Structure Determination of PR772 Virus from Single-particle XFEL Data

A. Hosseinizadeh[1], P. Schwander[1], G. Mashayekhi[1], J. Copperman[1], A. Ourmazd[1]
and the SPI collaboration lead by A. Aquila[2]
[1]University of Wisconsin-Milwaukee, WI 53211, USA; [2]LCLS, SLAC National Laboratory, CA 94025, USA

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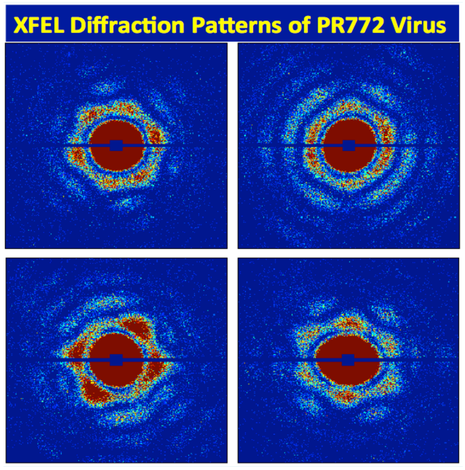
1. Outline

- In the absence of stochastic artifacts, XFEL single-particle snapshots can differ in particle orientations and conformations.
- Using experimental data from PR772 virus (SPI-AMO86615), we show that manifold embedding is able to determine the 3D structure of this virus to the resolution corresponding to the detector edge.

2. XFEL Snapshots of PR772 Virus

- Large viruses scatter millions of photons per shot
- This is much larger than needed for 3D reconstruction in the absence of strong stochastic effects
- However, diffraction patterns are strongly affected by extraneous effects such as shot-to-shot variations in intensity, aperture scattering, and background stochastic noise, etc.

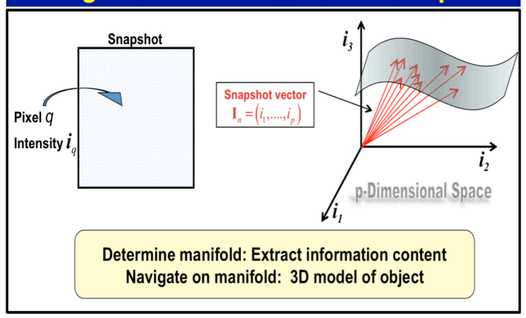
XFEL Diffraction Patterns of PR772 Virus



3. Manifold Embedding Approach

- Manifold Embedding = nonlinear PCA
- All we have is ensemble of diffracted intensities.
- Ensemble of snapshots produces a cloud of points
- Manifold embedding by Diffusion Map extracts (nonlinear) manifold describing the signal.
- Diffusion Map provides mathematical link to "cloud of points" via Laplace-Beltrami operator.

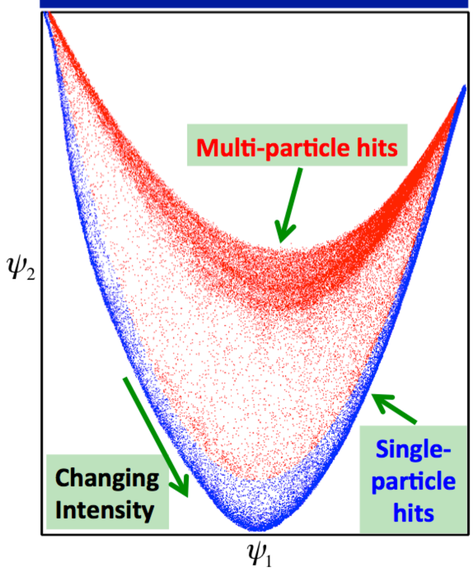
Finding the Manifold of Diffraction Snapshots



4. Single-particle Hit Finding

- Manifold of raw data projected onto the first two Laplace-Beltrami eigenfunctions ψ_1 and ψ_2 reveals a parabolic characteristic.
- Parabola reveals shot-to-shot intensity variations of XFEL pulses.
- Diffusion Map identifies ~38,000 single-particle hits located at the lower edge of the manifold
- The single-particle data set is used for orientation recovery after appropriate preprocessing steps

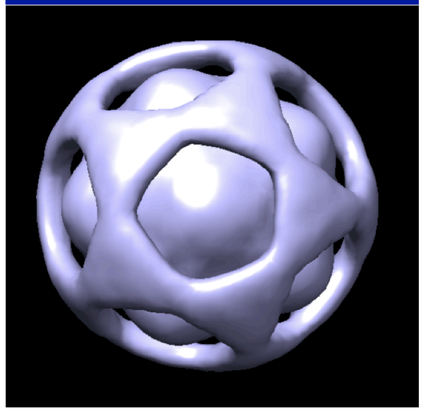
Manifold of Raw Diffraction Patterns from PR772 Virus



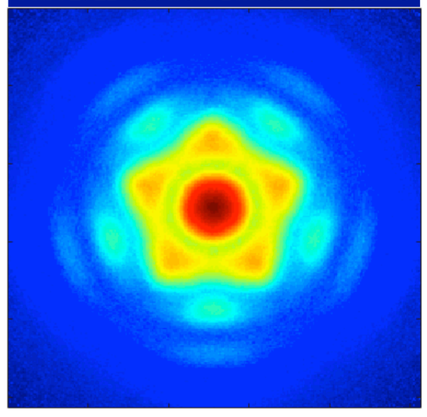
5. Diffraction Volume from Manifold

- After removing imaging artifacts (background noise, intensity variations, etc.), manifold of single-particle snapshots from PR772 virus is consistent with Icosahedral Wigner D-functions.
- The object orientation is determined for each snapshot.
- 3D diffraction volume from ~38,000 2D snapshots is recovered.
- Resolution of the recovered 3D volume extends to edge of detector (11nm determined by FSC) .

Reconstructed Diffraction Volume of PR772



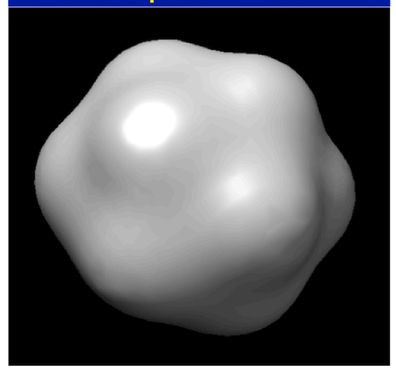
PR772: 2D Cut from Diffraction Volume



6. 3D Structure from Manifold

- 3D electron density obtained from diffraction volume via iterative phasing.

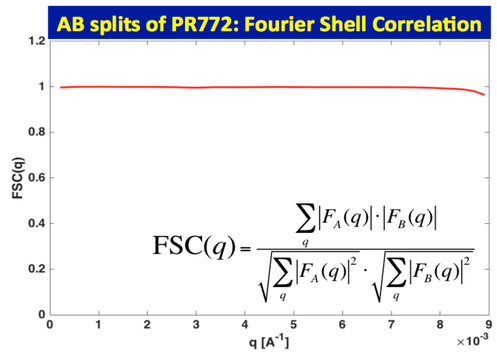
Phased Real-Space Structure of PR772



7. Validation of Orientation Recovery

- Fourier-Shell correlation (FSC) is computed between electron densities of two random subsets.
- Reveals statistically significant information out to detector edge (11nm).

AB splits of PR772: Fourier Shell Correlation



8. Summary

- Demonstrated single-particle 3D structure recovery to detector edge
- Manifold embedding a powerful end-to-end platform for single-particle structure recovery

Acknowledgements

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