Distance-Dependent Blur Correction in 3D Electron and Soft X-ray Microscopy

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Minisymposium on Computational Methods for Three-Dimensional Microscopy Reconstruction

November 8, 2010
Outline

Motivation and some background

Nature of Blurring
Bluring and its effects in SXM
Correction using DGCBP

Our Results

3D version of FDR
Application of FDR to SXM model

FDR in practice - work in progress
Blurring: PSF

Point Spread Function (PSF)

The point spread function (PSF) describes the response of an imaging system to a point source or point object. The degree of spreading (blurring) of the point object is a measure of the quality of an imaging system. In electron microscopy an image obtained resembles a convolution of the ideal projection image with a point spread function.
Blurring
Blurring: Distance-dependent PSF
Blurring: CTF

Contrast Transfer Function

A contrast transfer function (CTF) is the Fourier transform of a point spread function. It alters the various frequencies that make up the signal:

- modulating the amplitude at some frequencies
- changing the sign at some frequencies
- completely eliminating some frequencies

As long as the contrast transfer function affects each layer of an object in the same way it does not cause a problem in reconstruction since the blurring and integration commute and one can deblur the projections and then reconstruct.
Blurring: CTF

\[
\int v(x_1, x_2, x_3) \ast h(x_1, x_2) \, dx_3
\]

\[
\int V(\xi_1, \xi_2, x_3) H(\xi_1, \xi_2) \, dx_3
\]

\[
H(\xi_1, \xi_2) \int V(\xi_1, \xi_2, x_3) \, dx_3
\]

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Blurring: Distance-dependent CTF

- CTF changes its shape with the distance from the electron source.
- Each object layer is blurred by a slightly different function.

The effects of distance dependence on CTF blurring become more significant as the desired resolution of reconstructions increases and when larger specimens are imaged.
\[ H (\xi_1, \xi_2, x_3) = H_{CTF} (\xi, x_3) E_s (\xi, x_3) E_t (\xi), \]

where

\[ H_{CTF} (\xi, x_3) = (1 - a) \sin(D(\xi, x_3)) - a \cos(D(\xi, x_3)), \]

\[ D (\xi, x_3) = 2\pi \lambda \xi^2 \left( -\frac{\Delta f (x_3)}{2} + \frac{\lambda^2 \xi^2 C_s}{4} \right), \]

\[ E_s (\xi, x_3) = e^{-\pi^2 q_0^2 (C_s \lambda^3 \xi^3 - \Delta f(x_3) \lambda \xi)^2}, \]

\[ E_t (\xi) = e^{-(\frac{1}{2} \pi F_s \lambda \xi^2)^2}, \]

and the parameters involved are:

- \( \xi \equiv \sqrt{\xi_1^2 + \xi_2^2} \) is a spatial frequency,
- \( a \) is a fraction of the amplitude contrast, \( 0 \leq a \leq 1 \),
- \( \lambda \) is the electron wavelength,
- \( C_s \) is the lens spherical aberration coefficient,
- \( \Delta f(x_3) \) is the value of the defocus,
- \( q_0 \) is a quantity of dimension 1/length specifying the size of the source as it appears in the back focal plane,
- \( F_s \) is the lens focal spread coefficient.

Note that the defocus \( \Delta f(x_3) \) depends explicitly on the distance from the electron source.
Model of CTF

\[ H(\xi_1, \xi_2, x_3) = H_{CTF}(\xi, x_3) E_s(\xi, x_3) E_t(\xi), \]

where

\[ H_{CTF}(\xi, x_3) = (1 - a) \sin(D(\xi, x_3)) - a \cos(D(\xi, x_3)), \]

\[ D(\xi, x_3) = 2\pi \lambda \xi^2 \left( -\frac{\Delta f(x_3)}{2} + \frac{\lambda^2 \xi^2 C_s}{4} \right), \]

\[ E_s(\xi, x_3) = e^{-\pi^2 q_0^2 (C_s \lambda^3 \xi^3 - \Delta f(x_3) \lambda \xi)^2}, \]

\[ E_t(\xi) = e^{-\left(\frac{1}{2} \pi F_s \lambda \xi^2\right)^2}, \]

- \( a = 0, \)
- \( \lambda = 0.033487 \text{ Å}, \)
- \( C_s = 22,000,000 \text{ Å}, \)
- \( \Delta f \in [1000, 3000] \text{ (in Å)}, \)
- \( q_0 = 0.00746558 \text{ Å}^{-1} \)
- \( F_s = 141.35 \text{ Å}. \)
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Blurring: Soft X-ray point spread function

Plots of the normalized soft X-ray point spread function near the geometric focal point of a zone plate objective for defocus 0, 5 and 10 microns. Inserted on the right are the corresponding images acquired by the X-ray microscope at the BESSY II electron storage ring (Berlin, Germany). [Figure courtesy of Roberto Marabini]
Blurring: Soft X-ray microscopy

(a) (b) (c) (d) (e) (f) (g) (h)

Phantom: an astrocyte of dimensions 20×15 microns in x-y and 8 microns in depth

Reconstruction: by a 3D-EM algorithm from SXM data.
(a) a central section of the cell (b) reconstruction by a standard EM algorithm from 131 simulated SXM images taken around a horizontal tilt axis using a 560-zones zone plate (c), (d) and (e) present results when that slice was displaced 1, 2, and 3 microns from the focus, respectively (using 560-zones zone plate) (f), (g) and (h) present results when that slice was displaced 1, 2, and 3 microns from the focus, respectively (using 900-zones zone plate)
Blurring: Soft X-ray microscopy

First Considerations on the Reconstruction of X-ray Tomography
Joaquín Oton, C.O.S. Sorzano, Jose M. Carazo, and Roberto Marabini
SCANDEEM 2010
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Example: reconstruction using DGCBP
Cross sections of (a) phantom, (b) reconstruction with correction for the distance-dependent contrast transfer function, (c) reconstruction with correction appropriate for the central layer of the specimen, and (d) reconstruction with no correction for the contrast transfer function.
Noise in projection data

We simulated **structural noise**, **shot noise** and **digitization noise** based on the guidance of Baxter *et al.*\(^1\).

**structural noise**

- originates from ice and often carbon film surrounding the molecule during imaging
- different for each molecule and is subject to distance-dependent blurring
- simulated by adding to each voxel value in the rotated molecule a random sample from a zero-mean Gaussian distribution with standard deviation \(\sigma_1\)

**shot noise** and **digitization noise**

- occure during image recording and digitization
- simulated by adding to each of the pixel values in the projection images a random sample from a zero-mean Gaussian distribution with standard deviation \(\sigma_2\)

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A single projection of the phantom: (a) ideal projection with no CTF blurring, (b) distance-dependently blurred projection, (c) distance-dependently blurred projection with added noise using $\sigma_1 = 0.3052$ and $\sigma_2 = 2.99$, (d) distance-dependently blurred projection with added noise using $\sigma_1 = 0.6103$ and $\sigma_2 = 6$. 
Reconstruction from noisy projections

Three different cross-sections of the phantom (first column), of the reconstructions from noisy projection data generated using $\sigma_1 = 0.3052$ and $\sigma_2 = 2.99$ obtained by DD backprojection (second column) and by CL backprojection (third column) and of the reconstructions from noisy projection data generated using $\sigma_1 = 0.6103$ and $\sigma_2 = 6$ obtained by DD backprojection (fourth column) and by CL backprojection (fifth column).
Surface renderings of two reconstructions from the noisy projection data generated using $\sigma_1 = 0.6103$ and $\sigma_2 = 6$. (a) and (b) are rendered for voxel values thresholded at 0.5; (c) and (d) are rendered for voxel value thresholded at 0.9. (a) and (c) were obtained using DD backprojection; (b) and (d) were obtained using CL backprojection.
The parameters of the CTF need to be estimated from the projection images before correction and reconstruction can be performed.

- simulate projections in which the defocus parameters were not the same as the ones used in reconstruction
- the defocus varies from 1000 Å to 3000 Å according to the function
  \[ \Delta f(x_3) = m(x_3 - 1/2) + b, \]
  where \( m = 2000/n \), \( b = 1000 \), and \( n = 128 \) is the number of discrete layers into which the molecule is subdivided
- introduce a random variation by adding to \( m \) a sample from a zero-mean Gaussian distribution with standard deviation \( \sigma_3 \) and to \( b \) a sample from a zero-mean Gaussian distribution with standard deviation \( \sigma_4 \)
Three different cross-sections of the phantom (first column), of the reconstructions from projection data with incorrectly determined defocus parameters using $\sigma_3 = 1$ and $\sigma_4 = 50$ obtained by DD backprojection (second column) and by CL backprojection (third column) and of the reconstructions from projection data with incorrectly determined defocus parameters using $\sigma_3 = 5$ and $\sigma_4 = 100$ obtained by DD backprojection (fourth column) and by CL backprojection (fifth column).
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Related Work: FDR


Frequency Distance Relation (FDR)

The values of the frequencies of each coefficient in the 3D Fourier transform of the projection data obtained from around a single axis rotation are directly related to the distance from the electron source at which the points in the specimen contribute to the coefficient.

This implies that the collected data can be corrected to approximate true projections.
EM Operators

- **Ideal:**
  - $\mathcal{R}$ rotation
    \[
    [\mathcal{R}v](\theta, \phi, x_1, x_2, x_3) = v\left(x_1^F(\theta, \phi), x_2^F(\theta, \phi), x_3^F(\phi)\right)
    \]
  - $\mathcal{C}$ compression
    \[
    [\mathcal{C}w](\theta, \phi, x_1, x_2) = \int_{\mathbb{R}} w(\theta, \phi, x_1, x_2, x_3) \, dx_3,
    \]
- $\mathcal{P} = \mathcal{CR}$ projection
- **Distance-dependent:**
  - $\mathcal{R}$ rotation
  - $\mathcal{C}_h$ compression
    \[
    C_h = C[w * h]
    \]
  - $\mathcal{P}_h = C_h\mathcal{R}$ projection
We image a molecule that is an impulse located at \((\hat{x}_1, \hat{x}_2, \hat{x}_3)^T:\)

\[\kappa(x_1, x_2, x_3) = \delta(\hat{x}_1 - x_1) \delta(\hat{x}_2 - x_2) \delta(\hat{x}_3 - x_3).\]
We image a molecule that is an impulse located at \((\hat{x}_1, \hat{x}_2, \hat{x}_3)^T\):

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\]

The blur-free projection data \(\mathcal{P}_\kappa\) of our molecule \(\kappa\) is

\[
[\mathcal{P}_\kappa](\theta, \phi, x_1, x_2) = \delta(\hat{x}_1^B(\theta, \phi) - x_1) \delta(\hat{x}_2^B(\theta) - x_2).
\]
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\[
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\]

The blur-free projection data \(P_\kappa\) of our molecule \(\kappa\) is

\[
[P_\kappa] (\theta, \phi, x_1, x_2) = \delta (\hat{x}_B^1 (\theta, \phi) - x_1) \delta (\hat{x}_B^2 (\theta) - x_2).
\]

The distance-dependently blurred projection data \(P_{h\kappa}\) of \(\kappa\) is

\[
[P_{h\kappa}] (\theta, \phi, x_1, x_2) = h (x_1 - \hat{x}_B^1 (\theta, \phi), x_2 - \hat{x}_B^2 (\theta), \hat{x}_B^3 (\theta, \phi)).
\]
The 4D Fourier transform of $\mathcal{P}_\kappa$ is

$$
[\mathcal{F}_4 \mathcal{P}_\kappa](\Theta, \Phi, \xi_1, \xi_2) = \frac{1}{(2\pi)^2} \int_0^{2\pi} \int_0^\pi e^{-i(\hat{\chi}_1^B(\theta, \phi)\xi_1 + \phi \phi)} d\phi \ e^{-i(\hat{\chi}_2^B(\theta)\xi_2 + \theta \Theta)} d\theta.
$$

The 4D Fourier transform of $\mathcal{P}_{h\kappa}$ is

$$
[\mathcal{F}_4 \mathcal{P}_{h\kappa}](\Theta, \Phi, \xi_1, \xi_2) = \frac{1}{(2\pi)^2} \int_0^{2\pi} \int_0^\pi H(\xi_1, \xi_2, \hat{\chi}_3^B(\theta, \phi)) e^{-i(\hat{\chi}_1^B(\theta, \phi)\xi_1 + \phi \phi)} d\phi \ e^{-i(\hat{\chi}_2^B(\theta)\xi_2 + \theta \Theta)} d\theta.
$$
Stationary phase theorem

The method of *stationary phase* is used for the evaluation of highly oscillatory integrals of the form

$$I(\xi) = \int_{c_1}^{c_2} G(\sigma) e^{i\xi F(\sigma)} d\sigma.$$ 

If

- $G$ is a smooth function,
- $F$ is twice differentiable,
- and all stationary points of $F$ are non-degenerate,

then, as $\xi \rightarrow \infty$, the above integral can be well approximated by a sum over the stationary points of $F$. 

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Blur-Correction in Microscopy
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It has been shown in many practical applications that the sum approximates the original integral very well even for small values of \( \xi \).
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It has been shown in many practical applications that the sum approximates the original integral very well even for small values of \( \xi \).

In our work, the actual summation formula is not relevant. We simply make use of the fact that such an approximation is possible.
The 4D Fourier transform of $\mathcal{P}_\kappa$ is

$$\mathcal{F}_4 \mathcal{P}_\kappa (\Theta, \Phi, \xi_1, \xi_2) = \frac{1}{(2\pi)^2} \int_0^{2\pi} \int_0^\pi e^{-i(\hat{\chi}_1^B(\theta,\phi)\xi_1 + \phi\Phi)} d\phi \ e^{-i(\hat{\chi}_2^B(\theta)\xi_2 + \theta\Theta)} d\theta.$$  

The 4D Fourier transform of $\mathcal{P}_{h\kappa}$ is

$$\mathcal{F}_4 \mathcal{P}_{h\kappa} (\Theta, \Phi, \xi_1, \xi_2) = \frac{1}{(2\pi)^2} \int_0^{2\pi} \int_0^\pi H(\xi_1, \xi_2, \hat{\chi}_3^B(\theta,\phi)) e^{-i(\hat{\chi}_1^B(\theta,\phi)\xi_1 + \phi\Phi)} d\phi \ e^{-i(\hat{\chi}_2^B(\theta)\xi_2 + \theta\Theta)} d\theta.$$  

At stationary points we have

$$\hat{\chi}_3^B (\theta, \tilde{\phi}) = -\frac{\Phi}{\xi_1}.$$
Theorem

The 4D Fourier transform of the distance-dependently blurred projection data is related to the 4D Fourier transform of blur-free projection data:

\[
\mathcal{F}_4 \mathcal{P}_{h\kappa} (\Theta, \Phi, \xi_1, \xi_2) \approx H \left( \xi_1, \xi_2, -\frac{\Phi}{\xi_1} \right) \mathcal{F}_4 \mathcal{P}_\kappa (\Theta, \Phi, \xi_1, \xi_2).
\]
The Fourier coefficients for the corrected projection data of \( \nu \) can be obtained by a careful division, so as not to amplify the noise in the data, of the Fourier coefficients of the distance-dependently blurred projection data \( P_h \nu \) by the CTF \( H \); i.e., as

\[
\left[ F_4 P_\kappa \right] (\Theta, \Phi, \xi_1, \xi_2) \\
\approx \left[ F_4 P_h \nu \right] (\Theta, \Phi, \xi_1, \xi_2) / H \left( \xi_1, \xi_2, -\frac{\Phi}{\xi_1} \right).
\]
\[
[F_4 P_k] (\Theta, \Phi, \xi_1, \xi_2) \\
\approx [F_4 P_h v] (\Theta, \Phi, \xi_1, \xi_2) / H \left( \xi_1, \xi_2, -\frac{\Phi}{\xi_1} \right).
\]

This correction gives us approximations on the true projection data through the molecule.

The molecule can be reconstructed, based on such corrected projection data, using ANY reconstruction algorithm.
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FDR in practice - work in progress
\( \mathbf{v} \) denotes the function of three variables that we wish to recover from the projection images.

The physical interpretation of \( \mathbf{v} \) is that \( \mathbf{v}(x_1, x_2, x_3) \) is the value of an attenuation coefficient at the point \((x_1, x_2, x_3)\), which in turn depends on the biological material occupying that point of space.

\( h \) is a function of three variables to describe the distance-dependent PSF of the soft X-ray microscope.

\( \mathcal{X}_h \mathbf{v} \) the projection data of \( \mathbf{v} \) obtained by a soft X-ray microscope whose PSF is described by \( h \).
The relation of $\mathcal{X}_h \nu$ to $\nu$ is much more complex than the relation of $\mathcal{P}_h \nu$ to $\nu$ (projections in EM).

To specify $\mathcal{X}_h \nu$ we introduce another function $u$ of three variables and a function $p$ of two variables:

- $u$ - the intuitive physical idea is that the rotated version $[\mathcal{R}u] (\theta, \phi, x_1, x_2, x_3)$ of $u$ is essentially the intensity in the soft X-ray microscope when imaging $[\mathcal{R}\nu] (\theta, \phi, x_1, x_2, x_3)$;

- $p$ - can be obtained by a calibration of the soft X-ray microscope with no biological object present in the beam
Our physical model of image formation in soft X-ray microscopy, leads us to:

\[
\begin{align*}
\mathcal{R}_v (\theta, \phi, x_1, x_2, x_3) e^{-x_3} & = \int_{-\infty}^{x_3} \mathcal{R}_v (\theta, \phi, x_1, x_2, x'_3) dx'_3 \\
\mathcal{R}_v (\theta, \phi, x_1, x_2, x_3) & = \mathcal{R}_u (\theta, \phi, x_1, x_2, x_3) p(x_1, x_2).
\end{align*}
\]

The measured X-ray microscopy projection data $\mathcal{X}_h$ has the following relationship to the distance-dependently blurred projections of $u$:

\[
\mathcal{X}_h \, v = -\mathcal{P}_h u.
\]

Since we have a method to recover $u$ from $\mathcal{P}_h u$, we can obtain $u$ from the soft X-ray microscopy data.
Inversion of the Soft X-ray Transform

\[
\begin{align*}
\mathcal{R} v (\theta, \phi, x_1, x_2, x_3) e^{-\int_{-\infty}^{x_3} \mathcal{R} v (\theta, \phi, x_1, x_2, x_3') \, dx_3'} & = \frac{\mathcal{R} u (\theta, \phi, x_1, x_2, x_3)}{p(x_1, x_2)}.
\end{align*}
\]

Noting that the left-hand side of the above equation is the negative of the derivative of its second term, we obtain by integrating with respect to \(x_3\) that

\[
\begin{align*}
1 - e^{\int_{\mathbb{R}} \mathcal{R} v (\theta, \phi, x_1, x_2, x_3') \, dx_3'} & = \int_{\mathbb{R}} \mathcal{R} u (\theta, \phi, x_1, x_2, x_3') \, dx_3' \\
\int_{\mathbb{R}} p(x_1, x_2) & .
\end{align*}
\]

Rearranging, taking logarithms and applying operator \(\mathcal{P}\) we get

\[
[\mathcal{P} v] (\theta, \phi, x_1, x_2) = -\ln \left( 1 - \frac{[\mathcal{P} u] (\theta, \phi, x_1, x_2)}{p(x_1, x_2)} \right).
\]

The right-hand side of the above is already known (because we know how to recover \(u\) from \(X_h v\) and \(p\) is known) and \(v\) can be recovered by any classical method for inverting the projection transform.
Cooley-Tukey algorithm ( = FFT ) requires **uniform sampling**. In an experimental setup it is impossible to get samples with uniformly distributed projection directions.
Sampling in $\theta, \phi$ space
Sampling in $\theta, \phi$ space

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Blur-Correction in Microscopy
Karsten Fourmont [2003, Journal of Fourier Analysis] discusses two variations of non-uniform Fourier transform:

- NER (non equispaced result)
- NED (non equispaced data)

\[
\hat{z}_k = \sum_{l=1}^{M} z_l e^{-2\pi i x_l k/N}, \quad k = -N/2, \ldots, N/2 - 1.
\]

For \( x_l = l \) and \( M = N \) this represents the usual equispaced discrete Fourier transform of length \( N \).
Non-uniform fast Fourier transform

Given non-uniformly distributed samples, perform fast Fourier transform.
Given non-uniformly distributed samples, perform fast Fourier transform.

- Based on available samples, compute the values of the function at uniformly distributed points.
- Perform FFT.
Non-uniform fast Fourier transform

Given non-uniformly distributed samples, perform fast Fourier transform.

- Based on available samples, compute the values of the function at uniformly distributed points.
- Perform FFT.

Problem: Simple interpolation techniques do not give good results.
In gridding methods, the interpolated uniform sampling is obtained by convolution of the known non-uniform samples with a window function, for example:

- Gaussian
- Kaiser-Bessel

whose values are small outside of some interval.

Once FFT is taken, the data has to be deconvoloved.
Future work

• Implementation of the 4D Fourier transform.
• Combination of data collected with different defocus to recover frequencies that are set to zero.
• Development of a weighting scheme that smoothly transitions between different methods of correction for high and low frequencies (since, in theory, stationary phase approximation is not valid for low frequencies).
• Testing on more sophisticated phantoms using more microscopy-like conditions (noise in the projection data, inaccuracies in determination of CTF parameters, etc.).
Related Work

- D.J. DeRosier, *Correction of high-resolution data for curvature of the Ewald sphere*, Ultramicroscopy (2000)
- J.N. Dubowy, G.T. Herman, *An approach to the correction of distance-dependent defocus in electron microscopic reconstruction*, ICIP 2005
- J.Klukowska, G.T.Herman, I.G.Kazantsev, *Correction of Distance-Dependent Blurring in Projection Data for Fully Three-Dimensional Electron Microscopic Reconstruction*. ISBI 2010
Questions?