Constrained optimisation methods for the retrieval of structural information in electron crystallography with limited tilt angles

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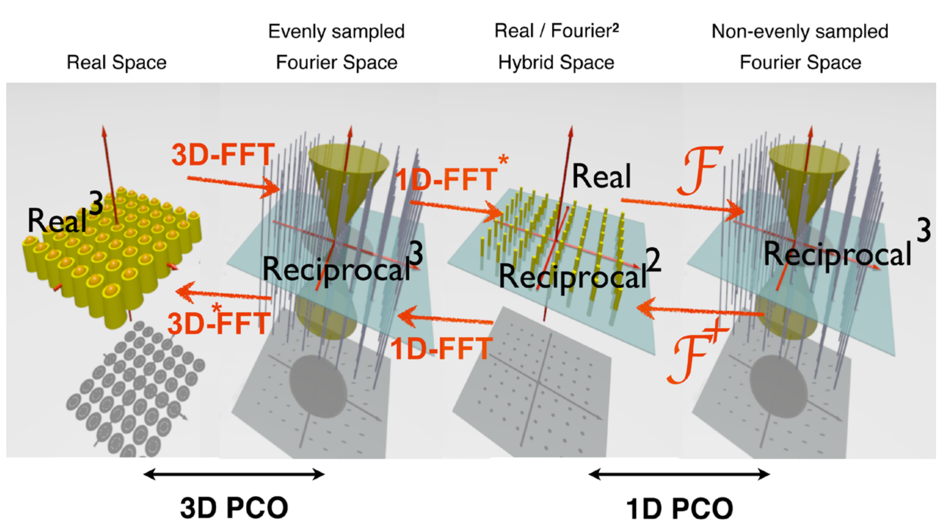
Abstract : Electron crystallography uses transmission electron microscopy (TEM) to determine the atomic structure of membrane proteins that exhibit preferential formation in two-dimensional crystals. For geometric reasons, data collection on tilted 2D crystals is limited to ~70º tilt, but for technical reasons the efficiency of data collection at tilt angles higher than 45º is low. Together with noisy data, the problem of three-dimensional reconstruction in electron crystallography is severely ill-posed and needs additional information to reduce the search space of solutions. The reconstruction is usually realized in the Fourier space, where the projected views, once averaged and corrected for the microscope's contrast transfer function (CTF), are merged. The tilt angle limitation results in zero information about the amplitude and phase values in a so-called "missing cone" of the Fourier domain. We present here two novel iterative reconstruction techniques combining projections onto convex/non convex sets (POCS) and mixed constraints, such as density support, positivity, maximal intensity and frequency achievable. The first presented method is an iterative Fienup-Gerchberg-Saxton algorithm that realizes the POCS, while enforcing the boundary and frequency constraints, respectively in the real and in Fourier-space. The second method recently emerged from the compressed sensing (CS) field, and optimizes separately the reliability to the data (unconstrained tomographic reconstruction) and sparsity cost functions in an alternative manner. The total-variation (TV) norm is chosen as cost function in order to encourage the convergence to solutions having smooth gradient. Each step of the optimization contributes to a convergence to the lowest possible raw data residue, while keeping cost functions at low values. The first method has been tested on an experimental data set of the Bacteriorhodopsin[1], showing full recovery of the missing cone data also from 45º-tilt-angle limited datasets. Both method are general and can be applied to any tomographic optimization problem, i.e for electron crystallography, single particle reconstruction, or for electron tomography on larger biological samples.

Spaces used in Projective Constraint Optimization (PCO)



Four different spaces are used :

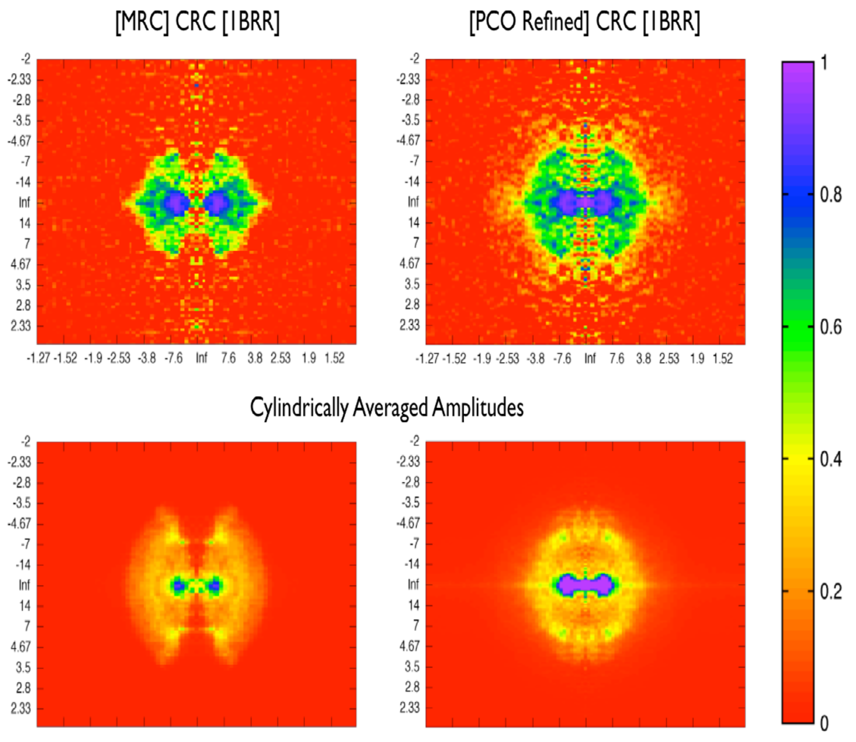
Left: The real space contains the protein structure.

Mid-left: The evenly sampled Fourier space contains reciprocal lattice lines.

Mid-right: A hybrid space was defined, where real-space representations of lattice line data are located in their reciprocal X/Y Fourier positions.

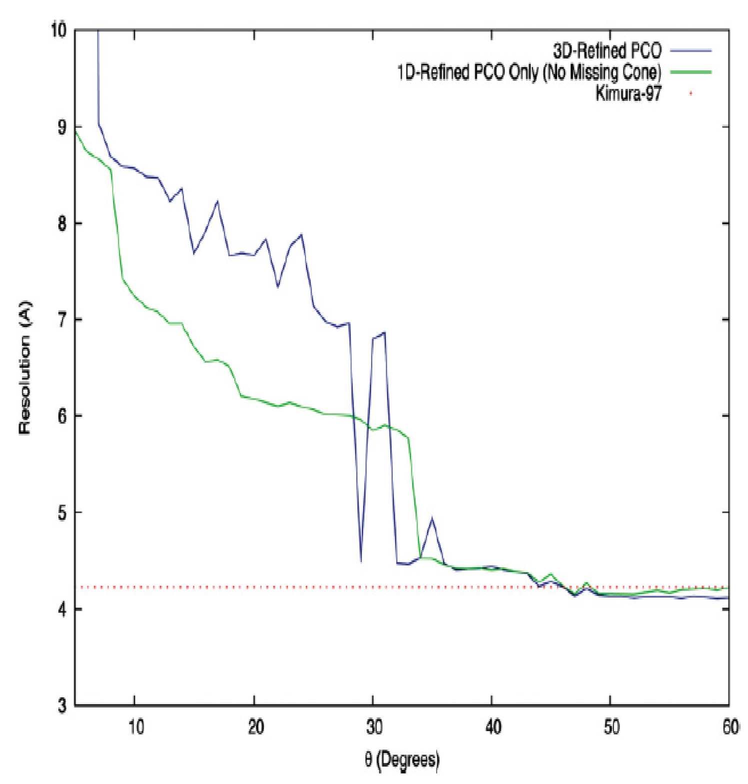
Right: The experimental 3D Fourier space contains the non-evenly sampled experimental measurements along the reciprocal lattice lines. The developed algorithm performed a one-dimensional PCO processing between the non-evenly sampled 3D Fourier space (right) and the Real/Fourier2 hybrid space (mid-right), operating on one lattice line at a time. The three-dimensional PCO performed a refinement between the real 3D space (left) and the evenly sampled Fourier space (mid-left). *All Illustration for PCO are taken from [1].*

Cylindrical Ring Correlation (CRC) comparison of tilt limited data



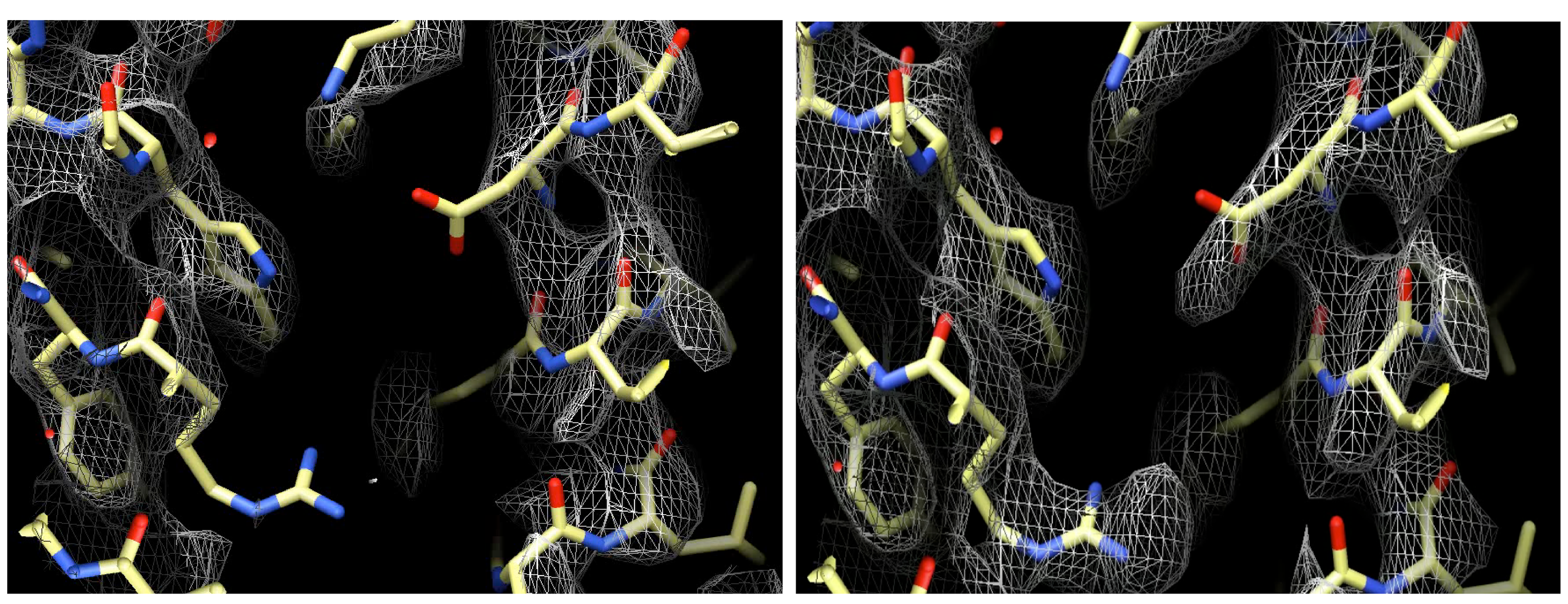
Cylindrical ring correlation plots (top) and normalized intensity profiles (bottom) and calculated relative to an atomic Bacteriorhodopsin crystallographic model (1BRR), without (left column) and with (right column) refinement by the here described PCO refinement algorithm. Plots are given as a function of ring of radius ρ (horizontal axis, in Å), and height z (in the vertical axis, in Å) in Fourier space.

Resolution of PCO as function of available tilt-range data



The average resolution is estimated by Fourier shell correlation (FSC, 0.5 threshold) with the 1BBR model. The original MRC reconstruction was based on data up to 60° specimen tilt, and showed a 4.4 Å res. The plot shows that a tilt limitation to only ±10°sample tilts still allows a 9 Å reconstruction. Inclusion of data up to only 35° tilts effectively allowed the full reconstruction of the structure up to a resolution of 4.5 Å . Inclusion of data beyond a 45° tilt range did not bring any further improvements.

Structural Comparison



An area from the transmembrane part of the Bacteriorhodopsin monomer close to the Schiffbase, shown before (left) and after (right) the iterative refinement by PCO. The grid surrounds the reconstructed volume. The atomic model of an XRD-determined structure of BR (PDB: 1BRR, Essen et al., 1998) is shown for comparison only. A first 100 rounds of PCO were applied, followed by 30 further rounds including edge detection and sharpening as additional masking constraints. After refinement with PCO, the wrong connections in the alpha-helical backbone of the electron density map are corrected, and side-chain densities were generated.

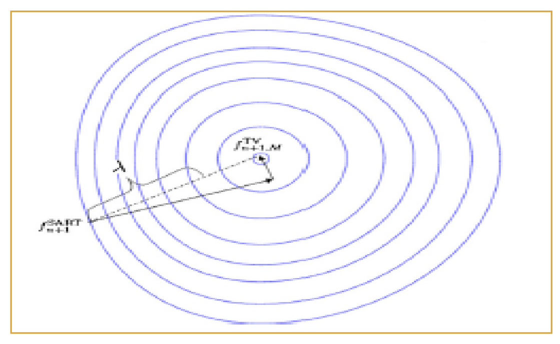
Constrained convex optimization from the compressed sensing view

The method seeks to minimize the total variation norm (TV-norm) of the image

$$\vec{f}^* = \arg\min \left\|\vec{f}\right\|_{TV} = \arg\min\left(\sum_{i,j,k}\sqrt{\left(f_{i,j,k}-f_{i-1,j,k}\right)^2+\left(f_{i,j,k}-f_{i,j-1,k}\right)^2+\left(f_{i,j,k}-f_{i,j,k-1}\right)^2}\right)$$

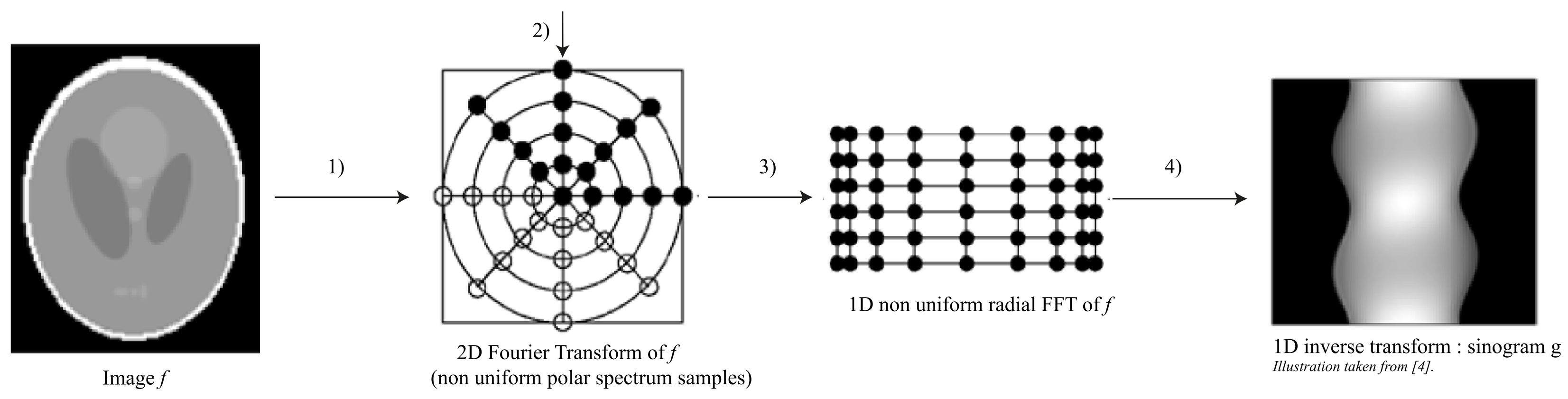
subject to the convex constraints in C

$$C:\begin{cases}\left|P\vec{f}-\vec{g}_{data}\right|\le\varepsilon_d & (1): \textit{reliability to measures}\\ \vec{f}\ge 0 & (2): \textit{positivity}\\ \vec{f}\left(x\in NullSup\right)=0 & (3): \textit{Zero Mask}\end{cases}$$



Adaptation of the Gradient step size. The new image iterate is a linear combination of the POCS optimisation and the TV minimization. The scalar λ is chosen so as to ensure a monotonic decrease of the data residual .
Illustration taken from [2].

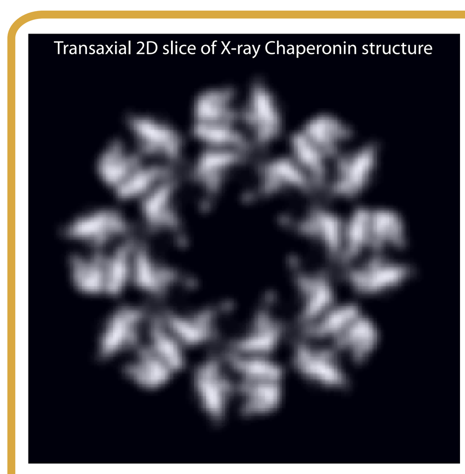
Acceleration of Projector/Backprojector in POCS with NUFFT

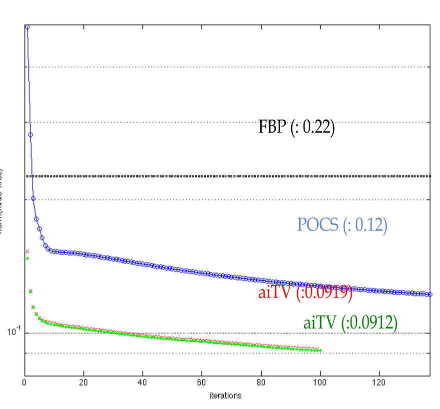


Basic steps of NUFFT forward projection in parellel-beam CT.

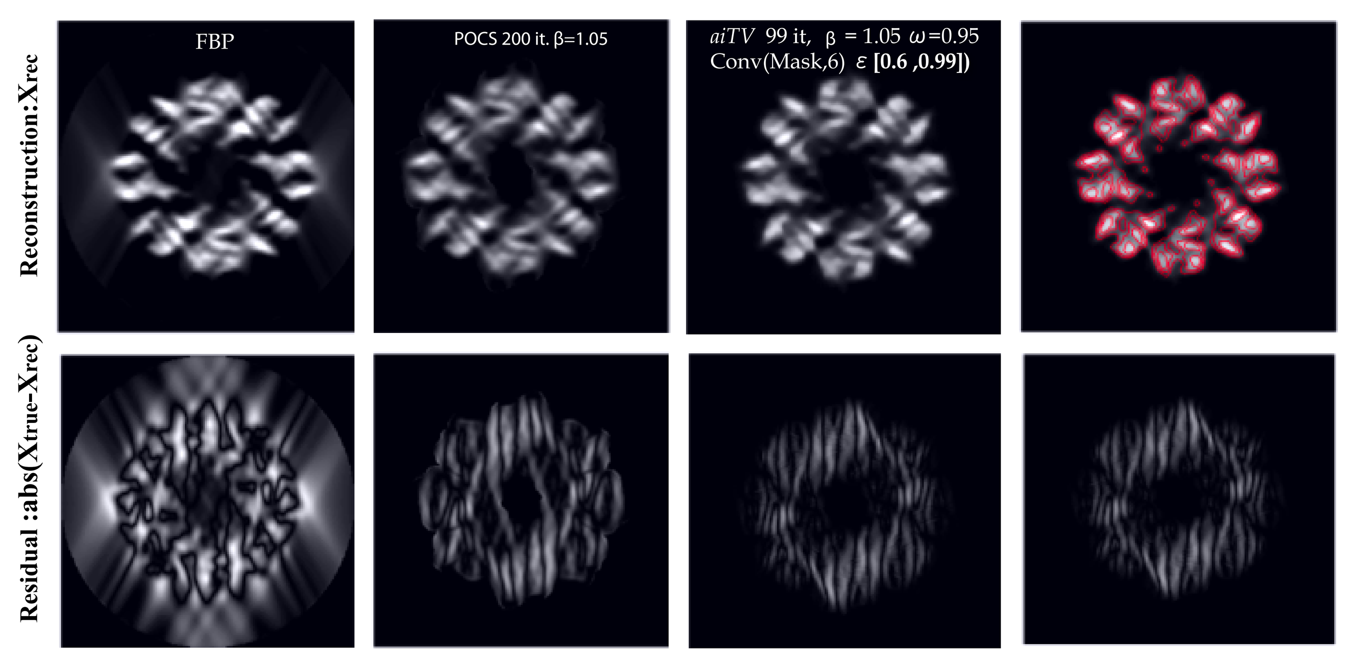
1) Two-dimensional NUFFT of image f to obtain polar spectrum samples.
(Fourier-slice theorem : θ-slice of $F_{2D}\,f = F_{1D}\,P_\theta f$, where P is the radon transform)
2) Multiply radially by the frequency response of the effective detector blur.
3) One-dimensional NUFFTs along radial direction r for each angle θ.
4) One-dimensional Inverse transform to get the sinogram (Projected image)

Comparison of reconstruction algorithm performance on a 2d-slice of an X-ray model structure of Chaperonin





iterations vs NMSE



60 noisy projections acquired on a 60-degree range

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[3] Y. Kumura *et al*, Nature (1997)
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