

Validation of 3D cryoEM single particle reconstruction correctness and handedness with Ewald's sphere correction

Zbyszek Otwinowski¹, Raquel Bromberg^{1,2}, Yirui Guo^{1,2}, Dominika Borek¹

¹*Department of Biophysics, UT Southwestern Medical Center, 5323 Harry Hines Blvd. MC8816, Dallas, TX 75390, USA;*

²*Ligo Analytics, 2207 Chunk Ct, Dallas, TX75206, USA*

Zbyszek.Otwinowski@UTSouthwestern.edu

We present an implementation of Ewald sphere correction that separates the chirality-sensitive component of the signal, enabling both automatic handedness determination and independent validation of 3D reconstructions. Quantum mechanical treatment of scattering places signal contributions on the Ewald sphere and its Friedel mate. In conventional cryo-EM analysis, these contributions are typically merged due to the large radius of the Ewald sphere. Our approach factorizes the signal into inversion-symmetric and inversion-antisymmetric components. Correlations between reconstructions derived from these components provide a reliable metric for handedness determination and reconstruction quality assessment.

This method is robust and can be applied routinely, even at resolutions below the threshold where Ewald sphere curvature begins to impact the signal-to-noise ratio. Our method also provides a complementary validation strategy independent of commonly used statistical measures such as the Fourier Shell Correlation between half-maps.