for this problem since the particles differ mainly in their orientations (they may also differ in position, and this does not affect the diffraction pattern intensities that are insensitive to the phases of the scattered amplitudes). This is relevant so long as it is a single particle in the beam at one time. Otherwise, the intensities are sensitive for the relative displacements of the particles in the same diffraction pattens. Even in that case, one might hope that, due to the random nature of these displacements, such relative phases are unimportant [8].

Angular pair correlations are related to measured quantities from

$$C_2(q,q',\Delta\phi) = < \int I_p(q,\phi) I_p(q',\phi+\Delta\phi) d\Delta\phi >_p, \tag{1}$$

where $I_p(q,\phi)$ is the measured intensity at resolution ring q and azimuthal angle ϕ on diffraction pattern p, and $I_p(q',\phi+\Delta\phi)$ the corresponding intensity at resolution ring q' azimuthal angle $\phi+\Delta\phi$. Similar to the pair correlations, two-point angular triple correlations may be defined [14]:

$$C_3(q,q',\Delta\phi) = < \int I_p^2(q,\phi) I_p(q',\phi+\Delta\phi) d\Delta\phi >_p . \tag{2}$$

The next step is to calculate these quantities to form other quantities related to them in the following way:

$$B_l(q,q') = \frac{2l+1}{2} \int C_2(q,q';\Delta\phi) P_l(x) dx \tag{3}$$

and

$$T_l(q,q') = \frac{2l+1}{2} \int C_3(q,q';\Delta\phi) P_l(x) dx, \tag{4}$$

where $x = \cos\Delta\phi$.

The sequence of operations is summarized next.

This method is well suited to the problem of a curved Ewald sphere, as pointed out in, e.g., [11]. A curved Ewald sphere is inevitable at high resolution.The point is that the quantities actually used to construct the 3D diffraction volume, namely, the $B_l(q,q')$ and the $T_l(q,q')$, are extracted from the quantities measurable in an experiment, namely, the $C_2(q,q';\Delta\phi)$ and $C_3(,q,q';\Delta\phi)$, by removing the effect of the details of the experiment, such as the X-ray energy, which are contained in the arguments of the Legendre polynomials P_l. This allows for a nice separation of the quantities that

depend on the experimental details, and the quantities that appear to contain only information about the 3D diffraction volume, which, in turn, gives information about the 3D structure of the particle. These arguments are equally applicable to pair correlation as to the two-point triple correlations defined by Kam [3]. Although Pedrini [15] and Kurta et al. [16] have proposed methods involving a three-point triple correlation function, the two-point triple correlation functions introduced by Kam [14] are easier to measure when photon counts are weak, as pixels with just two non-zero pixels make contributions. If the probability of a high resolution pixel is non-zero is 10^{-2} [12], the probability that two of them are non-zero is 10^{-4}. However, reasonable statistics are expected if one has the number of diffraction patterns expected to be measured in a typical experimental shift at even a present-day XFEL. In other words, in Kam's method [3], one calculated correlations from samples of many randomly oriented molecules. Nevertheless, and this is our crucial point, the correlations are similar to those from a single particle [17] We admit that this demonstration is only for rotations about a single axis, and for delta function like intensities. We investigate here whether it is still likely to hold for a random particle orientation in 3D, and for realistic amplitudes scattered from each particle. The angular pair correlations can be related to their angular momentum depompostions B_l by

$$C_2(q, q', \Delta\phi) = \sum_l F_l(q, q', \Delta\phi) B_l(q, q'), \tag{5}$$

where

$$F_l(q, q', \Delta\phi) = \frac{1}{4\pi} P_l[\cos\theta(q)\cos\theta(q') + \sin\theta(q)\sin\theta(q')\cos(\Delta\phi)], \tag{6}$$

where

$$\theta(q) = \pi/2 - \sin^{-1}[q/(2\kappa)] \tag{7}$$

and P_l is a Legendre polynomial.

Likewise, it can be shown that the triple correlations defined by (2) can be written as [14]

$$C_3(q, q'\Delta\phi) \quad = \quad \sum_l F_l(q, q', \Delta\phi) T_l(q, q'), \tag{8}$$

where

$$T_l(q, q) = \sum_{l_1, l_2, m_1, m_2, m}^{l_{max}} (-1)^m \left[\frac{(2l+1)(2l_2+1)(2l_1+1)}{4\pi}\right]^{1/2} \times \begin{pmatrix} l_1 & l_2 & l \\ 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} l_1 & l_2 & l \\ m_1 & m_2 & m \end{pmatrix} I_{l_2 m_2}(q) I_{l_1 m_1}(q) I^*_{lm}(q) = \sum_{l_1, l_2, m_1, m_2, m}^{l_{max}} (-1)^m G(l_1 m_1; l_2 m_2; lm) I_{l_2 m_2}(q) I_{l_1 m_1}(q) I^*_{lm}(q), \tag{9}$$

where G is a Gaunt coeffcient [18,19] and

$$I_{lm}(q) = \int I(q, \theta, \phi) Y^*_{lm}(\theta, \phi) d\Omega. \tag{10}$$

Although, in general, the $I_{lm}(q)$'s are complex quantities, the quantities we are concerned with this paper, namely, the $I_{l0}(q)$'s, are real, and all m values are zeros for the azimuthal symmetry.

For a flat Ewald sphere, the quantities $B_l(q, q')$ and $T_l(q, q')$, which contain the structural information, may be found assuming that $\cos(\theta(q))$ and $\cos(\theta(q'))$ tend towards zero and $\sin(\theta(q))$ and $\sin(\theta(q'))$ tend toward unity. In this case, the quantities $B_l(q, q')$ and $T_l(q, q')$ that contain true

structural information about the molecule can be found, for instance, (to within an arbitrary scaling factor) from the integrals

$$B_l(q,q) = \frac{2l+1}{2}\int C_2(q,q,\Delta\phi)P_l(\cos\Delta\phi)sin\Delta\phi d\Delta\phi \quad (11)$$

and

$$T_l(q,q) = \frac{2l+1}{2}\int C_3(q,q,\Delta\phi)P_l(\cos\Delta\phi)sin\Delta\phi d\Delta\phi \quad (12)$$

over the measured quantities C_2, and C_3. It should be stressed that, even in the case of a curved Ewald sphere the quantities containing the structural information about the particle, the $B_l(q,q)$ and $T_l(q,q)$ may be found by matrix inversion of Equations (5) and (8). Due to its expected azimuthal symmetry, nanorice particles have diffraction volumes characterized by spherical harmonic expansion coefficients of only azimuthal quantum numbers $m = 0$. In this case, the only relevant spherical harmonic expansion coefficients, I_{l0}, are real. Thus, the computations in this case ($q' = q$) may be performed entirely with real coefficients and only the diagonal parts of $B_l(q,q')$ and $T_l(q,q')$ are adopted.

It has been shown that these equations may be solved easily for particular common symmetries. It was pointed out by Caspar and Klug [20] that viruses tend to possess mainly icosahedral and helical symmetry. We have shown how to solve these equations for both icosahedral [7] and helical [21] cases, and are working on extending the method to particles that deviate a little from exact symmetry, using a form of perturbation theory [22]. In the case of a helical virus, we exploited the fact that if the virus is oriented with a helix axis parallel to the z-axis, the diffraction volume has exact cylindrical symmetry up to a certain resolution and may therefore be characterized by azimuthal quantum number $m = 0$.

Another case includes where the diffraction volume could be characterized by an $m = 0$ quantum number that is a diffraction volume of a nanorice particle. Experiments have been done on such particles at an XFEL and diffraction data from nanorice has been deposited in the cxidb.org web site. We investigate here whether this data may also be used to reconstruct the structure of nanorice by making use of the $m = 0$ condition. Because of the azimuthal symmetry of a nanorice particle about the major axis, it would be expected that such particles would also be characterized by $m = 0$. A question is if both a helical virus and a nanorice particle may be characterized by $m = 0$, how is it possible to distinguish them? The reason is that the intensities in the case of a helical virus must reflect the periodicity along the azimuthal axis and consequently split up into layer lines, but it does not do so in the case of a nanorice particle.

3. Simulation of Diffraction Patterns of Nanorice in Random Orientations

We tried to make the simulation as realistic as possible by assuming an X-ray beam of width 1000 Å, the design specification of the minimum focus of the Linac Coherence Light Source, the worlds' first X-ray free electron laser (XFEL). As for the size of a nanorice particle, we assumed each particle to be of a width about 50 Å to simulate a small protein. This is a realistic circumstance in which one expects a number of proteins to be illuminated. For the purposes of our test, we assumed only two nanorice particles illuminated in random relative orientations. We note this is a circumstance that is beyond the capabilities of all other algorithms that have been proposed for the XFEL problem, but is a realistic circumstance which is well suited to our method. There is an advantage to illuminating multiple particles as the total scattered signal goes up—with single particles, one is always struggling with few scattered photons.

For an initial model of nanorice, we assumed a ellipsoidal mask on a 3D Cartesian grid in real space, that is, we took the electron density of the nanorice particle to be uniform inside the particle and zero outside. As for the size of the particle in our solutions, the particle was entirely enclosed in a volume of 19 × 19 × 19 central voxels. This model is shown on Figure 1. We then simulated diffraction patterns due to random orientations of nanorice particles as follows.

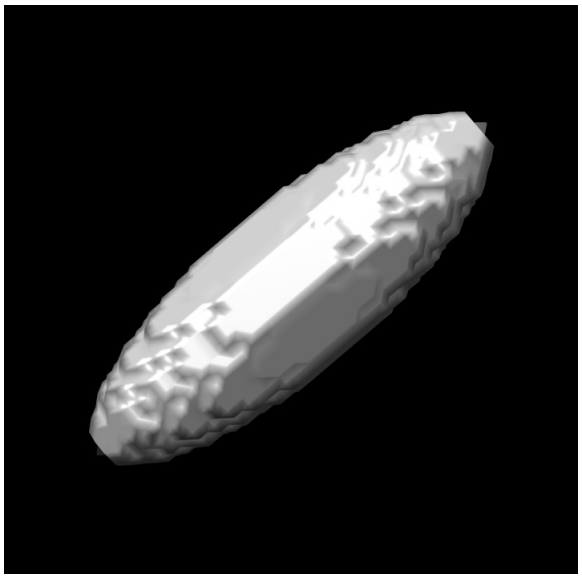

Figure 1. Model of a nanorice particle.

Since the angular correlations are quantities that are independent from all orientations of the particles, they are essentially independent from the number of particles contributing to each diffraction pattern, provided each takes all orientations. This has great importance for XFEL work, as often great pains are taken to restrict the number of particles in each of the measured diffraction patterns to just one, as required by all other methods of reconstructing particles from their diffraction patterns by the use of "hit-finder" software [10], for example. However, it might be argued that if not too much accuracy is required, this is unnecessary as angular correlations are independent from the number of particles illuminated and are the same for the same type of particle. This gives rise to the possibility that one might gain the same advantage. There seems to be no reason to not use multiple particles incident from a beam and actually get rid of the "hit-finder" [10] software. This paper demonstrates at least in theory that one may avoid "hit-finder" software altogether by this means. The only caveat is if the particles inevitably cluster, then the particles will differ in structure as well as orientation and it may be better to ensure that the same particle is hit always from different orientations by admitting only one particle at a time. This is a question that can only be answered by an experiment, but it is useful to know that there is an another, and possibly preferable option if the particles do not cluster. We reconstructed the object with about 2× oversampling in a region consisting of 41 × 41 × 41 voxels.

1. First, we calculated the 3D amplitude distribution in reciprocal space from their correlations, by the following steps:

$$A(\mathbf{q}) = \int \rho(\mathbf{r}) \exp(2\pi i \mathbf{q}.\mathbf{r}) d\mathbf{r}, \quad (13)$$

$$I(\mathbf{q}) = |A(\mathbf{q})|^2 = I(q, \theta, \phi). \quad (14)$$

2. We then calculated the spherical harmonics expansion of the 3D diffraction volume via

$$I_{lm}(q) = \int I(q, \theta, \phi) Y^*_{lm}(\theta, \phi) d\Omega. \quad (15)$$

3. We then found the values of the expansion coefficients I_{lm} for random orientations of the diffraction volume using a Wigner D-matrix for random Euler angles α, β and γ

$$I_{lm}(q) = \sum_{m'} D^l_{m,m'}(\alpha, \beta, \gamma) I_{lm'}(\theta, \phi). \quad (16)$$

4. We then calculated the diffraction pattern expected by slicing through the diffraction volume in each case through the plane $q_z = 0$

$$I(q, \theta = \frac{\pi}{2}, \phi) \quad = \quad \sum_{lm} I_{lm}(q) Y_{lm}(\theta = \frac{\pi}{2}, \phi). \tag{17}$$

This represents the intensity distribution expected of diffraction patterns from random orientations of a nanorice particle. For the two-particle case, we repeated these steps and either added ampitudes of scattering from each of the particles to simulate coherent illumination (assuming random interparticle displacements) or added scattered intensities to simulate incoherent illumination of multiple particles. Such typical diffraction patterns are shown in Figure 2.

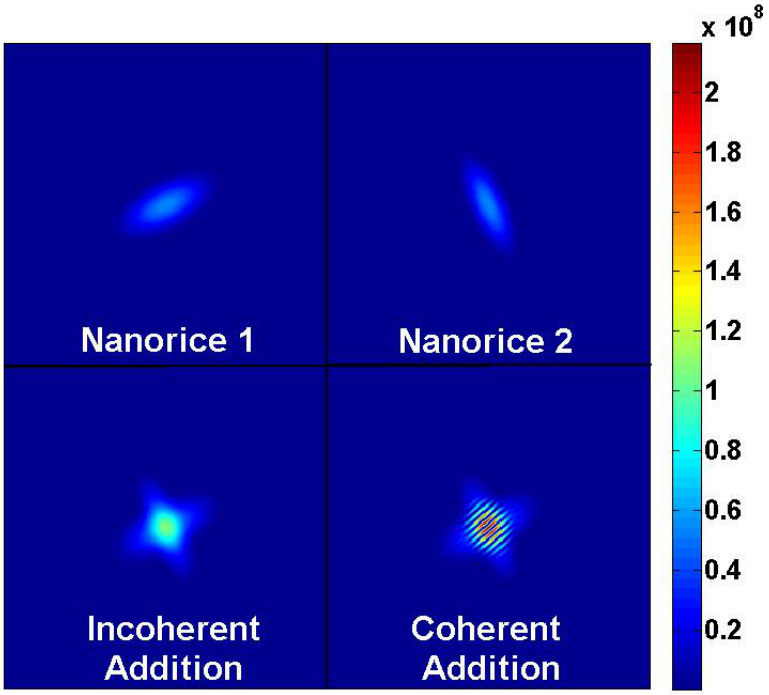

Figure 2. Simulated X-ray free electron laser (XFEL) diffraction patterns of one and two nanorice particles in random orientations.

4. Reconstruction of Diffraction Pattern of Nanorice

Having thus simulated diffraction patterns expected of random orientations of nanorice, our next step was to demonstrate that it is possible to reconstruct our model of nanorice from those patterns using the method outlined above. To this end, we calculated $B_l(q, q)$ and $T_l(q, q)$ as outlined above from the data in the simulated diffraction patterns.

The coefficients I_{lm} of a spherical harmonic expansion of the diffraction volume clearly depend on the orientation of the diffraction volume relative to the chosen z-axis. Two such 3D intensity distributions are displayed on Figures 3 and 4. By choosing a z-axis at the center of azimuthal symmetry, we eliminate the other components of I_{lm} except $m = 0$. Note that this is no loss of generality since the correlations do not determine the particle's orientation. As a matter of fact, the correlations are the same independent of the particles' orientation. On reconstruction of a real-space image of the particle made from the orentation-independent correlations, one is free to choose the particle's orientation for one's convenience .

It has been claimed that the recently proposed multi-tiered itrerative phasing (MTIP) algorithm [23] would not need to assume azimuthal symmetry. Since it is supposed to determine the the magnitudes of the quantities characterized by different magnetic quantum numbers, in principle,

it is capable of deducing that $m = 0$. However, in their recent paper, Donatelli et al. [23] definitely found much better results on the assumption of the degree of angular symmetry. Following Starodub et al. [24], we obtain much the same information by performing an singular value decomposition (SVD) matrix decomposition of the pair correlations.

The quantities B_l and T_l depend on the angular momentum quantum number l but not on the azimuthal quantum number m. However, in general, the spherical harmonic expansion coefficients $I_{lm}(q)$ depend on both sets of quantum numbers. However, there is one orientation when m is fixed and the the I_{lm} coefficients depend additionally only on l, and that is when a major axis of the ellipsoid representing the nanorice is coincident with the z-axis. Under these conditions, the particle, and also the diffraction volume, has azimuthal symmetry about the z-axis, and then can be characterized exactly by $m = 0$ for all l.

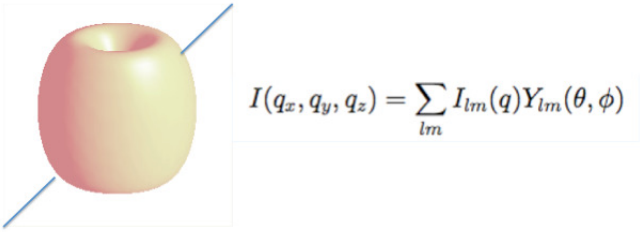

Figure 3. Expansion in spherical harmonics with respect to an arbitrary axis.

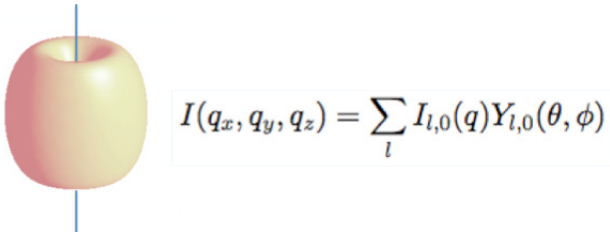

Figure 4. Expansion in spherical harmonics with respect to the z-axis.

We are not trying to reconstruct the particle in any particular orientation. Due to the random orientation of the particles contributing to the data, any orientation of reconstruction is as good as any other. We choose the orientation with the major axis of the ellipsoid along the z-axis. We can choose this orientation by assuming that only the $m = 0$ components of the $I_{lm}(q)$s exist. At this point, these coefficients depend only on l. The magnitudes of these spherical harmonic coefficients determined from

$$|I_{l0}(q)| = \sqrt{B_l(q,q)}. \tag{18}$$

Since I_{l0} is real, its only uncertainty is one of sign. These signs are found by an exhaustive search through

$$T_l(q,q) = \sum_{l_1,l_2}^{l_{max}} G(l_1 0, l_2 0, l0) I_{l_1 0}(q) I_{l_2 0}(q) I_{l_0}(q), \tag{19}$$

where G is a Gaunt coefficient [18,19], and basically we search through all possible signs of the $I_{l0}(q)$ on the RHS (right hand side) (the magnitudes of the I_{l0} are known from the pair correlations as mentioned above) to get best agreement with the experimentally-determined LHS (left hand side). Again, ms are zeros for azimuthal symmetry.

Since an ellipsoid has azimuthal symmetry about a particular axis, we can choose that particular axis as the z-axis, thus eliminating any other components of the magnetic quantum number except $m = 0$. $|I_{l0}(q)|$ can be obtained directly from $B_l(q,q)$ via (18).

The only unknown here is sign of $I_{l0}(q)$. The sign can be determined by fitting all possible signs of $I_{l0}(q)$ to the values $T_l(q)$ of the triple correlations calculated directly from the diffraction patterns of random particle orientations. It should be stressed that the number of equations is equal to the number of distinct $T_l(q,q)$ values, namely, the numbers of q and l values, as is the number of unknowns $I_{l0}(q)$, so there is no information deficit.

After obtaining the signs of the $I_{l0}(q)$, the diffraction volume can be calculated from

$$I(\mathbf{q}) = \sum_l I_{l0}(q) Y_{l0}(\hat{\mathbf{q}}). \tag{20}$$

An iterative phasing algorithm [25,26] applied to these diffraction volumes can then recover the electron density of the particle, the real space object giving rise to the diffraction volume. The calculated intensities are displayed on Figures 5 and 6 and reconstructed electron density after phasing is displayed on Figures 7 and 8.

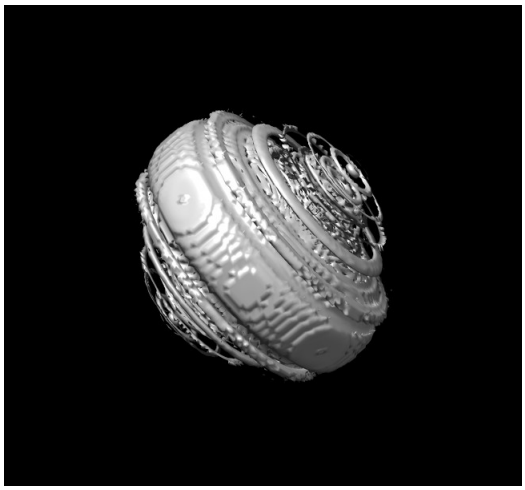

Figure 5. Reconstructed diffraction volume from one particle per shot (randomly oriented).

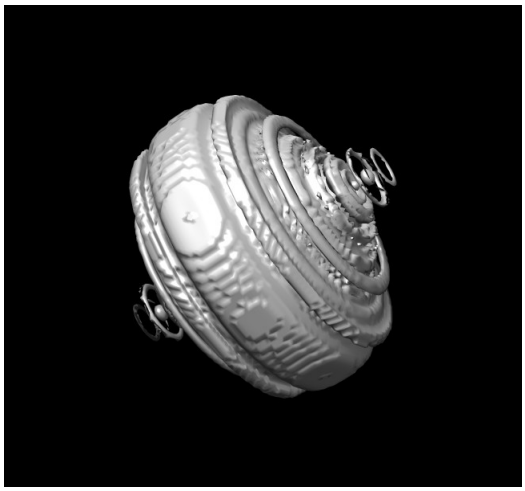

Figure 6. Reconstructed diffraction volume from two particles per shot (randomly oriented).

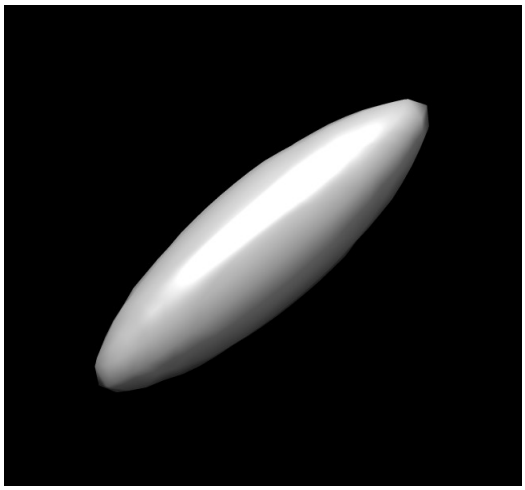

Figure 7. Image reconstructed from one particle per diffraction patten.

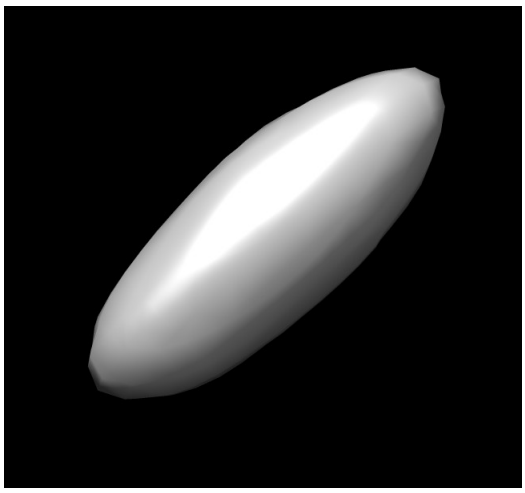

Figure 8. Image reconstructed from two particles per diffraction patten.

5. Angular Dependence of Scattering

We also investigated the effects of the angular dependence of the scattering. Our nanorice particles are about are about 50 Å in diameter. They are constituted of the chemical molecules Fe_2O_3 and thus on average are much stronger scatterers than a typical bioparticle. In fact, we estimate $\log|F(q)|^2$ to be about 10^8 at low resolutions, much higher than for a bioparticle. Of course, we see the precipitous fall off with angle, and Fung et al. estimated the number of high-resolution photons per Shannon pixel to be about 10^{-2} for a 100 kD protein. Since the nanoparticle contains many more electrons, we expect the scattering power of our nanoparticles to not need Poisson statistics. Consequently, we have plotted only $\log|F(q)|^2$, which will give the angular dependence of the measured signal (Figure 9).

6. Conclusions

It should be noted that the images computed from the diffraction patterns of single particles do not agree perfectly with those from two particles. This to be expected, as there will be extra interparticle interfences in the two-particle case. It is only if the number of particles contributing to each diffraction pattern becomes large that the arguments made by [27], for suggesting that the correlations from multiple particles, approach those of a single particle, due to the fact that the phases are random, allowing interparticle inteferences to be ignored.

The method of angular correlations [3,11] is shown to be very capable of recovering accurate 3D images of any particle of azimuthal symmetry as injected into an X-ray free electron laser (XFEL), even if there are multiple particles in random orientations. We tested this by means of a simulations in the case of nanorice, one of the early particles used as a test case with an XFEL. To this end, we simulated diffraction patterns expected of random orientations of the nanorice. From this data, one is able to find quantities that enable the reconstruction of an accurate image of nanorice particles. Note that this method is also applicable for reconstruction of artificial dumbbells, as demonstrated recently in a paper by Starodub et al. [24].

It should be noted that $m = 0$ is also assumed for a helical virus. The difference with that case is that the diffraction volume tends to be concentrated on "layer planes" implying a periodicity of the particle structure along the azimuthal axis. It is remarkable that the combination of "layer plane" intensities and azimuthal symmetry yield a helical structure in real space on phasing [21]. In the present case, the intensities are not concentrated on "layer planes" and yield a structure that is genuinely azimuthally symmetric, with no hint of helicity. Both are indications of the power of modern iterative phasing algorithms to find the correct structure from diffraction volumes.

Our next step will be to apply this method to nanorice experimental data from an XFEL, as deposited on [28], for example.

A problem with experimental data is that a huge number of diffraction patterns are measured, only a subset of which ("good" patterns) correspond to actual particle hits. In addition to all other methods that have been proposed for structure determination from XFEL diffraction patterns, one needs to filter out diffraction patterns corresponding to multiple particle hits. This is not necessary with our method. Only an elimination of patterns corresponding to no particle hits at all. Due to the huge number of diffraction patterns measured in an experiments, this needs to be done by computer. Fortunately, there has already been at least one machine learning algorithm [9] applied to discriminate between measured [9] "good" and "bad" patterns. We are collaborating with the first author of that paper [9] with an aim to apply our method to experimental data.

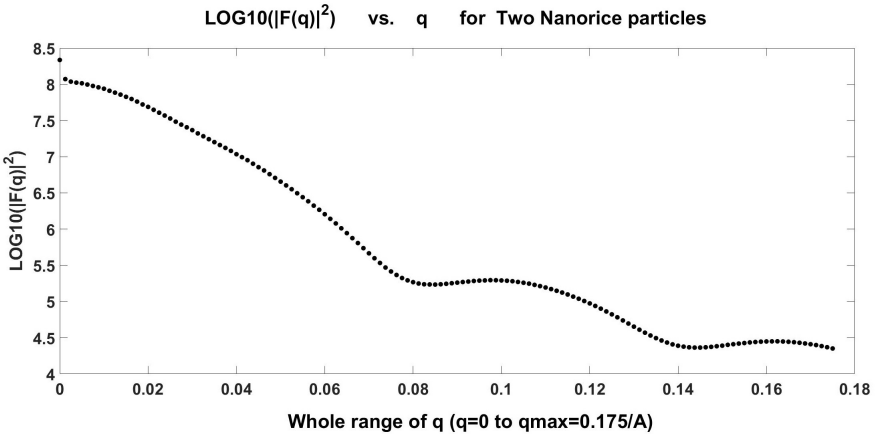

Figure 9. Logarithm of the intensities reconstructed from the angular correlations of two randomly spaced and oriented nanorice particles with widths of about 50 Å.

Acknowledgments: We acknowledge a grant from the U.S. National Science Foundation to fund the bioXFEL Science and Technology Center under Grant No. STC 1231306.

Author Contributions: Sung Soon Kim developed the 2 nanorice model, applied correlation method to this model with azimuthal symmetry, generated all figures in the manuscript though rekted caculateds and corrected minor mistakes in the manuscript. Sandi Wibowo conceived of the original theory of calculating nanorice from the angular correlations and developed the theory thereof. Diano Kerzaman Saldin was the supervisor of the project and wrote the first draft of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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