

# An Accelerated ISAF Algorithm with the Fast Mapping Strategy

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**Abstract**—ISAF (icosahedral symmetry-adapted functions) is a new approach used for 3D reconstruction of icosahedral macromolecules, which can achieve better resolutions compared with the commonly used Fourier-Bessel algorithm. However, ISAF is significantly slow. Among all reasons, the operation that rotating each image sixty times costs lots of running time. By analyzing the sixty symmetrical characteristics of icosahedrons in Fourier space, we proved that the positions of sixty mapped points derived from one sampling point are all the same in asymmetric unit. Thus, the sixty symmetrical rotating of each image can be removed and the reconstruction speed is increased. This accelerating strategy was validated by means of experiments with simulated data and experimental Cryo-EM data. The results showed that the speedup of this strategy was up to 41 times and it could grow up with the increase of the maximum Fourier radius and the number of images at the premise of maintenance of accuracy. Its running speed is about 9 times as great as that of Fourier-Bessel algorithm and is about 3 times than that of recISAFs algorithm.

**Index Terms**—3D reconstruction, mapping, asymmetric unit, ISAF, speedup

## I. INTRODUCTION

Crowther and Klug et.al proposed the theories of 3D reconstruction for protein molecules based on EM (electron microscopy) images in late 1960s [1]. After about 50 years development, the EM reconstruction methods have formed three main kinds: electron crystallography, electron tomography, single particle technology.

Currently, single particle technology based Cryo-EM (cryo-electron microscopy) has played an increasingly important role for determining structures of frozen and non-crystalline state macromolecules [2,3]. Among all

kinds of molecules, the icosahedral molecule has been widely studied [4]. The icosahedral single particle reconstruction methods are divided into two kinds [5]. One is called direct Fourier inversion method which is the most computationally efficient, but may have large memory requirement and is generally more sensitive to noise. The other is called the synthesis method which is less sensitive to image noise and has less memory requirement, but less computationally efficient. Two representatives of synthesis method are the Fourier-Bessel algorithm [1] and the ISAF algorithm [6, 7]. The difference between them is the basis function and interpolation coordinate system. The Fourier-Bessel algorithm uses the cylindrical coordinate system and exponential function, while the ISAF algorithm uses spherical coordinate system and symmetry-adapted functions. Because icosahedral molecules have sixty symmetrical characteristics in Fourier space [1,5, 6], one FFT image is rotated sixty times to get the sixty equivalent FFT images in ISAF algorithm. Subsequently, the sampling points in the sixty FFT images must be mapped onto the asymmetric unit. The purpose of this rotating operation is increasing SNR (Signal to Noise Ratio). Then the resolution of reconstructed map is improved [6]. However, since the number of processed images has to be increased sixty times, significantly more computing resources are required. Some previous studies have shown an exponential relationship between the number of Cryo-EM images and the resolution [9,10]. Therefore, rotating each image sixty times would become a bottle-neck in 3D reconstruction. So, it is very important to reduce the complexity from this rotating operation.

For ISAF algorithm, the sixty equivalent points generated from sixty symmetry operations must be

mapped onto asymmetric unit in Fourier space. With the help of sixty symmetrical characteristics of icosahedral molecules in Fourier space, we proved that the positions of sixty mapped points in asymmetric unit are all the same. That is to say, after rotating sixty times, the structure information in asymmetric unit is similar to that without rotating sixty times. Thus, this complex rotating operation can be removed at the premise of maintenance of accuracy. The accelerated ISAF algorithm is proposed according to this point. With this method, the speed of mapping sampling points onto asymmetric unit can be improved 60 times. The speed of the whole 3D reconstruction could be enhanced to a certain extent. To verify this point, the experiments were carried out with simulated data and experimental Cryo-EM data. The Fourier-Bessel algorithm and recISAFs [6] algorithm were used for comparison with our accelerated ISAF algorithm. The experimental results show that the reconstructed map without rotating sixty times is identical with the one with rotating sixty times. The speedup in the stage of mapping molecular images is 60 times and the whole speedup is up to 41 times. Its running speed is about 9 times as great as that of Fourier-Bessel algorithm and is about 3 times than that of recISAFs algorithm. In addition, the speedup of this method is increasing with the increase of the maximum Fourier radius and the number of images. This method can also be extended for 3D reconstruction of other polyhedral molecules.

II. METHODS

The processing steps of synthesis method include three stages: mapping molecular images, solving fitting coefficients and calculating density function. The main operation in mapping molecular images is rotating each FFT images sixty times and then mapping sampling points in the sixty FFT images onto asymmetric unit. It is known that this operation seriously affect the speed of ISAF algorithm. If the rotating operation is simplified, the speed of ISAF algorithm can be accelerated significantly. In this section, this accelerated ISAF algorithm and its mathematical proof are proposed.

A. Uniqueness Mapping Analysis

In the course of 3D reconstruction based on synthesis algorithm, the above rotating operation is common used both in the cylindrical coordinate and in the spherical coordinate systems. The purpose of this operation is enhancing SNR by averaging a number of images [1,6]. However, with the help of theories of crystal symmetry group, it is found that this operation is redundant in spherical coordinate system as follows.

In real space, icosahedral molecule has 5-2 symmetrical characteristics in the cylindrical coordinate system and has 5-3-2 symmetrical characteristics in the spherical coordinate system. In Fourier space, icosahedral molecule has the identical symmetrical characteristics in the cylindrical coordinate and in the spherical coordinate systems because Fourier transform has the rotational invariance.

In the cylindrical coordinate system, the sixty symmetrical points  $(R_i, \Phi_i, Z_i) (1 \leq i \leq 60)$  are mapped onto the 5-2 symmetrical unit  $\{(R, \Phi, Z) | (R > 0, 0 \leq \Phi \leq 72)\}$ . The positions of them in 5-2 symmetrical unit are different according to the difference between 5-3-2 symmetrical characteristics and the 5-2 symmetrical characteristics of icosahedral molecule. Thus, the SNR is enhanced because the structure information in 5-2 symmetrical unit increases sixty times.

On the contrary, for one sampling point in spherical coordinate system, it is found that the positions of the sixty mapped points are all the same in the 5-3-2 symmetrical unit (commonly referred to as 'asymmetric unit')  $\{(R, \Theta, \Phi) | (R \geq 0, 69.09 \leq \Theta \leq 90, -31.72 \leq \Phi \leq 31.72)\}$ . The brief analysis is as follows.

The spherical surface whose radius is  $R$  can be divided into 60 identical regions  $U_i (1 \leq i \leq 60)$ . The area of each region is the same as the area of the asymmetrical triangle:

$$U = \{(R, \Theta, \Phi) | (R \geq 0, 69.09 \leq \Theta \leq 90, -31.72 \leq \Phi \leq 31.72)\}$$

Here,  $U$  is one of the sixty regions and is denoted as  $U_1$ . The point from one region can be mapped onto another and the number of mapped points in the corresponding region is only one.  $A_1 = (R, \Theta, \Phi)$  denotes one point in the region  $U_1$ . Its 59 symmetrical points  $A_2, A_3 \dots A_{60}$  is distributed to the rest of the 59 regions and one point belongs to one region.  $A_k$  denotes the symmetrical points of  $A_1$  in region  $U_1$ . According to the sixty symmetrical characteristics, the sixty symmetrical points  $A_1, A_2 \dots A_{60}$  can be all mapped onto the region  $U_1$ . From the uniqueness mapping property, it is found that the 60 mapped points are all  $A_k$ . That is to say, the positions of 60 mapped points are all the same.

B. The Accelerating Strategy Based on Uniqueness Mapping

As describe above, all positions of the sixty mapped points are identical in the asymmetric unit. Therefore, the equivalent point of the sixty mapped points by averaging is equal to one of them. So, the SNR has not increased as shown in (1).

$$\bar{P} = \frac{\sum_{i=1}^{60} P_i}{60} = P_i \quad \bar{F} = \frac{\sum_{i=1}^{60} F_i}{60} = F_i \quad (1 \leq i \leq 60) \quad (1)$$

The formula equation (1) is used for calculating the equivalent position ( ) and structure factor value ( $\bar{F}$ ) of the sixty mapped points in asymmetrical unit. In this formula,  $P_i (1 \leq i \leq 60)$  and  $F_i (1 \leq i \leq 60)$  denote positions and structure factor values of the sixty mapped points respectively. All  $F_i (1 \leq i \leq 60)$  are all the same because the sixty points derive from the same sampling point in the original image. Similarly, all

$P_i (1 \leq i \leq 60)$  are the same due to the proof of above section. Under this condition, formula 1 is hold.

The positions and structure factor values of mapped points are used for constructing fitting coefficients equation set. From the above statement, it is known that getting rid of this operation has no effect on constructing fitting coefficients equation set. Thus, whether carrying out the sixty symmetrical operations or not has no effect on the following step. Besides, removing this redundant operation can speed up the computation process.

For the purpose of expression briefly, the ISAF algorithm with sixty symmetrical operations is called original ISAF algorithm and the one without sixty symmetrical operations is called accelerated ISAF algorithm for short.

### III. RESULTS

The theorem of uniqueness mapping is proved and the accelerated ISAF algorithm is proposed in the previous section. In this section, the 3D reconstruction experiments are carried out with simulated data and experimental Cryo-EM data, respectively. The goal is to validate accelerated ISAF algorithm. The recISAFs algorithm proposed by Hongrong liu [6] is selected for comparison because it can achieve the resolution of 3.88 Å [8]. The Fourier-Bessel algorithm is selected for comparison also. The experimental results are analyzed in terms of accuracy and speed. Besides, the Fourier-Bessel algorithm is compared with our new algorithm to demonstrate the advantages of 3D reconstruction for icosahedral molecular in spherical coordinate system. The following experiments are all implemented on one PC whose CPU is AMD Dual-Core 2.6G Hz, with DDR 2G.Byte memory.

#### A. The reconstruction experiment with simulated data

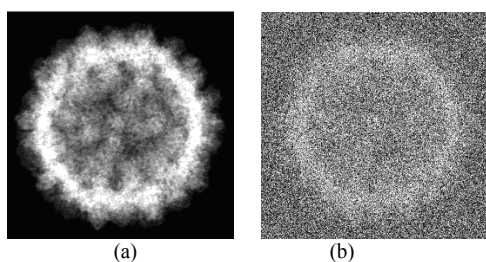


Figure 1. Simulated projection images of the melon necrotic spot virus. (a) Without noise. (b) with an SNR of 0.1.

The simulated data are the projections of the map generated from the melon necrotic spot virus structure (PDB ID 2ZAH), which is solved by X-ray crystallography to 2.81 Å [11] and has icosahedral symmetrical characteristics. Firstly, this model was generated at a resolution of 4 angstrom by using pdb2mrc [12] from EMAN's procedure library. The key parameters were apix=1.4 and res=4. The size of model is  $264 \times 264 \times 264$  pixels. Secondly, 2000 projections with random orientations were generated by using prjectIcosFile [13] from the EMAN's package. Finally, the random noise whose SNR was equal to 0.1 is added to each of the

projections. The images with and without noises were shown in Fig. 1 respectively. In the following experiments, original ISAF algorithm, accelerated ISAF algorithm, Fourier-Bessel algorithm and recISAFs algorithm were all tested. The maximum Fourier radius was  $1/33.6\text{\AA}^{-1}$ ,  $1/18.48\text{\AA}^{-1}$ ,  $1/9.24\text{\AA}^{-1}$  and  $1/4.2\text{\AA}^{-1}$ , respectively. For the purpose of comparison, the Fourier transform was applied to the original structure of melon necrotic spot virus. After that, the Fourier coefficients were disposed if the corresponding Fourier radius was greater than  $1/33.6\text{\AA}^{-1}$ ,  $1/18.48\text{\AA}^{-1}$ ,  $1/9.24\text{\AA}^{-1}$  and  $1/4.2\text{\AA}^{-1}$ , respectively. Finally, the Fourier inverse transform was performed on the truncated Fourier coefficients. Thus, the original maps at a resolution of 33.6Å, 18.48Å, 9.24Å and 4.2Å were generated.

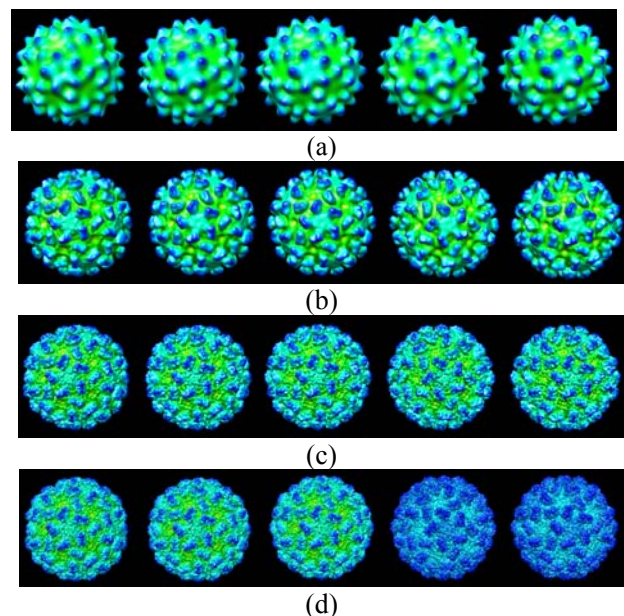


Figure 2. The reconstructed maps at different maximum Fourier radii. From left to right, the first column is the original map. The other columns are the reconstructed maps with original ISAF algorithm, accelerated ISAF algorithm, Fourier-Bessel algorithm and recISAFs algorithm respectively. From top to bottom, the corresponding maximum Fourier radii are  $1/33.6\text{\AA}^{-1}$ ,  $1/18.48\text{\AA}^{-1}$ ,  $1/9.24\text{\AA}^{-1}$  and  $1/4.2\text{\AA}^{-1}$ .

Fig. 2 show the comparison of reconstructed maps by the original ISAF algorithm (the second column), the accelerated ISAF algorithm (the third column), the Fourier-Bessel algorithm (the fourth column) and the recISAFs algorithm (the last column), and the maximum Fourier radius used in testing equals to  $1/33.6\text{\AA}^{-1}$ ,  $1/18.48\text{\AA}^{-1}$ ,  $1/9.24\text{\AA}^{-1}$  and  $1/4.2\text{\AA}^{-1}$ , respectively. From this picture, it is found that the reconstruction results with and without sixty symmetrical operations are both the same. It can be seen clearly now that the accuracy of reconstruction in spherical coordinate system is better than that of reconstruction in the cylindrical coordinate system.

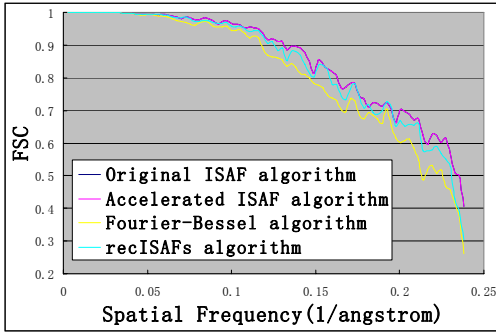


Figure 3. The FSC curves of the reconstructed maps with four methods at the maximum Fourier radius of  $1/4.2\text{\AA}^{-1}$

The FSC (Fourier Shell Correlation) [14] curves at the maximum Fourier radius of  $1/4.2\text{\AA}^{-1}$  were used for calculating the resolution of maps generated using original ISAF algorithm, accelerated ISAF algorithm, Fourier-Bessel algorithm and recISAFs algorithm, which are shown in Fig. 3. The FSC curves using original ISAF algorithm and accelerated ISAF algorithm are coincident with each other and they are drop to 0.5 at  $1/4.23\text{\AA}^{-1}$ . The FSC curve using Fourier-Bessel algorithm is below them and it drops to 0.5 at  $1/4.66\text{\AA}^{-1}$ . The FSC curve using recISAFs algorithm is between them and it drops to 0.5 at  $1/4.28\text{\AA}^{-1}$ . It indicates that the effective resolution using original ISAF algorithm and accelerated ISAF algorithm are both  $4.23\text{\AA}$ , the one using Fourier-Bessel algorithm is  $4.66\text{\AA}$  and the one using recISAFs algorithm is  $4.28\text{\AA}$ . In light of this, the maps generated using original ISAF algorithm and accelerated ISAF algorithm are identical, which are better than that using Fourier-Bessel algorithm and recISAFs algorithm.

Equation (2) is used for computing the speedup of our acceleration strategy. In this formula,  $t_{icos}$  is the time spent calculating the structure factors with rotating sixty times and  $t_{nonicos}$  is the time spent on anything else (e.g. reading and writing images).

$$A = \frac{t_{icos} + t_{nonicos}}{t_{icos} / 60 + t_{nonicos}} \quad (2)$$

The reconstruction speedups with different number of projections at the maximum Fourier radius of  $1/4.2\text{\AA}^{-1}$  are shown in Tab. 1. The reconstruction speedups with 2000 projections at the different maximum Fourier radius are shown in Tab. 2. In Tab. 1 and Tab. 2, the meanings of the column items are as follows: No is the number of projections,  $t_{icos}$ ,  $t_{nonicos}$  and  $A$  are the meanings in equation (2),  $t_A$ ,  $t_F$  and  $t_R$  are the running times with accelerated ISAF algorithm, Fourier-Bessel algorithm, recISAFs algorithm respectively. The unit in Tab. 1 and Tab. 2 is second. From the two tables, it is found that the speedup of accelerated ISAF algorithm is increasing with the increase of the maximum Fourier radius and the number of projections. Under the circumstance of high resolution (less than  $10\text{\AA}$ ), the speedup is often over 20 times and up to 26 times. Its running speed is 6 times as fast as Fourier-Bessel algorithm and is about 2.5 times than that of recISAFs algorithm.

TABLE 1 THE SPEEDUP WITH DIFFERENT NUMBERS OF PROJECTIONS AT THE MAXIMUM FOURIER RADIUS OF  $1/4.2\text{\AA}^{-1}$

No.	$t_{icos}$	$t_{nonicos}$	$t_A$	$A$	$t_F$	$t_R$
250	305.42	111.97	117.06	3.57	329.19	362.27
500	612.31	117.02	127.22	5.73	419.31	369.70
1000	1237.55	123.53	144.157	9.44	597.71	375.92
2000	2533.83	134.77	177.77	15.01	1033.52	396.77

TABLE 2 THE SPEEDUP AT THE DIFFERENT MAXIMUM FOURIER RADIUS WITH 2000 PROJECTIONS

Res	$t_{icos}$	$t_{nonicos}$	$t_A$	$A$	$t_F$	$t_R$
$1/33.6\text{\AA}^{-1}$	333.75	15.33	20.89	16.71	15.02	110.66
$1/18.48\text{\AA}^{-1}$	459.24	17.03	24.69	19.29	30.99	118.67
$1/9.24\text{\AA}^{-1}$	1084.02	24.31	42.38	26.15	125.74	144.70
$1/4.2\text{\AA}^{-1}$	2533.83	134.77	177.77	15.01	1033.52	396.77

*B. The reconstruction experiment with experimental Cryo-EM data*

In this section, the experimental data are the Cryo-EM images of HBV (Human Hepatitis B Virus) from R.A.Crowther, which are consist of 4588 HBV particles recorded on 11 electron micrographs. The triangulation is  $T=4$  and the diameter is about  $360\text{\AA}$ . Fig.4 shows 12 images at the different defocus. The imaging parameters are as follows: the size of each particle is  $256 \times 256$  pixels, the step of sampling is  $2.5\text{\AA}/\text{pixel}$ , the type of electron microscopy is FEI F30, the speed voltage is  $300\text{kV}$ , the spherical-aberration coefficient is  $2\text{mm}$ , the defocus is between  $1.3\mu\text{m}$  and  $2.4\mu\text{m}$ . These parameters are used for CTF correction [15].

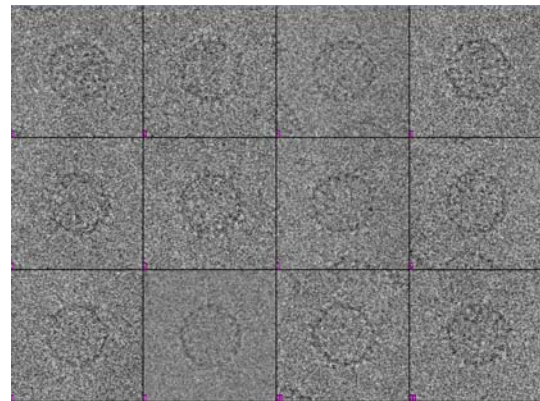


Figure 4. 12 CryoEM images of HBV at different defocus.

Like the previous section, the 3D reconstruction experiments are carried out by using four algorithms (the original ISAF algorithm, the accelerated ISAF algorithm, the Fourier-Bessel algorithm and the recISAFs algorithm) at the different maximum Fourier radius ( $1/30.36\text{\AA}^{-1}$ ,  $1/21.25\text{\AA}^{-1}$ ,  $1/12.5\text{\AA}^{-1}$ , and  $1/7.5\text{\AA}^{-1}$ ). The experimental results are shown in Fig. 5. It is found that the reconstructed maps with and without sixty symmetrical operations are both identical and are better than that using Fourier-Bessel algorithm and recISAFs algorithm.

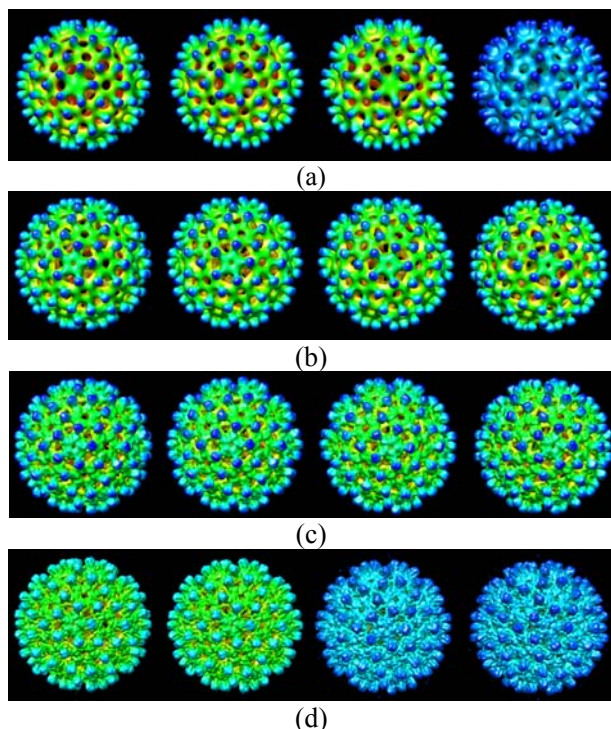


Figure 5. The reconstructed maps at different maximum Fourier radiuses. From left to right, they are the reconstructed maps with original ISAF algorithm, accelerated ISAF algorithm, Fourier-Bessel algorithm, and recISAFs algorithm respectively. From above to below, the corresponding maximum Fourier radiuses are  $1/30.36\text{\AA}^{-1}$ ,  $1/21.25\text{\AA}^{-1}$ ,  $1/12.5\text{\AA}^{-1}$ ,  $1/7.5\text{\AA}^{-1}$ .

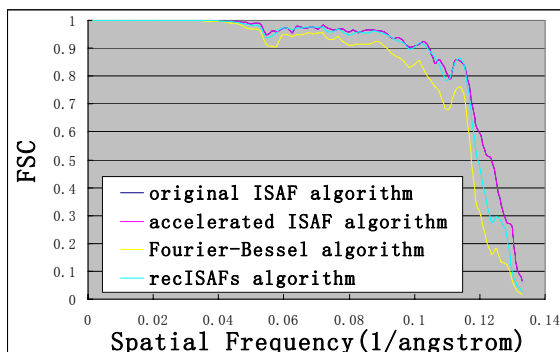


Figure 6. The FSC curves of the reconstructed maps with four methods at the maximum Fourier radius of  $1/7.5\text{\AA}^{-1}$

TABLE 3 THE SPEEDUP WITH DIFFERENT NUMBERS OF CRYO-EM IMAGES AT THE MAXIMUM FOURIER RADIUS OF  $1/7.5\text{\AA}^{-1}$

No.	$t_{icos}$	$t_{nonicos}$	$t_A$	$A$	$t_F$	$t_R$
573	975.24	13.93	30.19	32.77	367.981	249.69
1147	1488.66	17.85	42.66	35.32	531.847	259.80
2294	2469.06	25.87	67.02	37.23	856.088	280.14
4588	5686.32	41.70	136.47	41.97	1545.77	313.52

The FSC curves of maps generated from these algorithms are shown in Fig. 6 at the maximum Fourier radius of  $1/7.5\text{\AA}^{-1}$ . The effective resolution using original ISAF algorithm and accelerated ISAF algorithm are both  $7.97\text{\AA}$ , the one using Fourier-Bessel algorithm is  $8.38\text{\AA}$ , and the one using recISAFs algorithm is  $8.27\text{\AA}$ . So, removing rotation sixty times has no influence on reconstruction and the reconstruction algorithm in the

spherical coordinate system is better than that in the cylindrical coordinate system.

Like the previous section, the equation (2) is used for computing the speedups of this accelerating strategy. The computing results are shown in Tab. 3 and Tab. 4. Tab. 3 illustrates the speedups with different number of projections at the maximum Fourier radius of  $1/7.5\text{\AA}^{-1}$ . Tab. 4 shows speedups with 4588 projections at the different maximum Fourier radius. The meanings of column items in Tab. 3 and Tab. 4 are equivalent to that in in Tab. 1 and Tab. 2. The unit in Tab. 3 and Tab. 4 is second. From Tab. 3 and Tab. 4, it is found that the speedup is increasing with the increase of the maximum Fourier radius and the number of images. Under the circumstance of high resolution, the speedup is often over 35 times and up to 41 times. Its running speed is about 9 times as great as that of Fourier-Bessel algorithm and is about 3 times than that of recISAFs algorithm.

In summary, removing sixty symmetrical operations of 3D reconstruction of icosahedral molecule in spherical coordinate system has no effect on accuracy. Thus removing this step can enhance the speed of reconstruction greatly. In addition, this accelerated ISAF algorithm is better than the Fourier-Bessel algorithm in terms of both speed and accuracy.

TABLE 4 THE SPEEDUP AT THE DIFFERENT MAXIMUM FOURIER RADIUS WITH 4588 CRYO-EM IMAGES

Res	$t_{icos}$	$t_{nonicos}$	$t_A$	$A$	$t_F$	$t_R$
$1/30.36\text{\AA}^{-1}$	186.48	4.61	7.72	24.76	60.44	117.06
$1/21.25\text{\AA}^{-1}$	641.7	6.86	17.55	36.95	118.00	128.48
$1/12.5\text{\AA}^{-1}$	1666.08	13.28	41.04	40.9	409.01	162.73
$1/7.5\text{\AA}^{-1}$	5402.52	41.88	131.92	41.3	1545.77	313.52

#### IV. CONCLUSIONS

In the field of 3D reconstruction of protein structures, the ISAF algorithm can achieve better resolutions but with a low running speed. The study had revealed rotating each image sixty times is a most time-consuming operation among all operations. Thus, simplifying this operation is can increase the running speed of ISAF algorithm. The sixty symmetrical characteristics of icosahedrons in Fourier space is very useful for this point. It is found the positions of sixty mapped points in 5-2 symmetrical unit aren't all the same, but the positions of them in 5-3-2 symmetrical unit are identical. So, the information content in 5-2 symmetrical unit is increasing but the one in 5-3-2 symmetrical unit is invariable. That is to say, the sixty symmetrical operations is necessary for Fourier-Bessel algorithm in cylindrical coordinate system because the SNR in 5-2 symmetrical unit is enhancing. But this operation is no use for ISAF algorithm in spherical coordiante system because the SNR in 5-3-2 symmetrical unit is constant. This phenomenon is proved theoretically and is called 'theorem of uniqueness mapping'. Based on this theorem, this complex operation can be removed and the accelerated ISAF algorithm is proposed subsequently. The simulated data of melon necrotic spot virus and the

experimental Cryo-EM data of HBV are used for validating our accelerated ISAF algorithm. The results show that this accelerated ISAF algorithm can speed up significantly at the premise of maintenance of accuracy. Its running speed is about 9 times as great as that of Fourier-Bessel algorithm and is about 3 times than that of recISAFs algorithm. More importantly, the speedup of this strategy is increasing with the increase of the maximum Fourier radius and the number of images.

In addition, the reconstruction process of other polyhedrons molecule -- such as tetrahedral molecule, hexahedral molecule, octahedral molecule and so on -- is similar to that of icosahedral molecule. In this sense, our accelerating strategy can be extended to these new circumstances. Therefore, we will study its application to other-hedron symmetrical molecules.

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