Map Challenge Assessment

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Abstract

The maps submitted for the map challenge feature different sizes, sampling and orientations, making comparisons non-trivial. I decided to pose all maps in the same orientation, size and sampling to allow comparison with a reference and using the same mask for all. I developed a method to scale and orient the maps to a reference, aiming at introducing a minimum of interpolation artifacts. While not error-free, it presents a fair relative assessment of the maps. So far this allowed me to identify good maps, as well as maps with potentially serious problems.

Introduction

Cryo-electron microscopy (cryoEM) is undergoing an enormous expansion due the recent introduction of direct electron detectors {Vinothkumar, 2016 #5131}{McMullan, 2016 #5133}. These detectors have a significantly enhanced signal-to-noise (SNR) ratio combined with fast image acquisition, enabling solving biomolecular structures to atomic resolution. This newfound popularity comes with a price: The methodologies need to be robust enough to ensure valid outcomes {Heymann, 2015 #4930}{Rosenthal, 2016 #5134}. The Map Challenge was conceived to start addressing these issues (http://challenges.emdatabank.org/?q=2015_map_challenge).

The most commonly used method for high resolution structure determination is single particle analysis (SPA). While data processing for SPA is well understood, there remain pitfalls that a user can encounter. The fundamental reason is that the problem of selecting and aligning the particles is ill-defined. The result is influenced by the decisions made by the user. The Map Challenge is therefore an opportunity to highlight key decisions in SPA, and provide guidance for users.

Here I report on the assessment of the reconstructions submitted to the Map Challenge. The maps are of different sizes, scales and orientations, making comparison challenging. To be fair, I developed a method to pose all the maps in a specific case in directly comparable form. I then analyzed them by Fourier shell correlation (FSC), comparing even-odd maps as well as full reconstructions with references derived from atomic models. The latter served as check for overfitting compared to the even-odd analysis. This investigation revealed issues with how maps were filtered and masked, in some cases leading to overfitting (i.e., spurious high frequency correlations leading to claims of high resolution). In addition, several of the maps have unexpected statistical features, suggesting inappropriate filtering that could produce artifacts.

Methods

All processing was done in Bsoft {Heymann, 2007 #3057}.

Visual comparison of reconstructions

The unfiltered and filtered reconstructions were processed to produce central slices and central sections of the power spectra. The latter were transformed to their natural logarithms because of their large dynamic ranges, using the following formula (implemented in the program bfft):

$$I'(k) = \ln\left[\frac{I(k) - I_{min}}{I_{avg} - I_{min}} + 0.001\right]$$

Comparing reconstructions scaled to the same physical size

The various submitted reconstructions have different sizes, samplings and orientations, making direct comparison impossible. I decided to standardize the analysis on maps rescaled to a reference with a voxel size (sampling) of 1 Å/voxel. The reference maps were calculated from atomic coordinates using electron scattering cross-sections (program bsf) and sized to have the particle occupy about 5-10% of the volume (Table 1).

Comparisons between the reference and reconstructions, as well as between different reconstructions, are only fair if extraneous parts are masked out. In each of the seven cases, I generated a mask from the reference (Table 1), smoothed it with an averaging kernel, low-pass filtered it to 10 Å, and truncated it to remove negative values. The resultant fuzzy mask shows some variation in values within the masking region due to the low-pass filtering, but this has little effect on the FSC curve. The main point is that the mask does not contain any frequencies beyond 10 Å resulting in spurious high frequency correlations, and is suitable for masked FSC analysis and correlations used in alignment.

Rescaling the maps in real space resulted in interpolation artifacts (including aliasing) in the power spectra. To avoid this, I rescaled each map using the following protocol (implemented in the program bscale):

1. The map was first aligned to the reference, rotated and positioned without scale change to minimize interpolation artifacts. In cases with symmetry, equivalent symmetric orientations were tested.
2. The map sampling was then refined iteratively by cross-correlation with the reference.
3. The map was resized to give the same physical size as the reference (pixel size times map dimensions).
4. Finally, the map was Fourier transformed, resized in frequency space, and backtransformed to give a result directly comparable to the reference map.

I used the rescaled maps to calculate FSC curves with respect to the reference (FSCref) and between half or even-odd maps (FSCeo). The cutoff for the former was set at 0.5 (5 cases) or 0.3 (2 cases), and for the latter at 0.143. Further details of the analysis are given in the Appendix.

Table 1 Listing of the cases with details relating to the reference maps and masks used.

Case	EMDB ID	Reference map source	Size (edge pixels)	Symmetry imposed	Mask volume (Å^3, %)
GroEL	-	4hel	236	D7	1673807 (12.7)
T20S proteasome	6287	1pma	236	D7	1273135 (9.7)
Apoferritin	2788	4v1w	210	O	903158 (9.8)
TRPV1 channel	5778	3j5p	210	C4	565518 (6.1)
80S ribosome	2660	3j79, 3j7a	440	C1	4948140 (5.8)
Brome mosaic virus	6000	3j7l	420	I	6450047 (8.7)
ß-Galactosidase	5995	5a1a	264	D2	834535 (4.5)

Results

Visual inspection of the submitted maps

The map challenge results are presented as the full reconstructions, filtered and unfiltered, and the two halfmaps (presumed unfiltered). In several case the "unfiltered" and "filtered" maps are identical (cases 111, 119, 156), rendering the assessment of filtering meaningless. I did a visual inspection of the maps and their power spectra to assess their features and identify potential problems (see the Appendices for details of each case). There are considerable differences in the maps reflecting the diverse approaches taken. Here I attempt to guess at the processing steps as a precursor to estimate the quality and validity.

In many cases the "unfiltered" reconstructions already show some form of filtering. It is common that SPA reconstructions are done to a resolution limit chosen by the user based on the expected level of detail in the images. An effective low-pass filter is often part of the reconstruction, suppressing higher frequencies and improving the appearance of the map. The tables in Appendix 1 gives an indication of such filtering on the original reconstructions.

Where the reconstructions were further filtered, I noted the obvious effects to get an understanding of the type of map modifications (see Appendix 1). Real space masking or low-pass filtering are easily detectable in the map and its power spectrum. In both instances the aim is to remove noise that may interfere with analysis (such as calculating an FSC curve) or interpretation (modeling). The amplitudes in resolution shells may also be modified to enhance detail in the maps, also easily detected in power spectra. The most important reason for these map adjustments is to make them more amenable to modeling (not further explored here).

Attempt at an unbiased approach to compare maps

The maps submitted by different participants have different sizes, sampling and orientations. To have a fair assessment, these maps need to be converted into directly comparable forms. Because we are targeting resolutions around 3 Å, rescaling all maps to a sampling of 1 Å/voxel is a

reasonable approach. I orientated and the positioned the reconstructions with respect to references calculated from atomic coordinates of the corresponding structures. I was careful to rescale and orient the maps with the goal of minimizing the introduction of artifacts (see Methods). Where the specimen has symmetry, the equivalent symmetric views were checked to find the correct orientation.

Rescaling the maps to the same dimensions in each case also allows the use of the same mask for all. The masks were low-pass fiiltered to exclude high frequency terms that could affect alignment and analysis.

The maps were compared to the reference (FSCref) or between halfmaps (FSCeo) (see Appendix1). The cutoff for FSCref was chosen as 0.5 (i.e., where the SNR is one) in five of the case. In two cases (GroEL and TRPV1) a cutoff of 0.3 was used because the curves show poorer correlation at low frequencies. The cutoff chosen for the FSCeo is relatively arbitrary, because the correlation in a shell is a function of several variables, including the number of particles used, the SNR in the contributing images in that shell, and the error in alignment due to the SNR and structural variation. Because the alignment of the particles was supposedly done using independent data sets, the cutoff of 0.143 was used (ref). The FSCeo (0.143 cutoff) is always a more optimistic assessment of the detail compared to the FSCref, although in some cases it comes close (e.g., see the beta-galactosidase case in Appendix 1).

Discussion

All the maps have about the same appearance in terms of protein structure (such as secondary structure elements and the overall shape). The micrographs were selected to contain a high number of particles of the desired specimen. This means that the challenge is highly biased to generate the correct structure in each case. Where correct means that most particles picked represent the specimen in a reasonably faithful way. The number and quality of particles selected and their alignment therefore determines the detail achieved. Because this is unknown at this stage, the possible causes of problems in the reconstructions are assessed using features of the maps.

I cannot assess several issues because I do not have the relevant information. These include particle image numbers and selection criteria, alignment strategies and filtering, and reconstruction details. This leaves an analysis of the resultant maps and what I can learn from inspection.

The types of problems that can be deduced from the reconstructions are:

1. Poorly represented views
2. Reconstruction artifacts
3. Inappropriate masking – FSCeo does not go to zero
4. Overfitting – FSCeo appears much better than FSCref

Poorly represented views

If the view distribution of the particle images is uniform, the power spectrum of the reconstruction appears isotropic. Cases 149 and 150 (ribosome), 133 and 135 (TRPV1) show

evidence of poorly represented views (i.e., poorly represented data). There are wedges in the power spectra with lower amplitudes than the rest. This is absent in the other maps, suggesting a problem with alignment.

Artifacts in the reconstruction

Artifacts can arise from a poor view distribution, symmetry axis enhancement of noise, or interpolation problems.

Case 167 (ßGal): The map has strange artifacts: Streaks emanating from the density. This is very different from the typical reconstruction that shows an evenly textured noise distribution in the background. While the FSCeo is similar to that for the other maps, the FSCref shows very poor correspondence with the reference structure. The conclusion is that this map has multiple problems, issues with reconstruction and filtering.

Cases 159 and 164 (ßGal): These maps are identical. The reconstructions also have odd features that are prominently reflected in the power spectra. Because the overall shape of the molecule is correct, the issues are probably due to the reconstruction algorithm or filtering done after reconstruction. The filtering removed all negative densities, but this does not account for the strange features. The FSCeo does not go to zero, likely due to the artifacts. The FSCref shows lower correspondence than the other maps over most of the frequency range.

The apo-ferritin maps are prone to symmetry artifacts in general, particularly cases 112, 118, and 121.

Inappropriate masking

A poor mask introduces high frequency terms. When the same mask is applied to two maps and the maps then compared by FSC, these high frequency terms correlate, giving the impression of detail in the maps. An FSC curve that does not approach zero at high frequencies is often an indication of inappropriate masking. The following cases show signs of poor masking: 110 (BMV), 119 (Ribosome), 157 (ßGal), 158 (GroEL), 163 (TRPV1).

This particular manifestation of poor masking can be erased by low-pass filtering. If such filtering is done after masking during alignment, the danger of overfitting is lessened. The latter is effectively equivalent to using an appropriately softened mask or resolution limits in alignment.

Overfitting

Cases 106 and 154 (ßGal): In both these cases the FSCeo is much better than the other maps, as well as the FSCref. The full 154 has very little information beyond 3.5 Å, suggesting it had been low-pass filtered at some point. The conclusion is that the correlation of the high frequency shells result from the application of an inappropriate mask. Case 106 has lower correlation to the reference structure even at lower frequencies, a clear sign of overfitting (influence of noise during alignment).

Case 152 and to a lesser extent case 110 (BMV) show poor correlation (FSCref) at very low frequencies. The 152 reconstruction was band-pass filtered to 4-20 Å to exclude the lowest frequencies.
Case 110 is curious: It shows low correlation with the reference at frequencies below 40 Å, and high correlation at frequencies above 4 Å for FSCeo. The latter could be due to poor masking, while the former suggests some deeper problem. Note that 5 other maps do not have the same problems.

Cases 156, 161 (TRPV1) and 107 (Proteasome) shows signs of overfitting.

Acknowledgements

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References

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Appendix 1

Here I provide a description of each case with my detailed impression of the results. The varied nature of the submissions makes it difficult to present it in a rigid format. In Appendix 2 I show central slices and central sections from the power spectra of all the maps. The intention is to assess the nature of the reconstructions and guess at the type of filtering done. Some of the comments below refer to these impressions.

I also calculated the FSC curves between a reference and the full unfiltered map (FSCref), as well as the FSC curves between halfmaps (FSCeo). In each case I indicated what I consider to be the consensus FSC values for that case. This provides an idea of what level of detail can be achieved given the input data. Where a corresponding map and halfmaps are available from the original study, I calculated the FSC curves to compare with the curves from the different maps. In some cases the submitters actually did better than the original study.

GroEL

The particle images provided were generated using an electron microscope imaging model {Vulovic, 2013 #5130}. Despite the effort to achieve accurate modeling, the synthetic nature of the data means that it inherently has statistical properties that are different from those of real data.

One specific issue is that the source structure to generate the images does not have exact D7 symmetry. Thus, to distinguish maps with and without symmetry imposed, I ran a symmetry detecting algorithm. Two maps do not show exact D7 symmetry: 158 and 168.

Because of the symmetry issue, the maps were aligned and scaled to a density calculated from 4HEL assuming no symmetry. The comparison therefore should be all taken as asymmetric.

The FSCref estimates are narrowly distributed suggesting that in all cases the same information was incorporated into the reconstructions. The asymmetric maps (158 and 168) are clearly not better than the symmetric maps, indicating how difficult it is to capture slight deviations from symmetry. A moderate better correspondence is seen in the FSCref below a cutoff of 0.4, apparently retrieving some more detailed information. However, since these maps were processed symmetrically, this level of detail still obscures any asymmetry.

FSCref(consensus) = 4.2 Å (3), FSCeo(consensus) = 3.9 Å (4).

Table A1: Reconstruction features for the GroEL maps.

Map	Reconstruction resolution limit (Å)	Real space mask	Low-pass filter (Å)	Amplitude modification	FSCref (0.3, Å)	FSCeo (0.143, Å)
104	2.8, Nyquest	No	~4 (soft)	No	4.6	3.9
120	2.8, Nyquest	No	3.0	Yes	4.6	4.1
132	2.8, Nyquest	Yes	No	Yes	4.2	3.9
143	~4	No	4.1	Yes	4.2	3.9
153	2.8	No	6.0	Yes	5.7	4.6
158	3.6	Yes	No	No	5.2	4.5
165	2.8, Nyquest	No	3.9	Yes	4.2	3.9
168	3.7	Yes	No	No	5.2	4.6
169	2.8, Nyquest	Yes	No	No	4.5	4.1

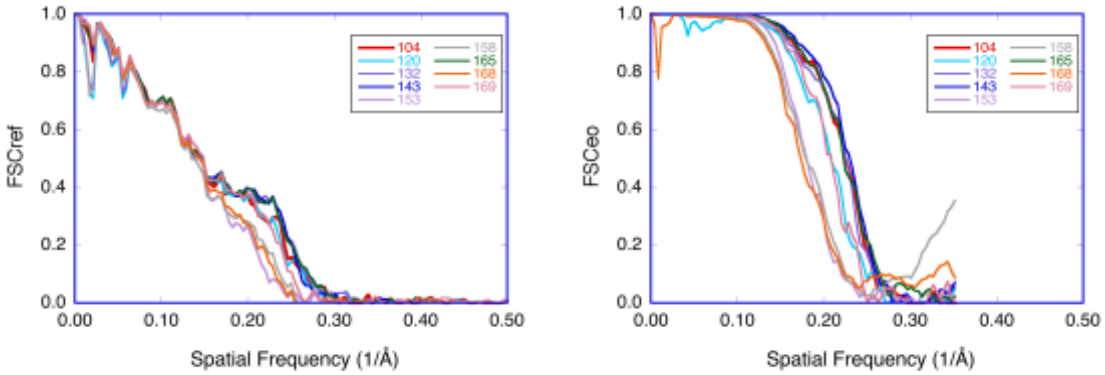


Figure A1: GroEL: (A) FSC curves against an asymmetric reference map calculated from 4HEL. (B) Even-odd FSC curves.

20S proteasome

For case 103, the "unfiltered" map has been low-pass filtered to 2.8 Å, but the poer spectra for the even-odd maps extends to Nyquest (2 Å).

The cases 130 and 131 have identical unfiltered maps, indicating a single source. 130 was low-pass filtered to 2 Å with amplitude modulation, while 131 appear to be real-space masked.

Cases 144 and 145 are near-identical, suggesting a very similar source. Both were low-pass filter to ~3 Å with amplitude modulation.

Only two cases show good agreement with the reference: 103 and 108. The other cases show poorer correspondence to the reference. Their FSCeo curves vary from lower (130, 131, 144, 145, 162), similar (141) and higher (107) than the curves for the reference.

The unfiltered 107 map appears to be real space masked. The FSCeo curve is greater than the reference while the FSCref curve is less. This suggests that the masking introduced high frequency features that showed up in the FSCeo curve, i.e., a case of overfitting.

EMD_6287: FSCref = 3.1 Å, FSCeo = 2.7 Å

FSCref(consensus) = 3.0 Å (2), FSCeo(consensus) = 2.6 Å (3).

Table A2: Reconstruction features for the T20S proteasome maps.

Map	Reconstruction resolution limit (Å)	Real space mask	Low-pass filter (Å)	Amplitude modification	FSCref (0.5, Å)	FSCeo (0.143, Å)
103	2.8 (2.0 for halfmaps)	Yes	No	No	3.0	2.8
107	2.6, Nyquest	No	No	Yes	4.3	2.6
108	2.0, Nyquest	No	No	Yes	3.0	2.6
130	~2.2	No	2.0	Yes	3.7	3.2
131	~2.2	Yes	No	Yes	3.7	3.2
141	2.0, Nyquest	No	2.8	Yes	3.6	2.6
144	2.1, Nyquest	No	3.1	Yes	3.9	3.0
145	2.1, Nyquest	No	3.0	Yes	3.9	2.9
162	2.0, Nyquest	No	2.8	Yes	3.9	2.8

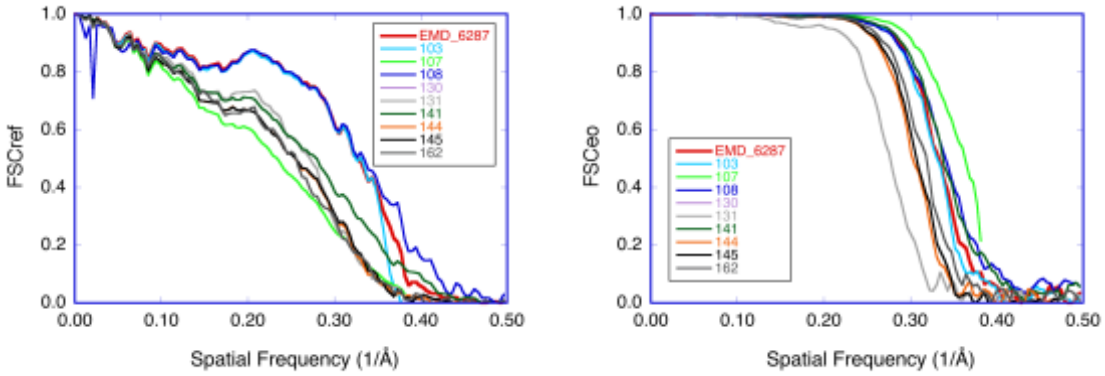


Figure A2: 20S proteasome: (A) FSC curves against an asymmetric reference map calculated from 1PMA. (B) Even-odd FSC curves.

Apo-ferritin

This case is somewhat complicated by the fact that the original map (EMD_2788) was calculated from a limited number of images. There is thus the potential for better reconstructions from more images. This is indeed what appears to have happened. A grouping of 4 cases performed much better than the original study (EMD_2788): (ordered from best to worst) 121, 112, 166 and 118. The assumption is that these were derived from larger sets of images. Case 124 is intermediate: similar to the original map but slightly better at higher frequencies. Case 147 is the worst both in comparison to the original map and in the even-odd analysis. Both cases 122 and 155 appear not to have been masked during reconstruction (from the unfiltered maps).

The main artifacts are associated with the 4-fold symmetry axes as seen in most of the maps.

EMD_2788: FSCref = 4.7, FSCeo =

FSCref(consensus) = 3.7 Å (3), FSCeo(consensus) = 3.4 Å (4).

Table A3: Reconstruction features for the apo-ferritin maps.

Map	Reconstruction resolution limit (Å)	Real space mask	Low-pass filter (Å)	Amplitude modification	FSCref (0.5, Å)	FSCeo (0.143, Å)
112	2.7, Nyquest	No	3.5	Yes	3.7	3.4
118	2.7, Nyquest	No	3.5	Yes	4.1	3.4
121	2.7, Nyquest	Yes	3.4	Yes	3.4	3.3
122	2.7, Nyquest	No	No	Yes	4.8	4.6
124	2.7, Nyquest	No	4.5	Yes	4.5	4.2
147	2.7, Nyquest	Yes	~5.5	Yes	6.6	6.3
155	2.7, Nyquest	Yes	~3.3	Yes	6.0	4.1
166	2.7, Nyquest	No	No	Yes	3.8	3.4

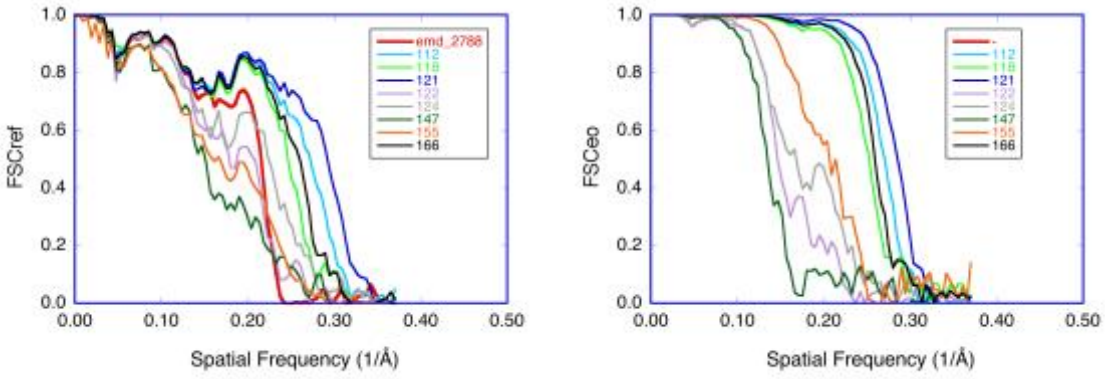


Figure A3: Apo-ferritin: (A) FSC curves against an asymmetric reference map calculated from 4V1W. (B) Even-odd FSC curves.

TRPV1

This specimen is complicated because extended parts of the structure are poorly represented. The mask calculated from the reference map gave uninterpretable FSC curves. A alternative mask was generated from the average of all the submitted maps to put all the reconstructions on the same footing. The effect of the poorly represented parts is evident from the FSCref curves. A cutoff of 0.3 was used because it more closely represent the observed detail in the maps. Given these issues, all the maps are comparable with respect to the reference. However, they differ remarkably in the FSCeo curves. Because the reference does not agree well with the maps, they are likely significantly better than the FSCref suggests, i.e., closer to the 3.3 Å estimated for EMD_5778.

Case 115 is slightly better than EMD_5778 in both FSCref and FSCeo, suggesting the quality is real. Cases 156 and 161 gave better FSCeo values, but similar FSCref values as EMD_5778. These may be cases of slight overfitting.

Cases 133, 135 are very similar, unfiltered more than filtered, both have radial artifacts in real space and varying representation of high frequencies beyond 4 Å.
No filtering was done in case 156.

EMD_5778: FSCref (0.3) = 4.6 Å, FSCeo = 3.3 Å

FSCref(consensus) = 4.1 Å (3), FSCeo(consensus) = 3.2 Å (3).

Table A4: Reconstruction features for the TRPV1 maps.

Map	Reconstruction resolution limit (Å)	Real space mask	Low-pass filter (Å)	Amplitude modification	FSCref (0.3, Å)	FSCeo (0.143, Å)
101	2.4, Nyquest	No	3.3	No	4.6	3.5
115	2.4, Nyquest	No	3.1	No	4.2	3.2
133	1.2, Nyquest	No	3.9	Yes	4.1	3.7
135	1.2, Nyquest	No	3.8	Yes	4.1	3.6
146	~1.8	Yes	No	No	4.4	4.1
156	2.4, Nyquest	No	No	No	4.6	3.1
161	2.4, Nyquest	No	3.2	??	4.5	3.2
163	3.0	Yes	No	No	4.3	4.1

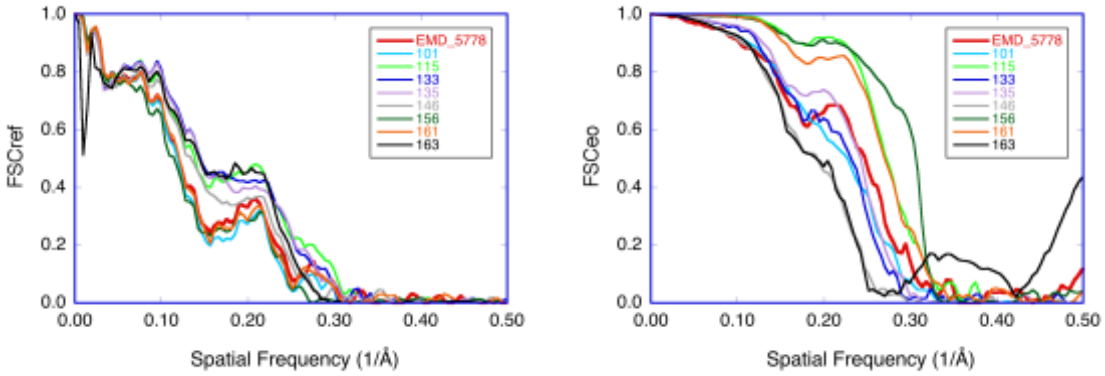


Figure A4: TRPV1: (A) FSC curves against an asymmetric reference map calculated from 3J5P. (B) Even-odd FSC curves.

Beta-galactosidase

The features of the maps allow them to be sorted into groups that most likely relate to the reconstruction algorithms used:
138 & 139
159 & 164 – these seem to be identical.

In general, most of the cases do not present truly unfiltered reconstructions, complicating statistical analysis. In one case (154), the apparently filtered map is identical to the unfiltered map, which appears to be filtered already.

Two of the cases (159 & 164) show power spectra with considerable anisotropy. Their real space histograms indicate that they were truncated to remove negative density, thus further complicating statistical analysis. Such truncation is considered a form of filtering. The power spectra also show an odd repeating pattern, as if it was reconstructed from a synthetic 2D crystal with high frequency terms originating from the edge transitions between panels.

Many of the maps were reconstructed to 2 Å or less, which is reasonable given the expected ~3 Å target resolution. One (116) shows an artifact at the cutoff resolution (2 Å) that is removed on filtering to a lower resolution.

EMD_5995: FSCref = 3.5 Å, FSCeo = 3.4 Å

FSCref(consensus) = 3.5 Å (4), FSCeo(consensus) = 3.3 Å (8).

Table A5: Reconstruction features for the beta-galactosidase maps.

Map	Reconstruction resolution limit (Å)	Real space mask	Low-pass filter (Å)	Amplitude modification	FSCref (0.5, Å)	FSCeo (0.143, Å)
106	2.6, Nyquest	No	2.5	Yes	4.0	2.8
113	2.6, Nyquest	No	3.7	Yes	4.2	3.4
116	2.0	No	3.2	Yes	3.6	3.4
134	1.8, Nyquest	Yes	No	No	3.4	3.3
138	1.3, Nyquest	No	3.0	Yes	3.6	3.3
139	1.3, Nyquest	No	3.0	Yes	3.5	3.3
154	~3.5	No	No	No	3.9	2.8
157	~3.0	Yes	No	No	4.1	3.5
159	2.4, Nyquest	Yes	No	No	4.6	3.2
160	~3.0	Yes	No	No	3.7	3.5
164	2.4, Nyquest	Yes	No	No	4.6	3.2
167	2.4, Nyquest	Yes	No	No	3.7	3.4

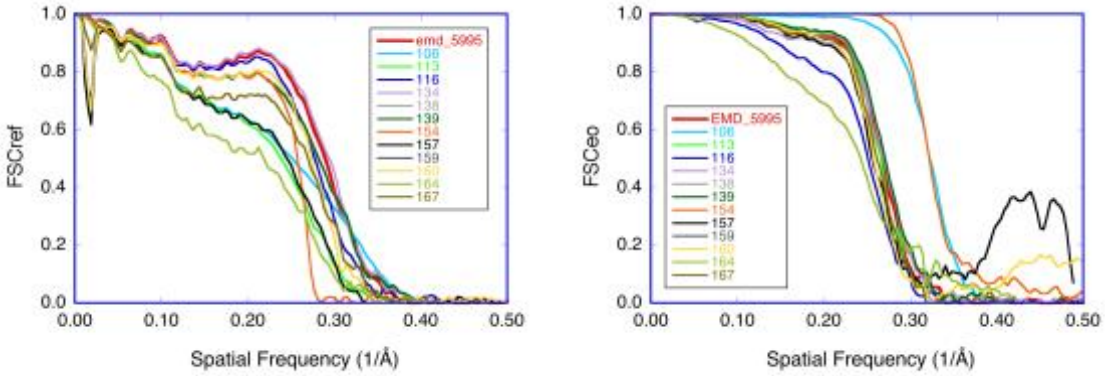


Figure A5: Beta-galactosidase: (A) FSC curves against an asymmetric reference map calculated from 5A1A. (B) Even-odd FSC curves.

BMV

All these reconstructions come out reasonably good in both the reference and even-odd comparisons, giving a resolution in the range of 3-4 Å. The best is case 102, closely resembling the EMD_6000 reference curve, but better in the FSCeo curve. My guess is that it did a better job of noise suppression in the reconstruction.

The maps have different orientations:
EMD_6000 and cases 102 and 110 show a 5-fold view.
Cases 136, 137, 140 and 142 show the standard 2-fold view.
Case 152 shows a 90° rotated version of the standard 2-fold view.

The original map, EMD_6000, was masked inside and outside, as well as low-passed limited to FS limit 2.7 Å.

102: 2Å (Nyquest), strong edge at 4 Å, FS filtered to 3.5 Å, amp mod

Case 110 shows clear evidence of inappropriate masking, resulting in the FSC curve not approaching zero at high frequencies.

Cases 136, 137, 140 and 142 were aggressively low-pass filtered.

Case 152 shows a strong limitation around 4 Å and was likely aggressively low-pass filtered. The FSC for the even-odd maps was truncated at 4 Å, with the correlation still much above 0.143. I could therefore not estimate a reasonable FSCeo.

EMD_6000: FSCref = 4.0 Å, FSCeo = 4.0 Å

FSCref(consensus) = 3.8 Å (3), FSCeo(consensus) = 3.5 Å (3).

Table A6: Reconstruction features for the brome mosaic virus maps.

Map	Reconstruction resolution limit (Å)	Real space mask	Low-pass filter (Å)	Amplitude modification	FSCref (0.5, Å)	FSCeo (0.143, Å)
102	2.0, Nyquest	No	3.5	Yes	3.7	3.4
110	~3.4	Yes	No	No	4.4	4.1
136	2.0, Nyquest	No	4.2	Yes	4.3	4.2
137	2.0, Nyquest	No	4.0	Yes	4.1	3.9
140	2.0, Nyquest	No	3.6	Yes	3.8	3.5
142	2.0, Nyquest	No	3.7	Yes	3.9	3.5
152	4 (- 20)	No	No	Yes	4.4	-

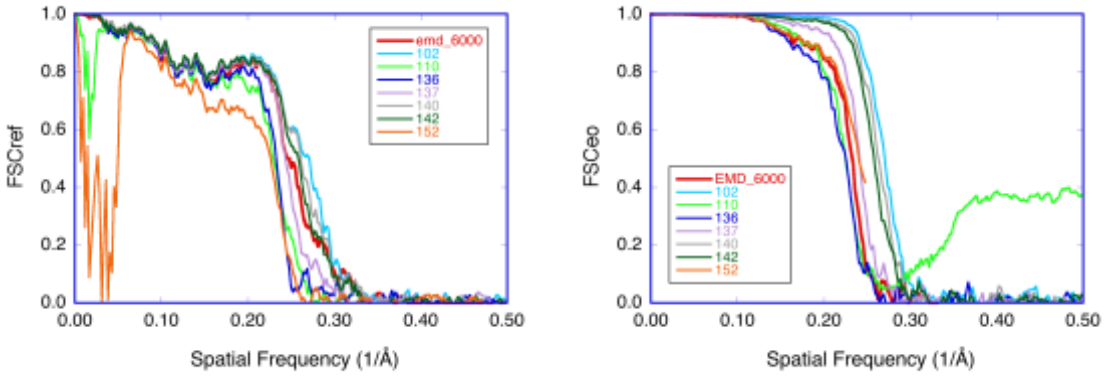


Figure A6: Brome masaic virus: (A) FSC curves against an asymmetric reference map calculated from 3J7L. (B) Even-odd FSC curves.

Ribosome

The small subunit is almost always of lower resolution, probably due to different conformations relative to the large subunit.

Cases 111 and 119 have diagonal artifacts in their power spectra that may be due to interpolation issues. The filtered and unfiltered maps are very similar and not low-pass filtered. Low frequencies up to 25 Å are poorly correlated, suggesting use of a low-resolution limit during alignment.

Cases 149 and 150 are very similar, with a diagonal line of low amplitude suggesting poor view representation.

EMD_2660: FSCref = 3.8 Å, FSCeo = 3.1 Å

FSCref(consensus) = 3.8 Å (7), FSCeo(consensus) = 3.2 Å (6).

Table A7: Reconstruction features for the ribosome maps.

Map	Reconstruction resolution limit (Å)	Real space mask	Low-pass filter (Å)	Amplitude modification	FSCref (0.5, Å)	FSCeo (0.143, Å)
111	2.7, Nyquest	No	No	No	4.2	3.2
114	2.7, Nyquest	No	3.3	Yes	3.8	3.2
119	2.7, Nyquest	No	No	No	4.4	-
123	2.7, Nyquest	No	3.0	Yes	3.7	3.0
125	2.7, Nyquest	No	3.2	Yes	3.7	3.7
126	2.7, Nyquest	No	3.3	Yes	3.8	3.2
127	2.7, Nyquest	No	3.5	Yes	4.1	3.4
128	2.7, Nyquest	No	3.6	Yes	4.2	3.5
129	2.7, Nyquest	No	4.3	Yes	6.1	4.1
148	2.7, Nyquest	No	3.9	Yes	4.5	3.8
149	2.7, Nyquest	No	3.3	Yes	3.9	3.2
150	2.7, Nyquest	No	3.2	Yes	3.9	3.1
151	2.7, Nyquest	No	3.3	Yes	3.8	3.2

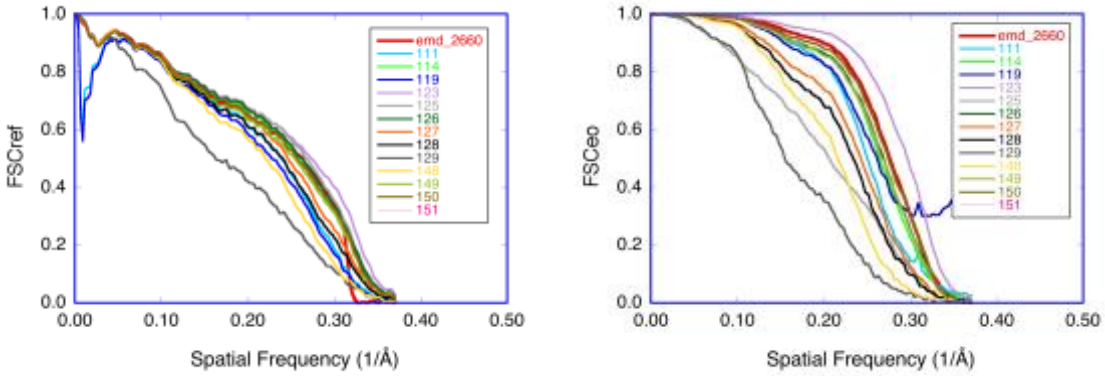
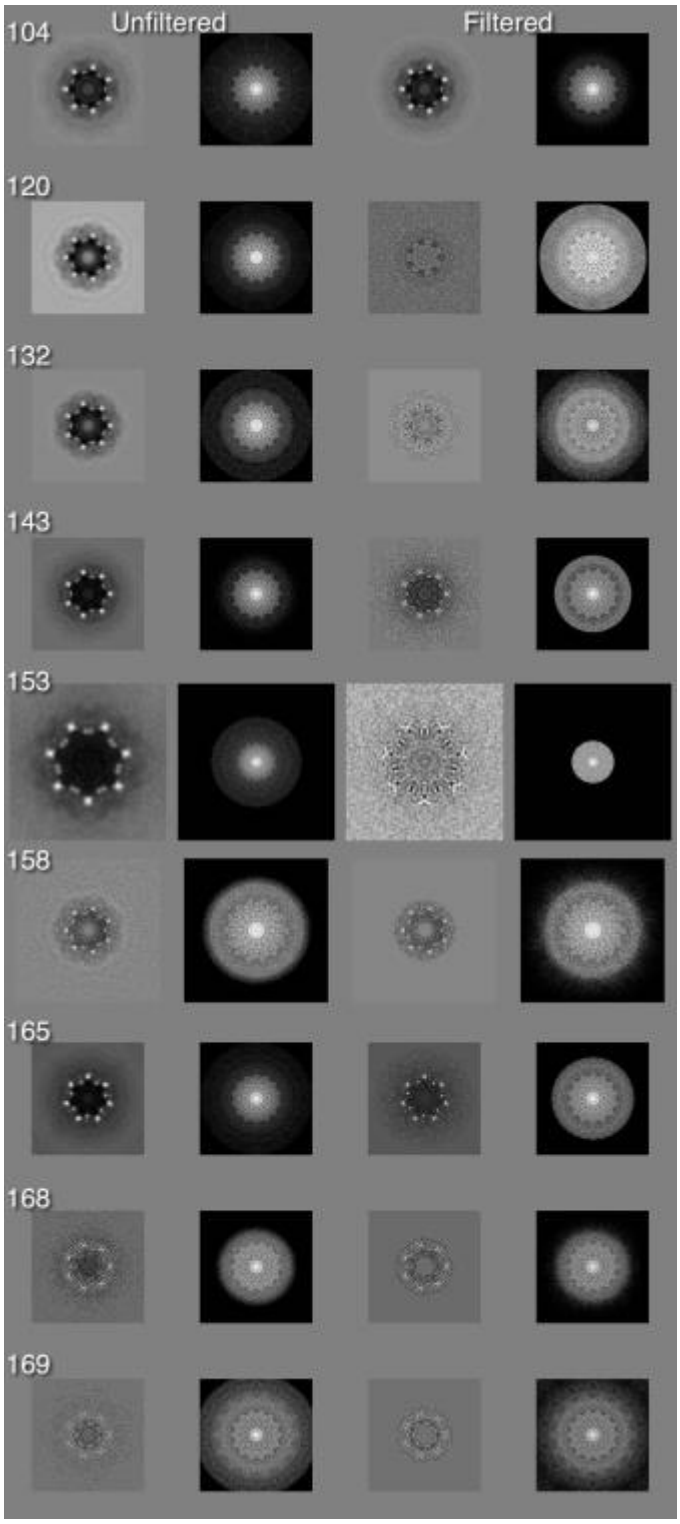


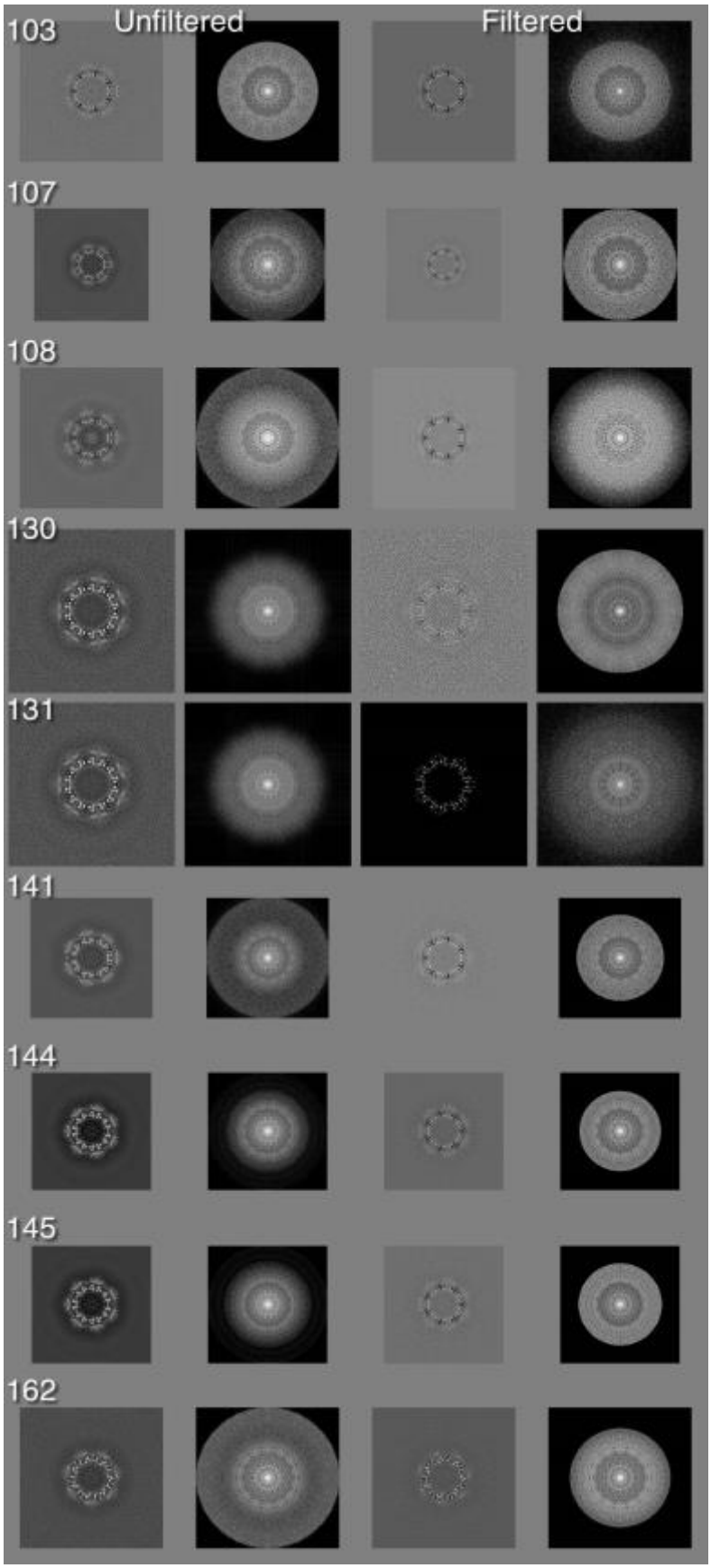
Figure A7: Ribosome: (A) FSC curves against an asymmetric reference map calculated from 3J79+3J7A. (B) Even-odd FSC curves.

Appendix 2: Central slices and sections of all maps

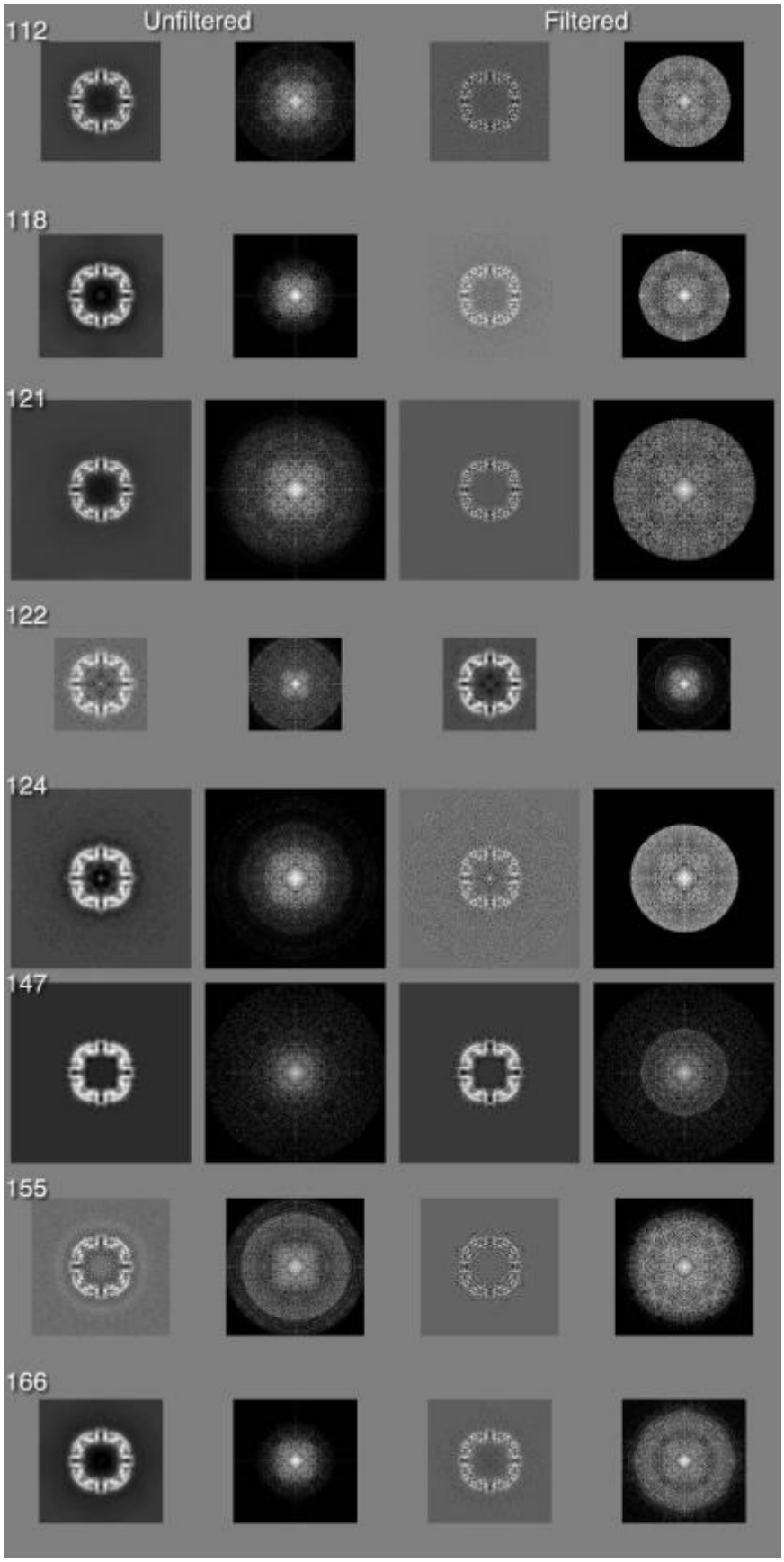
GroEL



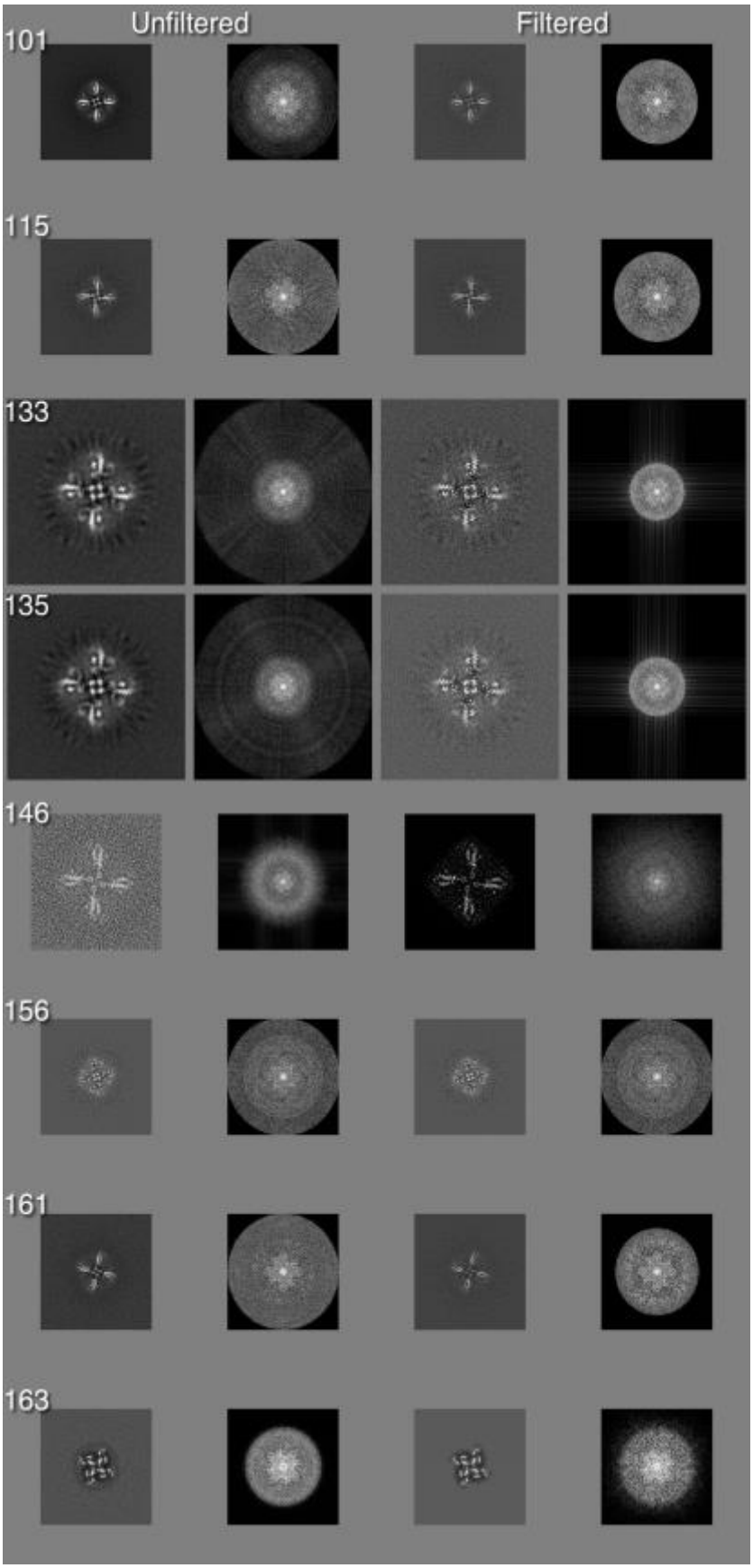
20S proteasome



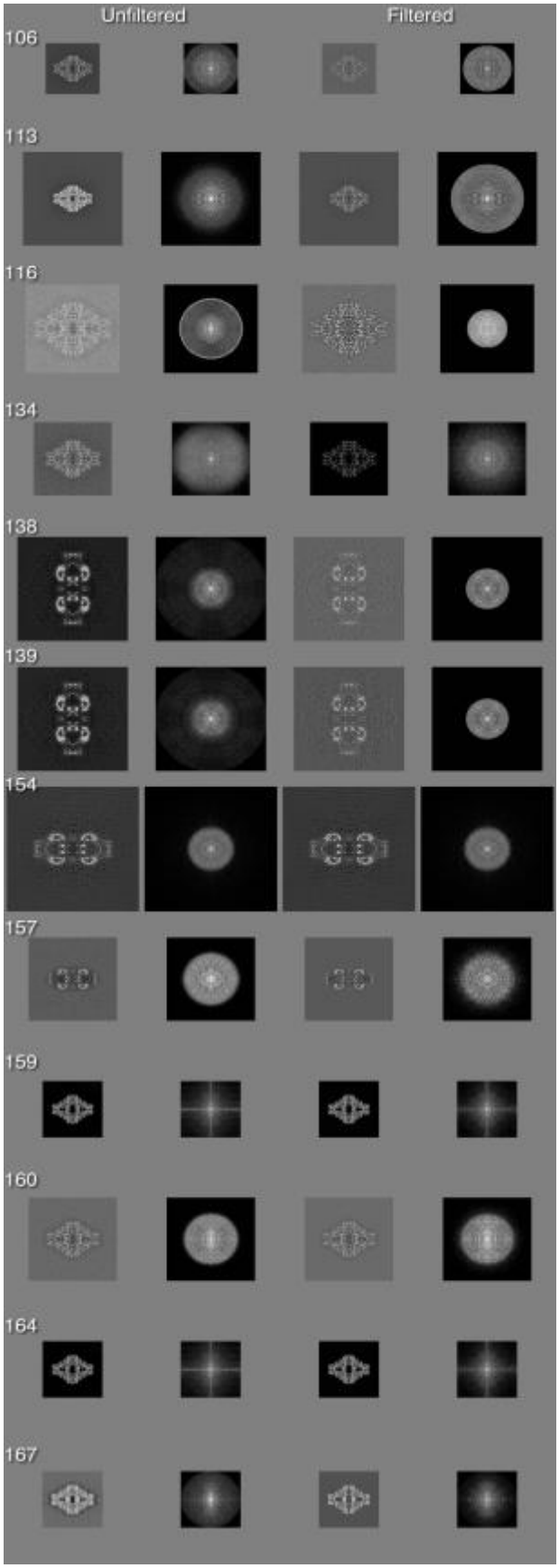
Apo-ferritin



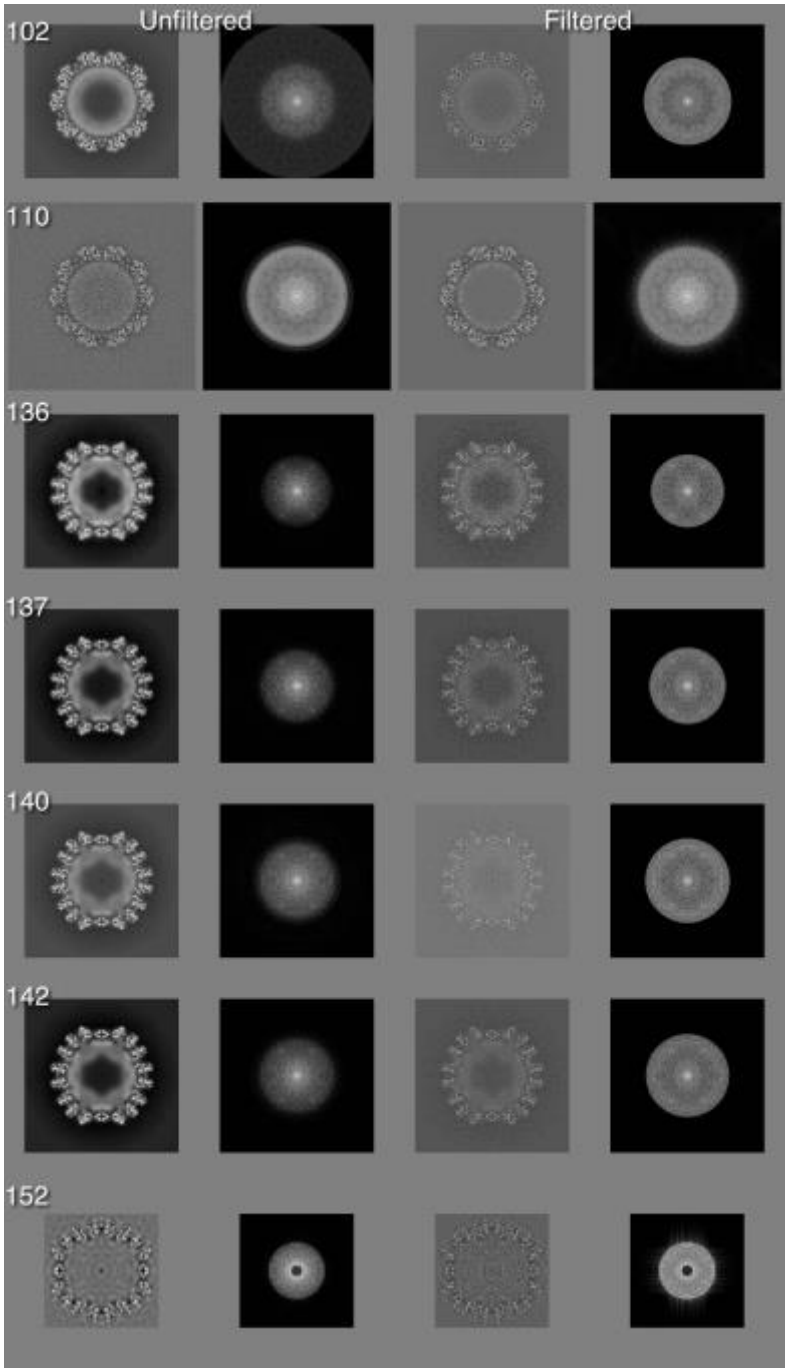
TRPV1



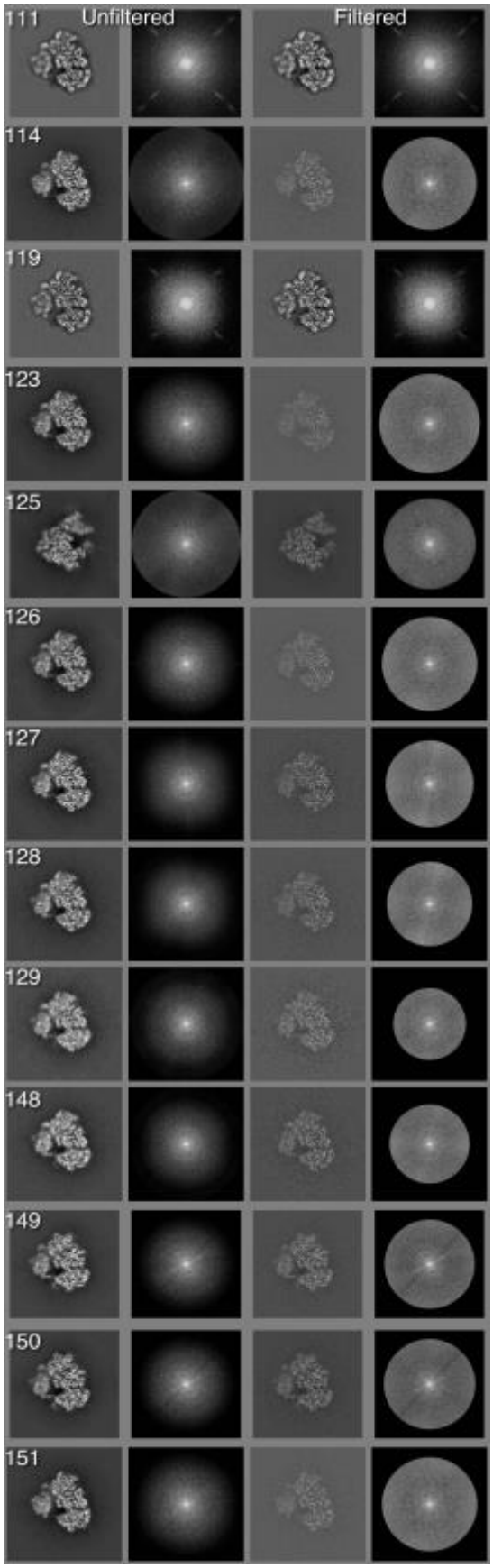
Beta-galactosidase



Brome mosaic virus



Ribosome


111
Unfiltered
Filtered
114
119
123
125
126
127
128
129
148
149
150
151