Chapter 4

Conclusion

4.1 Progression of Single Particle Analysis

To avoid the challenges of crystallization and the size limitations of nuclear magnetic resonance spectroscopy, it has long been hoped that single-particle cryo-electron microscopy would eventually produce atomically interpretable maps. Steady progress towards this goal has been made (Frank 2002), led by reconstructions of large icosahedral viruses, whose 60-fold symmetry, size, and rigid architecture all facilitate precise image alignment.   3D single-particle reconstructions of virus particles from electron micrographs were first accomplished by Fourier synthesis in 1970 (Crowther, Amos et al. 1970). By the turn of the 21st Century, single particle techniques had already achieved sub-nanometer resolutions (Bottcher, Wynne et al. 1997; Conway, Cheng et al. 1997; Trus, Roden et al. 1997) but were still limited in resolution by various factors (Baker, Olson et al. 1999; van Heel, Gowen et al. 2000). The difficulty in modeling some of these factors led to the lack of accurate predictions about the severity of each of these limits and it was unclear which was the most dominant limit. Thus, when I began my thesis work in 2002, I chose to address two of these problems, namely the lack of computing power for high-resolution reconstructions and the depth of field or, equivalently, the Ewald sphere curvature problem (DeRosier 2000), as they were best suited to my interests and abilities.

4.2 Hybrid Approach to Address Lack of Computational Power

I have addressed the lack of computational power using a hybrid computational approach (parallel computation used in conjunction with a distributed computation system), which utilizes untapped resources to effectively increase computational power. This approach consisted of (1) Parallel implementations of conventional and paraboloid reconstruction algorithms (Chapter 3), which are also compatible with distributed computation systems and (2) Development of a distributed computation system (Chapter 2) designed specifically for (but not limited to) large scale image processing.

Thus, the hybrid approach, when applied to single particle reconstructions, allows for the utilization of all cores on each computer and all available computers participating in the distributed computation system. This leads to a massive computational speedup and is necessary for high-resolution reconstructions of large virus particles.

4.3 Paraboloid Reconstruction Algorithm to Address Ewald Sphere Curvature

I have addressed the Ewald sphere curvature problem, or equivalently the depth of field problem, by development of the *Prec* algorithm. The algorithm, unlike conventional reconstruction algorithms that are based on the projection theorem, takes into account the curvature of the Ewald sphere and is able to correct for this resolution limitation completely (Chapter 3).

The *Prec* algorithm was applied to simulated images and recent experimental data sets of three 700–750 Å diameter viruses, which had been reconstructed to ~ 4 Å resolution

(Jiang, Baker et al. 2008; Yu, Jin et al. 2008; Zhang, Settembre et al. 2008) by conventional methods. Two main conclusions could be drawn from the results: (1) The Ewald sphere curvature problem has been completely solved and (2) The curvature of the Ewald Sphere is currently not the dominant resolution limit.

Thus, in order for the effects of the Ewald sphere curvature correction to be significant, higher resolution reconstructions of larger virus particles have to be achieved. It would seem that with the rapid improvements in single-particle reconstruction resolutions over the past decade, it is just a matter of time before these resolutions become sufficiently high. When this occurs, the application of the Prec algorithm will be necessary for high-resolution reconstructions of large virus particles.

4.4 References

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