

Supplementary Information

Energy filtering enables macromolecular MicroED data at sub-atomic resolution

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Supplementary Table 1. Data processing and refinement statistics.

Data collection	
No. of crystals	17
Space group	<i>P</i> 4 ₃ 2 ₁ 2
Unit cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	66.92, 66.92, 107.56
α , β , γ (°)	90, 90, 90
Resolution (Å)*	56.90-1.09 (1.12-1.09)
Observed reflections	2,811,895 (95,185)
Unique reflections	98,530 (6,664)
Multiplicity	28.5 (14.3)
Completeness (%)	97.5 (90.3)
R _{merge}	0.284 (1.154)
$\langle I/\sigma I \rangle$	7.8 (1.4)
CC _{1/2} (%)	99.5 (33.0)
Refinement	
Resolution (Å)	56.82-1.09
No. of reflections	98,240
R _{work} /R _{free}	0.1498/0.1831
No. of atoms	
Protein	2,197
Ligand/ions	6
Waters	334
Hydrogen omit maps	
No. of hydrogen atoms	1,937
Peaks at $\geq 3\sigma$	767 (39.6%)
Peaks at $\geq 2\sigma$	1,354 (69.9%)
$\langle B \rangle$ factors (Å ²)	
Overall	11.29
Protein	9.36
Ligand/ions	10.86
Waters	25.19
R.m.s. deviations	
Bond lengths (Å)	0.012
Bond angles (°)	1.80

*Values in parenthesis are for the highest resolution shell. Data were truncated at a mean $I/\sigma I \geq 1.0$ and a cross correlation between two random half sets that is statistically significant at CC_{1/2} ~ 33%.

Supplementary Table 2. Comparison of data processing and refinement statistics of filtered and unfiltered MicroED data limited to 1.4 Å resolution.

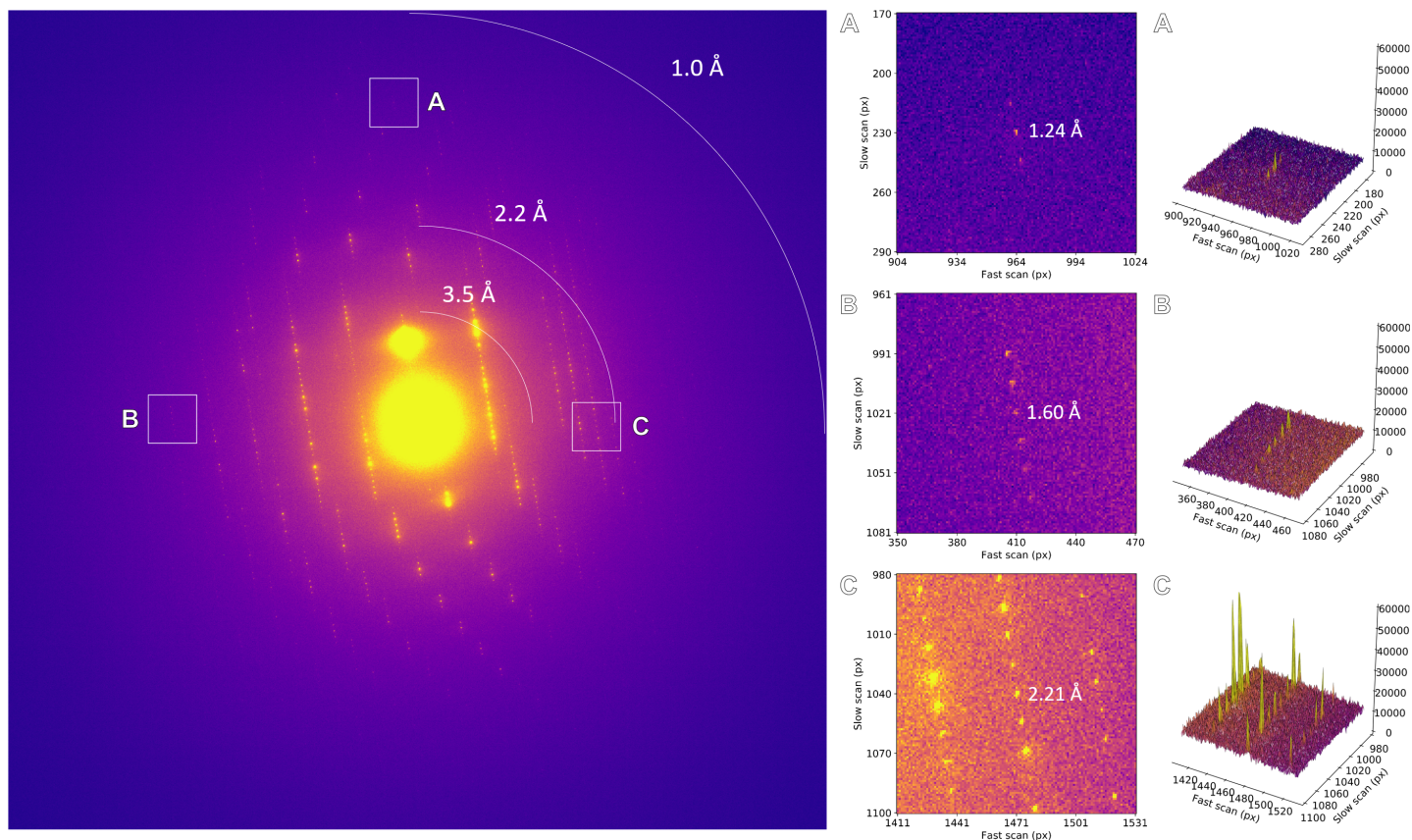
	Unfiltered [†]	Unfiltered [#]	Filtered
Data collection			
No. of crystals	5	12	17
Rotation range (°)	63.0	20.0	20.0
Flux density (e ⁻ /Å ² /s)	0.002	0.002	0.002
Fluence (e ⁻ /Å ²)	1.00	0.84	0.84
Dose (MGy) [‡]	3.66	3.08	3.08
Data integration			
Space group	<i>P</i> 4 ₃ 2 ₁ 2	<i>P</i> 4 ₃ 2 ₁ 2	<i>P</i> 4 ₃ 2 ₁ 2
Unit cell dimensions			
<i>a</i> , <i>b</i> , <i>c</i> (Å)	67.02, 67.02, 107.53	67.62, 67.62, 106.59	66.92, 66.92, 107.56
<i>α</i> , <i>β</i> , <i>γ</i> (°)	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)*	19.55-1.40 (1.45-1.40)	57.10-1.40 (1.44-1.40)	56.90-1.40 (1.44-1.40)
Observed reflections	1,231,502 (69,985)	1,101,042 (82,782)	1,371,884 (108,136)
Unique reflections	47,744 (3,442)	47,420 (3,467)	47,927 (3,494)
Multiplicity	25.8 (20.7)	23.4 (23.9)	28.6 (30.9)
Completeness (%)	97.3 (96.2)	96.0 (96.3)	98.2 (98.4)
R _{merge}	0.324 (1.784)	0.441 (1.987)	0.226 (0.598)
< <i>I</i> /σ <i>I</i> >	7.6 (1.4)	6.2 (1.4)	12.1 (5.3)
CC _{1/2} (%)	99.4 (27.0)	95.9 (23.2)	99.4 (85.7)
Refinement			
Resolution (Å)	19.77-1.40	57.10-1.40	56.82-1.40
No. of reflections	47,738	46,737	47,907
R _{work} /R _{free}	0.1374/0.1735	0.1538/0.1856	0.1208/0.1427
No. of atoms			
Protein	2,063	2,197	2,197
Ligand/ions	10	6	6
Waters	307	334	334
< <i>B</i> > factors (Å ²)			
Overall	14.47	12.19	10.61
Protein	12.51	10.11	8.52
Ligand/ions	27.44	10.15	10.19
Waters	27.21	25.92	24.39
R.m.s. deviations			
Bond lengths (Å)	0.015	0.016	0.013
Bond angles (°)	1.10	1.80	1.79

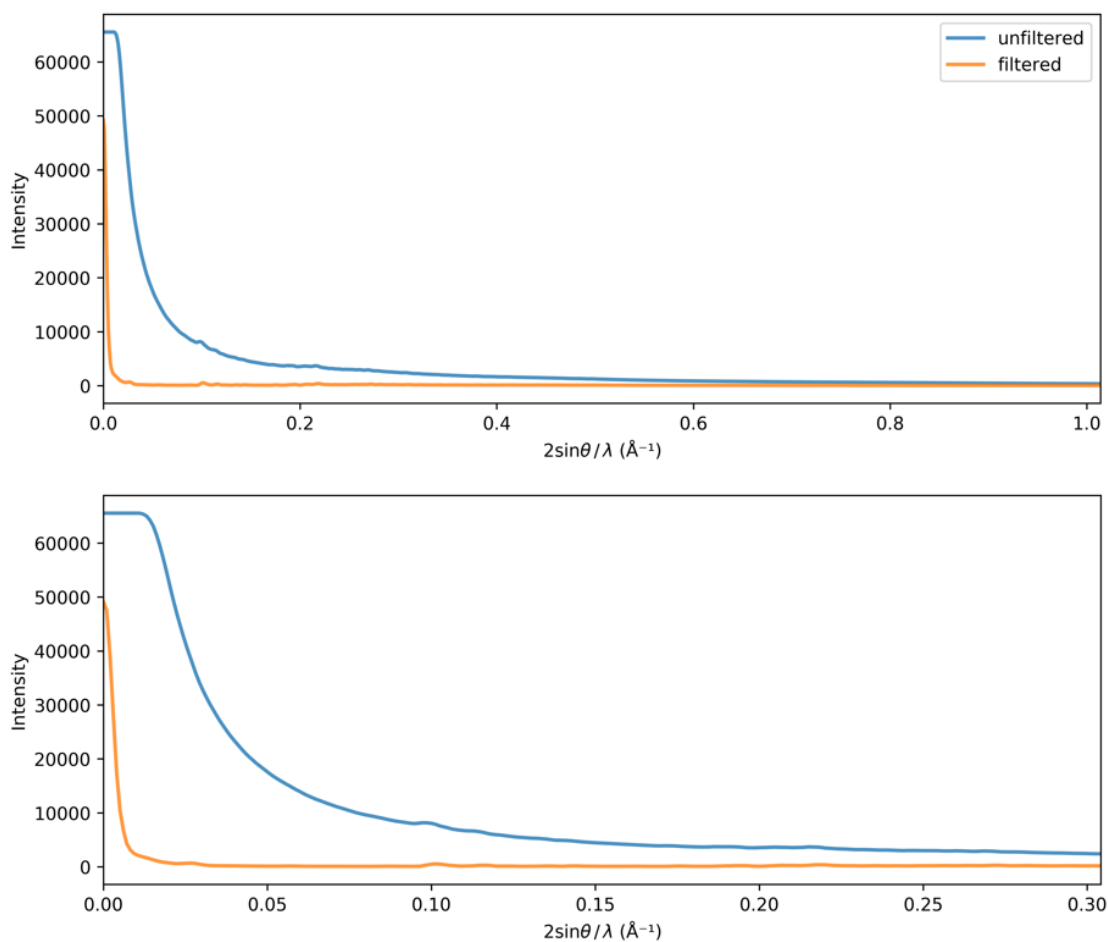
[†]Unfiltered MicroED data collected from 63° sweeps were previously published in Martynowycz *et al.*, 2023.

[#]An equivalent unfiltered MicroED dataset collected utilizing a 20° sweeps strategy, identical to the filtered data.

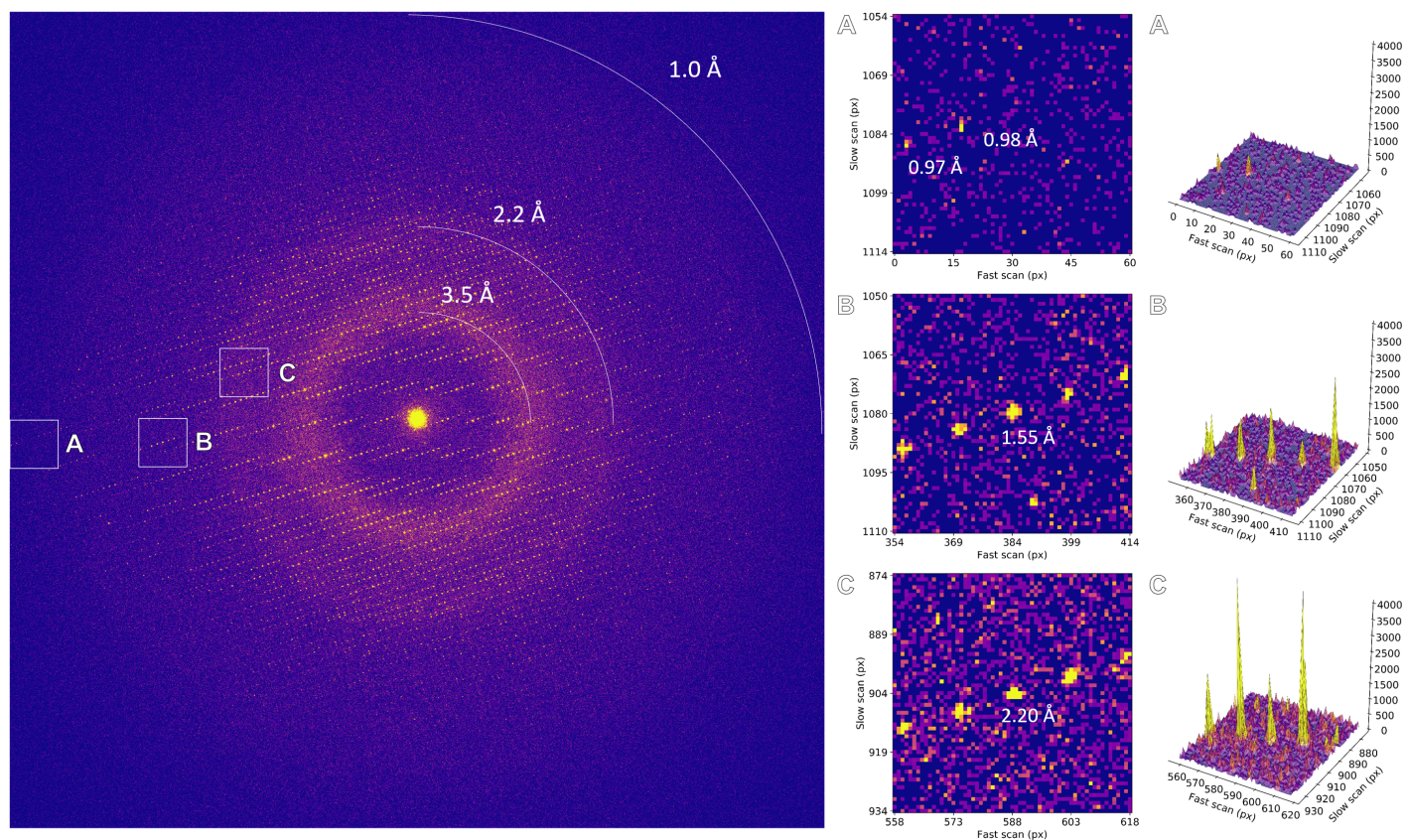
*Values in parenthesis are for the highest resolution shell. For an equal comparison, all data were truncated at 1.4 Å resolution.

[‡] Calculated with the EMED subprogram of RADDOS-3D (5.0.1047)²⁰.

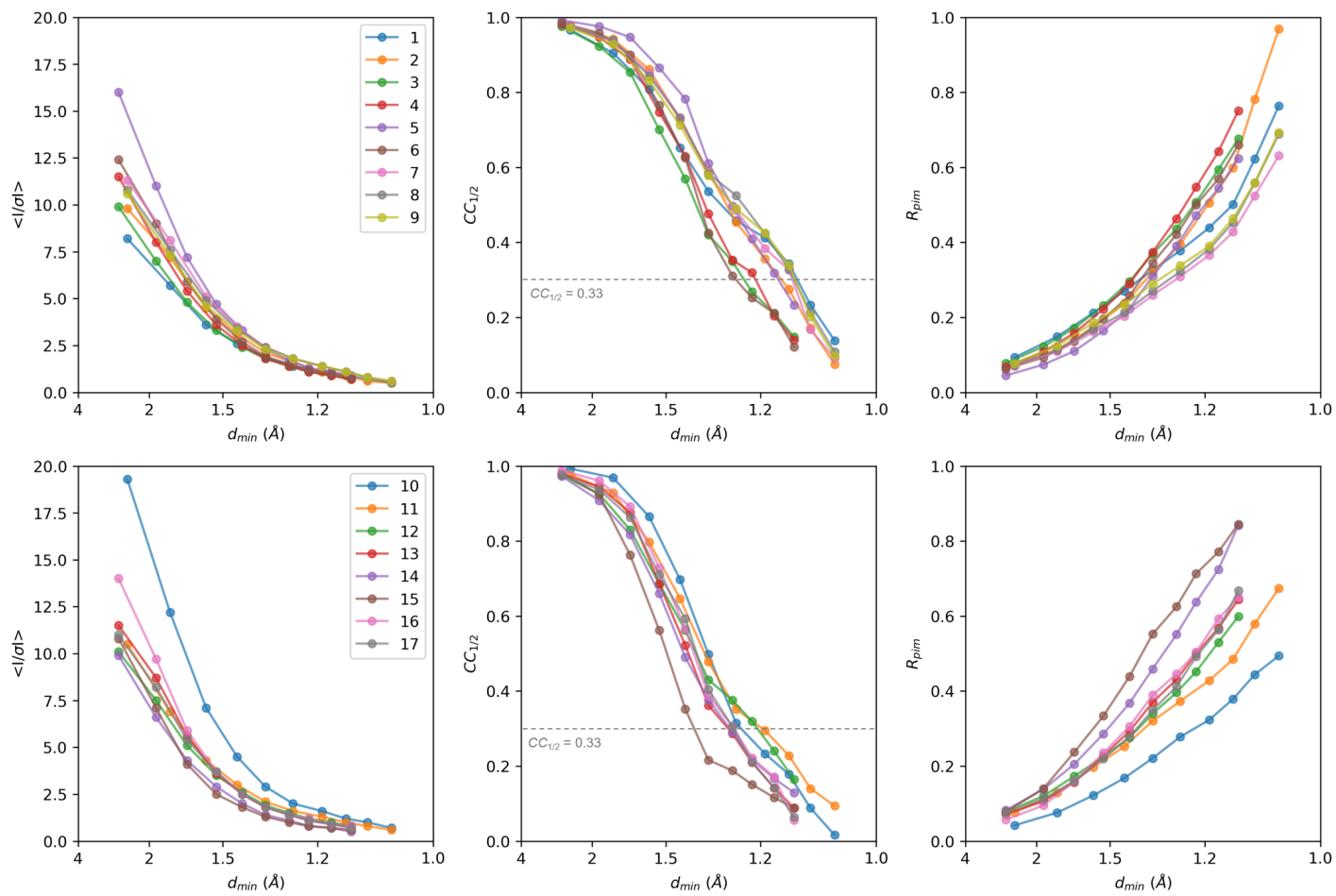




Supplementary Figure 2. Radial averages of unfiltered and filtered MicroED data. Radial averages of an energy-filtered diffraction frame (shown in Figure 1) and unfiltered data (shown in Supplementary Figure 1). Top: radial plots extending from the center peak to the edge of the detector, at approximately 1 \AA resolution. Bottom: Same radial plots focusing on the lower angle data, extending to ~ 3 \AA resolution.



Supplementary Figure 3. Energy-filtered continuous rotation MicroED data. Initial frame from energy-filtered and electron-counted MicroED data collection with a 5 s exposure time and a total fluence of $0.01 \text{ e}^-/\text{\AA}^2$. Highlighted areas are magnified in the right panels and the corresponding peak profiles are plotted.



Supplementary Figure 4. Intensity statistics for individual datasets. Intensity statistics for individual MicroED datasets, featuring from left to right I/σ , $CC_{1/2}$, and R_{pim} as function of resolution. Individual datasets were truncated ranging from 1.13 to 1.06 Å resolution at a $CC_{1/2}$ that was still significant at the 0.1% level.