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Improving data quality for 3D electron diffraction (3DED) by Gatan Image Filter (GIF)

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During the last two decades, 3D electron diffraction (3DED) is developing a regular and reliable technique for structure determination. This technique is becoming a complementary technique to single-crystal X-ray diffraction (SCXD) and single particle analysis. The development of 3DED as a method for structure determination is pioneered by electron diffraction tomography (EDT)[1] and rotation electron diffraction (RED)[2], which utilize stepwise rotation along a single axis. More recently, new protocols, such as microcrystal electron diffraction (MicroED)[3], fast-EDT[4] and continuous rotation electron diffraction (cRED)[5,6] are merging for determining structure of beam-sensitive materials. They are based on rotating the sample stage at constant speed while collecting ED patterns.

The strong interaction between electrons and materials brings dynamical scattering. With kinematic approximation used in most of the current methods, lots of structure details can be revealed already. However, usually there are still some unexplainable residue potentials. These residue potentials may hinder further discovery of finer structures during refinement and the final R1 value is usually larger than 10%, which will consider questionable for many small molecule X-ray crystallographers.

Another issue for electron diffraction is inelastic scattering, which brings background in the diffraction patterns. This background is most obvious for electron diffraction patterns from protein crystals, especially at low angles. Even though modern diffraction data software has sophisticated background removal algorithms to deal with this, the existence of inelastic scattering will still add errors in the diffraction experiment. The inelastically scattered electrons can be removed by energy filters. Yonekura et.al. utilized an in-column omega energy filter and did a systematic investigation on partial charges in protein crystals[7]. Gemmi et.al reported energy filtered EDT and they found the obtained structure from filtered datasets was closer to the X-ray refinement[8]. However, these experiments require an in-column omega energy filter, which is not commonly equipped in the microscopes. Therefore, the accessibility for this method is limited.

Here, we implemented energy-filtered 3DED using GIF in both TEM and STEM mode. The schematics were shown in Figure 1. Nowadays many TEMs are equipped with a GIF, ensured the accessibility of this method. The main advantage for this method is removing the inelastically scattered electrons, which removing a part of dynamical scattered electrons at the same time[7]. In addition, in order to track the crystal in STEM mode, we developed a tracking method based on monitoring live HAADF image stream. This can avoid crystal moving out of the beam during the tilting and the tilt range can always reach the maximum tilt range of the microscope (in our case ~150°). Datasets from NaCl and $NH_4H_2PO_4$ crystals were tested in energy-filtered 3DED experiments in STEM mode and the results were summarized in Table 1 and Table 2. The results showed that the final R_1 values have been improved after energy filtration.

Table 1 Structure refinement results for NaCl crystals

Dataset no.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Energy-filtered	Yes	Yes	Yes	Yes	No	No	No	No
Rotation Range (°)	137.2	150.4	150.6	133.4	136.1	144.3	135.2	137.9
Resolution (Å)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Reflection No. (Fo > 4sig(Fo))	21	19	21	21	21	18	19	16
Reflection No. (all unique)	21	20	21	21	21	19	20	18
R_1 (Fo > 4sig(Fo))	7.9%	7.5%	10.1%	8.3%	11.7%	13.8%	15.6%	14.7%
R_1 (all reflections)	7.9%	7.9%	10.1%	8.3%	11.7%	15.2%	15.7%	15.9%
Goof	1.34	1.461	1.464	1.217	1.34	1.383	1.481	1.038
No. of Parameters	4	4	4	4	4	4	4	4

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Table 2 Structure refinement results for $NH_4H_2PO_4$ crystals

Dataset no.	**1**	**2**	**3**	**4**	**5**	**6**
Energy-filtered	Yes	Yes	Yes	No	No	No
Rotation Range (°)	144.3	140.5	137.0	130.9	140.9	143.1
Resolution (Å)	0.85	0.85	0.85	0.85	0.85	0.85
Reflection No. (Fo > 4sig(Fo))	174	173	166	171	166	155
Reflection No. (all unique)	189	188	183	179	184	183
R_1 (Fo > 4sig(Fo))	8.3%	10.5%	11.9%	11.0%	11.6%	14.5%
R_1 (all reflections)	8.8%	12.6%	12.7%	11.1%	14.2%	18.9%
Goof	0.979	1.158	1.193	1.240	1.352	1.442
No. of Parameters	21	21	21	17	21	21

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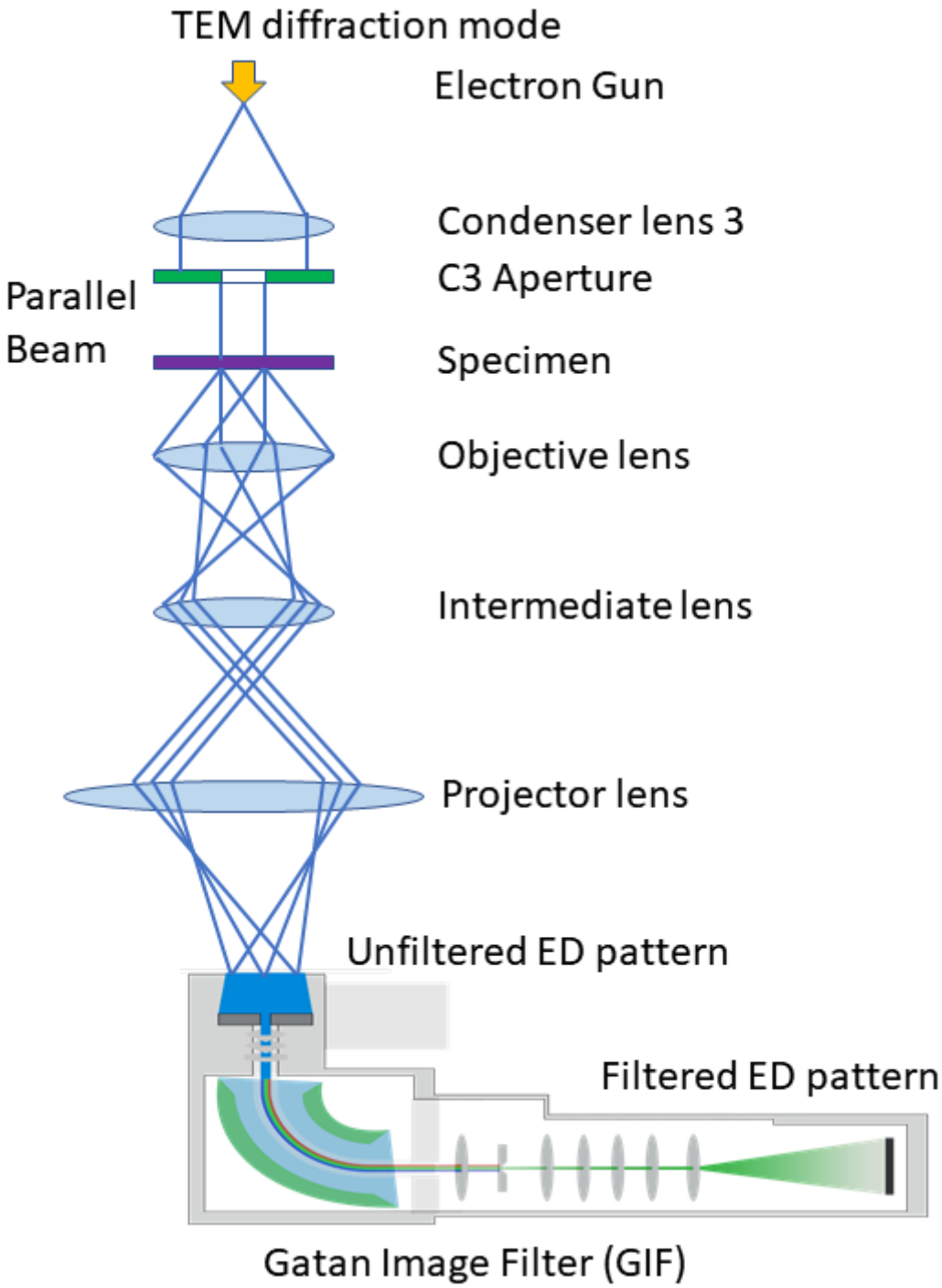

Figure 1. Figure 1. Schematics of energy-filtered 3DED experiments implemented in TEM mode

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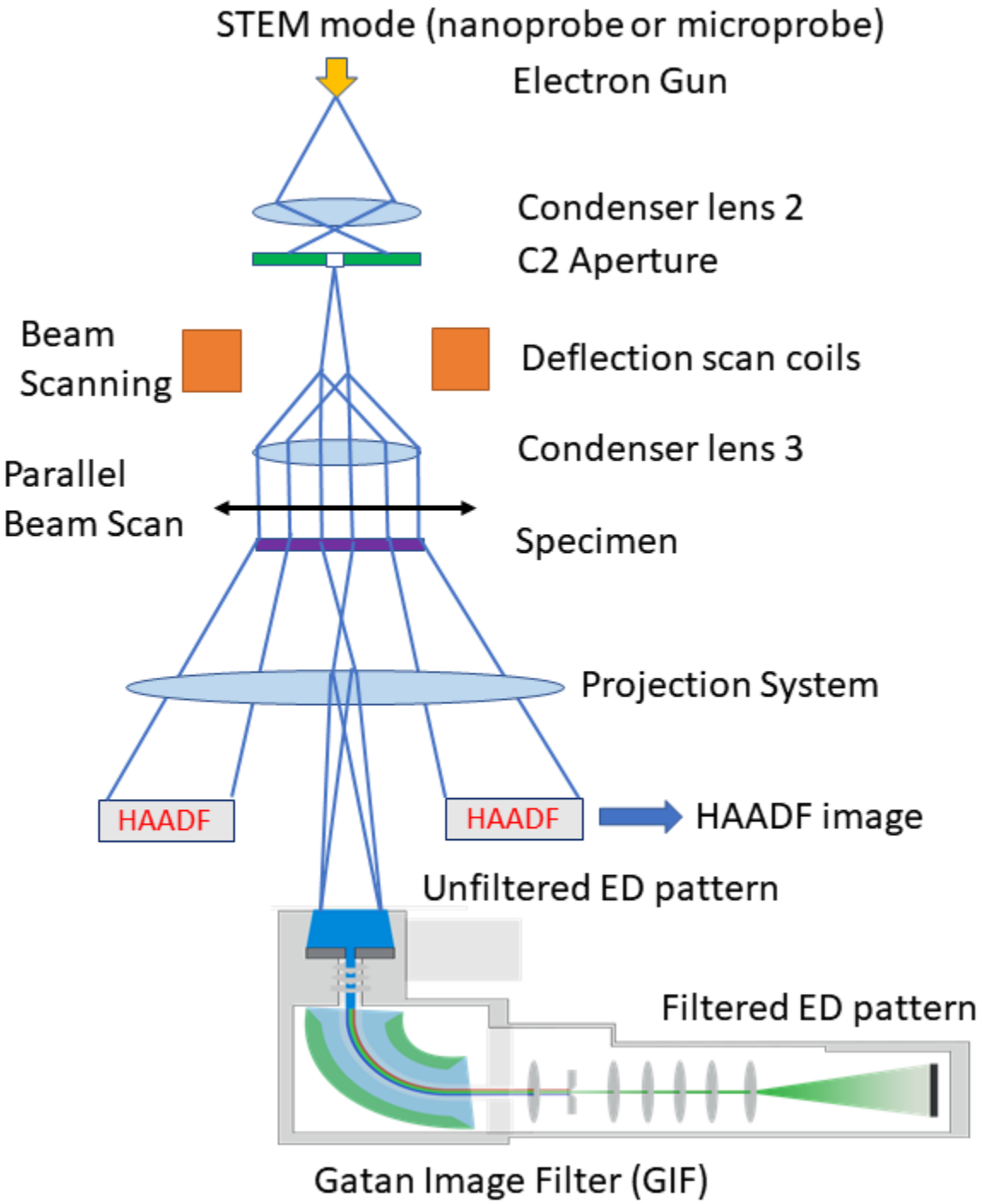

Figure 2. Figure 2. Schematics of energy-filtered 3DED experiments conducted in STEM mode

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