Beam absorption correction for electron cryotomography data

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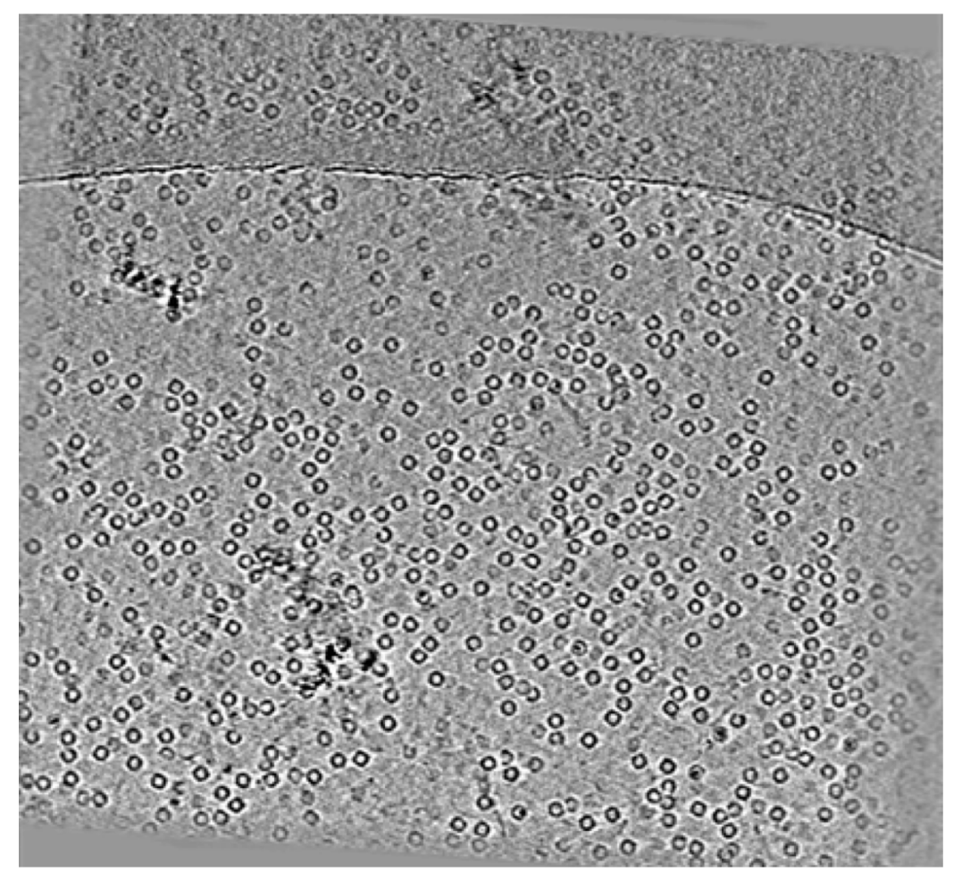
Abstract

Typical specimens for single-particle electron cryomicroscopy (cryoEM) span only a few hundred nanometers in thickness; however, it is increasingly common to study thicker specimens such as whole cells using electron cryotomography (cryoET). While image formation through thin specimens can be described adequately by the weak-phase approximation, doing so ignores the influence of beam absorption in samples thicker than ~1µm. The impact of ignoring such effects is reduced contrast proportional to the mass-thickness of the sample, causing projection images to appear darkest where the beam traverses the most material and resulting in a significant amount of uninterpretable content within recorded images. Since an increasing number of specimens fall into such an intermediate-thickness regime, our lab is investigating the influence of absorption or amplitude contrast on image formation and exploring strategies to enhance contrast in thick samples. Here we discuss our simulations as well as an iterative strategy for increasing image contrast by modeling specimen transmittance as a sum of amplitude and phase-contrast terms calculated from projection images at various tilt angles. Once the contribution of beam absorption is determined, raw data can be reweighted to more closely resemble pure-phase contrast and ultimately improves the visibility of low-resolution features in cryoET data.

Motivation

Samples appear darkest in regions of high density or thickness. This is due to phase contrast as well as absorption of the electron beam by the specimen. The resulting loss of intensity diminishes one's ability to distinguish features of interest.



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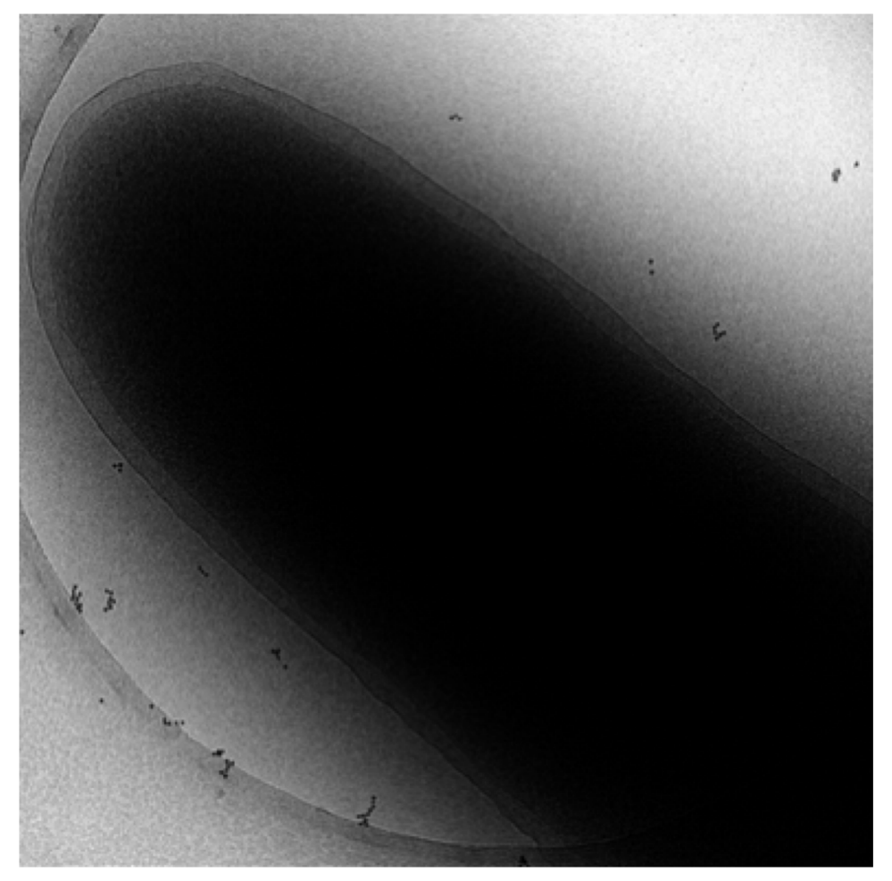

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TEM Image Contrast

In electron optics, the beam is modeled as a plane wave traveling from the source through the specimen and to a detector. Because the majority of incident electrons scatter elastically, i.e. without energy loss, image contrast is primarily due to a phase difference between the incident and scattered electron waves. This "phase contrast" is represented mathematically as

$$\psi_{exit}(x,y) = \psi_0(x,y)e^{-i\,\alpha \int \varphi(x,y,z)dz}$$

where $\varphi(x,y,z)$ represents the 3D electrostatic potential of the specimen. Assuming the specimen is very thin, the complex exponential can be simplified using the weak phase approximation:

$$\psi_{exit}(x,y) = \psi_0(x,y)\big(1 - i\,\alpha\,\varphi(x,y)\big)$$

where the exit wave is represented intuitively as the phase difference between the incident and scattered electron beam.

As specimen thickness increases, this approximation breaks down and absorbance begins to modulate the amplitude of incident electrons. This "amplitude contrast" produces a loss of image intensity proportionate to a specimen's mass-thickness, i.e.

$$I = I_0 e^{-\beta \int \varphi(x,y,z)\,dz}$$

where μ describes the molecular composition and density of the specimen.

Including phase and amplitude effects, we obtain the full, complex description of the scattered wave:

$$\psi_{full} = \psi_0 e^{-i\,\alpha \int \varphi(x,y,z)\,dz} e^{-\beta \int \varphi(x,y,z)\,dz}$$

Since this cannot be approximated via the weak-phase approximation, conventional contrast-transfer theory cannot be used to interpret the resulting images unless corrections can be applied to reduce the influence of beam absorption. To accomplish this, numerical methods are required to evaluate the beam-specimen interaction to achieve a physically accurate representation of the specimen in the microscope. Here, we describe a simplified model to reduce intensity decay at low resolution without formal simulation experiments.

Correction Strategies

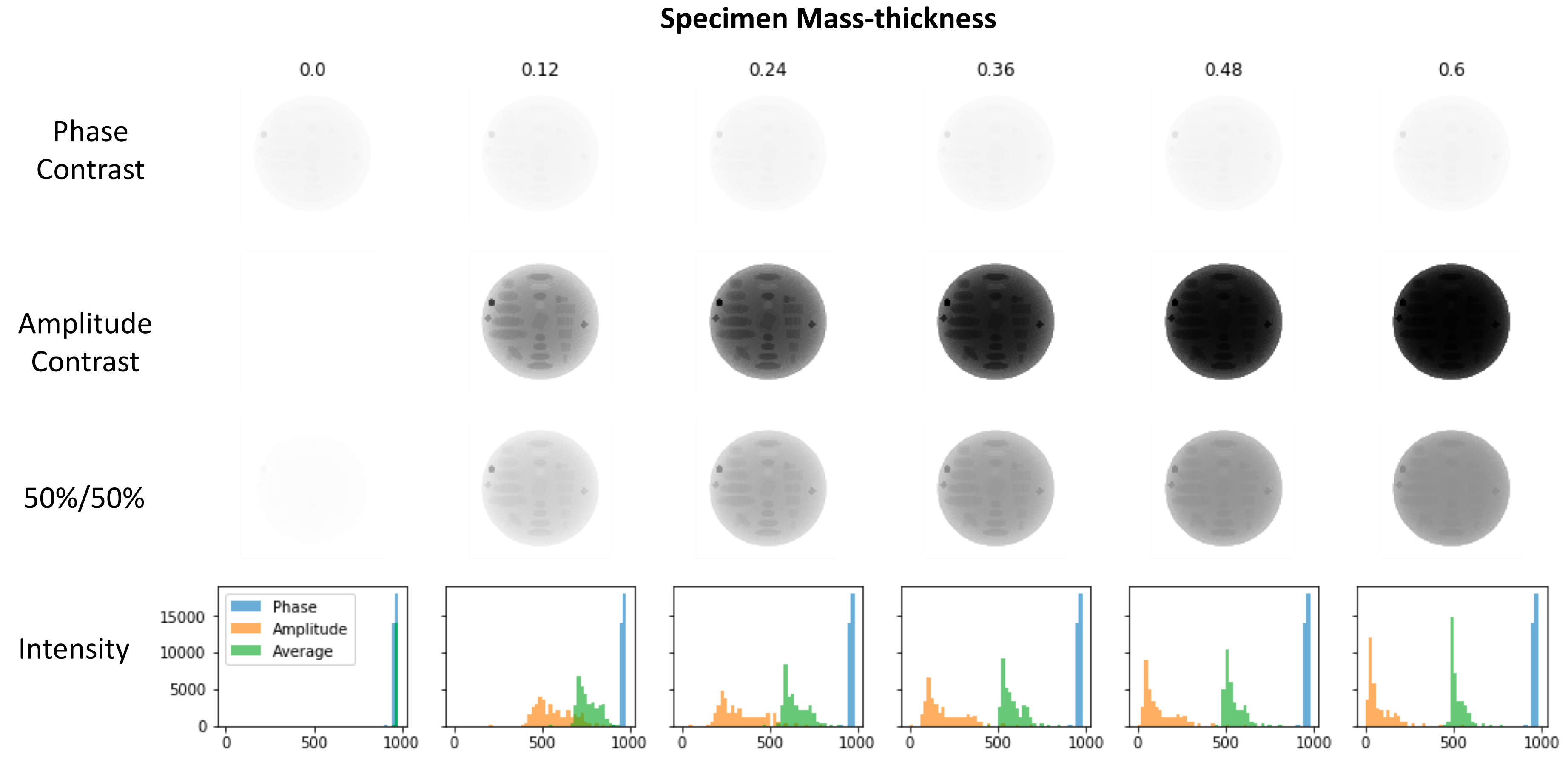
	Transmittance Model	Linear Potential Model
Premise	$-\log\left(\frac{\psi}{\psi_0}\right) = \frac{\mu z}{2} + i\lambda\varphi$	$\varphi = R_{phase} = a * R_{amp} + b$
Correction	$-\frac{\mu z}{2}$	$\min_{a,b} \lVert R_{phase} - a\,R_{amp} + b \rVert^2$
Pros	Accurate physical model describing amplitude and phase effects.	Iterative approach that can be easily computed from the raw data.
Cons	Requires accurate simulation of specimen-beam and lens interactions. Sensitive to detector shot noise and reconstruction artifacts.	Semi-physical. Not robust to alignment errors, specimen motion, and radiation damage. Heavily influenced by CTF and other optical effects.

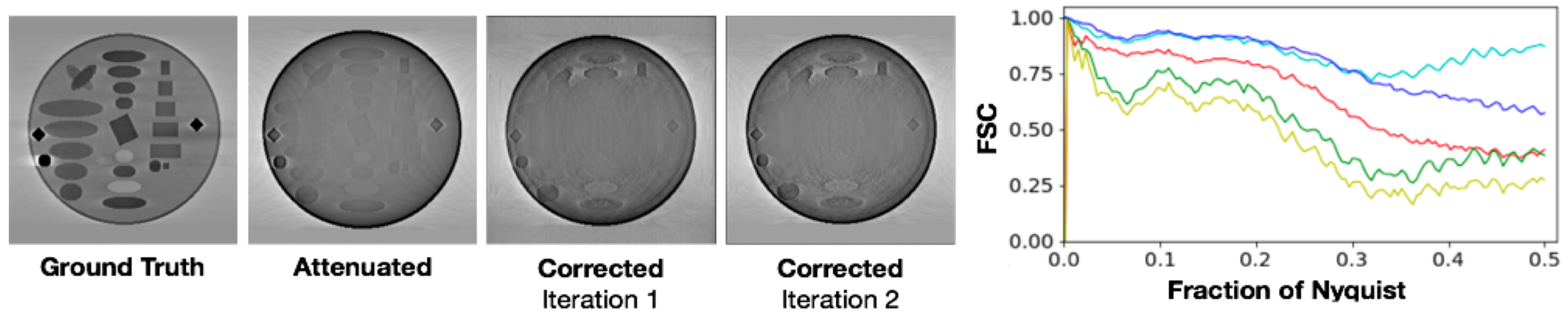
Our initial approach was to simulate the interaction of the electron beam and our sample. This would allow us to represent the specimen as a dispersive medium with a complex index of refraction, $n = \beta\varphi + i\alpha\varphi$. Calculated transmittance, ψ/ψ_0, could be related to the refractive index, allowing us to subtract amplitude attenuating effects. However, further analysis led to the realization that not only would such an approach be highly sensitive to detector noise, but also it would be computationally expensive to perform iterative wave propagation simulations through large tomographic volumes.

Instead, we have devised an alternative strategy. We can think of phase contrast as a linear projection through the sample, and under this assumption, the theoretical outcome of a 3D reconstruction, say R_{phase}, is directly proportional to the electrostatic potential of the specimen, φ(x,y,z). Conversely we can think of amplitude contrast as applying a negative exponential to the incident intensity, which is also related to φ(x,y,z). To obtain such non-linear images, we can calculate the residual between linear projections of a prior reconstruction and raw recorded tilt images. Taking the log and performing a 3D reconstruction, we obtain a volume, R_{amp}, that is linearly proportional to φ(x,y,z). Having two representations of the specimen potential, φ, we can optimize a set of coefficients to linearly relate the volumes. Through averaging and subsequent iterations, we hope to obtain more accurate representations of φ(x,y,z).

Simulations

To examine the influence of absorption on image contrast, we simulated projections with varying levels of absorbance. To accomplish this, phase contrast was treated as a linear sum and subtracted from the incident beam intensity. Absorbance was introduced by multiplying the incident beam intensity by a negative exponential with a decay coefficient proportionate to a linear projection through the specimen. The resulting images were combined with equal weight to simulate the influence of absorption on observed intensity.

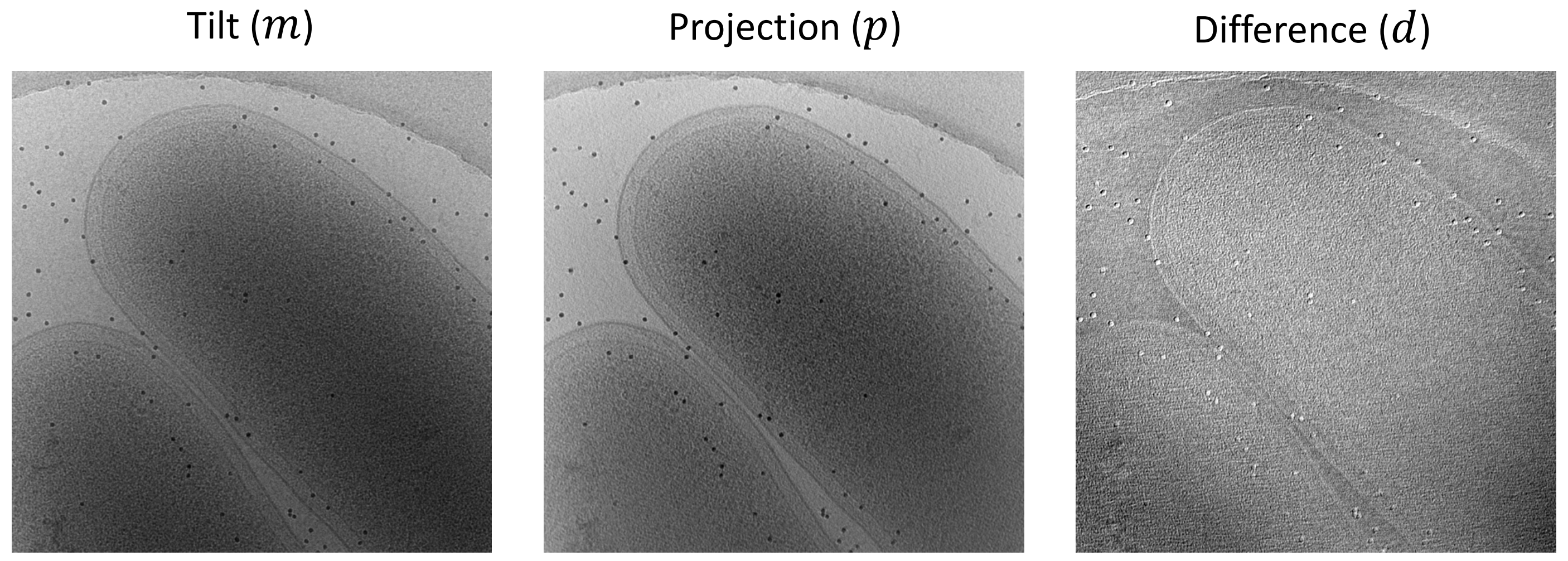


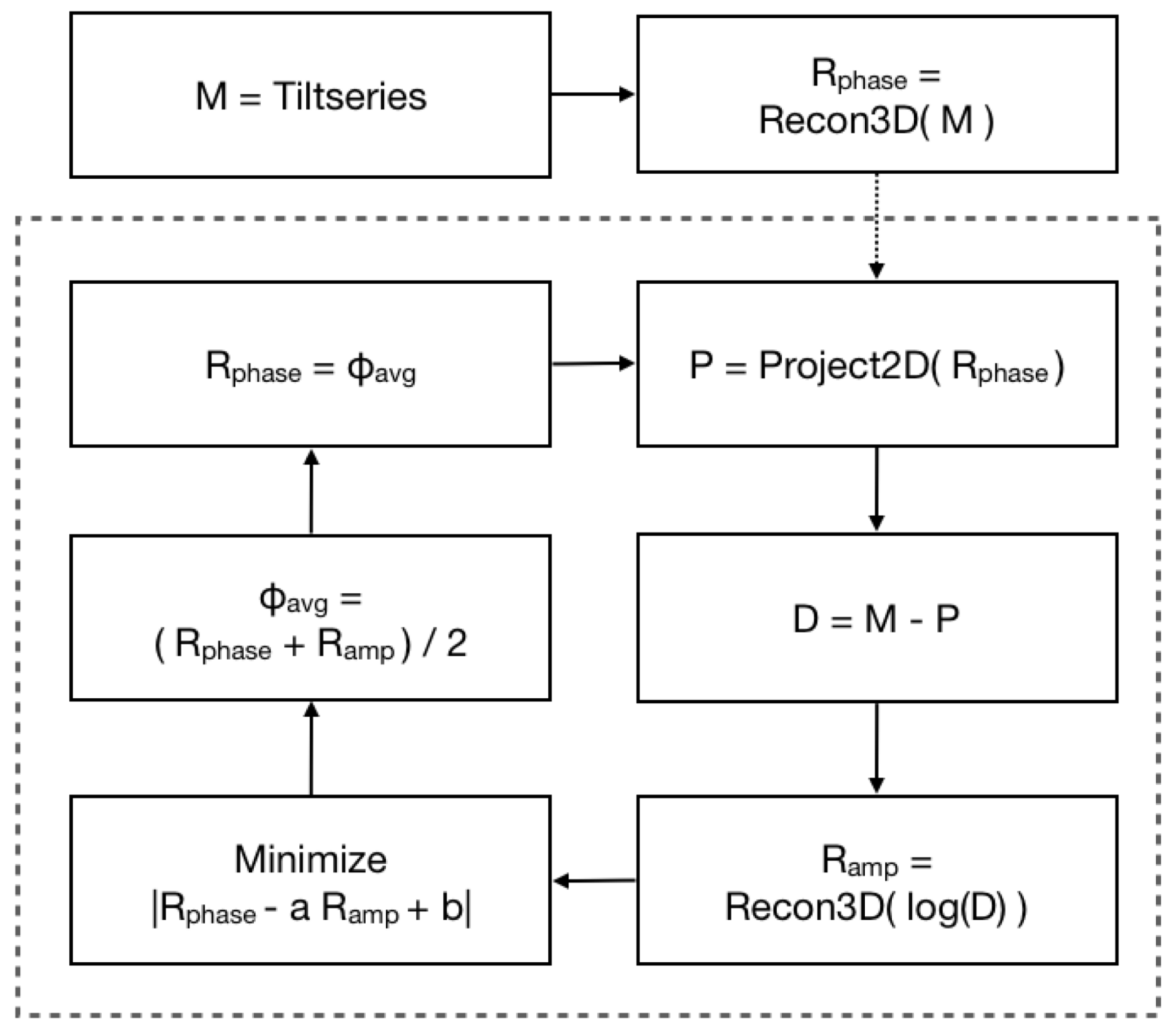


Taking the same phantom volume, we simulated 3D reconstructions without correction and also applied our proposed correction via the algorithm described in panel 6. On the left, we show a central slice through the ground truth reconstruction. Next is the attenuated reconstruction, corresponding to an initial reconstruction from real data. The last two images show results from the first and second iteration of our proposed algorithm. **FSC Legend**: Cyan: Ground Truth Vs Attenuated, Blue: Attenuated Vs Iteration 1, Red: Attenuated Vs Iteration 2, Green: Ground Truth Vs Iteration 1, Yellow: Ground Truth Vs Iteration 2.

Algorithm

Our first-order strategy for recovering lost amplitude is to iteratively maximize the similarity of 3D reconstructions calculated from tilt images and the log-difference between tilt images and 2D re-projections from prior 3D reconstructions. Since these representations of the electrostatic potential of the sample are related by a linear transformation, we propose that averaging optimally transformed volumes will restore a portion of lost amplitude, yielding a 3D volume that more accurately represents the specimen.





M = Tiltseries

R_{phase} = Recon3D(M)

$R_{phase} = \phi_{avg}$

P = Project2D(R_{phase})

D = M - P

R_{amp} = Recon3D(log(D))

Minimize $|R_{phase}$ - a R_{amp} + b|

ϕ_{avg} = (R_{phase} + R_{amp}) / 2

Model:

$$R_{phase} = a\,R_{amp} + b$$

$$R_{phase} = F^{-1}\left[\frac{1}{N}\sum_N T(F[m])\right]$$

$$R_{amp} = F^{-1}\left[\frac{1}{N}\sum_N T(F[\log(m-p)])\right]$$

Objective:

$$\min_{a,b} \lVert R_{phase} - a\,R_{amp} + b \rVert^2$$

R_{phase} and R_{amp} are calculated via direct Fourier insertion following the projection-slice theorem.

Conclusions

Correcting absorbance in cryoET remains a challenging task. Even simulated data presents modeling hurdles to be overcome, and we do not possess a complete understanding of the influence of amplitude contrast in thick cryoET specimens. While our initial strategy has been unsuccessful in restoring lost amplitude in attenuated reconstructions with respect to ground truth, this may be because reconstruction artifacts strongly influence the results obtained from our approach. To address this we will examine the influence of alternate reconstruction algorithms such as SIRT to understand the influence of various types of reconstruction artifacts. We may also explore simpler, geometric cases that introduce fewer artifacts.

Our next step will be to assess the feasibility of performing wave propagation simulations that use quantum mechanics to model the electron beam while the specimen is handled classically. If determined to be a viable approach, we will feed these simulations into the transmittance model discussed in panel 4 and attempt to enhance amplitude contrast in simulated and, eventually, real cryoET data. We anticipate real data to present additional challenges including strong background noise, convolution with the contrast transfer function, and imprecise orientation determination.

References & Acknowledgements

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