

Toward defocus estimation per movie frame of cryo-EM

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Workshop at ISSAS, Taipei

References (1)

- [1] Wu S, Armache JP, Cheng Y. Single-particle cryo-EM data acquisition by using direct electron detection camera. *Microscopy (Oxf)*. 2016;65(1):35-41.
- [2] K. Zhang. Gctf: Real-time CTF determination and correction. *Journal of Structural Biology*. 2016; 193(1):1-12.
- [3] A. Rohou, N. Grigorieff. CTFFIND4: Fast and accurate defocus estimation from electron micrographs *Journal of Structural Biology*. 2015; 192:216-221
- [4] Y. Fan, Z. Zhao. Cryo-electron microscopy image Analysis using multi-frequency vector diffusion maps. Arxiv. 1904.07772
- [5] L.S. Jacqueline, et al. Cryo-electron microscopy – a primer for the non-microscopist. FEBS Journal 2013; 280:28-45
- [6] J. Frank. Three-Dimensinal Electron Microscopy of Macromolecular Assemblies: Visulaization of Biological Molecules in Their Native State. Oxford university press. 2006
- [7] E. J. Kirkland. Advanced Computing in Electron Microscopy. Springer. 2010
- [8] A. Heimowitz et al. Reducing bias and variance for CTF estimation in single particle cryoEM. *Ultramicroscopy* 2020; 212:112950
- [9] J. James. A Student's Guide to Fourier Transforms: With applications in Physics and Engineering. Cambridge University Press. 2011
- [10] G. Plonka. Numerical Fourier Anlaysis. Springer International Publishing. 2018

Motivation

Since the development of the direct electron detection device, DDD [1], and its application to cryo-EM, movie data are available for image data analysis in the single particle analysis. One of the analysis stages is to estimate the contrast transfer function, CTF, for the cryo-EM data.

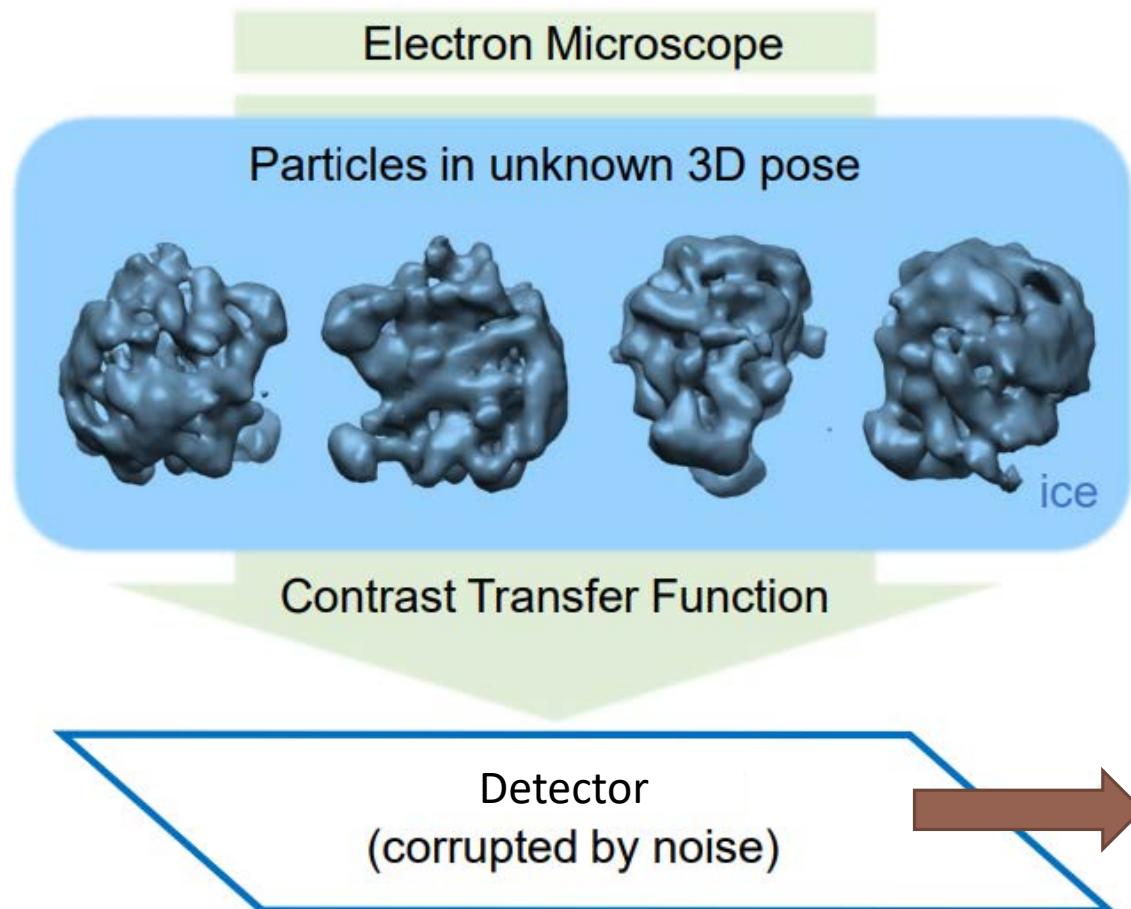
Common approaches for estimation of CTF are Gctf [2] and CTFFIND4 [3]. We review the approaches' capability of estimating CTF for movie data. We also look for an opportunity to enhance the accuracy of the CTF estimation for movie-frame.

Content

- Image formation (model) in cryo-EM
- Procedure of CTF estimation for cryo-EM micrograph
- Case study using common CTF approaches
- Progress on simulation
- Outlook

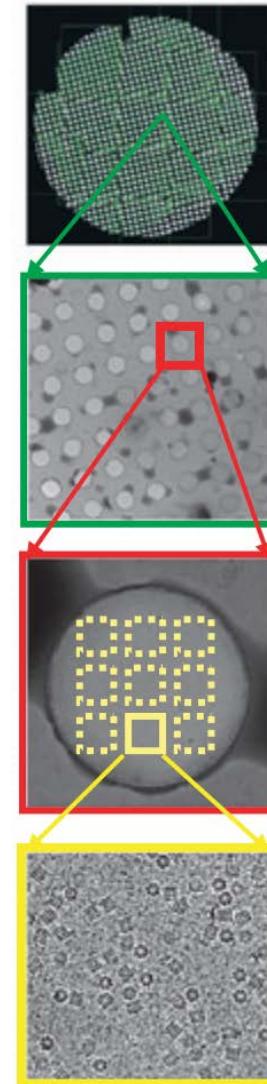
Image formation in cryo-EM

Visualization modified from [4]



The electron beam (green) projects randomly oriented particles in ice.

Figure from [5]



The projected image is modulated by CTF and contaminated by noise.

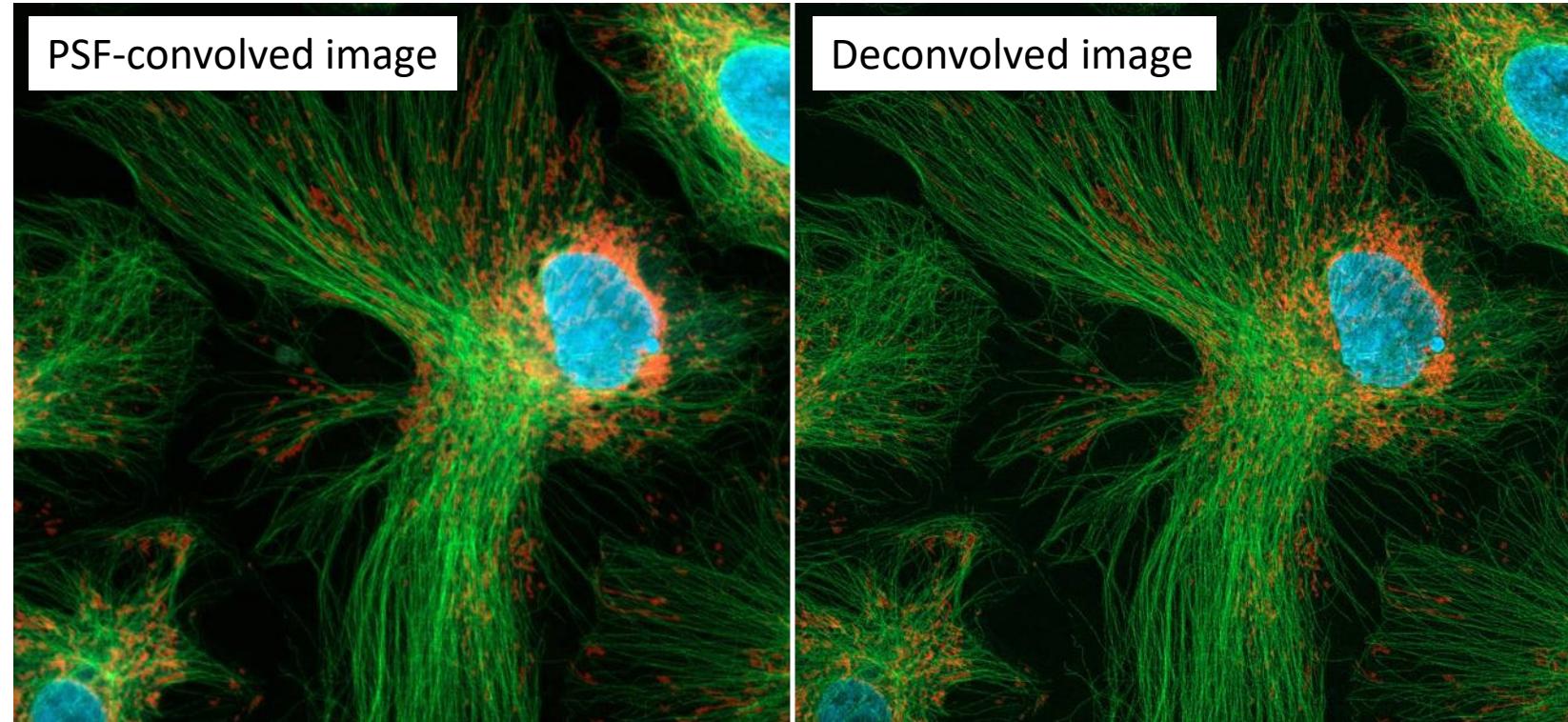
Image formation model in cryo-EM

Following the image formation, a cryo-EM micrograph containing 2D projections of particles can be described by linear model:

$$I_{\text{data}} = I_{\text{PSF}} \circledast I_{\text{obj}} + I_{\text{noise}}$$

Observed micrograph, I_{data}
Projection, I_{obj}
Noise, I_{noise}
Point spread function, I_{PSF}

Figures of kidney cells for showing effect of point spread function. Figures from [https://bitesizebio.com/22166/a-beginners-guide-to-the-point-spread-function-2/]



Spherical aberration and depth of focus

The effect of the spherical aberration C_s [6].

- a) the ideal lens with $C_s = 0$
- b) the real lens with finite C_s . The focal points f_o and f_j are different.

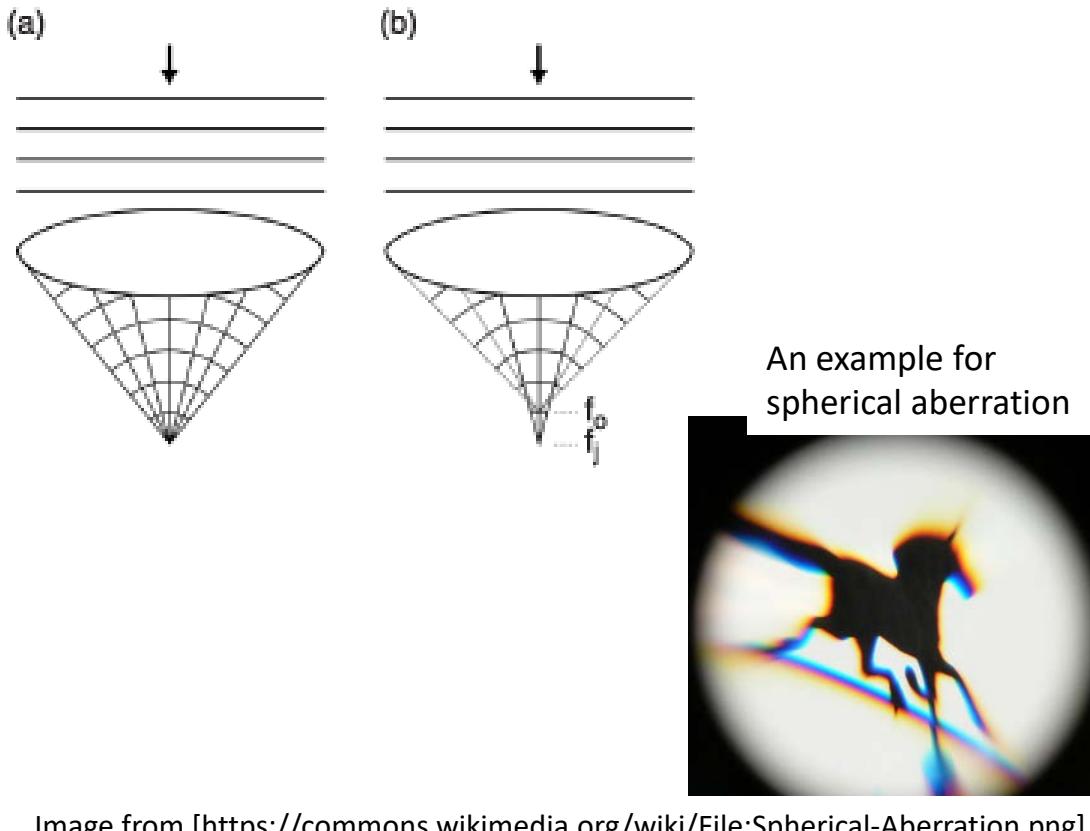
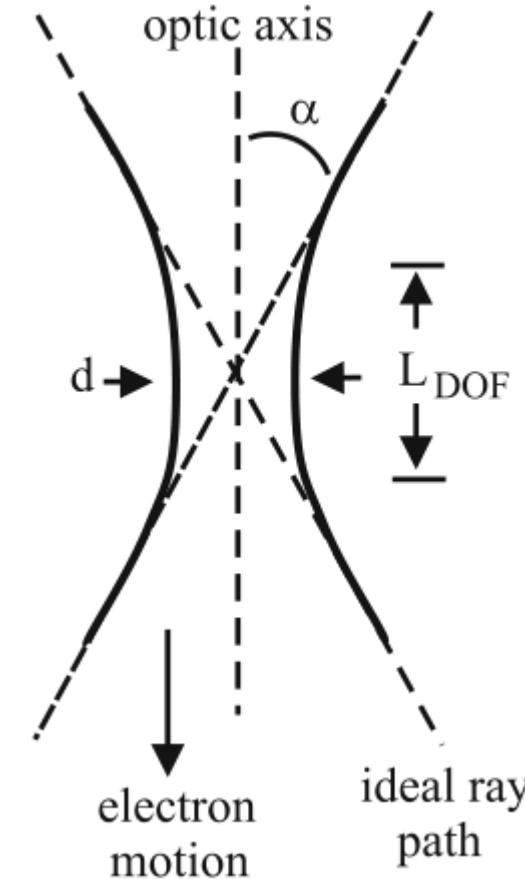


Image from [<https://commons.wikimedia.org/wiki/File:Spherical-Aberration.png>]

Depth of focus of a focused probe. Figure from [7]



A disk of diameter d , depth of focus, L_{DOF} . For small angles, $\alpha \sim d/L_{DOF}$

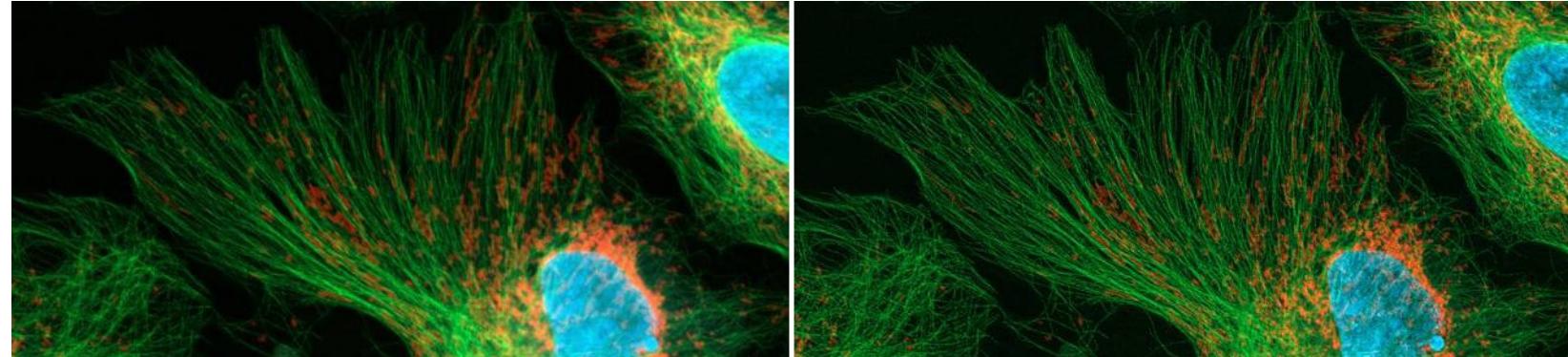
Image formation model in cryo-EM

Following the image formation, a cryo-EM micrograph containing 2D projections of particles can be described by linear model:

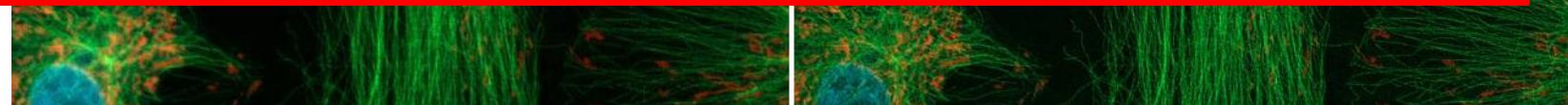
$$I_{\text{data}} = I_{\text{PSF}} \circledast I_{\text{obj}} + I_{\text{noise}}$$

Observed micrograph, I_{data}
Projection, I_{obj}
Noise, I_{noise}
Point spread function, I_{PSF}

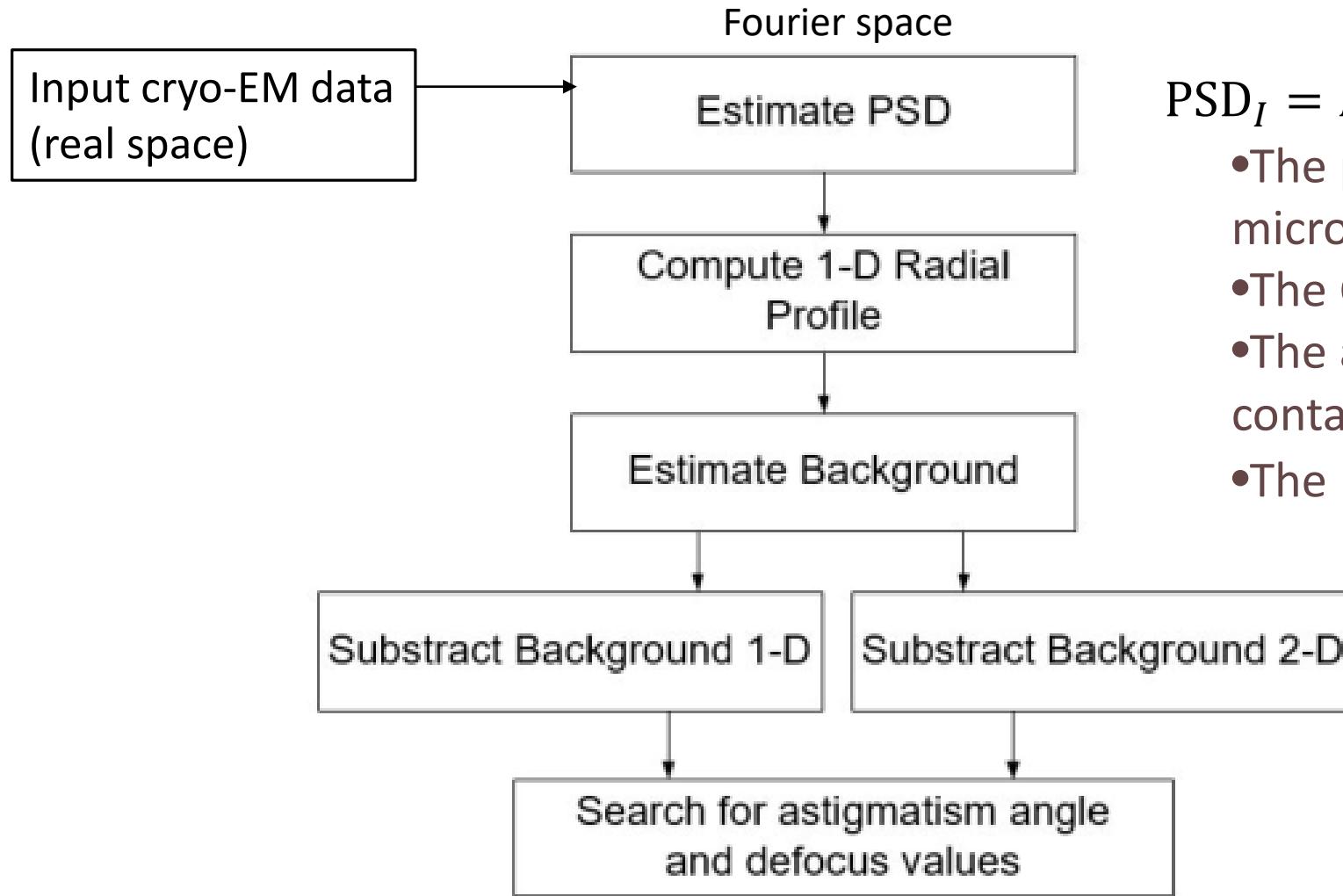
Figures of kidney cells for showing effect of point spread function. Figures from [https://bitesizebio.com/22166/a-beginners-guide-to-the-point-spread-function-2/]



We are interested in I_{obj} which is one of the ingredients for 3D particle reconstruction. To access I_{obj} , we usually take advantage of Fourier transformation to deconvolve.



Common procedure of CTF estimation in micrograph



$$\text{PSD}_I = H^2 \cdot \text{PSD}_f + \text{PSD}_b \text{ (in Fourier space) [8]}$$

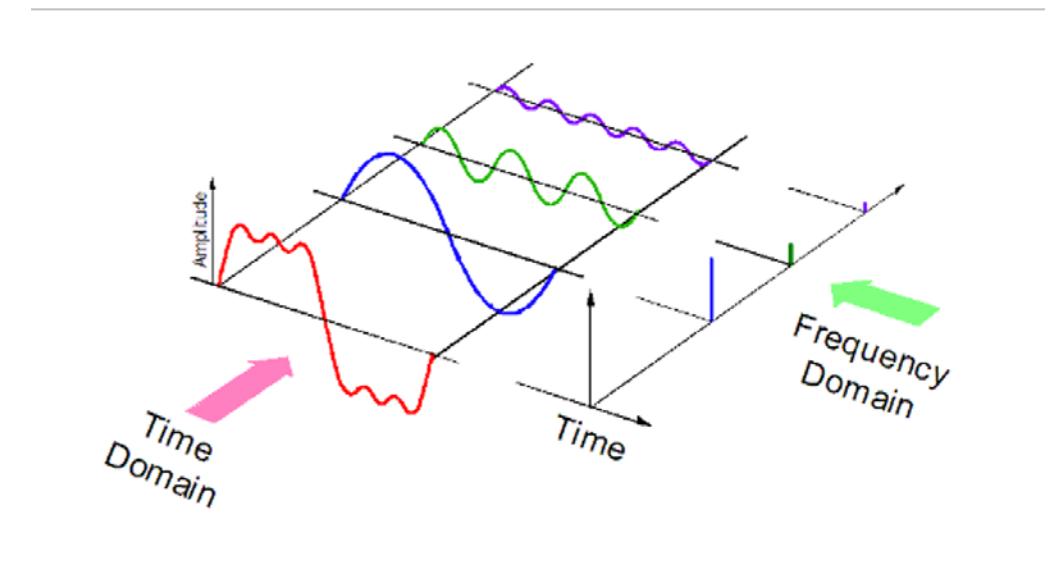
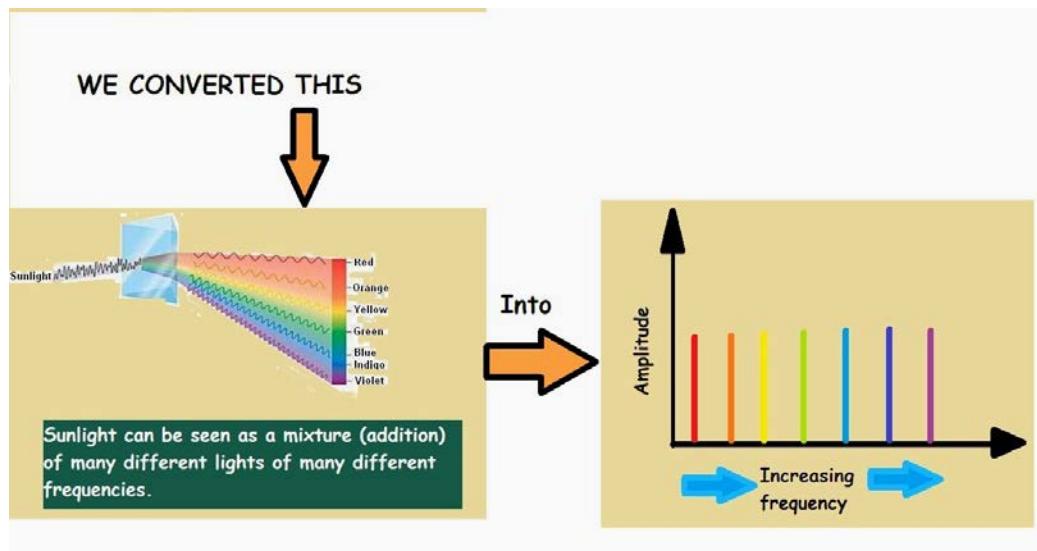
- The power spectral density of the micrograph PSD_I ;
- The CTF, H ;
- The average PSD of the particles and other contaminants PSD_f ;
- The PSD of the background noise PSD_b .

Introduction to Fourier transform and its application

We define the Fourier transform, $\mathcal{F}\{ \cdot \}(k)$, of a function, $f = f(x)$, which is continuous and integrable,

$$\mathcal{F}\{f(x)\}(k) = \int_{-\infty}^{\infty} f(x)e^{-2\pi ikx}dx$$

The function, f , is transformed from x space to k space. In Physics, we can apply the concept of the Fourier transform into wave in time and frequency domains.



Visualizations by [<http://visualizingmathsandphysics.blogspot.com/2015/06/fourier-transforms-intuitively.html>] (left) and [<https://kinder-chen.medium.com/denoising-data-with-fast-fourier-transform-a81d9f38cc4c>] (right)

Properties of the Fourier transform (1)

Suppose we have two integrable functions, $f(x)$ and $g(x)$.

Linearity [9, 10]:

$$\int [af(x) + bg(x)]e^{-2\pi ikx}dx = a \int_{-\infty}^{\infty} f(x)e^{-2\pi ikx}dx + b \int_{-\infty}^{\infty} g(x)e^{-2\pi ikx}dx$$

where a and b are complex numbers.

Properties of the Fourier transform (2)

● Convolution theorem [9]

Let $f \circledast g$ denote the convolution of two functions, f and g . We then write down the Fourier transform of the convolution (see below)

$$\mathcal{F}\{f \circledast g\} = \int_{-\infty}^{\infty} \left\{ \int_{-\infty}^{\infty} f(p)g(x-p)dp \right\} e^{-2\pi ikx} dx$$

If we define $q=x-p$, and $dq = dx$, we can rewrite the above equation into:

$$\begin{aligned} \mathcal{F}\{f \circledast g\} &= \int_{-\infty}^{\infty} \left\{ \int_{-\infty}^{\infty} f(p)g(q)dp \right\} e^{-2\pi ik(p+q)} dq \\ &= \int_{-\infty}^{\infty} f(p)e^{-2\pi ikp} dp \int_{-\infty}^{\infty} g(q)e^{-2\pi i kq} dq \end{aligned}$$

That is a production of Fourier transforms of f and of g , which is written:

$$\mathcal{F}\{f \circledast g\} = \mathcal{F}\{f\}\mathcal{F}\{g\} \text{ (Space convolution = frequency multiplication)}$$

Back to the cryo-EM image model (1)

Today we have an image captured by cryo-EM,

$$I_{\text{data}}(x, y) = I_{\text{PSF}} \odot I_{\text{obj}} + I_{\text{noise}}$$

Specifically, we can write the convolution in its integral mathematical formation:

$$I_{\text{data}}(x, y) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} I_{\text{PSF}}(m, n) I_{\text{obj}}(x - m, y - n) dm dn + I_{\text{noise}}(x, y)$$

In order to deconvolve $I_{\text{PSF}} \odot I_{\text{obj}}$, we take a Fourier transformation to $I_{\text{data}}(x, y)$ and we can write down:

$$\mathcal{F}\{I_{\text{data}}(x, y)\}(u, v) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \left\{ \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} I_{\text{PSF}}(m, n) I_{\text{obj}}(x - m, y - n) dm dn + I_{\text{noise}}(x, y) \right\} e^{-i2\pi(ux+vy)} dx dy$$

According to the linearity introduced previously, we can separate convolution and noise terms.

$$\begin{aligned} & \mathcal{F}\{I_{\text{data}}(x, y)\}(u, v) \\ &= \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \left\{ \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} I_{\text{PSF}}(m, n) I_{\text{obj}}(x - m, y - n) dm dn \right\} e^{-i2\pi(ux+vy)} dx dy + \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \{I_{\text{noise}}(x, y)\} e^{-i2\pi(ux+vy)} dx dy \end{aligned}$$

Then the convolution term is transformed into a double-integral product by using $p=x-m$, $dp=dx$, $q=y-n$ and $dq=dy$.

$$\begin{aligned} & \mathcal{F}\{I_{\text{data}}(x, y)\}(u, v) \\ &= \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} I_{\text{PSF}}(m, n) e^{-i2\pi(um+vm)} dm dn \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} I_{\text{obj}}(p, q) e^{-i2\pi(up+uq)} dp dq + \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \{I_{\text{noise}}(x, y)\} e^{-i2\pi(ux+vy)} dx dy \\ &= CTF \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} I_{\text{obj}}(p, q) e^{-i2\pi(up+uq)} dp dq + \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \{I_{\text{noise}}(x, y)\} e^{-i2\pi(ux+vy)} dx dy \end{aligned}$$

where contrast transfer function, CTF, is the Fourier-transformed point spread function, $\mathcal{F}\{\text{PSF}\}$.

Back to the cryo-EM image model (2)

$$\mathcal{F}\{I_{\text{data}}(x, y)\}(u, v) = CTF \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} I_{\text{obj}}(p, q) e^{-i2\pi(up+uq)} dp dq + \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \{I_{\text{noise}}(x, y)\} e^{-i2\pi(ux+vy)} dx dy$$

The outcomes of the Fourier transform of I_{obj} and I_{noise} are $\hat{F}_{\text{obj}} = |\hat{F}_{\text{obj}}| e^{i \arg \hat{F}_{\text{obj}}}$ and $\hat{F}_{\text{noise}} = |\hat{F}_{\text{noise}}| e^{i \arg \hat{F}_{\text{noise}}}$, [10] respectively. $|\hat{F}_{\text{obj}}|$ and $|\hat{F}_{\text{noise}}|$ are **modulus (magnitude)**. $\arg \hat{F}_{\text{obj}}$ and $\arg \hat{F}_{\text{noise}}$ are **phase**.

we can substitute them into the above formula:

$$F(u, v) = \mathcal{F}\{I_{\text{data}}(x, y)\}(u, v) = CTF \times |\hat{F}_{\text{obj}}| e^{i \arg \hat{F}_{\text{obj}}} + |\hat{F}_{\text{noise}}| e^{i \arg \hat{F}_{\text{noise}}}$$

In Fourier analysis [10], power spectrum (power spectral density) is squared modulus ie.

$$\begin{aligned} |F(u, v)|^2 &= |CTF \times |\hat{F}_{\text{obj}}| e^{i \arg \hat{F}_{\text{obj}}} + |\hat{F}_{\text{noise}}| e^{i \arg \hat{F}_{\text{noise}}}|^2 \\ &= CTF^2 |\hat{F}_{\text{obj}}|^2 + |\hat{F}_{\text{noise}}|^2 + 2CTF(|\hat{F}_{\text{obj}}| |\hat{F}_{\text{noise}}| e^{-2 \arg \hat{F}_{\text{obj}} \arg \hat{F}_{\text{noise}}}) \end{aligned}$$

Common approaches to estimate CTF

- **CTFFIND4** [3] (**Gctf** [2]) estimates accurately the CTF by maximizing the cross-correlation of a simulated CTF with the (logarithmic) amplitude spectra of observed micrographs.
 - Gctf involves B-factor into CTF and Gctf is also featured by using GPU-acceleration.
- **ASPIRE** [8] is a new method for CTF estimation based on multitaper techniques to reduce bias and variance in the estimate.

CTF estimation with and without motion correction

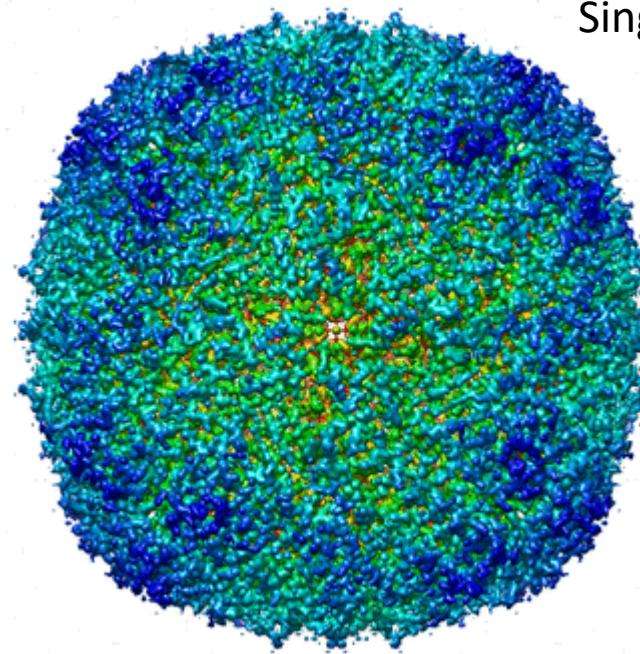
An experiment is constructed to observe the correlation of molecule between frames.

- **(case A)** $\text{CTF}(|\mathcal{F}\{\sum_{i=1}^n I_i^*\}|^2) \rightarrow$ this CTF is expected to have whole rings.
- **(case B)** $\text{CTF}(|\mathcal{F}\{\sum_{i=1}^n I_i\}|^2) \rightarrow$ this CTF is expected to have broken rings.
- **(case C)** $\sum_{i=1}^n \text{CTF}(|\mathcal{F}\{I_i\}|^2) \rightarrow$ the resulting CTF should have whole rings.

where I_i^* (I_i) is an (un)aligned frame, n is the maximum number of frames, $\mathcal{F}\{ \}$ is a Fourier transform operation, and $\text{CTF}()$ is a function which estimates CTF quantities, defocus, astigmatic angle, etc...

Data set for the CTF experiment

[[EMPIAR-10200](#)] Human apo-ferritin



Single particle at 1.65 Å

Micrographs were taken with the following conditions:

- Acceleration voltage of 300 kV, Cs=2.7 mm and calibrated defocus 0.5 – 8.0 µm.
- Detector: GATAN K2 SUMMIT which is a direct detection camera
- Micrographs are unaligned and multi-framed (40 frames per micrograph).

Two micrographs applied no alignment

1 out of 40 frames
in micrograph A:
99_003_Oct04_20.
12.12.tif

No alignment

Sum over 40 frames of
micrograph A

No alignment

1 out of 40 frames in
micrograph B:
99_004_Oct04_20.12.3
0.tif

No alignment

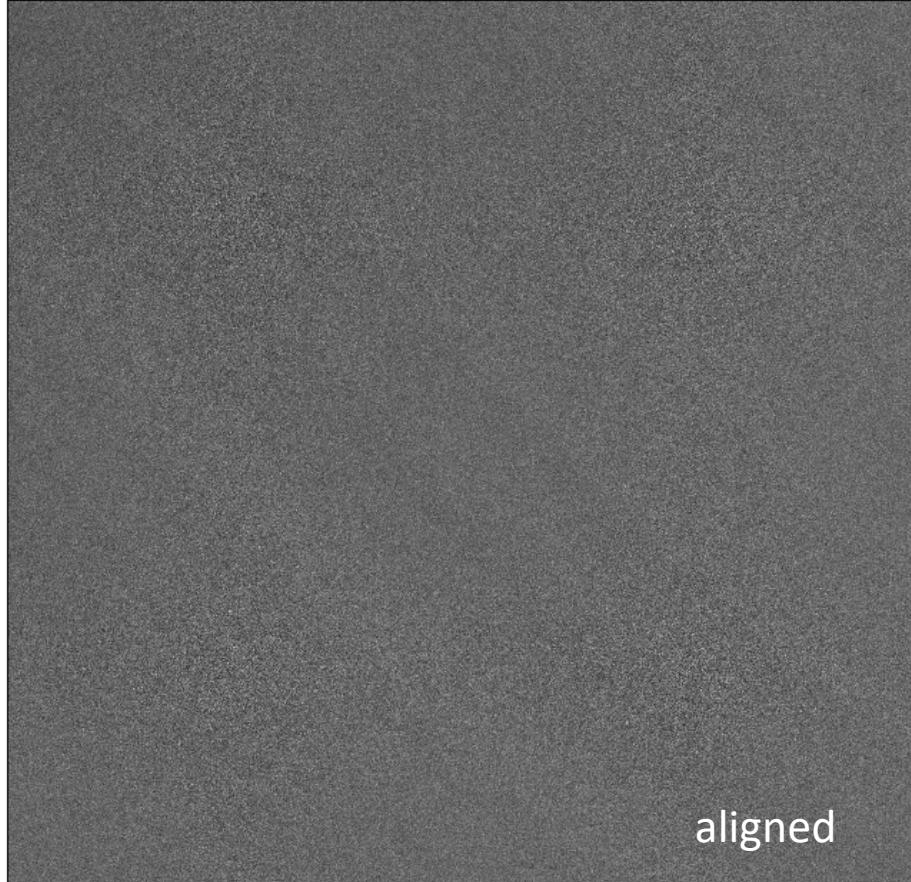
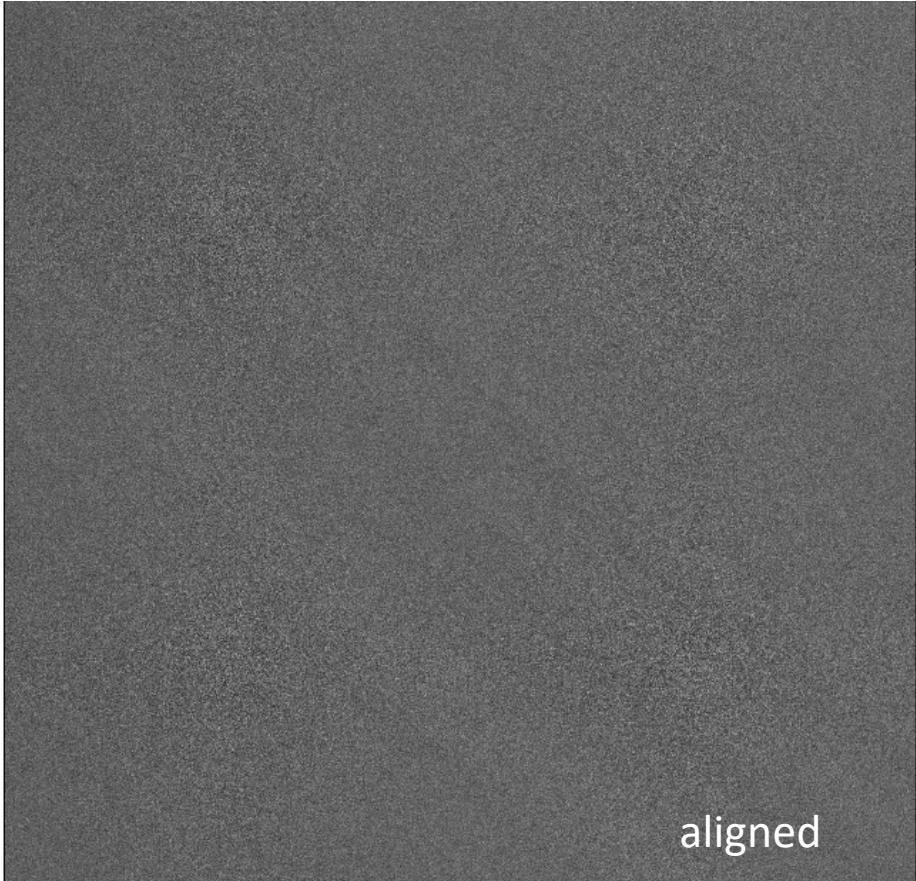
Sum over 40 frames of
micrograph B

No alignment

1. 2 micrographs, M1 and M2, are used as inputs to ctffind4

M1 and M2 are experimental data and are used in the following slides.

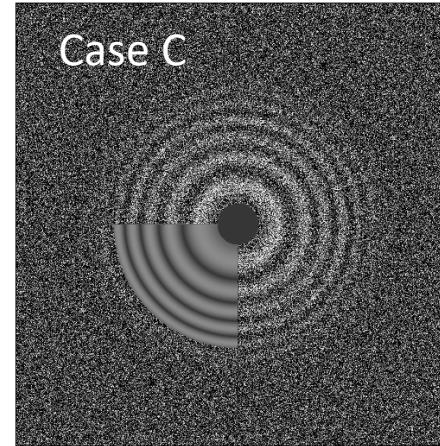
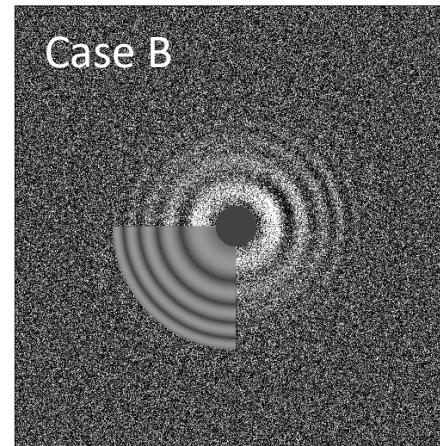
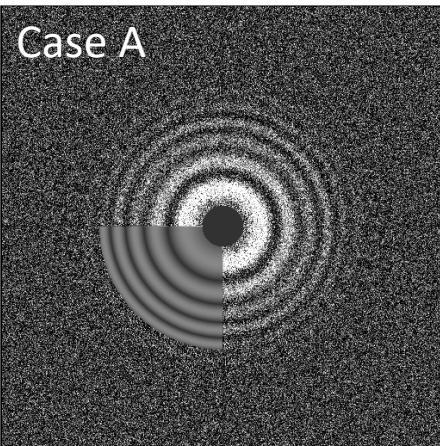
Two micrographs applied alignment



*: the file names are 99_003_Oct04_20.12.12 (left) and 99_004_Oct04_20.12.30 (right)

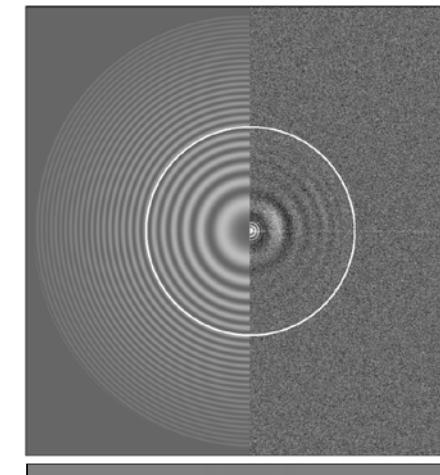
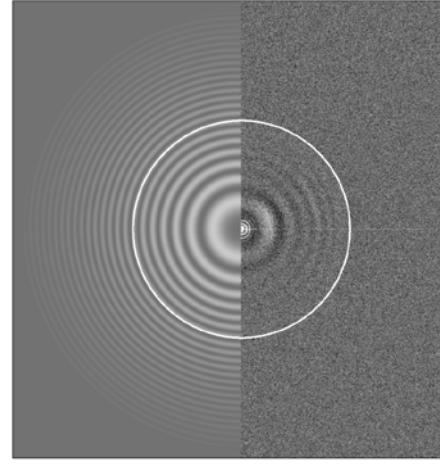
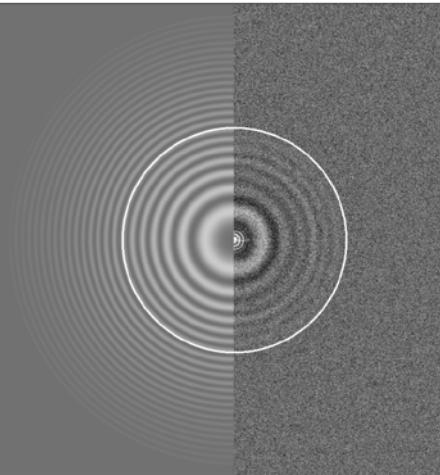
1. 2 micrographs* M1 and M2 are aligned by Relion in Scipion3

CTFFIND4



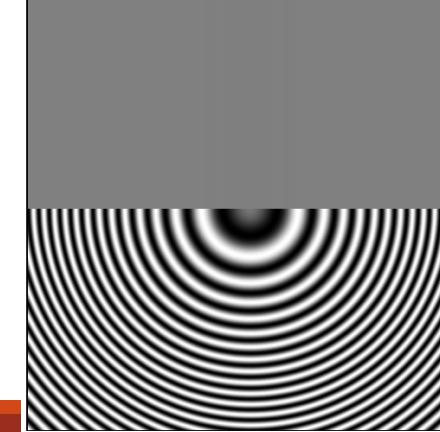
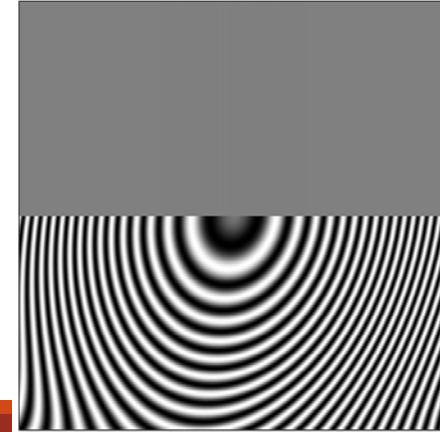
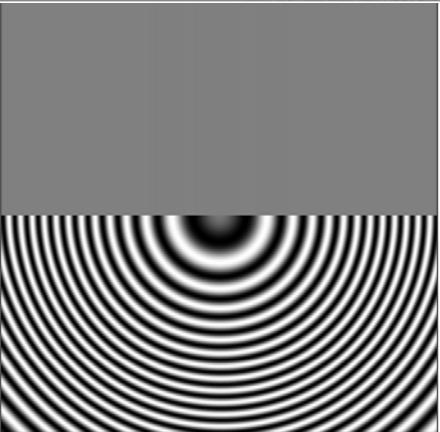
A visualization in a big panel about CTF estimation using the three approaches on M1

Gctf



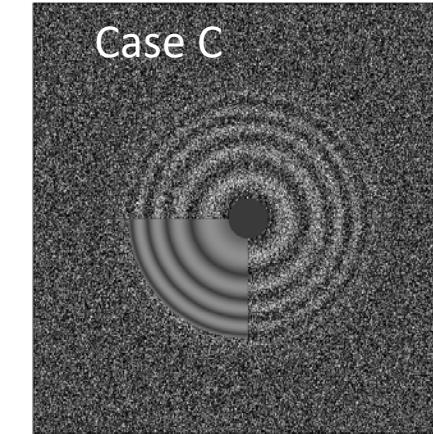
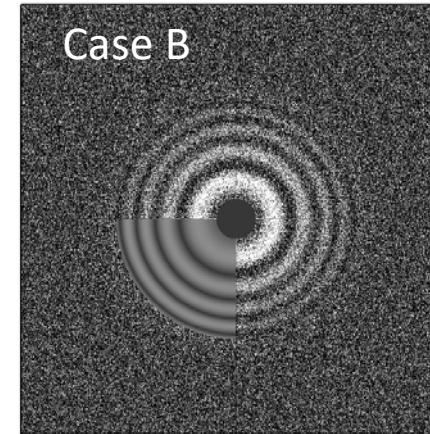
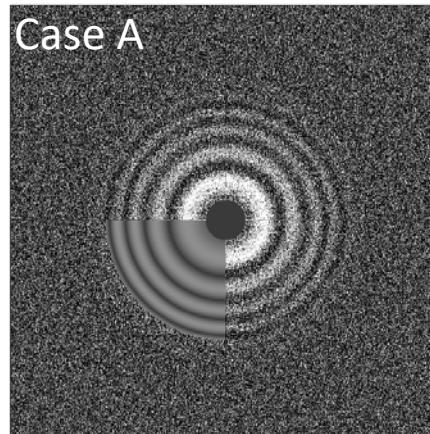
Gctf is able to take a single movie frame: average number of movie frames for movie or particle stack CTF refinement.

ASPIRE



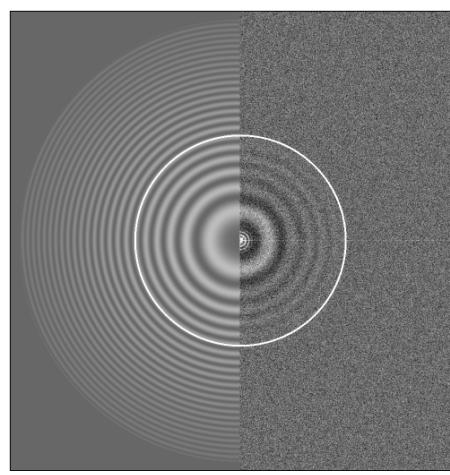
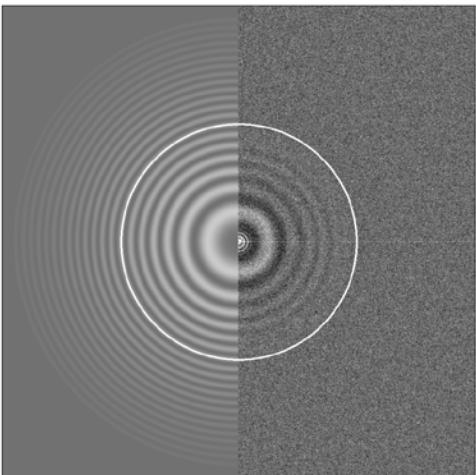
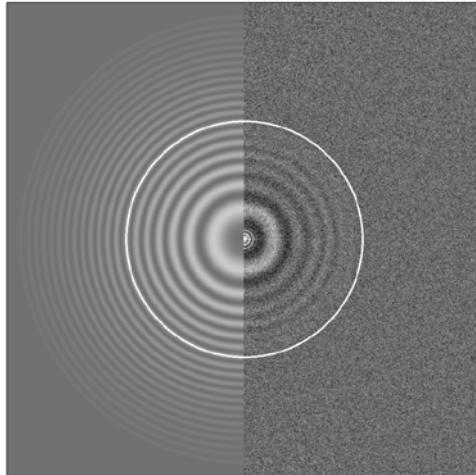
- Case A: $\text{CTF}(|\mathcal{F}\{\sum_{i=1}^n I_i^*\}|^2)$
- Case B: $\text{CTF}(|\mathcal{F}\{\sum_{i=1}^n I_i\}|^2)$
- Case C: $\sum_{i=1}^n \text{CTF}(|\mathcal{F}\{I_i\}|^2)$

CTFFIND4

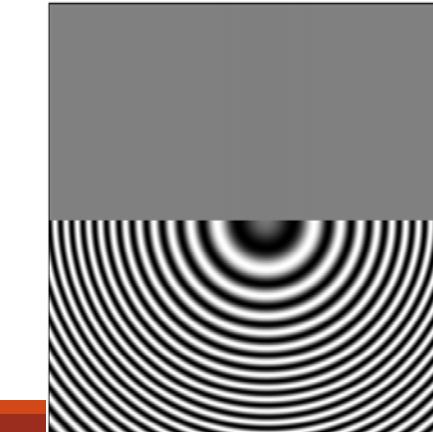
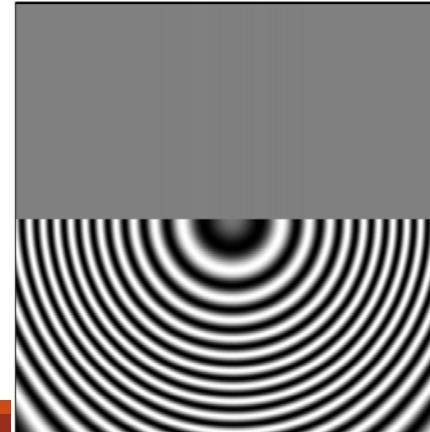
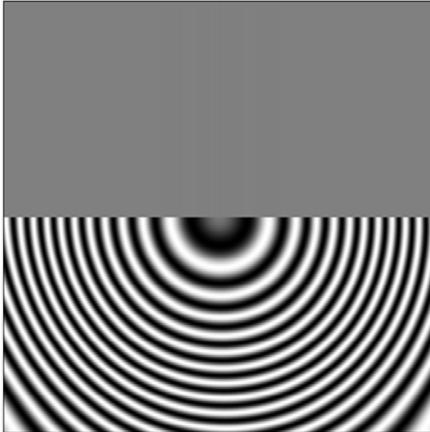


A visualization in a big panel about CTF estimation using the three approaches on M2

Gctf



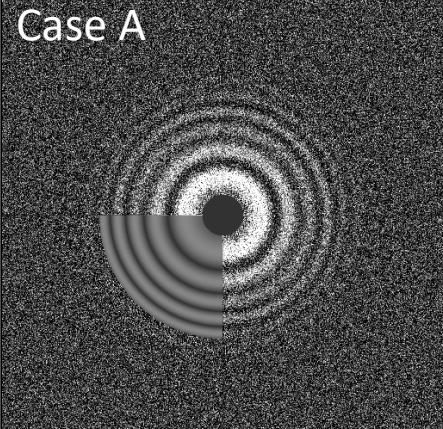
ASPIRE



Gctf is able to take a single movie frame: average number of movie frames for movie or particle stack CTF refinement.

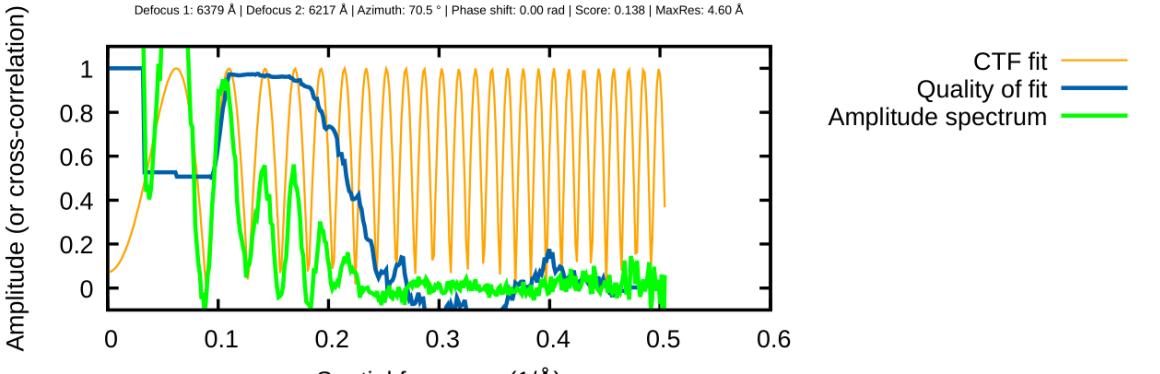
- Case A: $\text{CTF}(|\mathcal{F}\{\sum_{i=1}^n I_i^*\}|^2)$
- Case B: $\text{CTF}(|\mathcal{F}\{\sum_{i=1}^n I_i\}|^2)$
- Case C: $\sum_{i=1}^n \text{CTF}(|\mathcal{F}\{I_i\}|^2)$

Case A



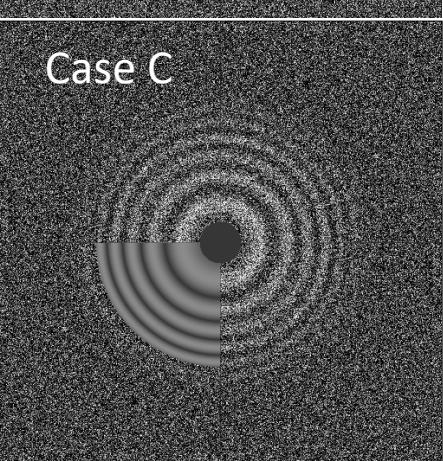
99_003_Oct04_20.12.12_aligned_mic_DW.mrc, 1 of 1 micrographs

Defocus 1: 6379 Å | Defocus 2: 6217 Å | Azimuth: 70.5 ° | Phase shift: 0.00 rad | Score: 0.138 | MaxRes: 4.60 Å

**M1 and CTFFIND4 are used.**

Cases	Defocus vector, $\Delta f_1, \Delta f_2, \alpha_{\text{ast}}$	Score
Case A	6379 Å, 6217 Å, 71°	0.138
Case B	6354 Å, 6241 Å, 71°	0.069
Case C	6338 Å, 6209 Å, 62°	0.092

Case C



Score function in CTFFIND4 [CTFFIND4]

- The score shown in the previous slide is the cross correlation coefficient minus a restrain term.

$$S = CC - \frac{\Delta\Delta f^2}{2\Delta\Delta f_{\text{res}}^2 N_{CC}}$$

N_{CC} is the number of pixels included in the computation of CC

g_{\min} and g_{\max} are minimum and maximum limits of spatial frequency. They are input parameters

where cross correlation coefficient, $CC = \frac{\sum_{g_{\min} < |g| \leq g_{\max}} A_d(g) |\text{CTF}(g)|}{\sqrt{\sum_{g_{\min} < |g| \leq g_{\max}} A_d^2(g) \sum_{g_{\min} < |g| \leq g_{\max}} \text{CTF}^2(g)}}$ between the

CTF and the experimental amplitude spectrum, A_d , whose background are subtracted. This CC measures how similar the two functions are.

The restrain term is currently not used in my studies.

The restrain term is a penalty for a high astigmatic CTF. Strength of the restrain term is strong when low $\Delta\Delta f_{\text{res}}$ and high $\Delta\Delta f$. $\Delta\Delta f$ and $\Delta\Delta f_{\text{res}}$ are the amplitude of astigmatism and a restrain parameter, respectively.

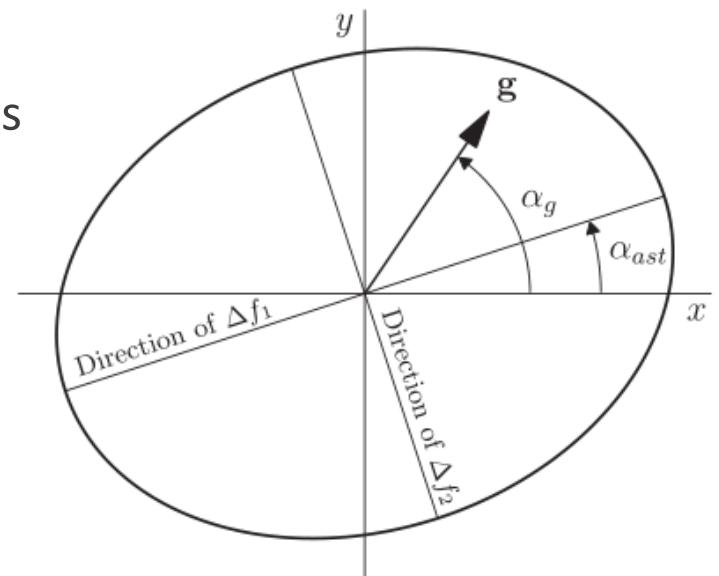
Procedure of computing the final score

1. Search for astigmatic angle

- Mirror the original spectrum along y axis.
- Rotate the mirrored spectrum, meanwhile compute the cross correlation of mirrored and original spectra
- The highest correlation determines the best rotation.
- The estimated astigmatic angle is the half of the best rotation.

2. Search for defocus values from Δf_{\min} to Δf_{\max} to find the values Δf_1 and Δf_2 maximizing S

3. Use conjugate-gradient minimizer for S. This computes the final estimates of the defocus parameters.



Computation of 1D profile

- Look for the CTF extrema and its spatial frequency by taking derivative of CTF which is →

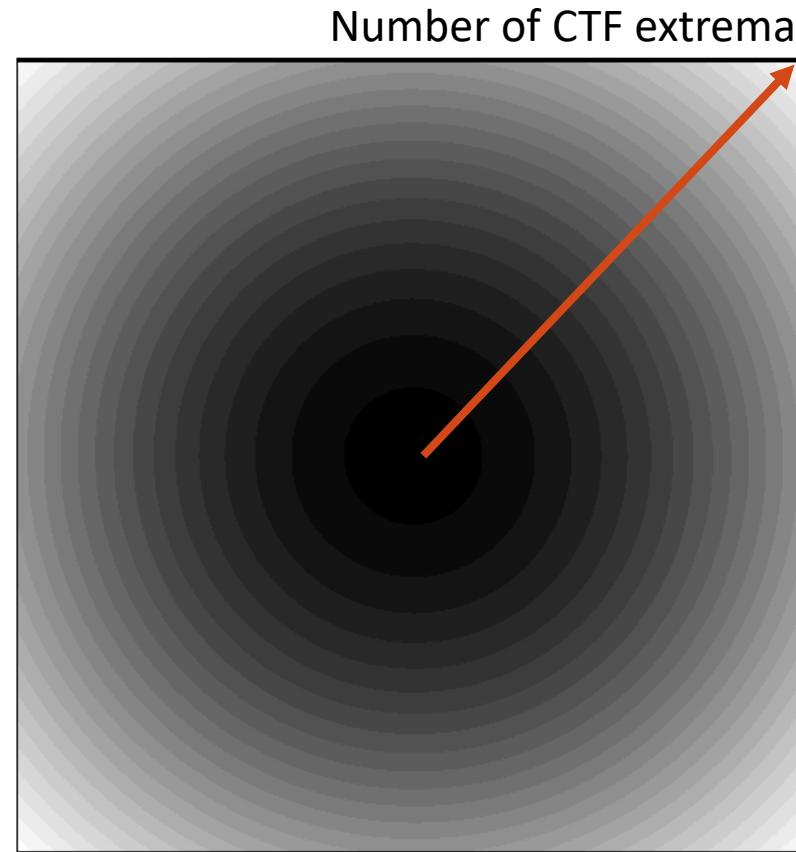
$$\cos \chi = 0$$

And χ is either $n\pi - \frac{\pi}{2}$ or $(n + 1)\pi - \frac{\pi}{2}$ where n is the number of CTF extrema

$$n = \left\lfloor \frac{1}{\pi} \chi + \frac{1}{2} \right\rfloor$$

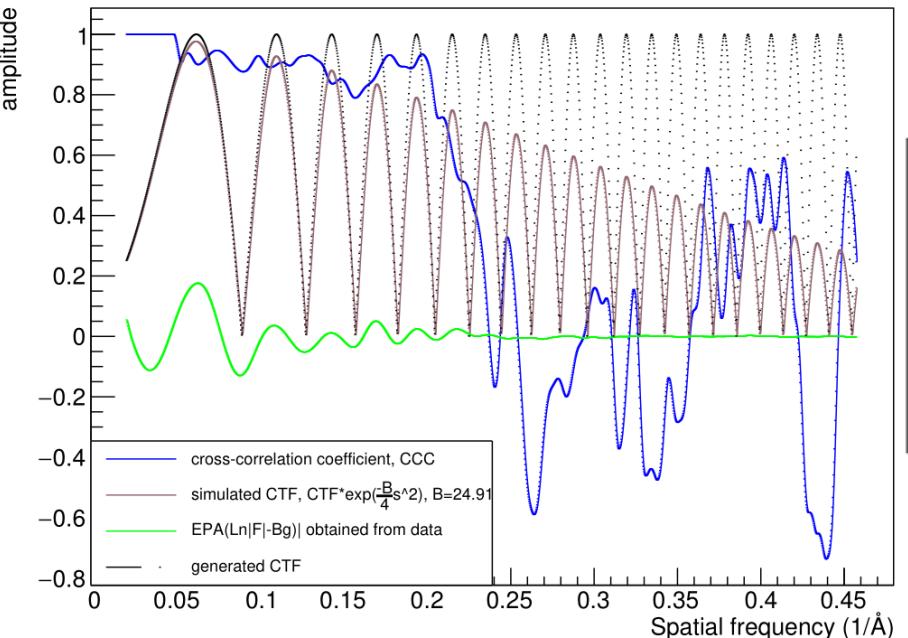
Compute the 1D profile along the direction of average defocus, $\alpha_{\text{mid}} = \alpha_{\text{ast.}} + \pi/4$. We can construct a set of \mathbf{g} which has the same number of CTF extrema, and the assigned CTF is closed to CTF along α_{mid} .

Estimate the quality of fit CC_{fit} (blue) by computing the correlation of CTF_{fit} (yellow) and \mathbf{A} (green) in 1D.

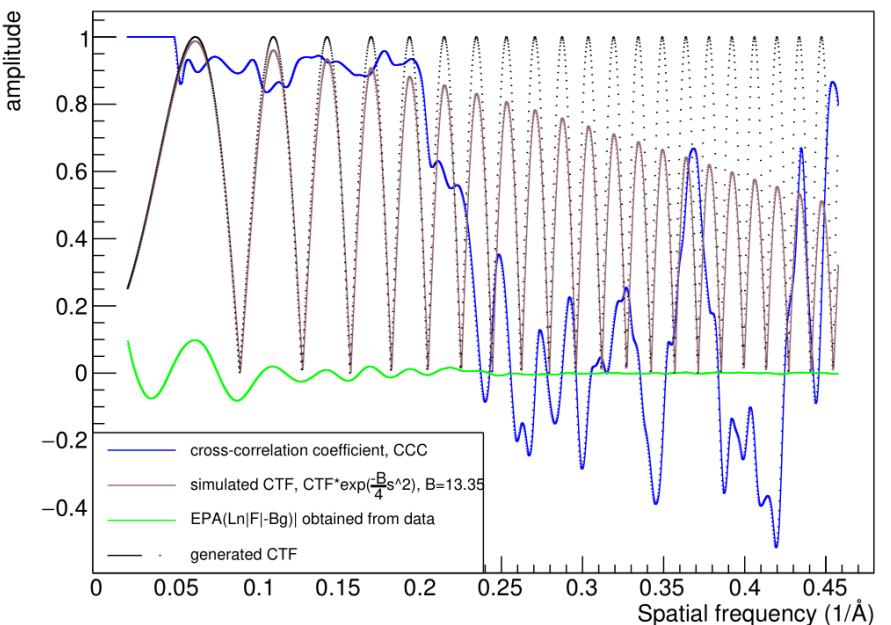


Black → white (increasing the number)

Case A



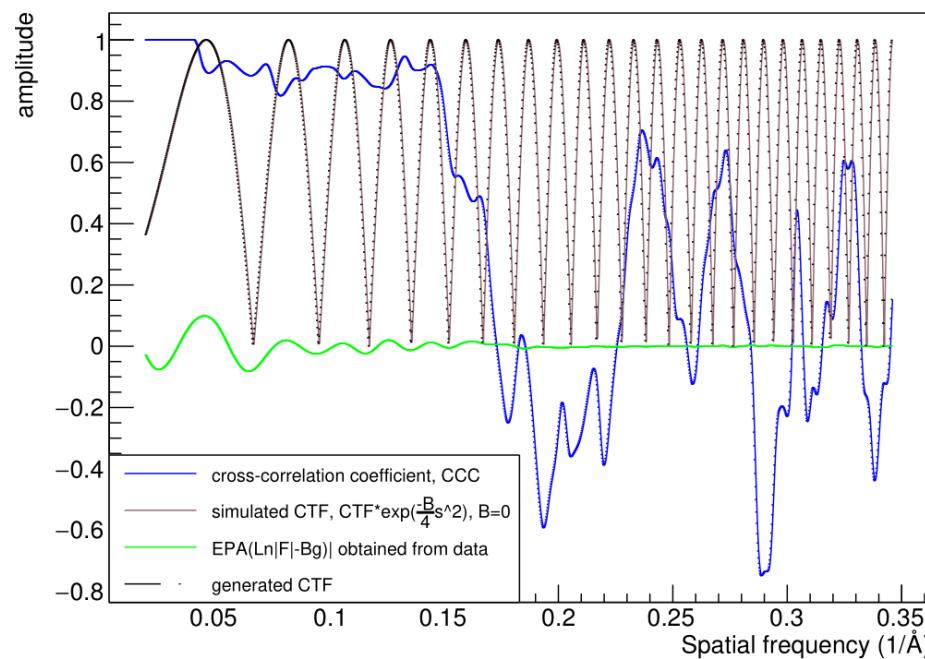
Case B



B factor, B, is estimated by Gctf

M1 and Gctf are used.

Case C



Cases	Defocus vector, $\Delta f_1, \Delta f_2, \alpha_{\text{ast.}}$	Final CCC	Estimated B factor
Case A	6309 Å, 6203 Å, 63°	0.14	24.91
Case B	6302 Å, 6150 Å, 44°	0.07	13.35
Case C	11065 Å, 10748 Å, 29°	0.004	0

Final score and EPA in Gctf

[Gctf]

- The final score in Gctf determines the final defocus vector:

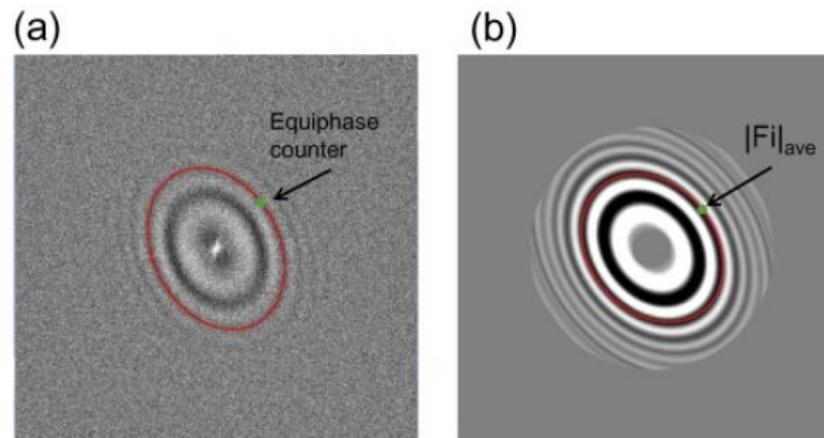
$$\text{Defocus vector} = \arg_z \max \left\{ CC \left(\ln|F(\mathbf{g})| - Bg(\ln|F(\mathbf{g})|), |\text{CTF}(\mathbf{g})| e^{-\frac{B}{4}\mathbf{g}^2} \right) \right\}$$

where $F(\mathbf{g})$ ($\ln|F(\mathbf{g})|$) is the (logarithmic) amplitude spectrum*. Bg is the estimated background. $\text{CTF}(\mathbf{g})$ is the simulated CTF with the B-factor. CC is the cross correlation function.

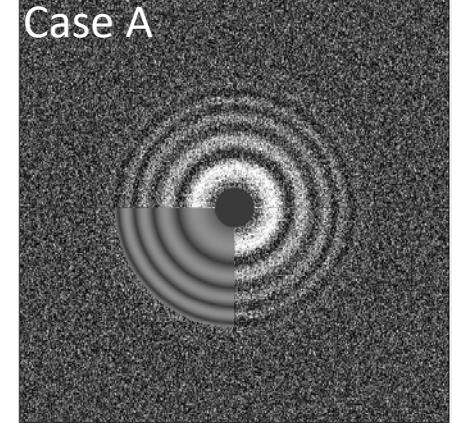
*: this is different from CTFFIND3 which uses square root of power spectra. In Gctf, $\ln|F(\mathbf{g})|$ decrease the strong signal at low frequency

The Equiphase averaging, EPA, method is proposed for the high astigmatic CTF. The idea is to average the amplitudes of the micrograph FFT which have the same CTF phase. Through this method, the ring-pattern is enhanced.

- (a) A logarithmic amplitude spectrum. The green point is the target pixel to be averaged. The red line is where all pixels with equiphases for the green point
- (b) The resulting averaged amplitude

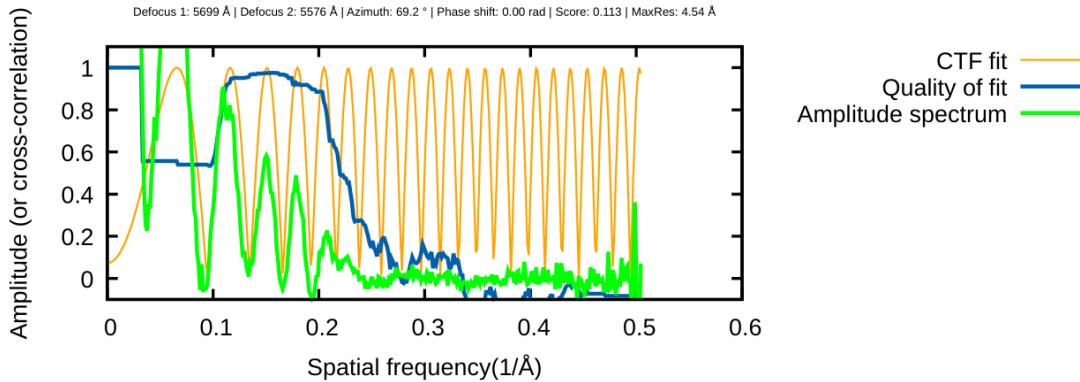


Case A

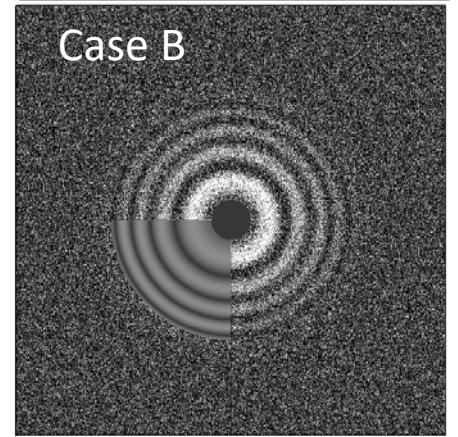


99_004_Oct04_20.12.30_aligned_mic_DW.mrc, 1 of 1 micrographs

Defocus 1: 5699 Å | Defocus 2: 5576 Å | Azimuth: 69.2 ° | Phase shift: 0.00 rad | Score: 0.113 | MaxRes: 4.54 Å

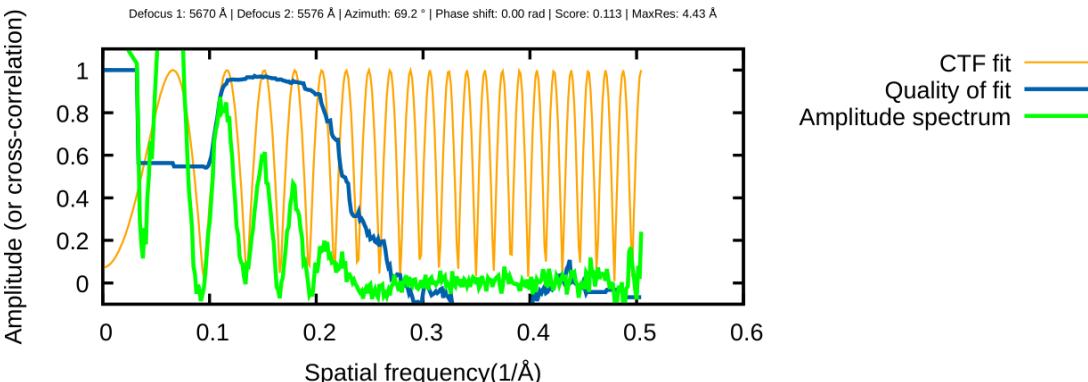
**M2 and CTFFIND4 are used.**

Case B

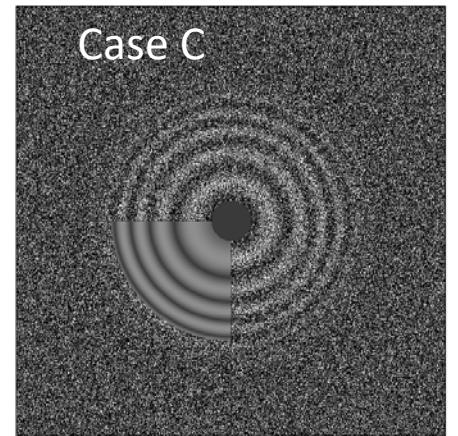


99_004_Oct04_20.12.30.tif, 1 of 1 micrographs

Defocus 1: 5670 Å | Defocus 2: 5576 Å | Azimuth: 69.2 ° | Phase shift: 0.00 rad | Score: 0.113 | MaxRes: 4.43 Å

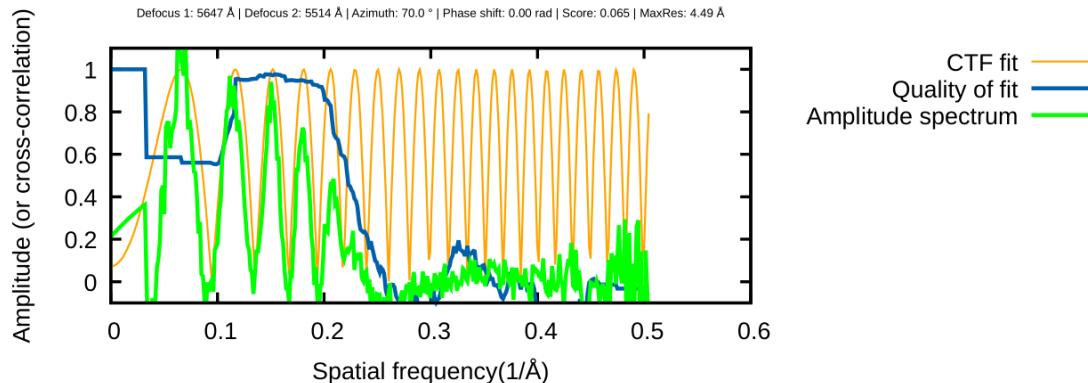


Case C



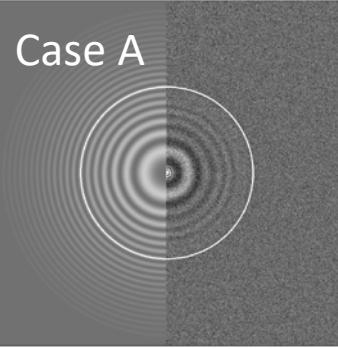
99_004_Oct04_20.12.30.tif, 1 of 1 micrographs

Defocus 1: 5647 Å | Defocus 2: 5514 Å | Azimuth: 70.0 ° | Phase shift: 0.00 rad | Score: 0.065 | MaxRes: 4.49 Å

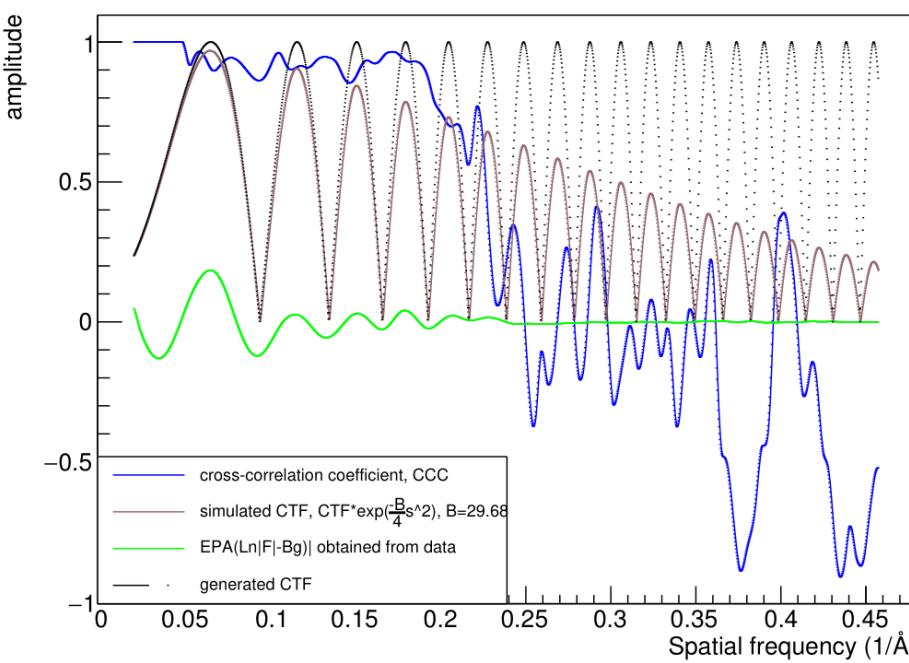


Cases	Defocus vector, $\Delta f_1, \Delta f_2, \alpha_{\text{ast.}}$	Score
Case A	5699 Å, 5576 Å, 69°	0.113
Case B	5670 Å, 5576 Å, 69°	0.113
Case C	5674 Å, 5514 Å, 70°	0.065

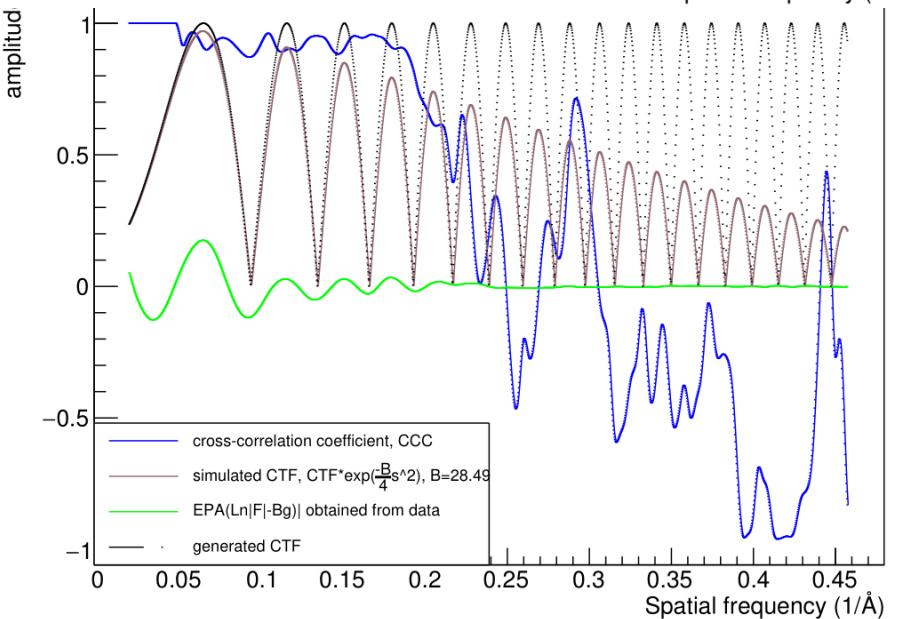
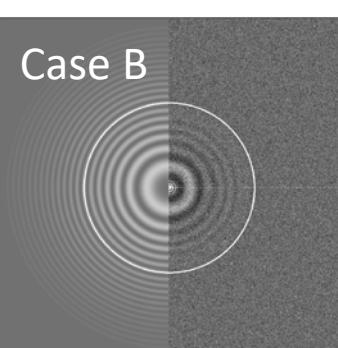
Case A



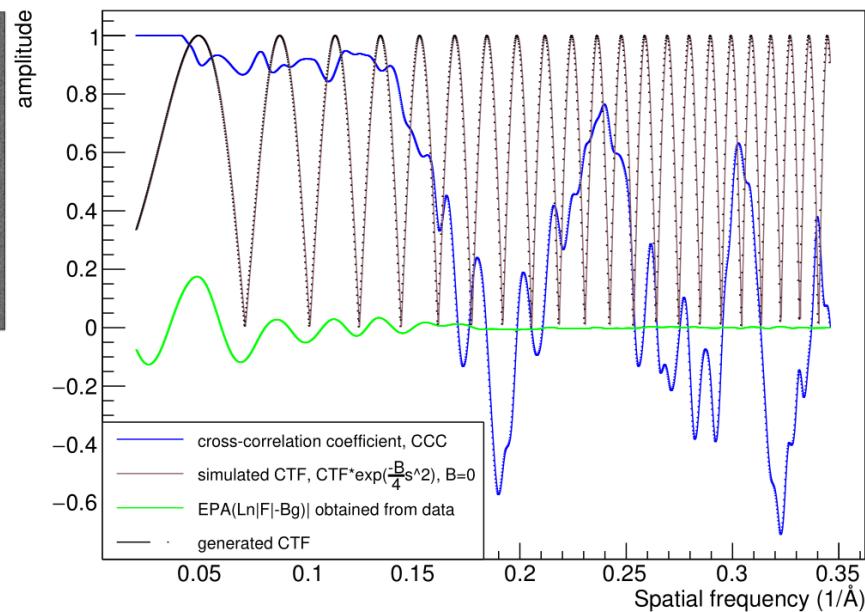
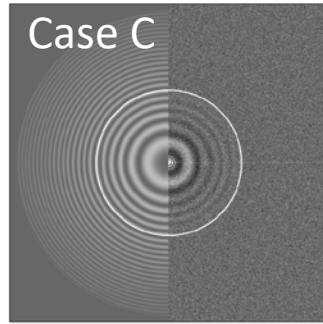
B factor, B, is estimated by Gctf



Case B



M2 and Gctf are used.



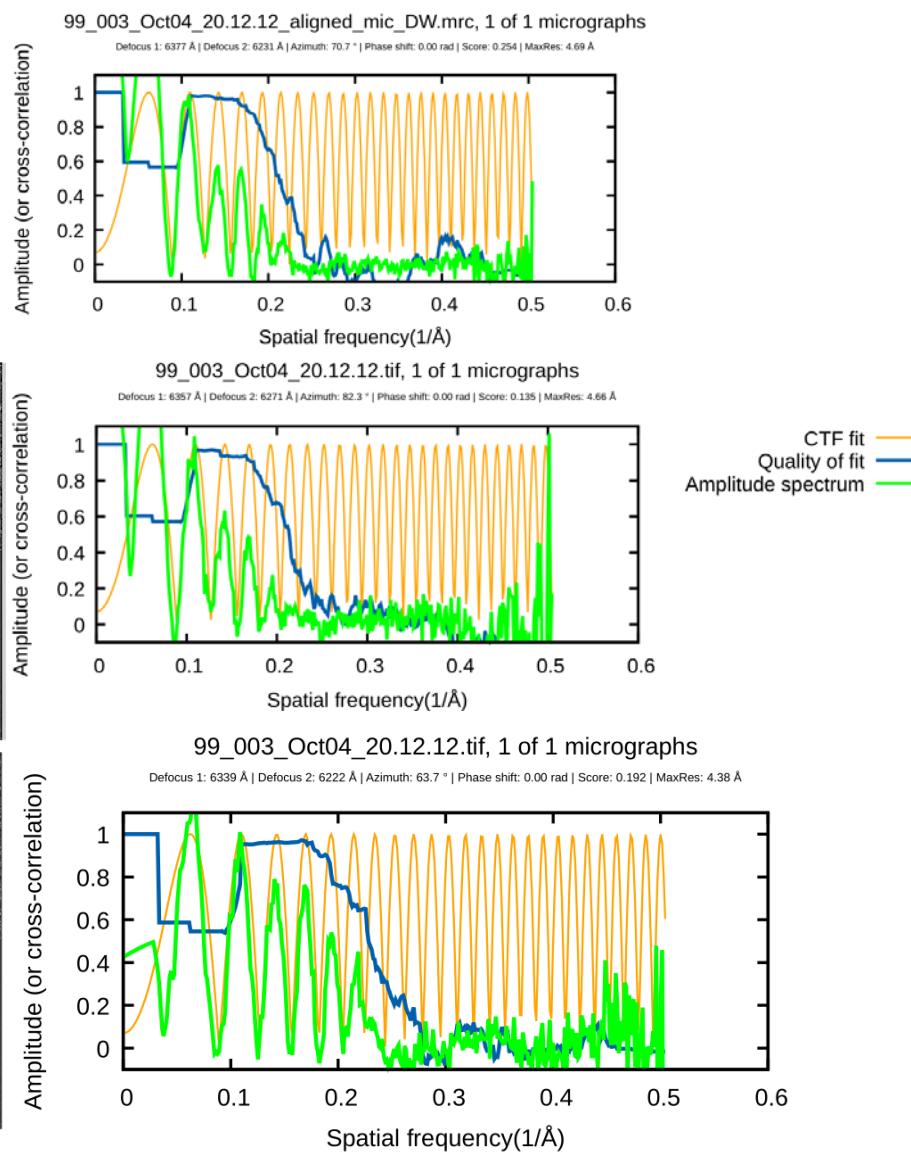
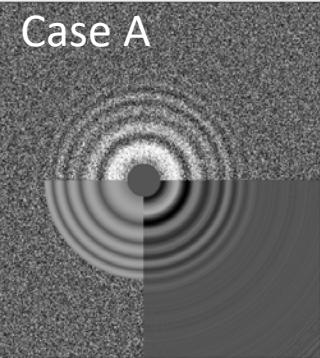
Cases	Defocus vector, $\Delta f_1, \Delta f_2, \alpha_{\text{ast}}$	Final CCC	Estimated B factor
Case A	5605 Å, 5629 Å, 23°	0.14	29.68
Case B	5656 Å, 5566 Å, 54°	0.136	28.49
Case C	9881 Å, 9764 Å, 65°	0.09	0

Hypothesis

Gctf uses $\text{Ln}|F(\mathbf{g})|$ to estimate CTF, which decreases the strong signal at low frequency meanwhile the weak signal at high frequency becomes weaker. In my option, using $\text{Ln}|F(\mathbf{g})|$ could be harmful for estimating CTF at high frequency.

CTFFIND4 uses amplitude spectrum, $F(\mathbf{g})$ for the CTF estimation. We can simply take $\text{Ln}|F(\mathbf{g})|$ which will be an input for CTFFIND4 approach to see the estimation with the new condition.

First experiment results (not final)



M1 and modified CTFFIND4 ($\ln(F)$) are used.

Cases	Defocus vector, $\Delta f_1, \Delta f_2, \alpha_{\text{ast.}}$	Score
Case A	6377 Å, 6231 Å, 71°	0.254
Case B	6357 Å, 6271 Å, 82°	0.135
Case C	6339 Å, 6222 Å, 64°	0.192

Scores in three cases are higher than the regular CTFFIND4.

- 1D amplitude are all positive as a function of spatial frequency, which is different from Gctf case.
- Besides, the B factor is not included into CTF in CTFFIND4.

Report on the progress of simulation

InSilicoTEM is used to produce two micrographs with the almost identical defocus and different positions of particles.

We want to see if CTF tools can estimate similar defocuses within the two micrographs.

Msim1	Msim2	Microgr aphs	CTFFIND4 Defocus, Δf_1(relative difference%), Δf_2(%)
Defocus of simulation: 1196 nm	Defocus of simulation: 1197 nm	Msim1	1197 (0.08%) nm, 1164 (-2.7%) nm
		Msim2	1185 (-1%) nm, 1179 (-1.5%) nm
Msim1	Msim2	Microgr aphs	Gctf Defocus, Δf_1, Δf_2
Defocus of simulation: 1196 nm	Defocus of simulation: 1197 nm	Msim1	8272 nm, 8360 nm
		Msim2	8357 nm, 8300 nm

Outlook

- Check the hypothesis by investigating the ingredients for CTF-estimation in CTFFIND4 and Gctf.
 - The 1D profile of amplitude.
- Simulation work.
 - Use InSilicoTEM to produce two micrographs with different particle positions, and check if their power spectra are the same or not → ongoing

Backup
