The autocorrelation functions in cryo–EM

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The 3-D Fourier transform of an L-bandlimited 3-D volume (e.g., particle) can be expanded into spherical harmonics:

$$\hat{V}(k,\theta,\phi) = \sum_{\ell=0}^{L}\sum_{m=-\ell}^{\ell} A_{\ell,m}(k) Y_\ell^m(\theta,\phi), \tag{1}$$

where $\theta \in [0,\pi)$ is the polar angle, $\phi \in [0,2\pi)$ is the azimuthal angle, k is the radial coordinate, $Y_\ell^m(\theta,\phi)$ is the spherical harmonic of degree ℓ and order m and $A_{\ell,m}(k)$ are the associated spherical harmonics coefficients. The goal is to estimate the functions $A_{l,m}$. A rotation of the volume by $\omega \in SO(3)$ can be described using the Wigner D-function $D^\ell_{m,m'}$:

$$\begin{aligned}(R_\omega \hat{V})(k,\theta,\phi) &= \sum_{\ell=0}^{L}\sum_{m=-\ell}^{\ell} A_{\ell,m}(k)(R_\omega Y_\ell^m)(\theta,\phi) \\ &= \sum_{\ell=0}^{L}\sum_{m=-\ell}^{\ell} A_{\ell,m}(k) \sum_{m'=-\ell}^{\ell} D^\ell_{m,m'}(\omega) Y_\ell^{m'}(\theta,\phi).\end{aligned} \tag{2}$$

By the Fourier slice theorem, the Fourier transform of each cryo-EM measurement (that is, each projection) is a slice of $\hat{V}$, associated with $\theta = \pi/2$, after $\hat{V}$ was rotated by $\omega \in SO(3)$. Explicitly, the Fourier transform of a projection from the viewing direction ω is related to the spherical harmonic coefficients of the object through:

$$\hat{P}_\omega(k,\phi) = \sum_{\ell=0}^{L}\sum_{m=-\ell}^{\ell} A_{\ell,m}(k) \sum_{m'=-\ell}^{\ell} D^\ell_{m,m'}(\omega) Y_\ell^{m'}(\pi/2,\phi). \tag{3}$$

Next, we relate the projections P_ω to the mean and the autocorrelation functions, both computable from the observed micrographs. The mean of the micrograph is proportional to

$$M_1 \propto \sum_{n=1}^{N}\sum_{x,y} P_{\omega_n}(x,y), \tag{4}$$

where ω_n denotes the viewing direction of the nth projection. By taking $n \to \infty$, we get

$$M_1 \propto \sum_{x,y}\int_\omega P_\omega(x,y)\rho(\omega)d\omega, \tag{5}$$

where $\rho(\omega)$ denotes the (possibly unknown) viewing direction distribution over $SO(3)$.

We assume the projections are sufficiently separated so that, in the limit $n \to \infty$, the (Δ_x, Δ_y) entry of the second-order autocorrelation of the micrograph is proportional to:

$$M_2(\Delta_x, \Delta_y) \propto \sum_{x,y} \int_\omega P_\omega(x, y) P_\omega(x + \Delta_x, y + \Delta_y) \rho(\omega) d\omega + \text{bias}. \tag{6}$$

The assumption here is that (Δ_x, Δ_y) are small enough so that, in computing the autocorrelation, points (x, y) and $(x + \Delta_x, y + \Delta_y)$ do not touch distinct particles. In the same way and under the same conditions, the third moment is given by

$$M_3(\Delta_x^1, \Delta_y^1; \Delta_x^2, \Delta_y^2) \propto \sum_{x,y} \int_\omega P_\omega(x, y) P_\omega(x+\Delta_x^1, y+\Delta_y^1) P_\omega(x+\Delta_x^2, y+\Delta_y^2) \rho(\omega) d\omega + \text{bias}. \tag{7}$$

In order to determine the particle, by (1) one needs to estimate order of L^3 spherical harmonics coefficients. If the pixel size is proportional to $1/L$ (to match the volume's resolution), then M_3 provides order of L^4 equations involving triple products of P_ω. However, since the in-plane rotation of each particle image is usually uniformly distributed, M_3 depends on only three parameters: the length of the vector (Δ_x^1, Δ_y^1), the length of the vector (Δ_x^2, Δ_y^2) and the angle between the two vectors. Therefore, M_3 provides only $\sim L^3$ equations. Since P_ω depends (after coordinate transformation) linearly on the spherical harmonic coefficients through (3), this means we have a system of $\sim L^3$ cubic equations in the $\sim L^3$ sought parameters. Importantly, the coefficients of these equations can be estimated from the micrographs directly, without particle picking.