

1 **Cryo-TEM simulations of amorphous radiation-sensitive samples using multislice** 2 **wave propagation.**

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7 8 9 **Abstract:**

10
11 Image simulation plays a central role in the development and practice of high-
12 resolution electron microscopy, including transmission electron microscopy of frozen-
13 hydrated specimens (cryo-EM). Simulating images with contrast that matches the
14 contrast observed in experimental images remains challenging, especially for
15 amorphous samples. Current state-of-the-art simulators apply post hoc scaling to
16 approximate empirical solvent contrast, attenuated image intensity due to specimen
17 thickness, and amplitude contrast. This practice fails for images that require spatially
18 variable scaling, e.g., simulations of a crowded or cellular environment. Modeling both
19 the signal and the noise accurately is necessary to simulate images of biological
20 specimens with contrast that is correct on an absolute scale. The “Frozen-Plasmon”
21 method is introduced which explicitly models spatially variable inelastic scattering
22 processes in cryo-EM specimens. This approach produces amplitude contrast that
23 depends on the atomic composition of the specimen, reproduces the total inelastic
24 mean free path as observed experimentally, and allows for the incorporation of radiation
25 damage in the simulation. These improvements are quantified using the matched-filter
26 concept to compare simulation and experiment. The Frozen-Plasmon method, in
27 combination with a new mathematical formulation for accurately sampling the tabulated
28 atomic scattering potentials onto a Cartesian grid, is implemented in the open-source
29 software package *cisTEM*.

30 31 **Introduction:**

32 The power (variance) of the noise in cryo-EM images outweighs the power of the
33 signal, often by a factor of 20 or more. The dominant source of noise in cryo-EM is
34 “shot” noise, arising from the stochastic nature of detecting an electron at a given
35 location and time due to low-dose imaging conditions. A detailed analysis by Baxter et
36 al [1] demonstrated the need to also consider structural noise, defined as any contrast
37 arising from sources other than the final object of interest: carbon film, crystalline ice,
38 radiation damaged particles, unwanted macromolecular conformers, the supporting
39 amorphous ice etc. Unlike shot noise, structural noise is affected by objective lens
40 aberrations, which give rise to the contrast transfer function (CTF). Baxter et al.

41 modelled both the structural noise and the shot noise as additive white Gaussian noise,
42 which fails to capture the artifacts and challenges that are commonly encountered
43 during image processing, as previously demonstrated by Scheres et al [2].

44 An improvement in how the structural noise is simulated, particularly that arising from
45 the supporting amorphous ice, can be found in TEM simulator [3] and inSilicoTEM [4].
46 They implement multislice wave propagation as described originally by Cowley and
47 Moodie [5], resulting in noise that is affected by the CTF. The result of a multislice
48 simulation is a probability distribution defined by the squared complex modulus of the
49 electron wave function at the detector $\psi_d(x, y)$. The simulated image is then formed by
50 drawing from a Poisson distribution unique to every pixel while incorporating the
51 influence of the detector quantum efficiency (DQE).

52 Most of the information transferred from the specimen to the image in high-resolution
53 cryo-EM is captured in phase contrast arising from interference between elastically
54 scattered and non-scattered electrons. Amplitude contrast is also present due to
55 electrons scattered outside the objective lens aperture, and loss of electrons from the
56 elastic image due to inelastic scattering. The latter source of amplitude contrast is
57 enhanced using an energy filter [6]. Amplitude contrast cannot be explained by linear
58 image formation theory [7] and is accounted for post hoc via a phase shift term added to
59 the CTF applied to the simulated image [8]. This treatment is also common practice in
60 solving the inverse problem of image reconstruction, which seeks to answer the
61 question "*what is the probability of the model given the observed data.*" However, in
62 forward modeling, which asks "*what is the probability of observing some data given a*
63 *particular model,*" it would be desirable to account for the fact that amplitude losses
64 depend both on atom type, and on local mass thickness. For example, heavy atoms like
65 gold scatter more electrons outside the objective lens aperture than light atoms. On the
66 other hand, light atoms produce more amplitude losses in energy filtered images than
67 heavy atoms due to their higher ratio of inelastic to elastic scattering [9][10].

68 The multislice formalism is essential for thick specimens where the projection
69 approximation fails, as it incorporates important effects like multiple scattering of
70 electrons and the curvature of the Ewald Sphere. Increasingly thick samples are also
71 less transparent to electrons, and all simulators we are aware of apply an implicit
72 "energy filter" to remove inelastically scattered electrons from the final image. To
73 account for inelastic losses, a single thickness parameter is used to attenuate the image
74 intensity according to

75
$$\frac{I}{I_0} = \exp\left[\frac{-Thickness}{\lambda}\right] \quad (1)$$

76 where I_0 is the unattenuated image intensity, and λ is the inelastic mean free path for
77 single scattering - the average distance an electron passes through the specimen
78 before scattered inelastically at least once. It is clear that these single filters cannot
79 work for specimens with variable mass thickness, *e.g.*, at the edge of a cell, for variable
80 atomic composition, *e.g.*, the increased phosphorus concentration in the nucleus, and
81 even for many single particle samples [11].

82 Even with a limited subset of atomic species, to which we will constrain the following
83 discussion, there are two very different environments that need to be simulated - the
84 molecule and the solvent. We will refer to how well the molecule stands out from the
85 solvent as “solvent signal-to-noise-ratio or $SNR_{solvent}$ ” as quantified by Yonekura et al
86 [6] where \bar{I} is the mean image intensity and $\sigma_{solvent}$ is the standard deviation in the
87 solvent region.

88

$$89 \quad SNR_{solvent} \equiv \frac{|\bar{I}_{protein} - \bar{I}_{solvent}|}{\sigma_{solvent}} \quad (2)$$

90 Typically, the solvent is added on top of the simulated molecules in projection, with a
91 single value given by the mean inner potential for aqueous water. This approach, which
92 we will refer to as “the continuum model”, is equivalent to using an infinite time average
93 of a collection of moving water atoms. One shortcoming of the continuum model is the
94 failure to account for the hydration radius of a molecule, which should be zero inside a
95 particle, higher than the bulk solvent immediately outside the particle envelope and
96 gradually falling off with distance [12]. Ignoring the fact that molecules displace the
97 solvent has been shown to produce $SNR_{solvent}$ that fails even visual inspection at
98 exposures of $100 \text{ e}^-/\text{\AA}^2$ [4].

99 We now know that the infinite time average used in the continuum model does not
100 adequately describe reality; even though the solvent is frozen low-density amorphous
101 ice (LDA), it is not static during the imaging process. McMullan and Henderson
102 quantified the motion of water molecules in LDA during imaging, estimating a RMSD of
103 $\sim \frac{1 \text{\AA}}{\text{e}^-/\text{\AA}^2}$ [13]. Importantly, this motion results in a blurring of the solvent over time, which
104 can be thought of as low pass filtering, and so $\sigma_{solvent}$ decreases with increasing
105 exposure. The net result is that $SNR_{solvent}$ is a function of the total exposure in an
106 image, gradually increasing as the solvent becomes more blurred. Of note, the increase
107 of $SNR_{solvent}$ with exposure is further amplified in experimental images by mass loss,
108 which also decreases $\sigma_{solvent}$ and increases the numerator in Equation 2 by reducing
109 $\bar{I}_{solvent}$. A more sophisticated version of our solvent model may implement this mass
110 loss in future work.

111 While $SNR_{solvent}$ is useful for its simplicity, a more detailed analysis requires another
112 metric to quantify how well the macromolecules we simulate match experimental
113 images. For this, we propose using the matched filter, which is the statistically optimal
114 realization of a cross-correlation detector. With image statistics characteristic of cryo-
115 EM data, the output of the matched filter can be simply defined as the ratio of the cross-
116 correlation coefficient (CCC) to the standard deviation of the CCC when only noise is
117 present (σ_n) [14] including any sources of structural noise as defined above.

$$118 \quad SNR_{mf} = \frac{CCC}{\sigma_n} \quad (3)$$

119 The upper bound on the SNR_{mf} is given by the ratio of the power of the input signal to
120 the power of the noise in the image [15]. This means, for example, that a larger
121 molecule will generally have a higher SNR_{mf} , while any disagreement between the
122 signal in the image and the simulated template reduces the SNR_{mf} from this maximal
123 value. As such, the relative accuracy to which the simulated molecular density matches
124 experimental data can be determined by searching images using a matched filter. To
125 evaluate Equation 3, we use the cross-correlation tools and relevant preprocessing as
126 available in *cisTEM* [16][17].

127

128 To understand why the continuum model fails to produce accurate $SNR_{solvent}$ it is
129 instructive to consider the source of the motion of the solvent upon exposure to the
130 imaging electrons. Along with radiation damage to the molecule of interest, sample
131 motion is the result of energy being transferred to the specimen via inelastic scattering.
132 For the samples in which we are interested, inelastic scattering is generally attributed to
133 plasmons, i.e., collective excitation of valence electrons by the electric field of the
134 imaging electrons. However, the extent to which these are bulk plasmons, which are
135 strongly delocalized, or more localized single-electron excitations remains unclear [18].
136 Independent of the exact form of the plasmons, their net effect is an alteration of the
137 system's Hamiltonian during imaging, such that product of a traditional multislice
138 simulation, $\psi_d(x, y)$, is no longer valid. Just as the original multislice method introduced
139 a division of the specimen potential into thin spatial slices to ensure the small angle
140 approximation is valid, we suggest dividing the simulated exposure into small temporal
141 slices, where the specimen does not change too much. While we refer to time here,
142 what is most practical from the microscopist's point of view is exposure measured in
143 $e^-/\text{\AA}^2$. Therefore, the time step in our simulator is specified as the desired exposure per
144 movie frame. Exposure rate dependent phenomena like detector DQE and beam
145 coherence are parameterized by an exposure rate with the exposure time implicitly set
146 by the software according to the user supplied exposure-per-frame divided by the

147 exposure rate. The DQE parameterization is based on the results of Ruskin et al [19]
148 while the coherence is parameterized based on the beam brightness of an Xfeg as
149 given in the Titan Condenser Manual [20].

150

151 **Theory:**

152 There are three main components in modeling the image formation process in high-
153 resolution transmission electron microscopy (HRTEM) of which cryo-EM is a subset:

- 154 1. The relativistic electron wave function and its modulation by the sample.
- 155 2. The exposure-dependent Coulomb potential of the specimen.
- 156 3. The microscope, including apertures, detector, lens optics and aberrations.

157 In this work, we are concerned primarily with how the Coulomb potential changes due to
158 energy being deposited in the specimen during imaging and will provide only a brief
159 summary of the other two components. The interested reader is referred to, in
160 increasing order of completeness, the treatments by Vulović et al [21], Kirkland [22],
161 Reimer and Kohl [23] Hawkes and Kasper [24].

162 Unlike photons, electrons have a spin quantum number and so their interaction with
163 matter is governed by solutions to the Dirac equation. Given reasonable approximations
164 [24], a relativistically corrected version of the Schrödinger wave equation, called the
165 Klein-Gordon equation is used in practice.

$$166 \quad \left[-\frac{\hbar^2}{2m} \nabla^2 + \mathcal{V}(\mathbf{r}) \right] \psi(\mathbf{r}) = E \left(\frac{m_0}{m} \right) \left[1 + \frac{E}{2m_0 c^2} \right] \psi(\mathbf{r}) \quad (3)$$

167 Analytical solutions to this equation are intractable for all but the simplest systems, so
168 we turn to multislice wave propagation [25], which produces an approximate numerical
169 solution to this equation. The first step in a multislice simulation is the calculation of the
170 specimen's Coulomb potential $\mathcal{V}(\mathbf{r}; t)$. The time dependence will be omitted assuming a
171 quasi-stationary solution for an exposure to a single electron. The potential is divided
172 into thin slices along the imaging axis, which can be approximated by two-dimensional
173 scattering potentials through which the electron wave function is sequentially
174 propagated. This subdivision ensures the potential varies slowly in the direction of the
175 electron wave propagation, such that the small angle approximation remains valid and
176 scattered spherical wave fronts may be approximated locally by a parabola (Fresnel
177 diffraction.) In the limit of infinitely thin slices, this results in an exact numerical solution
178 to the Klein-Gordon equation [26].

179 Multislice simulations can model both elastic and inelastic scattering processes,
180 provided that the respective Coulomb potentials can be calculated. In analogy to the
181 optical potential in light microscopy, inelastic scattering is incorporated into the wave
182 theory via a complex term in the specimen potential as introduced by J.C. Slater in 1937
183 [27].

$$184 \quad \mathcal{V}(\mathbf{r}) = \mathcal{V}(\mathbf{r})_{elastic} + i\mathcal{V}(\mathbf{r})_{inelastic} \quad (4)$$

185 The isolated atom superposition approximation states that the specimen potential $\mathcal{V}(\mathbf{r})$
186 may be represented as the sum of the individual atomic potentials $\varphi(\mathbf{r})_i$. We introduce a
187 scaling factor β to compensate for the contribution of bonds among those atoms to
188 maintain the correct total scattering cross section:

$$189 \quad \mathcal{V}(\mathbf{r}) \cong \beta \sum \varphi(\mathbf{r})_i \quad (5)$$

190 The elastic atomic potential can be calculated using relativistic Hartree-Fock wave
191 functions [28]. The solutions for isolated atoms, having isotropic distributions, are
192 commonly parameterized by a sum of four or five Gaussian functions [29]. Typically, the
193 potential is recorded indirectly as these fits are tabulated as elastic electron scattering
194 factors, defined as the Fourier transform of the elastic potential [30].

$$195 \quad f^{(e)}(\theta) = \frac{2\pi me}{h^2} \mathcal{F}\{\varphi(\mathbf{r})\} \quad (6)$$

196 The numerator describes the product of the electron charge and relativistic mass, h is
197 the Planck constant, and \mathcal{F} denotes the Fourier transform operator. An important
198 relation we will return to later equates the spectral distribution of the scattering factor to
199 the differential scattering cross section - the probability of an electron being scattered
200 through a solid angle Ω :

$$201 \quad \frac{d\sigma}{d\Omega} = |f^{(e)}(\theta)|^2 \quad (7)$$

202 While $\mathcal{V}(\mathbf{r})_{elastic}$ is straightforward to calculate from first principles, $\mathcal{V}(\mathbf{r})_{inelastic}$ is more
203 problematic given the varied mechanisms with which an incident electron may transfer
204 energy to the specimen: ionization, excitation, dissociative attachment, vibrational and
205 rotational excitations, bremsstrahlung, etc. [31]. One example where $\mathcal{V}(\mathbf{r})_{inelastic}$ is well
206 defined is for radiation-insensitive crystalline specimens, where thermal diffuse
207 scattering (TDS) caused by phonon excitation is the primary contributor to the complex
208 potential [29]. One model to calculate the TDS potential treats the time average atomic
209 displacement through Debye-Waller factors and improves the accuracy of dynamic
210 RHEED calculations [32]. This time average approach is analogous to how the solvent

211 calculation, specimen motion, radiation damage and alignment errors are accounted for
212 in HRTEM simulations of biological specimens by B-factors, which are related to Debye-
213 Waller factors by a factor of 4.

214 While this time-averaged approach preserves the total intensity of the projected
215 interaction potential [33], it is well known that the image contrast produced in this way is
216 systematically wrong, often by a factor of three or more. The error, known as the Stobbs
217 factor [34], becomes worse with an increasing strength of the electron specimen
218 interaction, which in turn, depends on the average mass thickness in the specimen.
219 Stobbs et al. proposed two likely causes for the observed contrast mismatch between
220 simulation and observation: a) existing simulators do not account properly for radiation
221 damage to the specimen, and/or b) they fail to model the inelastic scattering with
222 sufficient accuracy. As recently shown empirically, these are related phenomena [35].

223 Van Dyck et al. demonstrated that the Stobbs factor could be largely corrected for by
224 using the "frozen phonon" method [36][37]. The approach is conceptually simple: A
225 series of simulations are carried out where each atom is displaced randomly based on
226 empirical TDS values. The intensities in the image plane as calculated from these
227 individual simulations are then averaged together. Here we propose a similar idea,
228 applied to radiation-sensitive frozen-hydrated specimens, where plasmons are the
229 primary form of inelastic scattering. The "frozen plasmon method" presents several
230 computational and theoretical challenges:

- 231 1. The number of solvent atoms ($O(10^9)$) greatly outweighs those of the
232 macromolecules we wish to simulate ($O(10^5)$), requiring careful algorithmic
233 design to make the computations tractable.
234
- 235 2. The solvent and macromolecules have very different elastic and inelastic total
236 scattering cross-sections, as well as different average mass densities (~ 0.94
237 g/cm^3 for low-density amorphous ice and $\sim 1.38 \text{ g/cm}^3$ on average for proteins)
238 [38]. This means that the amplitude contrast and inelastic losses cannot be
239 applied ad hoc to the final simulated image and must be considered on a per-
240 atom basis.
241
- 242 3. The preceding points also place a requirement on the accuracy of the calculation
243 of each atomic scattering potential, which can no longer simply be rescaled and
244 so must be correct from the start.
245
- 246 4. The scattering factor for plasmons in low-density amorphous ice is needed to
247 achieve the appropriate contrast, which depends on the appropriate spectral
248 distribution. To obtain an expression $\phi^{(\text{inelastic})}(\mathbf{r})_i$, we start from the double

249 differential scattering cross-section for plasmons [39]. The essential form is
250 Lorentzian

$$251 \quad \frac{d^2\sigma}{d\Omega dE} \propto \frac{1}{\theta^2 + \theta_E^2} \quad (8)$$

252 with the angular dependence θ and the energy dependence captured in the
253 characteristic angle

$$254 \quad \theta_E = \frac{m\omega_{plasmon}}{\hbar k_0^2} \quad (9)$$

255 $\hbar\omega_{plasmon}$ is the energy loss of the plasmon, and k_0 the incident electron's wave
256 vector. To calculate a scattering factor for plasmons, we first form an empirical
257 probability distribution for plasmons arising from singly scattered electrons in
258 amorphous ice, derived from EELS published by Du and Jacobsen [40]. We then
259 numerically integrate Equation 8 over energies in the low-loss spectrum (7.5 -
260 100 eV) for each angle.

261 We then combine this spectrum with empirical measurements of the ratio of
262 inelastic to elastic total scattering cross sections, which are inversely proportional
263 to atomic molecular weight [10]. As we calculate the elastic potential during
264 simulation, we separately accumulate an inelastic potential scaled per atom by
265 these total probabilities. During wave function propagation, this inelastic potential
266 is given the correct Lorentzian form, taken to be the square root of the values
267 above.

268 Plasmons scatter strongly at low angles and are generally referred to as being
269 delocalized. This is reflected in Figure 1 where the inelastic scattering factor we derived
270 for plasmons is compared to the elastic scattering factor for a glutamine molecule. While
271 plasmon scattering dominates at low resolution compared to elastic scattering, it still
272 contributes significantly at high angles as can be seen by the red hash marks in Figure
273 1 that demarcate bins of 20% total inelastic scattering probability. The precise nature of
274 inelastic scattering in amorphous materials is not well understood, such that the
275 relationship between this high-resolution information and the underlying specimen
276 structure is not defined.

277

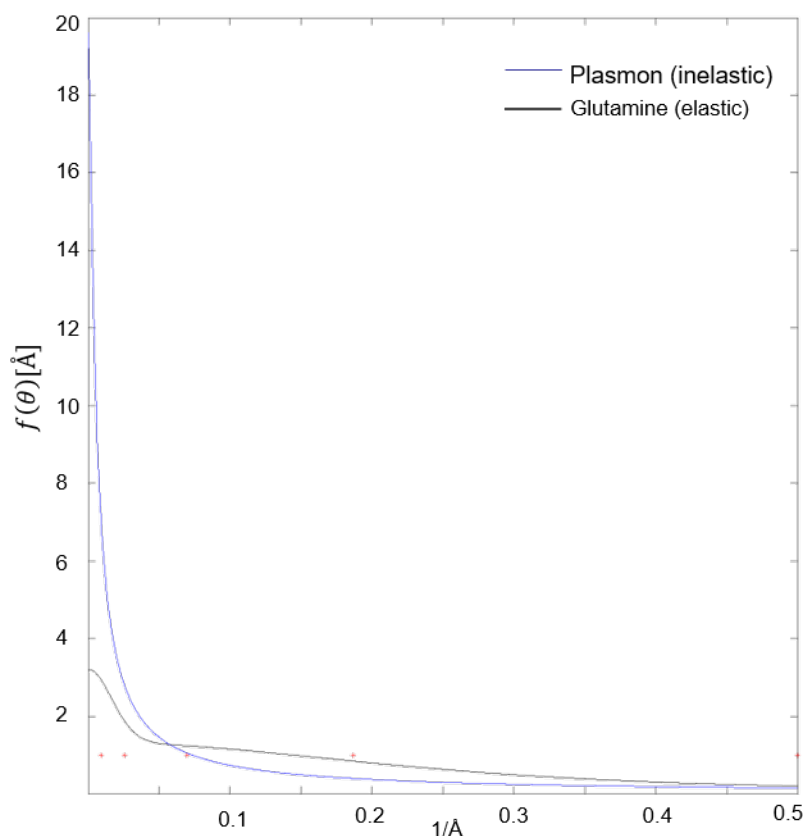


Figure 1 – Comparison of elastic scattering factor for a glutamine molecule (black line) vs. the inelastic scattering factor for a plasmon pseudo-particle (blue line.) Red markers indicate the right edge of bins comprising 20% total scattering probability for the inelastic Plasmon scattering factor as calculated from the squared norm of the scattering factor.

278
279

280 **Results:**

281 *Accurate representation of molecular density:*

282 For isolated neutral atoms, the scattering potential, defined as the Fourier transform of
283 the parameterized scattering factors, can be written as

$$284 \quad \phi(\mathbf{r}) = \frac{\hbar^2}{2\pi m_0 e} \sum_i a_i \left(\frac{4\pi}{b_i + B} \right)^{\frac{3}{2}} \exp \left[\frac{-4\pi^2((x - x_0)^2 + (y - y_0)^2 + (z - z_0)^2)}{b_i + B} \right] \quad (10)$$

285 This atomic potential is sharply peaked in real space, requiring a high sampling rate
286 when discretizing in order to maintain the total projected potential. This high sampling

287 rate effectively produces a numerical integration of Equation 10. To allow for coarser
288 sampling, and hence improve the efficiency of our simulator, we analytically integrate
289 the expression from Equation 10.

290

$$291 \quad \phi(\mathbf{r}) = \frac{\hbar^2}{2\pi m_0 e} \sum_i a_i \left(\frac{4\pi}{b_i + B} \right)^{\frac{3}{2}} \prod_j \int_0^\infty dx_j \exp \left[\frac{-4\pi^2 (x_j - x_{j0})^2}{b_i + B} \right] \quad (11)$$

292 resulting in

$$293 \quad \phi(\mathbf{r}) = \frac{\hbar^2}{2\pi m_0 e} \sum_i a_i \prod_j \operatorname{erf} \left[\frac{2\pi (x_j - x_{j0})^2}{\sqrt{b_i + B}} \right] \quad (12)$$

294

295 While the potential in each voxel is marginally more complex to calculate (to evaluate
296 the limits of integration, the error function must be evaluated six times per voxel,
297 compared to a single exponential) this is more than compensated by the reduced
298 number of voxels needed. For example, simulating at 0.5 Å voxel pitch is 125x less
299 computationally expensive than simulating at 0.1 Å voxel pitch. While the voxel pitch is
300 the same in the z-dimension, the slab thickness is a free parameter which also affects
301 computational efficiency. A simple test to determine the maximum allowable thickness,
302 as suggested by Kirkland [22], is to search for the point where the results of the
303 simulation become dependent on slab thickness. Our simulations begin to show a
304 dependence on slab thickness around 7 Å (data not shown) and, therefore, we typically
305 use 5 Å. Even more important than computational speed, using Equation 12 in our
306 simulations also means the sampled potential still has the correct magnitude and is not
307 simply proportional to the continuous potential, as discussed in the following section.

308

309 *Compensating for the isolated atom superposition approximation:*

310 While the integral formulation of the scattering potential preserves the calculated
311 potential of all the individual atomic contributions, there is still a systematic
312 underestimation of the scattering potential due to bonding interactions. This is generally
313 estimated to be between 5-10% of the total potential [41], and ignoring this difference is
314 referred to as the isolated atom superposition approximation. Given that we want to
315 obtain images that are quantitative on an absolute scale, we sought to measure and
316 calibrate this error. To approximate the redistribution of the scattering potential due to
317 bonding in a biological specimen, we use the available data for amorphous carbon

318 comparing to results from electron holography as follows. The average phase shift in a
319 material depends on the mean inner potential of the material (V_0), the thickness (t), and
320 an interaction constant C_E [23].

$$321 \quad C_E = \frac{2\pi}{\lambda} \frac{E_0 + E}{2EE_0 + E^2} \quad (13)$$

322 Additionally, surface boundary effects are also known to be important in cryo-EM
323 imaging, so we compared our calculated phase shift ($\delta\varphi$) to empirical results obtained
324 using electron holography, which measures both the mean inner potential of carbon
325 ($V_0 = 9.04\text{eV}$ for $1.75 \frac{\text{g}}{\text{cm}^3}$ density) and an additional thickness-independent surface-
326 induced phase shift φ_{add} (0.497 radians)[42].

$$327 \quad \delta\varphi = C_E V_0 t + \varphi_{add} \quad (14)$$

328 Considering the principle of a Zernike phase plate, we simulated an amorphous carbon
329 sheet that should produce a phase shift of $\pi/2$ radians (Figure 2A) with a density of
330 $1.75 \frac{\text{g}}{\text{cm}^3}$ and 348.6 \AA thickness per Equation 14. Our simulation suggested that the
331 average phase shift is $\sim 3.8\%$ too small. To correct for this error, we introduce a constant
332 scaling factor (β) of 1.038 to the isolated atomic potentials. The simulated phase plate
333 also serves as a sanity check that the calculation of the elastic scattering potential is
334 consistent across different pixel sizes (Figure 2B).

335 +

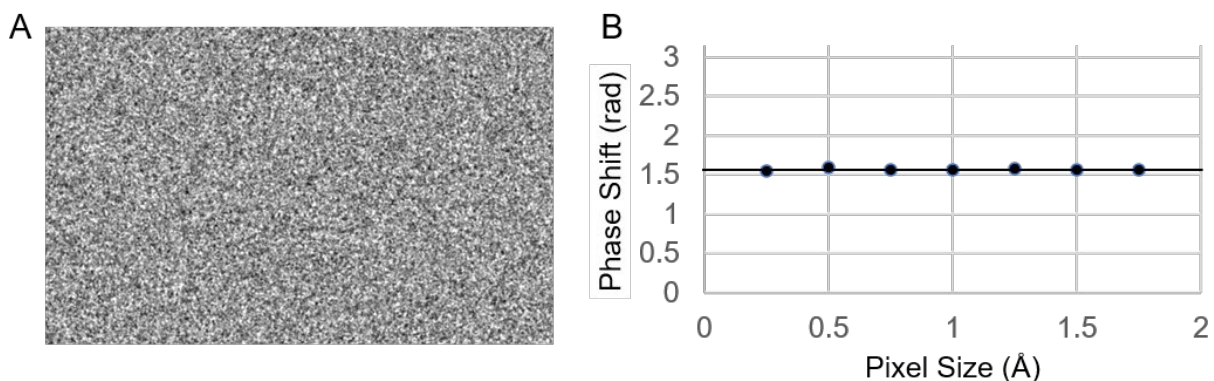


Figure 2 –A) “phase plate” simulated from amorphous layer of carbon atoms 348.6\AA thick with a density of 1.75 g/cm^3 B) Mean phase shift for the simulated phase plate as a function of pixel sampling rate during simulation. Black line plotted at $\pi/2$ radians for visual reference.

336

337

338

339 *Modeling the solvent envelope*

340 In the previous section, we demonstrated that we could accurately calculate the contrast
341 for a collection of randomly distributed atoms of a given density. Next, we show that in
342 order to accurately compare a simulated protein density to experimental data, we must
343 also consider the solvent displaced by the protein. This creates a low-resolution “hole”
344 that impacts subsequent analysis as discussed in detail by Zhang and Sigworth [12].
345 We incorporate their hydration radius model into the simulator by tracking the smallest
346 distance to any non-solvent molecule and weighting any nearby solvent with a
347 probability distribution defined by normalizing Equation 1 from their paper. (We note that
348 the parameter “r3” in table 1 of Zhang and Sigworth should be ~3.0, not 1.7, personal
349 communication.)

350 When simulating isolated molecules to use for comparison to experimental images, we
351 weight the average water potential by this probability distribution, with an exponential
352 decay beyond 4 Å. This exponential decay is added because our knowledge of the
353 sample rapidly decays to zero beyond the particle of interest. This produces an effect
354 similar to the ad hoc model suggested previously by Henderson and McMullan [43].
355 When simulating images, the probability distribution is applied to individual pseudo-
356 water molecules as described next.

357

358

359 *Modeling radiation damage*

360 As soon as the electron beam is “switched on” the sample begins to accumulate
361 radiation damage. This has long been known to be *the* limiting factor in cryo-EM [44]
362 and an analytical function describing the effects of radiation damage as a Fourier space
363 filter - $\xi(\mathbf{q})$ - was described by Grant and Grigorieff [45]. Since radiation damage is
364 specimen dependent, the analytical model of Grant and Grigorieff will only strictly apply
365 to rotavirus VP6 capsid protein, and not to nucleic acids, for example. Alternatively,
366 radiation damage combined with other errors, for example uncorrected motion blur, may
367 be fit using exposure-dependent B-factors [46][47]. The latter approach has the
368 advantage that it does not try to separate the blurring due to radiation damage from
369 other sources of blurring, which can be difficult in practice.

370 To quantify the accuracy in modeling radiation damage using the analytical model, $\xi(\mathbf{q})$,
371 we employ the matched filter concept which is sensitive to the spectral distribution of the
372 template when the noise does not have a flat power spectrum. Using the rotavirus DLP
373 images originally used by Grant & Grigorieff to derive their radiation damage model, we
374 investigated several sources of such noise including the water shell, as described in the

375 next section, the detector modulation transfer function (MTF), CTF coherence envelope,
376 residual intra-frame motion, atomic modeling uncertainty and defocus uncertainty.

377 By looking at a single early frame from each of 18 movies, each with $\sim 1.5 e^-/\text{\AA}^2$ total
378 exposure ($0.77 e^-/\text{\AA}^2$ intra frame exposure), we could quantify how well we modelled
379 these sources of noise, while assuming no significant radiation damage. In Figure 3A
380 we show the projected scattering potential of our simulated DLP (PDB 3gzv) along with
381 the average SNR_{mf} from 180 DLPs overlaid. Each source of noise is included
382 cumulatively, showing the relative improvement in detection as the model becomes
383 increasingly more detailed. Applying additional positive or negative B-factors only made
384 the score worse. To illustrate the effect of each of these modifications on the template's
385 spectral distribution, we also plot the ratio of the rotationally averaged power spectra of
386 each simulation to the previous one. A representative image for frame 2 is shown in
387 Figure 3B, while an average of frames 2-91 is shown in Figure 3C.

388 To assess the radiation damage model, we averaged movie frames 2-N such that the
389 accumulated exposure ranged from $10 - 100 e^-/\text{\AA}^2$ either with, or without, exposure
390 filtering applied to the images. We then measured the SNR_{mf} of the DLPs in these two
391 sets of images using two sets of references calculated with, and without, exposure
392 filtering applied during simulation, and plotted the results in Figure 3D. We found the
393 largest increase in SNR_{mf} using a total exposure of $50 e^-/\text{\AA}^2$ when applying the
394 exposure filter to both the image and during simulation of the reference. To account for
395 blurring due to residual intra-frame specimen motion in this experiment, the exposure
396 filter was additionally modified to include a damping envelop which, for uniform motion,
397 is trivial to show is a sinc function. Less trivial is determining the intra-frame specimen
398 motion (\mathbf{d}_i) for which we only know a lower bound, estimated as the average of the
399 displacements between the two neighboring frames, and applied as a sinc modulation
400 along with the exposure filter (if included) for that frame.

401
$$\frac{1}{N-1} \sum_{i=2}^N \text{sinc}(\mathbf{q} \cdot \mathbf{d}_i) \xi(\mathbf{q})_i \quad (15)$$

402 Taken together, these results suggest that other modifications to the template that result
403 in a better match to the experimental data can further improve the SNR_{mf} ; for example,
404 some amino acid side chains are affected more strongly by radiation damage than
405 others, e.g., aspartate and the disulfide bond of cystine. These details could be
406 incorporated into a new atom specific damage model in future work.

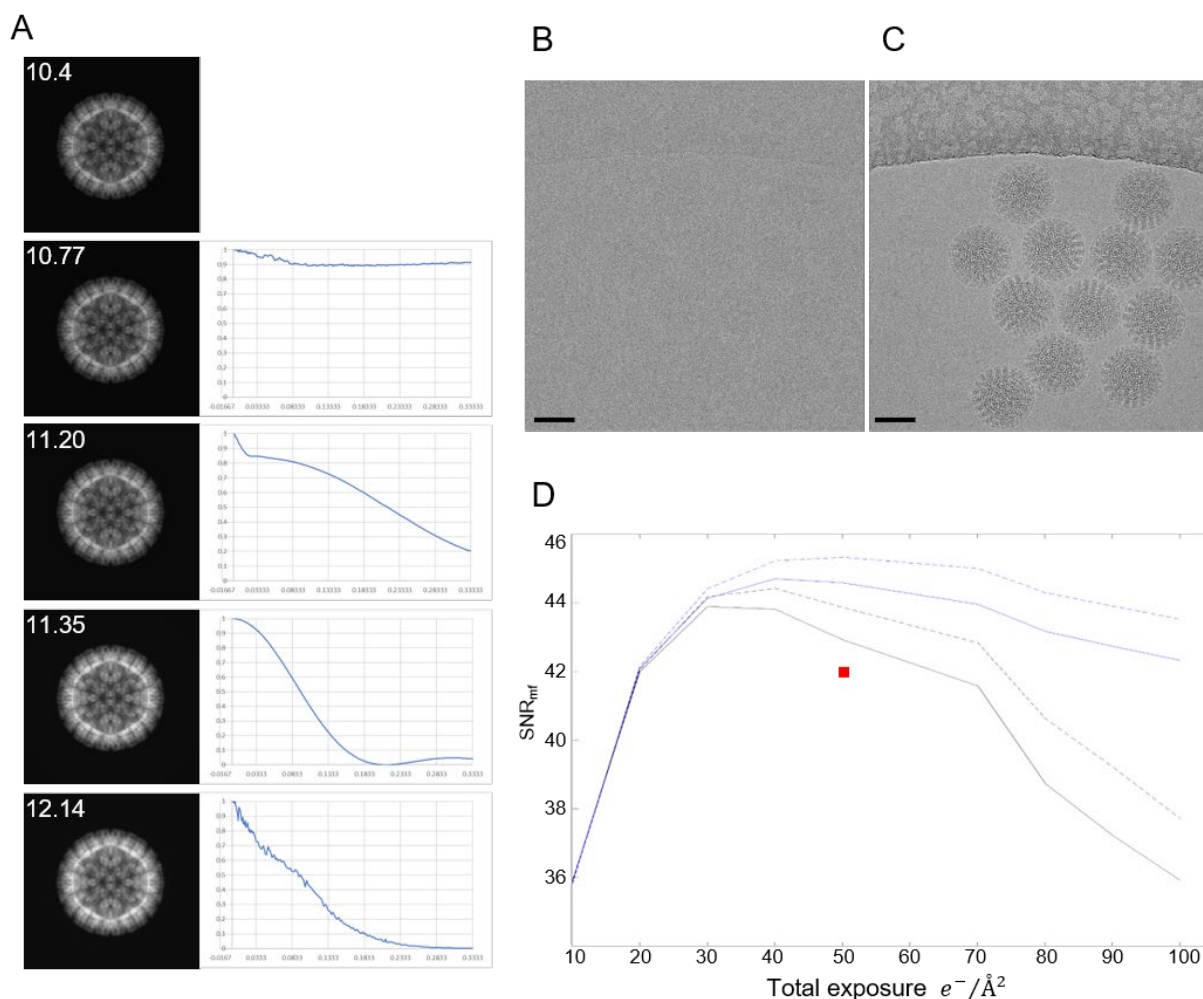


Figure 3 – A) Left column, projections of simulated rotavirus DLPs from PDB-3gzu with each of the blurring process applied cumulatively. Average peak SNR_{mf} from 180 DLPs over 18 images overlaid in white. Right column, the shape of the individual envelopes produced by the respective blurring as calculated by dividing the rotationally averaged power spectrum by the preceding image. B) Example image from frame 2 and C) frames 2-91 of DLPs from the data set used in this analysis, kindly shared by Dr. Tim Grant. D) Average SNR values as a function of total exposure, frames averaged from 2-N, where N is on the total exposure on the horizontal axis. Solid black line, no filtering. Dashed black line, image summed from exposure weighted movie frames. Solid blue line, reference simulated with cumulative exposure filter as would normally be applied to a movie. Dashed blue line, both image and reference exposure filtered. Red square is the maximum SNR obtained by using a single B-factor to represent all the envelopes in (A). Scale bars 500 Å.

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411 *Accurate representation of solvent noise:*

412 Because the number of water molecules is very large, we elected to calculate a coarse-
413 grained model for water, where each water molecule is represented as a single,
414 isotropic scattering center. We based the scattering factor for our pseudo-waters on the
415 elastic scattering factor tabulated for oxygen, but scaled by the ratio of the total elastic
416 scattering cross-section of oxygen:water, which we know from experiment [31]. These
417 pseudo-molecules are seeded randomly at the proper density for low density
418 amorphous ice ($\sim 0.94\text{g/cm}^3$). A movie is then simulated, where each time step (movie
419 frame) is defined by a user-specified exposure and the specimen is held constant within
420 that time. The simulated probability density for the constant potential is shown in Figure
421 4A while our coarse-grained all-atom model is shown in Figure 4B. The average
422 intensity in the solvent region is the same in both images.

423 In Figure 4D we show selected time points from a movie simulated using the continuous
424 solvent potential model (top row), the coarse-grained all-atom model (middle row), and
425 experimental data in the bottom row (EMPIAR-10061 [48]). As can be seen visually, the
426 $SNR_{solvent}$ is stronger for the continuum model, because the potential only has a DC
427 component. To quantify this effect, we calculated $SNR_{solvent}$ as in Equation 2, defining
428 the solvent region by the white portion of the mask in Figure 4C and the protein as the
429 central black region. The results are plotted in Figure 4F where the final $SNR_{solvent}$ is
430 about a factor of two too strong using the continuum model, while our model closely
431 matches that of experimental data.

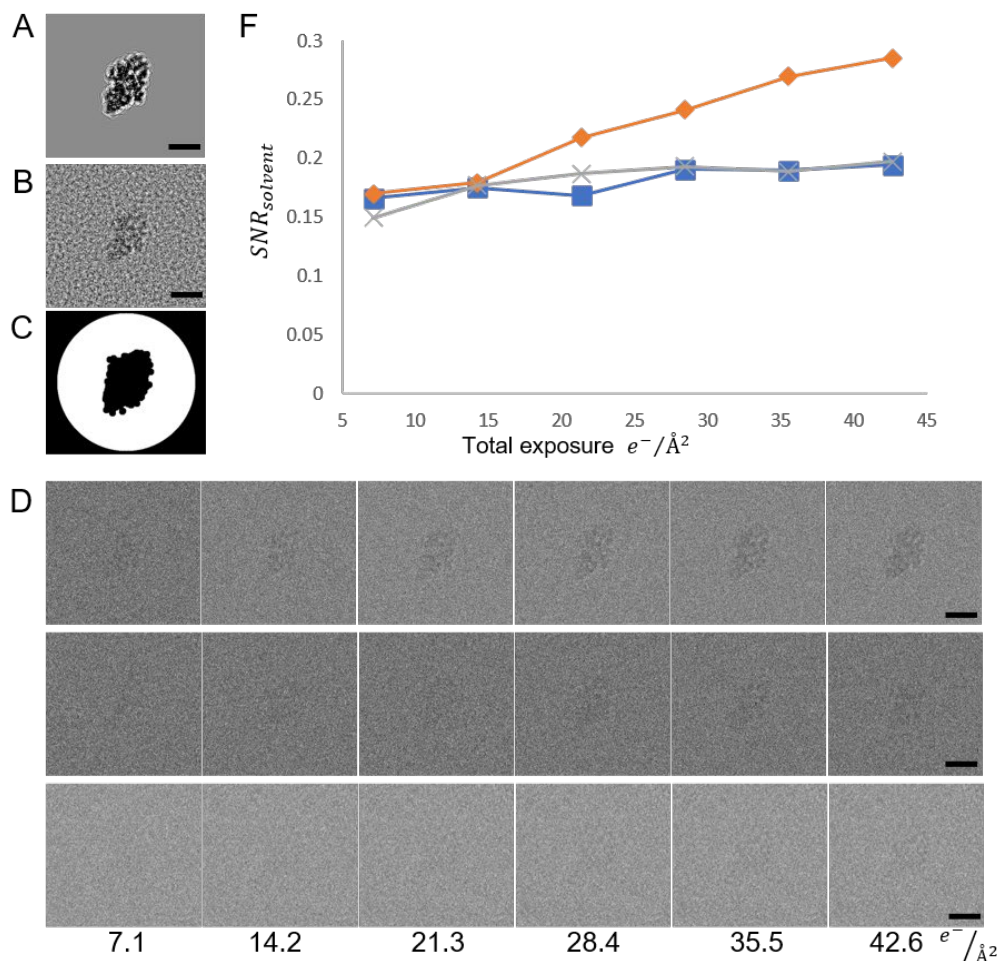


Figure 4 – Comparing the continuum and coarse-grained all-atom solvent model to experimental data. A) $|\Psi_{detector}|^2$ with a constant potential added for the solvent. B) $|\Psi_{detector}|^2$ with coarse-grained all-atom water model. The average intensity in A and B in the solvent region is identical within numerical precision. C) Mask used in calculating $SNR_{solvent}$ (equation 2 main text) where the white region was used for the solvent, and the central black region for the protein. D) Plot of $SNR_{solvent}$ as a function of accumulated exposure for experimental data (Blue line, square marker) coarse-grained all-atom solvent model (grey, x-marker) and constant solvent potential (orange, diamond marker). E) Images used in calculating (D) with the same color/marker scheme, and total exposure indicated along the bottom. Experimental data taken from EMPIAR-10061, beta-galactosidase. Scale bars 100 Å

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434 *Amplitude contrast*

435 Amplitude contrast can arise from electrons being scattered outside the objective lens
 436 aperture, or by removing inelastically scattered electrons using an energy filter. The
 437 former is incorporated by applying an aperture function directly to the complex wave
 438 function prior to image formation, which results in an attenuation of the expected
 439 number of electrons at the detector. This is demonstrated in Figure 5 for a series of

440 aperture diameters and a simulated amorphous specimen with density and thickness as
441 used previously for the “phase plate” with atomic potentials of either carbon (orange
442 circles), phosphorous (grey x’s) or gold (blue squares). The smallest aperture used
443 (0.01 μ) excludes all but the unscattered beam and so is a measure of total
444 transmittance of the simulated layer.

445 Amplitude losses due to inelastic scattering are incorporated into the multislice
446 formalism via a complex scattering potential, commonly defined as linearly proportional
447 to the real (elastic) potential, for example as in inSilicoTEM. A detailed analysis of why
448 this proportional model is inadequate is found in Dudarev et al. [29]. Our atomically
449 specific inelastic scattering potential is described in the theory section. To demonstrate
450 that it produces the correct amplitude losses, we rearrange Equation 1 to plot the
451 negative-natural logarithm of the expected electron count vs solvent thickness. Fitting
452 this with linear regression gives a readout of the simulated inelastic mean-free path in
453 Figure 6 which closely matches experimental numbers [49].

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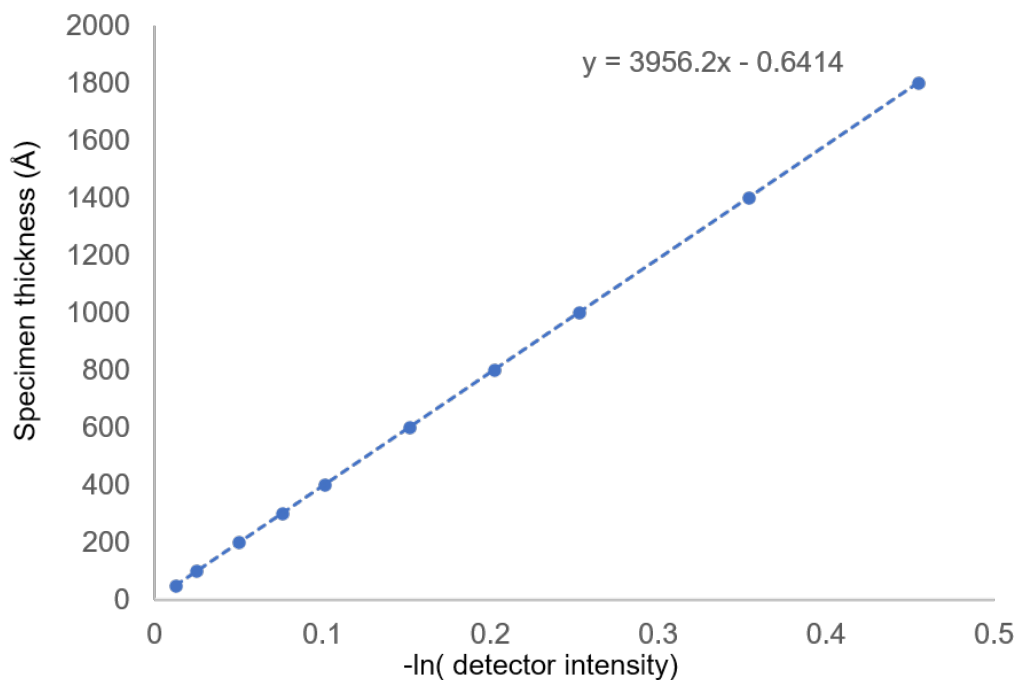


Figure 6 – The coarse-grained all-atom solvent model in combination with the inelastic scattering factor for plasmons we derived produce amplitude losses via the complex potential that do not need to be scaled post hoc. The slope is a read out for the inelastic (single-scatter) mean free path in our simulated solvent.

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457 Discussion:

458 Our simulator implements the most thorough forward model for calculating the
459 interaction between high-energy electrons and radiation-sensitive biological samples
460 demonstrated to date. The improvements described here result from an approximate
461 description of the changes in the specimen due to deposition of energy via inelastic
462 scattering during imaging. This added accuracy in simulating the molecular density
463 produces more realistic image simulations for algorithmic development, but just as
464 importantly, it provides a means to investigate the behavior of complex biological
465 specimens in atomic detail using matched filtering via 2D template matching.

466 Since the output of the matched filter is sensitive to the spectral distribution of the
467 signal, we can quantify the accuracy of our image formation/damage model by
468 measuring the change in SNR_{mf} . We found that modeling the water envelop, detector
469 MTF, residual motion blur, and PDB model uncertainty resulted in a higher SNR_{mf} than
470 could be obtained by optimizing a single B-factor. This analysis is limited by the fact that
471 we cannot strictly disentangle changes to the signal from different envelopes that could
472 be mutually compensatory, though this may not be too severe a problem given the
473 differences in the envelopes shown in Figure 3A. A more careful consideration of the
474 impact of different spatial frequencies on SNR_{mf} may prove useful in addressing this
475 limitation in future work.

476 Our explicit solvent model, while coarse-grained, allows us to accurately reproduce
477 attenuation due to inelastic losses and amplitude contrast that is spatially variable, and
478 based only on the atomic species and local mass thickness in the simulated
479 specimen. In principle, any configuration of atoms can be simulated by supplying an
480 appropriate PDB file to the simulator. In practice, variable solvent thickness, or other
481 sources of structural noise like regions of hexagonal ice could be included directly into
482 the simulator, however, we leave this for future work. We show a considerable
483 improvement in matching $SNR_{solvent}$ to experimental data and expect this to improve the
484 ability of models (artificial neural networks especially) trained on simulated data to
485 generalize more readily to experimental data. On visual inspection, the granularity in the
486 solvent appears a bit different than that observed in experiment; we suspect including
487 solvated ions might account for this and plan to include this in future work.

488 Simulations using an explicit solvent model are computationally very demanding,
489 increasing the number of scattering centers to be considered by a factor of ~ 200 when
490 simulating single particle image stacks, and up to a factor of 10^4 when simulating
491 micrographs with well-spaced particles. We have addressed this computational demand
492 via multi-threading in C++. Most of the time taken by the wave-propagation calculation
493 is spent on Fourier transforms. The calculation is currently limited to 4 threads per

494 simulation, independent of the number of threads used in calculating the real-space
495 potentials. To simulate tilted samples, which will have a substantially larger number of
496 slices to propagate, the Fourier transform can become a bottleneck, suggesting a GPU
497 implementation may be beneficial for future work.

498 Conclusion:

499 Here we have presented an accurate forward model describing sources of signal
500 attenuation and show how the spectral characteristics of that attenuation improve the
501 output of the matched filter (SNR_{mf}) as used in template matching for the detection of
502 molecules in cryo-EM images. The SNR_{mf} is in turn directly related to the mass limit for
503 detection; any improvement in our forward model results in being able to detect smaller
504 particles, which will expand the capacity of template matching in visual proteomics. The
505 increased SNR_{mf} due to modeling radiation damage is encouraging but should likely be
506 modeled more accurately for the purpose of template matching. We also suggest that
507 our model for inelastic scattering could be improved by direct comparison to experiment
508 using the matched filter. If properly accounted for, we could in principle use this
509 scattering information in particle detection, rather than discarding it using an energy
510 filter.

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