Reference-enhanced x-ray single particle imaging

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High energy x-ray free electron laser (XFEL) sources facilitate the 3D structure determination of nanoscale biological entities such as viruses and cluster of proteins, from a large number of diffraction patterns. This technique of single particle imaging (SPI) enables collection of millions of patterns from copies of these objects in random orientations. However, the resolution of 3D structure is limited by relatively low signal due to background noise, total amount of patterns collected and heterogeneity of the target particle. We proposed two holographic-principle based experimental methodologies by attaching a strong scattering reference to target object for obtaining high-quality diffraction patterns, facilitating in gain of signal-to-noise by including scattering from a strongly scattering reference[1].

Here we conduct reference-SPI experiment by conjugating gold nanospheres (AuNP) with biological objects, in this case encapsulin proteins and MS2 phages, to capitalize on high scattering cross-section of AuNP enabling high collection rate. The composite system adds heterogeneity to the dataset, since the diffraction patterns vary not only in the orientation of the particles in the beam but also in the properties and relative position of the reference. To overcome the additional heterogeneity, we also develop a reconstruction algorithm [2] based on maximum likelihood estimation to recover the full 3D structure of a mostly reproducible object from a large number of patterns of composite structures consisting of the target object as well as a reference introduced by the attachment process, to obtain a high resolution (sub 2 nm) structure of the target particles.

![Fig. 1. Methodologies to attach a reference to a target object [1]. (a) random sphere cluster depicting biological like entities attached to spherical AuNP as reference used as the test object for illustration. (b) same cluster within a unit cell of 2d lattice. (c) Simulated diffraction pattern for a random object in a normal single-particle imaging (SPI) case with 4810 photons. (d) Diffraction pattern from the same particle with a small AuNP attached (10,000 photons). If background is present, structural information can be discerned at higher scattering angles in the holographic case [2].](image-url)

[1] Kartik Ayyer, "Reference-enhanced x-ray single-particle imaging," Optica 7, 593-601 (2020).
[2] Mall, Abhishek, and Kartik Ayyer. "Holographic single particle imaging for weakly scattering, heterogeneous nanoscale objects." arXiv preprint arXiv:2210.10611 (2022).