Denoising and Covariance Estimation of Cryo-EM Images

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Joint work with Teng Zhang (UCF) and Amit Singer (Princeton)

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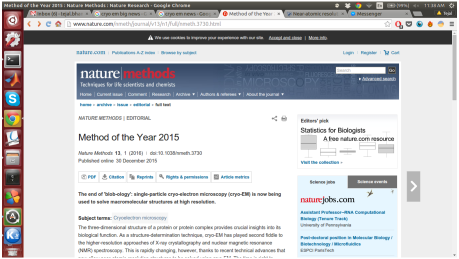
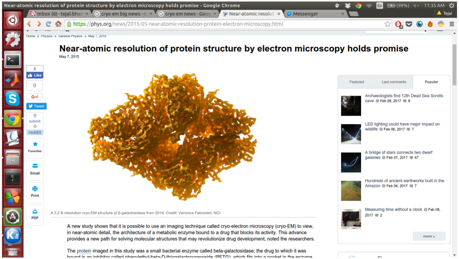
Why cryo-EM?

- 3D structure of macromolecules - understand function - drug discovery for cancer, AIDS, HIV, etc.
- X ray crystallography: many proteins, viruses resistant to crystallization (HIV virus)
- Cryo-EM: native state
- Can study structures impossible before!

Nature Method of the Year 2015



Cancer


Near-atomic resolution of protein structure by electron microscopy holds promise - Google Chrome
https://phys.org/news/2015-05-near-atomic-resolution-protein-electron-microscopy.html
Home > Physics > General Physics > May 7, 2015
Near-atomic resolution of protein structure by electron microscopy holds promise
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A 3.2 Å resolution cryo-EM structure of β-galactosidase from 2014. Credit: Veronica Falconieri, NCI
A new study shows that it is possible to use an imaging technique called cryo-electron microscopy (cryo-EM) to view, in near-atomic detail, the architecture of a metabolic enzyme bound to a drug that blocks its activity. This advance provides a new path for solving molecular structures that may revolutionize drug development, noted the researchers.
The protein imaged in this study was a small bacterial enzyme called beta-galactosidase; the drug to which it was
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Zika


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These authors contributed equally to this work.
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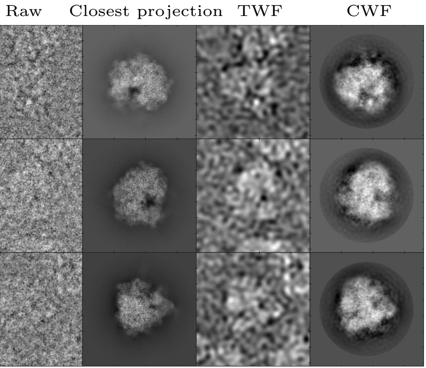
3D from 2D

- Flash freeze sample in an ice layer
- Image with an electron microscope
- 2D images at unkown orientations -¿ 3D structure
- Sounds like Structure from Motion (SfM)?

Sounds like SfM?

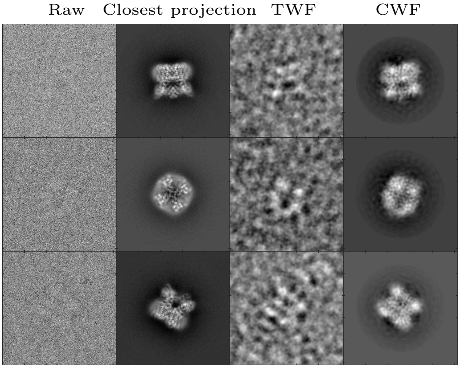
- Can't detect features from raw images, unlike SfM
- Need new algorithms to handle noise level!

Experimental data - 80S ribosome



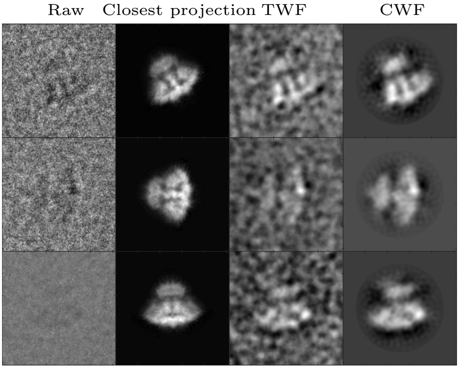
- FALCON II 4k×4k direct electron detector
- 105247 motion corrected, picked particle images of 360×360 pixels

Experimental data - TRPV1



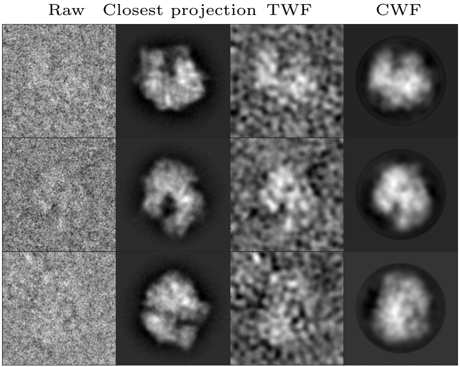
- K2 direct electron detector
- 35645 motion corrected, picked particle images of 256×256 pixels

Experimental data -IP_3R1



- Gatan 4k×4k CCD
- 37382 picked particle images of 256×256 pixels

Experimental data - 70S ribosome



- TVIPS TEMCAM-F415 (4k x 4k) CCD
- 216517 picked particle images of 250×250 pixels

Cryo-EM Pipeline

- Particle picking from micrographs
- **Preprocessing**
- **2D classification (Class averaging): inspect underlying particles, estimate viewing angles**
- 3D classification
- 3D refinement

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Motivation

- Visualization of underlying particles without class averaging
- Image restoration (CTF correction and denoising) in a single step
- Outlier detection

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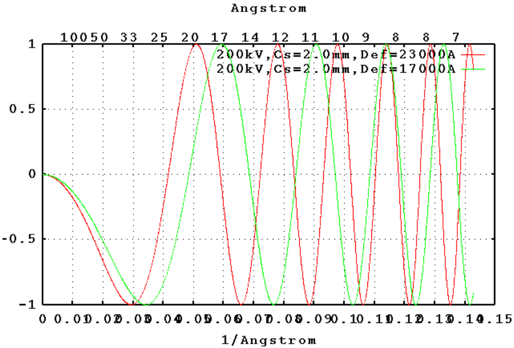
CTF Correction

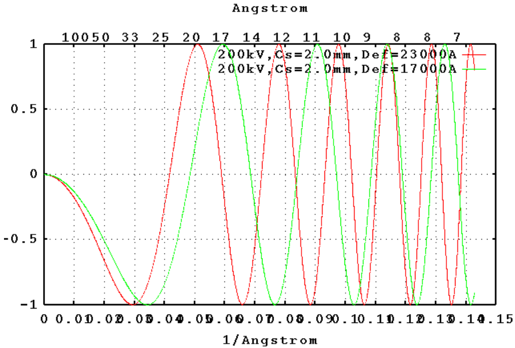


[1]

- CTF suppresses information and inverts contrast
- Challenge: CTF cannot be trivially inverted (zero crossings)
- Information lost from one defocus group could be recovered from another group that has different zero crossings.

[1] http://www.bio.brandeis.edu/~shaikh/lab/ctf.htm

CTF Correction



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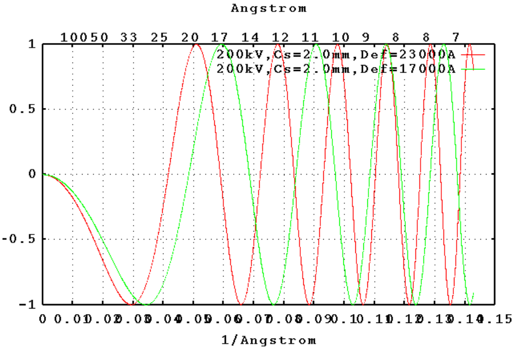

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CTF Correction

- Do nothing (Denial)
- Correct Fourier phases but not amplitudes
- Correct both Fourier phases and amplitudes

Current Image Restoration Techniques

- **Phase flipping + steerable PCA (sPCA)**:
  - Flip sign of the Fourier coefficients at frequencies for which the CTF is negative
  - Preserves noise statistics
  - Data adaptive basis: eigenvectors of the sample covariance matrix
  - Phase flipping corrects only phases
- **Traditional Wiener Filtering (TWF)**:
  - Corrects both phases and amplitudes
  - Requires prior estimation of the spectral signal to noise ratio (SSNR)
  - Cannot restore information at zero crossings of the CTF
  - Not in a data adaptive basis (restricted to Fourier basis)

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Covariance Wiener Filtering (CWF)

- Estimate the CTF-corrected covariance matrix of the underlying clean 2D projection images
- Wiener filtering to solve the image restoration deconvolution problem
- No averaging, act on each image separately
- CTF correction and denoising in a single step

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Covariance Wiener Filtering (CWF)

Table : Comparison of CTF Correction/Denoising Methods

Property	Phaseflip + sPCA	TWF	CWF
Applicable at preliminary stage	✓	✓	✓
Data dependent basis	✓	✗	✓
Correct both phases and amplitudes	✗	✓	✓
CTF corrected covariance estimate	✗	✗	✓

Fourier-Bessel Steerable Basis

- The population covariance matrix Σ must be invariant under in-plane rotation of the projection images
- Block diagonal in any steerable basis in which the basis elements are outer products of radial functions and angular Fourier modes [2]
- CTF and the whitening filter are also block diagonal in the Fourier Bessel basis (radial isotropy)
- Suffices to estimate each diagonal block of Σ, corresponding to the angular frequency k, separately
- Nearly unitary transformation

[2] Fast Steerable Principal Component Analysis, Z. Zhao and Y. Shkolnisky and A. Singer, IEEE Transactions on Computational Imaging

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The Model: Real space

Linear, weak phase approximation

$$y_i = a_i * x_i + \epsilon_i, \quad i = 1, 2, \ldots, n \tag{1}$$

n: number of images
$*$: convolution operation
y_i: noisy, CTF filtered i'th image in real space
x_i: underlying clean projection image in real space
a_i: the point spread function of the microscope
ϵ_i: additive Gaussian noise that corrupts the image

The Model: Fourier space

$$Y_i = A_i X_i + \xi_i, \quad i = 1, 2, \ldots, n \tag{2}$$

A_i: diagonal operator, whose diagonal consists of the Fourier transform of the point spread function

$X_1, \ldots, X_n$: vectors in $\mathbb{C}^p$, where p is the number of pixels
i.i.d. samples from a distribution with mean $\mathbb{E}[\mathbf{X}] = \mu$ and covariance $\mathbb{E}[(\mathbf{X} - \mu)(\mathbf{X} - \mu)^T] = \Sigma$

"All models are wrong but some are useful" - George Box

The Model

$$\mathbb{E}[\mathbf{Y}_i] = A_i \mathbb{E}[\mathbf{X}_i], \quad i = 1, 2, \ldots, n. \tag{3}$$

$$\begin{aligned} \mathbb{E}[(\mathbf{Y}_i - \mathbb{E}[\mathbf{Y}_i])(\mathbf{Y}_i - \mathbb{E}[\mathbf{Y}_i])^T] &= \mathbb{E}[A_i(\mathbf{X}_i - \mu)(\mathbf{X}_i - \mu)^T A_i^T] + \sigma^2 I \\ &= A_i \Sigma A_i^T + \sigma^2 I. \end{aligned} \tag{4}$$

Relates the second order statistics of the noisy images with the population covariance Σ of the clean images

Mean Estimation

$$\hat{\mu} = \arg\min_{\mu} \sum_{i=1}^{n} ||(Y_i - A_i\mu)||_2^2 + \lambda||\mu||_2^2 \tag{5}$$

$$\hat{\mu} = (\sum_{i=1}^{n} A_i^T A_i + \lambda I)^{-1}(\sum_{i=1}^{n} A_i^T Y_i). \tag{6}$$

Covariance Estimation

$$
\begin{aligned}
\hat{\Sigma} &= \arg\min_{\Sigma} \sum_{i=1}^{n} ||(Y_i - \mathbb{E}[\mathbf{Y}_i])(Y_i - \mathbb{E}[\mathbf{Y}_i])^T - (A_i \Sigma A_i^T + \sigma^2 I)||_F^2 \\
&= \arg\min_{\Sigma} \sum_{i=1}^{n} ||A_i \Sigma A_i^T + \sigma^2 I - C_i||_F^2
\end{aligned} \tag{7}
$$

where $C_i = (Y_i - A_i\mu)(Y_i - A_i\mu)^T$ and $||.||_F$ is the Frobenius matrix norm.

Solving using Conjugate Gradient

System of linear equations for the elements of the matrix $\hat{\Sigma}$

$$\sum_{i=1}^{n} A_i^T A_i \hat{\Sigma} A_i^T A_i = \sum_{i=1}^{n} A_i^T C_i A_i - \sum_{i=1}^{n} \sigma^2 A_i^T A_i \tag{8}$$

$$L(\hat{\Sigma}) = B \tag{9}$$

where $L : \mathbb{R}^{p\times p} \to \mathbb{R}^{p\times p}$ is the linear operator acting on $\hat{\Sigma}$ defined by the left hand side of eqn. 8, and B is the right hand side.

- Direct inversion of this linear system is slow for large image sizes
- Applying L only involves matrix multiplications: fast!
- Conjugate gradient

Eigenvalue Thresholding

- $L(\hat{\Sigma})$ is a PSD matrix whenever $\hat{\Sigma}$ is PSD (as a sum of PSD matrices)
- B may not necessarily be PSD due to finite sample fluctuations (n is finite)
- Project B onto the cone of PSD matrices
- Compute the spectral decomposition of B and set all negative eigenvalues to 0 (eigenvalue thresholding)
- $n \gg p$

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Eigenvalue Shrinkage: Spiked Covariance Model

Analyze B when $X_i = 0$ for all i (input images are white noise images)

- $$M = \sum_{i=1}^{n} A_i^T C_i A_i = \sum_{i=1}^{n} A_i^T Y_i Y_i^T A_i. \tag{10}$$
- $\mathbb{E}[M] = \sigma^2 \sum_{i=1}^{n} A_i^T A_i$ and $B = M - \mathbb{E}[M]$
- $S = (\mathbb{E}[M])^{1/2}$, i.e. S is PSD and $\mathbb{E}[M] = S^2$
- $$S^{-1} L(\hat{\Sigma}) S^{-1} = S^{-1}(M - \mathbb{E}[M]) S^{-1} = S^{-1} M S^{-1} - I. \tag{11}$$

Eigenvalue Shrinkage: Spiked Covariance Model

- $S^{-1}MS^{-1}$ can be viewed as a sample covariance matrix of n vectors in $\mathbb{R}^p$ whose population covariance is the identity matrix
- Eigenvalues corresponding to the signal can only be detected if they reside outside of the support of the Marčenko Pastur (MP) distribution
- Kritchman Nadler (KN) rank estimation to determine the number of eigenvalues corresponding to the signal [3]
- Apply operator norm eigenvalue shrinkage procedure (Donoho et al.) to those eigenvalues, while setting all other eigenvalues to 0 [4]

[3] Determining the number of components in a factor model from limited noisy data, Shira Kritchman and Boaz Nadler, Chemometrics and Intelligent Laboratory Systems

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Wiener Filtering

- White noise: estimate X_i as

$$\hat{X}_i = (I - H_i A_i)\hat{\mu} + H_i Y_i \tag{12}$$

where $H_i = \hat{\Sigma} A_i^T (A_i \hat{\Sigma} A_i^T + \sigma^2 I)^{-1}$ is the linear Wiener filter

- Colored noise: estimate X_i as

$$\hat{X}_i = (I - H_i W A_i)\hat{\mu} + H_i Y_i \tag{13}$$

with $H_i = \hat{\Sigma} A_i^T W^T (W A_i \hat{\Sigma} A_i^T W^T + \sigma^2 I)^{-1}$

Computational Complexity

$O(TDL^4 + nL^3)$, where T is the number of conjugate gradient iterations

- D defocus groups with d_i images in group i
- Images of size $L \times L$
- n images

Computational Complexity: Timings

n images of size $L \times L$

UNIX environment with 60 cores, running at 2.3 GHz, with total RAM of 1.5TB

Table : Timing in seconds

Dataset	L	n	Basis coeffs	CWF
TRPV1	256	35645	312s	574s
80s	360	30000	731s	385s
IP3R1	256	37382	429s	589s
70s	250	99979	1174s	113s

Relative error of estimated clean images

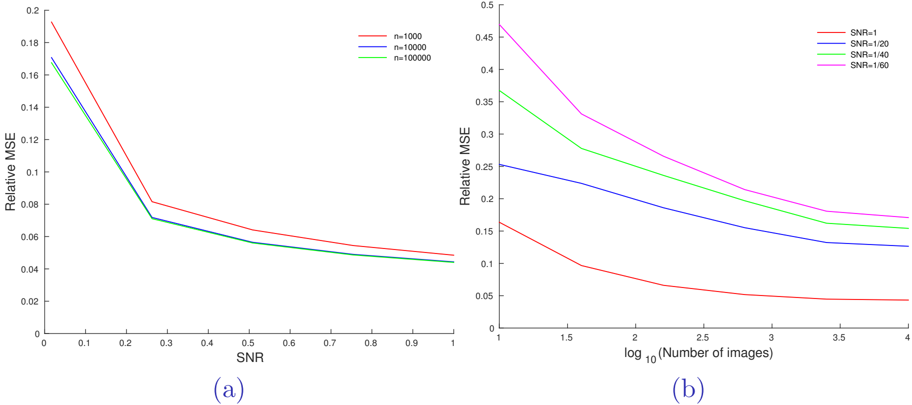


Figure : (a) Fixed number of images (b) Fixed SNR

Relative error of estimated covariance

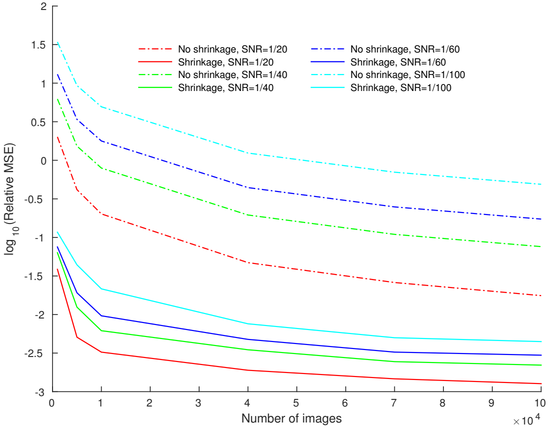
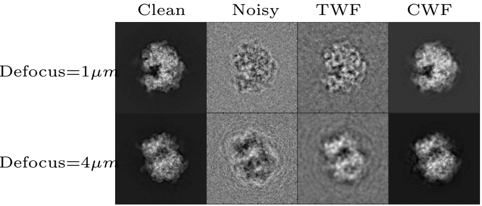
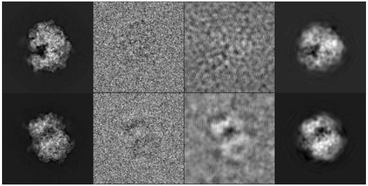


Figure : The estimator $\hat{\Sigma}$ can be shown to be consistent in the large sample limit $n \to \infty$

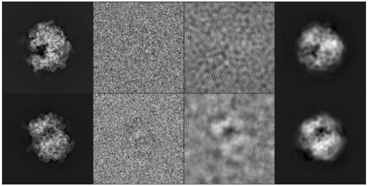
Simulations with white noise: 80S ribosome (EMDB-6454)



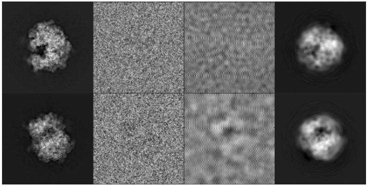
(a) SNR=1



(b) SNR=1/20

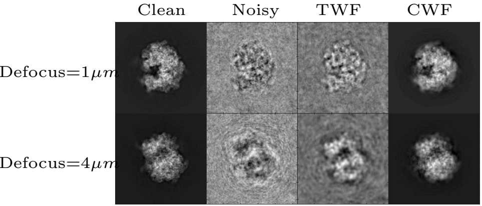


(c) SNR=1/40

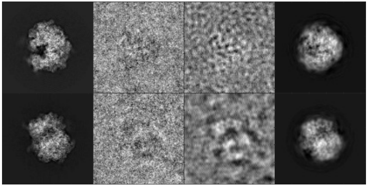


(d) SNR=1/60

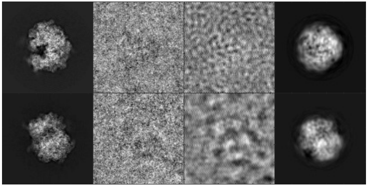
Simulations with colored noise: 80S ribosome (EMDB-6454)



(e) SNR=1



(f) SNR=1/10



(g) SNR=1/20

Outlier Detection

- Significant amount of time is spent on discarding outliers by visual inspection after the particle picking
- CWF: automatic way to classify picked particles
- Specimen particles at various depths in the ice layer: acquired projection images can have different contrasts
- $$Y_i = \alpha_i A_i X_i + \xi_i, \quad i = 1, 2, \ldots, n \tag{14}$$
- Absorb α into $\mathbf{X}$ and estimate $\alpha_i X_i$
- Outlier images typically have low contrast after denoising: linear classifier after CWF

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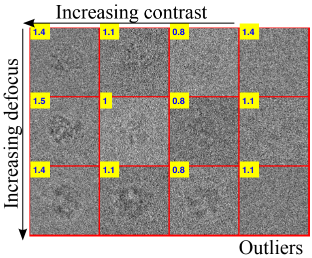
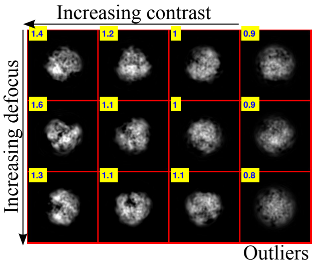
Outlier Detection: 80S ribosome (EMDB-6454)

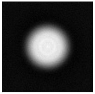
SNR=1/20 $\alpha \in [0.75, 1.5]$ 10% images are pure noise



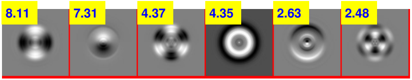
(h)



(i)



(j)



(k)

Current and Future Work

- Better class averages
- Covariance matrix: Orthogonal Replacement using Kam's method
- 3D reconstruction from denoised images, without class averaging

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Resources

- Code: `https://github.com/PrincetonUniversity/cwf_denoise`
- Paper: Journal of Structural Biology: `10.1016/j.jsb.2016.04.013`

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