Sparseness and Smoothness Regularized Imaging for improving the resolution of Cryo-EM single-particle reconstruction

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In this paper, we present a refinement method for cryo-electron microscopy (cryo-EM) single-particle reconstruction, termed as OPUS-SSRI (Sparseness and Smoothness Regularized Imaging). In OPUS-SSRI, spatially varying sparseness and smoothness priors are incorporated to improve the regularity of electron density map, and a type of real space penalty function is designed. Moreover, we define the back-projection step as a local kernel regression and propose a first-order method to solve the resulting optimization problem. On the seven cryo-EM datasets that we tested, the average improvement in resolution by OPUS-SSRI over that from RELION 3.0, the commonly used image-processing software for single-particle cryo-EM, was 0.64 Å, with the largest improvement being 1.25 Å. We expect OPUS-SSRI to be an invaluable tool to the broad field of cryo-EM single-particle analysis. The implementation of OPUS-SSRI can be found at https://github.com/opus-ssri.

Cryo-EM | 3D reconstruction | ill-posed inverse problem | smoothness | sparseness

Cryo-electron microscopy (Cryo-EM) single-particle analysis is a powerful method for determining macromolecular structures. The major advantages of cryo-EM over the traditional X-ray crystallography are that it does not require crystallization and is not plagued by the phase problem. However, there remain many new challenges in this promising technique. The central problem of cryo-EM single-particle analysis is the incompleteness of experimental observations. More specifically, the information of the relative orientations and translations of all particles is missing. Furthermore, in a dataset with multiple conformations (or substates), the membership of a specific class needs to be defined. Moreover, the signal-to-noise ratio (SNR) of a cryo-EM dataset is often very low since the electron exposure of the sample needs to be strictly limited to reduce radiation damage (1). Other problems often present in cryo-EM datasets include the nonuniform angular sampling, which frequently results in inadequate sampling or even no sampling in certain orientations (2). Therefore, the problem of cryo-EM three-dimensional (3D) reconstruction is an extremely ill-posed problem. To alleviate the ill-posedness of this problem, prior assumptions must be incorporated into the reconstruction process to ensure the uniqueness of solution and the objectivity of the final maps.

Two outstanding features of 3D density maps are sparseness and smoothness. Specifically, since the atoms in macromolecules only occupy part of the 3D maps, the macromolecular maps are often sparse in space. On the other hand, because the atoms in macromolecules are connected through chemical bonds, the electron densities of macromolecules vary smoothly across the space (3). Though sparseness is a popular prior in solving inverse problems, it is a relatively novel notion to cryo-EM 3D reconstruction. In contrast, the importance of smoothness prior is widely recognized in cryo-EM 3D refinement. An early attempt to enforce the smoothness of the density map was to apply the Wiener filter by using Bayesian statistics (3). Scheres et al. assumed that the Fourier components of the density map are distributed according to Gaussian distributions (3) a priori and derived a maximum a posteriori estimation for reconstruction. This approach, as implemented in REgularised Likelihood Optimisation (RELION) (5), is referred to as the traditional approach in this context of the paper. Except for incorporating priors into the reconstruction process, another line of efforts aims to enhance cryo-EM 3D refinement by optimizing the defocus parameter and class membership for each particle, as exemplified by THUNDER (6). THUNDER has been shown to improve cryo-EM refinement by providing more accurate contrast transfer function and membership for each particle.

In this paper, we continued the direction used in RELION and proposed an approach to regularize the 3D maps. Our approach, named OPUS-SSRI (Sparseness and Smoothness Regularized Imaging), focuses on imposing sparseness and smoothness priors (i.e., $l_1$ regularization) (7) and total variation (TV) (8). To encourage sparseness and smoothness of the density map while suppressing bias, we proposed a nonconcave, nonsmooth, real-space restraint by combining $l_1$ regularization and TV norm. Since such target function is difficult to optimize, we designed a reweighted scheme to approximately optimize it with a sequence of weighted $l_1$ and TV problem. The major differences between the traditional approach in RELION and our approach in OPUS-SSRI are outlined in detail in the Materials and Methods section. In essence, the traditional approach in RELION can be viewed as applying a translation-invariant isotropic kernel to smooth the 3D map, whereas OPUS-SSRI applies a spatially varying anisotropic kernel. Another challenge of 3D map reconstruction in real space is the prohibitive computation cost as a result of its ultrahigh dimensionality. For example, a common $512 \times 512 \times 512$ 3D volume contains hundreds of millions of floating-point operations, and the computation cost of the reconstruction process is then extremely high.

In this paper, we present a refinement method for cryo-electron microscopy (cryo-EM) single-particle reconstruction, termed as OPUS-SSRI (Sparseness and Smoothness Regularized Imaging). In OPUS-SSRI, spatially varying sparseness and smoothness priors are incorporated to improve the regularity of electron density map, and a type of real space penalty function is designed. Moreover, we define the back-projection step as a local kernel regression and propose a first-order method to solve the resulting optimization problem. On the seven cryo-EM datasets that we tested, the average improvement in resolution by OPUS-SSRI over that from RELION 3.0, the commonly used image-processing software for single-particle cryo-EM, was 0.64 Å, with the largest improvement being 1.25 Å. We expect OPUS-SSRI to be an invaluable tool to the broad field of cryo-EM single-particle analysis. The implementation of OPUS-SSRI can be found at https://github.com/opus-ssri.

Materials and Methods

Three-dimensional refinement is a critical component of cryo-EM single-particle reconstruction. In this paper, we report the development of a computational method, OPUS-SSRI, and its application to seven real cryo-EM datasets. Our data clearly demonstrated that OPUS-SSRI can improve the final resolutions and structural details in cryo-EM single-particle analysis.

Significance

Three-dimensional refinement is a critical component of cryo-EM single-particle reconstruction. In this paper, we report the development of a computational method, OPUS-SSRI, and its application to seven real cryo-EM datasets. Our data clearly demonstrated that OPUS-SSRI can improve the final resolutions and structural details in cryo-EM single-particle analysis.

Author contributions: Z.L., Q.W., and J.M. designed research; Z.L., A.A.C.-A., and L.L. performed research; Z.L., Q.W., and J.M. analyzed data; and Z.L., Q.W., and J.M. wrote the paper.

The authors declare no competing interest.

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variables. We addressed this challenge by leveraging a Compute Unified Device Architecture (CUDA)-accelerated implementation. In addition, we cast the back-projection as a local kernel regression problem, thus paving a way to promote the smoothness of the 3D map (9). By applying it to seven real cryo-EM datasets, we demonstrated that our OPUS-SSRI supports a significant improvement over RELION 3.0 or THUNDER, especially for systems with significant flexibility.

Results

We tested OPUS-SSRI by performing 3D refinement on a total of seven real datasets and comparing the refinement results with those obtained using RELION 3.0 or THUNDER. The detailed experimental process and optimal parameters are reported in the SI Appendix.

According to the gold-standard Fourier shell correlation (FSC) at 0.143, the final density maps reconstructed by OPUS-SSRI clearly have higher SNRs compared to those generated by RELION 3.0 in most resolution shells for β-galactosidase (10, 11) (Fig. 1A), 80S ribosome (12) (Fig. 1B), influenza hemagglutinin (HA) (13) (Fig. 1C), transient receptor potential melastatin (TRPM4) (14) (Fig. 1D), protein-conducting ERAD channel Hrd1/Hrd3 complex (15) (Fig. 2A), transient receptor potential vanilloid 5 (TRPV5) (16) (Fig. 3A), and calcium-activated chloride channel (TMEM16A) in nanodisc (17) (Fig. 4A). The final maps refined by OPUS-SSRI have resolutions that are 0.15 to 1.25 Å better than those refined by RELION 3.0, with averaged resolution improvement of 0.64 Å for all seven systems (Table 1). The improvement of the density maps reconstructed by OPUS-SSRI is also confirmed by the model versus map FSCs. The postprocessed maps of OPUS-SSRI have much higher correlations with respect to the corresponding rigid-body fitted atomic models in most resolution shells than RELION 3.0 (SI Appendix, Fig. S1). Overall, for the seven systems, the improvements in resolution for the postprocessed maps of OPUS-SSRI are in the range of 0.14 to 0.73 Å, with an average of 0.30 Å, over those refined by RELION 3.0 (SI Appendix, Table S1).

THUNDER was also run on five of these seven systems (it failed to execute on two datasets due to incompatibility with our computing facility). According to the gold-standard FSC at 0.143, the improvements in resolution by THUNDER over RELION 3.0 are in the range of −0.09 to 0.28 Å with an average of 0.07 Å (Table 1). If judged by the model versus map FSCs at 0.143, the improvements in resolution of THUNDER over RELION 3.0 are in the range of −0.18 to 0.17 Å with an average of 0.07 Å on the five systems (SI Appendix, Table S1).

Fig. 1. Gold-standard unmasked and masked FSC curves for the final 3D reconstructions refined by OPUS-SSRI (in red color) or RELION 3.0 (in blue color) for (A) β-galactosidase, (B) 80S ribosome, (C) influenza hemagglutinin, and (D) TRPM4. In all panels, the dashed black line represents FSC = 0.143.
five systems, OPUS-SSRI constantly outperforms THUNDER on four systems and only slightly underperforms THUNDER on one system (80S ribosome) as gauged by the gold-standard 
FSC = 0.143 and model versus map FSC = 0.143. Overall, 
OPUS-SSRI produces an average improvement of 0.47 Å in 
resolution over THUNDER for all five systems if judged by the 
gold-standard FSC = 0.143, with the largest improvement being 
1.20 Å (Table 1 and SI Appendix, Table S1), and of 0.20 Å in 
resolution if judged by the model versus map FSC = 0.143, with 
the largest improvement being 0.63 Å (SI Appendix, Table S1 and 
Fig. S3).

Fig. 2 shows some of the structural improvements for Hrd1/
Hrd3 complex in more detail. Clearly, compared to the density 
map reconstructed by RELION 3.0 (Fig. 2B), the density map 
from OPUS-SSRI is much sharper and cleaner (Fig. 2C). In fact, 
out of the seven systems studied, OPUS-SSRI refinement on 
Hrd1/Hrd3 complex results in the largest improvements in res-
solution (Table 1 and SI Appendix, Table S1). For instance, in 
the density map from RELION 3.0, there is a gap in the main-chain 
density between residues 147 and 148 (Fig. 2D). However, in the 
density map from OPUS-SSRI, the density in this region 
becomes continuous and strong (Fig. 2E).

Similarly, for TRPV5, comparing to the final map obtained by 
RELION 3.0 (Fig. 3B), the density map from OPUS-SSRI 
becomes much sharper with improved SNRs (Fig. 3C). Most 
impressively, the density map from OPUS-SSRI even allows 
retracing of the structural model in the region of residues 374 to 
380 that was out of the density map in the original structure 
(highlighted in dashed red circle in Fig. 3D). After the manual 
adjustment in the crystallographic object-oriented toolkit COOT 
(18) and structural refinement using Python-based Hierarchical 
ENVironment for Integrated Xtallography (PHENIX) (19), the 
mesh between the model and map is substantially improved 
(highlighted by dashed red circle in Fig. 3E).

In addition, for TMEM16A, in contrast to the density map 
from RELION 3.0 (Fig. 4B), the density map obtained by OPUS-
SSRI (Fig. 4C) shows sharper and smoother densities with less 
noise throughout. The improvement from OPUS-SSRI is high-
lighted for two helices in the regions of residues 408 to 440 
(Fig. 4D and E) and 848 to 884 (Fig. 4F and G). Most im-
pressively, in the density map refined by OPUS-SSRI, the den-
sities for side chains of residues F412, M416, W419, and F423 
(Fig. 4E) and F863, I865, F867, and N869 (Fig. 4G) become very 
well separated, in marked contrast to the blobs of densities from 
RELION 3.0 in Fig. 4D and F, respectively.

Discussion
In this paper, we proposed OPUS-SSRI, a 3D refinement
method for cryo-EM single-particle analysis. The improvement
of our method in gold-standard FSC of the final reconstructions
is the most noticeable, which can be largely attributed to the
superior denoising effect of the sparseness and smoothness pri-
ors that we introduced. By setting relatively small components in
the 3D map to zero and filtering components to be more
consistent with their neighbors, the sparseness and smoothness restraints can suppress the noisy densities that do not belong to the molecules in the map, thus producing cleaner reconstructions. The cleaner map in turn leads to more accurate pose estimation for each particle. These improvements brought about by our method result in an overall much-improved final reconstruction. Furthermore, the relatively large improvements for structures with heterogeneous flexibility such as Hrd1/Hrd3 and TMEM16A confirm the theoretical difference between the traditional smoothness prior in RELION and our smoothness prior in OPUS-SSRI. For structures with heterogeneous flexibilities in different regions, the traditional approach in RELION enforces translation-invariant isotropic smoothness to the 3D maps, thus smearing the rigid regions and creating large biases in the reconstructions. In contrast, OPUS-SSRI can adapt to different flexibilities in different regions in the maps, thus greatly reducing biases and improving the final reconstructions. Another approach we explored to promote smoothness is by casting the back-projection as a local kernel regression problem. This formulation enables us to embed the 3D maps in a reproducing kernel Hilbert space (RKHS) with specific smoothness.

Although our method introduces five more parameters, their optimal values can be easily determined. First of all, we can set $\epsilon$ to the level of density values corresponding to molecular content in the 3D volume. This level can be easily obtained from the intermediate volumes generated by the refinement using RELION 3.0. The optimal values of $\alpha$, $\beta$, $\gamma$, and $\epsilon'$ can be found by grid search as detailed in SI Appendix, Experiment process and exemplified in SI Appendix, Fig. S4. The parameters were searched in the order $\alpha \rightarrow \beta \rightarrow \gamma \rightarrow \epsilon'$. The complexity of the grid search depends linearly on the number of parameters. The ranges of parameters where optimal settings were found in our tests are summarized as follows: $\alpha$ are in the range of $[0.4, 0.8][\|A^TDy\|_2]$, $\beta$ are in the range of $[0.5, 4][\|A^TDy\|_2]$, $\gamma$ are in the range of $[0.05, 0.2]N(n)$, and $\epsilon'$ are in the range of $[\frac{1}{4}, 2][\epsilon]$.

These ranges can serve as useful guidance for future applications of OPUS-SSRI to other systems. In practice, a reasonable set of parameters can be obtained with just a few trials.

It is worth noting that OPUS-SSRI focuses on improving accuracies of pose parameters for each particle in the maximization step, which is complementary to the approach explored by THUNDER that targets other latent variables, such as defocus parameters and class membership. Hence, these two approaches can be readily combined. In fact, accurate determination of pose parameters are the prerequisites for a better per-particle defocus.

Fig. 3. Refinement of TRPV5. (A) Gold-standard unmasked and masked FSC curves calculated from two independent reconstructions by OPUS-SSRI or RELION 3.0. The dashed black line represents FSC = 0.143. (B) Final reconstructed cryo-EM map using RELION 3.0. (C) Final reconstructed cryo-EM map using OPUS-SSRI. The red rectangles in B and C define a region of the EM map to be enlarged in D for RELION 3.0 and E for OPUS-SSRI for residues 374 to 409, respectively. The dash red circles highlight a region in the model before (D) and after (E) the manual adjustments in COOT and structural refinement using PHENIX. The EM density is represented in mesh (blue), and the structural model is represented by a ribbons diagram with side chains in stick presentation. Both density maps are contoured at the same level.
parameter refinement. This is exemplified by the limited improvement of THUNDER on the highly noisy dataset Hrd1/Hrd3, in which the pose of each particle was of large errors (SI Appendix, Figs. S2 and S3), yielding inaccurate reference two-dimensional (2D) projections and adversely affecting the per-particle contrast transfer function (CTF) refinement. Therefore,

Table 1. Comparison of the final reconstructions refined by RELION 3.0, THUNDER, or OPUS-SSRI

| Proteins          | RELION     | THUNDER | ∆Å over RELION* | OPUS-SSRI       | ∆Å over RELION* | ∆Å over THUNDER† |
|-------------------|------------|---------|-----------------|----------------|----------------|-----------------|
| β-galactosidase   | 4.16       | 4.25    | −0.09           | 3.93           | 0.23           | 0.33            |
| 80S ribosome      | 4.08       | 3.80    | 0.28            | 3.93           | 0.15           | −0.13           |
| Hemagglutinin     | 4.19       | 4.11    | 0.08            | 3.77           | 0.42           | 0.34            |
| TRPM4 (EMPIAR-10126) | 3.48 | /       | /               | 2.74           | 0.74           | /               |
| Hrd1/Hrd3 (EMPIAR-10099) | 4.80 | 4.75    | 0.05            | 3.55           | 1.25           | 1.20            |
| TRPV5 (EMPIAR-10254) | 3.12 | 3.09    | 0.03            | 2.47           | 0.65           | 0.62            |
| TMEM16A (EMPIAR-10123) | 3.90 | /       | /               | 2.84           | 1.06           | /               |
| Average improvement | 0.07       |         |                 | 0.64           | 0.64           | 0.47            |

*The value in negative indicates the resultant resolution is worse than that from RELION, while the value in positive indicates the resultant resolution is better than that from RELION.
†The value in negative indicates the resultant resolution is worse than that from THUNDER, while the value in positive indicates the resultant resolution is better than that from THUNDER.

* indicates that the comparison was unavailable in two cases in which THUNDER failed to execute due to computer incompatibility.
our OPUS-SSRI might enhance the per-particle CTF refinement on some noisy datasets by improving the pose determination of these datasets.

Finally, our tests of OPUS-SSRI on seven real datasets support that OPUS-SSRI can greatly improve the resolution of the final density map, thus allowing more accurate building of atomic models. We expect OPUS-SSRI to be an invaluable tool to the general field of cryo-EM single-particle analysis.

Materials and Methods

We clarify some notations here. For a vector $x \in \mathbb{R}^d$, we use $\|x\|_p$ to represent the $L_p$ norm of the vector $x$, which is defined as $\|x\|_p = \left( \sum_{i=1}^{d} |x_i|^p \right)^{1/p}$. $Z$ represents the set of integers, while $\mathbb{R}$ stands for the set of reals. We use $x$ to represent the 3D map, $V$ to represent the Fourier transform (FT) of $x$, and $X_j$ to represent the FT of the jth image. For simplicity, we often use vector representation for multidimensional data (i.e., a point with multidimensional index $[i, \ldots, k]$ is mapped to the jth component $x_i$ of $x$).

Introduction to Cryo-EM Refinement. Formally, the FT of 3D map $V$ to be reconstructed in cryo-EM refinement can be defined as the maximizer of the penalized log marginal likelihood function (3) $\sum_{l=1}^{N} \log P(X_l|V) - \sum_{l=1}^{N} \text{SNR}(r) \left| V_{l,3} \right|^2$, where $\text{SNR}(r)$ is the SNR estimated by gold-standard FSC (23).

To understand the effect of the smoothness restraint of the traditional method, we consider the use of the restraint in the gradient ascent iteration, which is of the form

$$V_{l,3} = V_{l,3} + \eta \left( \frac{\partial}{\partial V_{l,3}} \log P(X_l|V) - \hat{\alpha}(r) V_{l,3} \right),$$

where $\hat{\alpha}(r) = \frac{\alpha r^2}{\text{SNR}(r)},$ is the damping weight for $V_{l,3},$ and $\eta > 0$ is the learning rate. The gradient of the traditional prior $\hat{\alpha}(r) V_{l,3}$ can be viewed as a smoothed map in real space. By convolution theorem, let the inverse FT of $\text{SNR}(r)$ of masking in the calculation of masked FSC, where the voxels which are outside these datasets.

Sparseness and Smoothness Priors in OPUS-SSRI. Sparseness resembles the idea of masking in the calculation of masked FSC, where the voxels which are below a certain threshold are setting to 0. The similar effects can be achieved by restraining the sum of the absolute values of densities, namely, the $L_1$ norm of the density map (7) during reconstruction. Hence, we can encode the sparseness using the $L_1$ norm (7) and the smoothness of 3D map using the TV regularization. Though these two priors can effectively guarantee both sparseness and smoothness of the map, they also introduce biases to the final solution (23). In particular, $L_1$ regularization tends to underestimate true nonzero elements (23) since the corresponding soft-threshold operator shrinks the volume by a global threshold. Fan et al. discovered that nonconcave penalty can prevent true nonzero elements from being overly shrunk while preserving sparseness (23). Therefore, we employ a nonconcave penalty log norm (24) to reduce biases in the solution. The penalized log likelihood function in OPUS-SSRI has the form

$$\sum_{l=1}^{N} \log P(X_l|V) - \sum_{l=1}^{N} \log (|x_i| + \epsilon) + \beta \log (\|V_{l,3}\|_2 + \epsilon).$$

where $\epsilon$ and $\beta$ are positive, and $\epsilon$ and $\epsilon$ are positive parameters to guard against the singularity of logarithm function at zero.

Optimization Methods in OPUS-SSRI. This subsection presents the algorithm to optimize the penalized log likelihood in Eq. 3. First, the log marginal likelihood function can be optimized by the expectation–maximization method (25) (see SI Appendix, Expectation maximization for derivation). The reconstruction process alternates between the expectation step in which the distribution of pose parameters for each particle is determined and the maximization step in which the 3D map is reconstructed. Secondly, to address the nonconcavity of log norm, we approximate the logarithm function by concave function and iteratively improve the approximation (24) at each maximization step (see SI Appendix, Weighted approximation for derivation). Lastly, to average 3D maps reconstructed in consecutive maximization steps, we consider leveraging implicit gradient ascent (26), which is a widely used technique to improve the stability of optimization method. The implicit gradient ascent restricts the Euclidean distance between the new solution and the 3D map of previous maximization step $x^{t-1}$. These choices yield a target function in Eq. 4 by optimizing which can improve Eq. 3 (24). Formally, at the $t+1$th iteration of the $t$th maximization step, let the solution obtained in the previous iteration be $x^t$. We approximate Eq. 3 with the expected log likelihood (the first term in Eq. 4) and the weighted $l_1$ and TV norms and define the solution at iteration $i+1$ as the maximizer of the following equation:

$$\max_x \sum_{l=1}^{N} \log P(X_l|V_{l,3}^{t-1}) - \text{CTF} \left( |\mathcal{X}| \right)^2 - \frac{\lambda}{2} \sum_{i=1}^{N} \left( |x_i| + \beta \|V_{l,3}^t\|_2 + \epsilon \right),$$

where $\text{CTF}(\mathcal{X})$ is the conditional probability of the pose parameters given the observation $X_l$ and the map $V_{l,3}^t$ from the previous maximization step, and $\lambda > 0$ is the weight of the implicit gradient ascent penalty. The effect of log norm becomes evident after converting it to the weighted norm in Eq. 4. Reweighting each voxel yields a spatially varying threshold (Eq. 6), which can reduce the bias of prior. Next, we will demonstrate that the gradient of TV norm enforces heterogenous smoothness to the 3D map, and the $l_1$ norm achieves sparseness by the soft-thresholding rule while preserving the optimization algorithm.

Though TV norm is nondifferentiable at zero, we can approximate its gradient by Nesterov smoothing (27). The approximate gradient of TV norm at a voxel $[i, j, k]$ (see SI Appendix, Nesterov smoothed TV norm for derivation) is of the form

$$\nabla f_k(x_k) = \frac{1}{c_{i,j,k}} (\|x_k\|_1 - \frac{1}{\Delta_k}) - \frac{2}{\Delta_k} c_{i,j,k} x_k k_e [i,j,k] \pm \Delta_k,$$

where $\Delta_k$ is a $1 \times 3$ vector with 1 on the ath entry and zeros elsewhere, $c_{i,j,k} = 3 \|V_{i,j,k}\|_1 + \frac{3}{\Delta_k} \|V_{i,k}\|_1$, $c_{i,j,k} \Delta_k = \|V_{i,j,k}\|_1$ and $c_{i,j,k} \Delta_k = \|V_{i,j,k}\|_1$, $\|V_{i,j,k}\|_1 = \left\{ \|V_{i,j,k}\|_1 + \epsilon \right\} \text{max}(\|V_{i,j,k}\|_1, 1)$, and $\mu$ is the Nesterov smoothing parameter. The gradient of the smoothed TV norm at the voxel $[i, j, k]$ depends on the gradients at this voxel and its neighboring voxels $[i, j, k] \pm \Delta_k$. Specifically, the voxel on the direction with smaller gradient has a larger contribution to the gradient of TV norm. We can write the continuous form of Eq. 5 as $x_k = \frac{1}{\mu} \sum_{i=0}^{\mu} g_k(x_k)$, where $g_k(x_k)$ is the Nesterov smoothed TV norm.
depends on the voxel $u$ and its neighboring voxel $v$ simultaneously. Therefore, the weighted TV norm smooths the map by a spatially varying anisotropic kernel $K(u, v)$, which adapts to heterogenous smoothness in the 3D map.

The differentiable function with $l_1$ penalty can be optimized by a joint application of gradient ascent and soft-thresholding operator (28). Denote the implicit gradient-restrained expected log likelihood

$$-\sum_{i=1}^{N} P(\phi(X_i, V_{n1}))-xi - CTF_P(V)|^2 - \frac{1}{2}||x - x_i^{n1}||^2_2$$

as $w_i$, and let the learning rate be $\eta$ for the $j$th component of $x$ at $t + 1$ iteration. Eq. 4 can be optimized by the following equation:

$$x^{t+1} = \begin{cases} 0, & x^{t+1} < \text{SNR} \\ x^{t+1} - \eta w_i \text{sign}(x^{t+1}), & x^{t+1} \geq \text{SNR} \\
\end{cases}$$

where $x^{t+1} = x^{t} + \eta(\nabla_f \alpha) - \mu\nabla_f \alpha(x^{t+1})$ is the 3D map updated by gradient ascent, and $\text{sign}()$ extracts the sign of a voxel value. Eq. 6 is referred to as the soft-thresholding operator. The sparseness of the volume is preserved by the soft-thresholding operator since it sets the voxels with relatively small values to zeros. Moreover, the threshold $\text{SNR}$ is inversely proportional to the strength of the voxel. Therefore, the voxels with small values are more likely to be shrunk to 0 s. Since the values of electron densities for molecules are often higher than the background noises, this soft-thresholding operator can suppress noises while leaving the electron densities of molecules intact. We hence demonstrate how our $l_1$ restraint yields an unbiased cleaner 3D map.

In summary, Eq. 6 is applied iteratively in the maximization step. The gradient of the TV norm enforces spatially varying smoothness in gradient ascent, while the soft-thresholding operator induced by the weighted $l_1$ norm guarantees the sparseness of the 3D map. In all of our experiments, convergences were achieved in a maximization step after 100 iterations.

Back-Projection as Local Kernel Regression. To reconstruct the 3D map, cryo-EM researchers introduced a back-projection operator, which puts the 2D FT of the image into the 3D map. As the inverse of slice operator $P^o$ (SI Appendix, Eq. S2), back-projection puts the data with 2D index $\{i, j\}$ to the 3D index $\{h, k, l\}$ of the 3D map $V$. Since the Gaussian kernel is quickly diminishing, we let $\text{SNR}$ be $\frac{\eta}{\text{SNR}}$.

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as $w_i$, and let the learning rate be $\eta$ for the $j$th component of $x$ at $t + 1$ iteration. Eq. 4 can be optimized by the following equation:

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\end{cases}$$

where $x^{t+1} = x^{t} + \eta(\nabla_f \alpha) - \mu\nabla_f \alpha(x^{t+1})$ is the 3D map updated by gradient ascent, and $\text{sign}()$ extracts the sign of a voxel value. Eq. 6 is referred to as the soft-thresholding operator. The sparseness of the volume is preserved by the soft-thresholding operator since it sets the voxels with relatively small values to zeros. Moreover, the threshold $\text{SNR}$ is inversely proportional to the strength of the voxel. Therefore, the voxels with small values are more likely to be shrunk to 0 s. Since the values of electron densities for molecules are often higher than the background noises, this soft-thresholding operator can suppress noises while leaving the electron densities of molecules intact. We hence demonstrate how our $l_1$ restraint yields an unbiased cleaner 3D map.

In summary, Eq. 6 is applied iteratively in the maximization step. The gradient of the TV norm enforces spatially varying smoothness in gradient ascent, while the soft-thresholding operator induced by the weighted $l_1$ norm guarantees the sparseness of the 3D map. In all of our experiments, convergences were achieved in a maximization step after 100 iterations.

Back-Projection as Local Kernel Regression. To reconstruct the 3D map, cryo-EM researchers introduced a back-projection operator, which puts the 2D FT of the image into the 3D map. As the inverse of slice operator $P^o$ (SI Appendix, Eq. S2), back-projection puts the data with 2D index $\{i, j\}$ to the 3D index $\{h, k, l\}$ of the 3D map $V$. Since the Gaussian kernel is quickly diminishing, we let $\text{SNR}$ be $\frac{\eta}{\text{SNR}}$.

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