Atomic resolution structures of biological macromolecules using microED on JEOL TEMs

Micro electron diffraction, or microED, is a technique aimed at solving structures of biological macromolecules by electron diffraction. Barn-storming work by the group from Prof. Gonen showed the impressive impact and promise of this technique[1]. The technique borrows from X-ray crystallography in that precession techniques are used for data collection and that much of the well-established software for solving structures by X-ray crystallography can be used for microED. However, it differs in a fundamental way in that electrons are used, which, owing to the substantially larger scattering cross-section of electrons with biological matter, means much smaller crystals can be used. Thus, crystals the size of a few microns are perfectly adequate for microED. The current crop of cameras and levels of microscope automation allow for the rapid collection of a full data set in a matter of minutes. By combining microED with the principle steps of SPA as employed in SerialEM[2], a single overnight run can yield hundreds of microED data sets[3]. Note that because diffraction data is collected, the acquisition process is immune to mechanical vibrations and drift; only a shift caused by poor eucentricity that would move the crystal out of the selected aperture field of view would impact the data collection.

MicroED can be applied on every new electron microscope in the JEOL TEM line-up. Precise control of the goniometer's tilting speed compatible with microED is standard and can be set through either SerialEM or Recorder, the latter being part of JEOL's TEMography package (SIF). SerialEM was first introduced on a JEOL JEM-2200FS in 2006 and is now fully compatible with all of JEOL's electron microscopes. A record-breaking 1.34Å structure of apo-ferritin was recently obtained from JEOL's latest generation of cryo-TEMs, the CRYO ARM™, a microscope available at 200 and 300 kV with a cold field emission gun and in-column energy filter[4].

This application note reports on microED results obtained on a JEOL JEM-F200 outfitted with a cold FEG and a Gatan Elsa holder to keep the sample at -177ºC for the sake of reducing radiation damage[5]. Figure 1 shows a gallery of principle checks whether crystals of L-histidine will deliver high-resolution diffraction patterns for microED. The micro-crystals are deposited on ultrathin carbon films overlaid on lacey carbon.

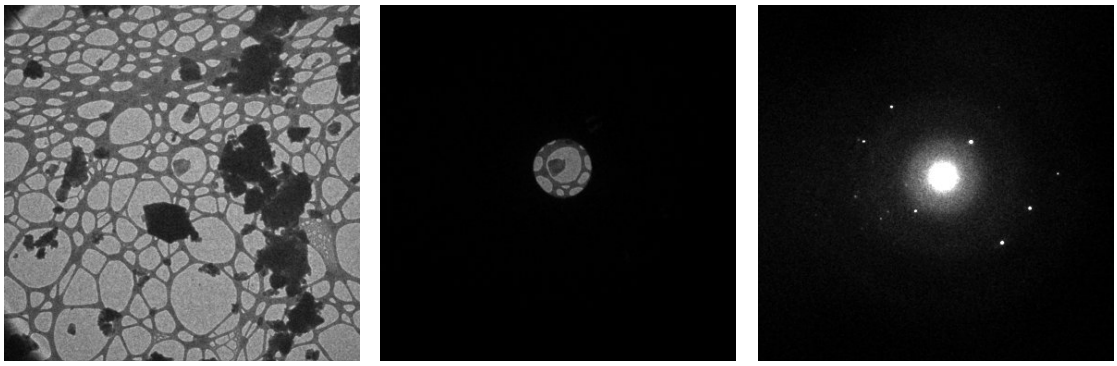

Fig. 1: Diffraction images of L-histidine in defocused diffraction mode without (left) and with (middle) a selected area aperture inserted showing the L-histidine crystals. The focused diffraction pattern is shown on the right showing strong Bragg reflections.

Figure 2 shows several frames from one of the microED data sets acquired under continuous rotation at 0.25°/sec from -30° to +30°. The beam stopper is used to prevent the central, undiffracted beam from hitting the camera's sensor.

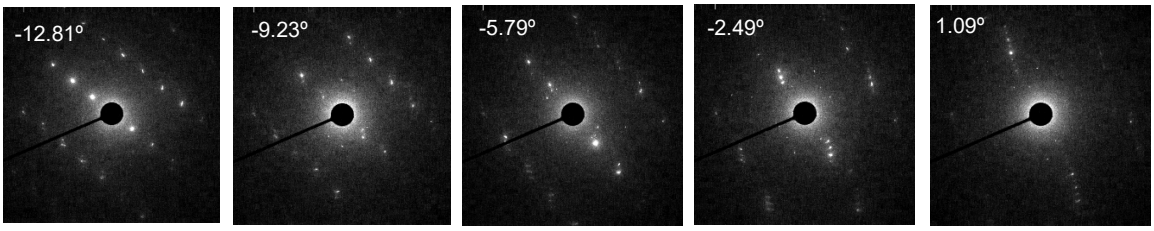

Fig. 2: Selected focused diffraction patterns from -30° to +30° microED series acquired at 0.25°/sec. Total dose was 2.4 el/Å².

After unpacking the movies, the individual frames are processed using standard X-ray crystallographic packages – SIR2019 and SHELXL. Table 1 shows the experimental results whereas Figure 3 shows the structures calculated from the data using direct methods. The refined map after running SHELXL shows clearly the expected atoms in the structure.

Table 1: experimental results	
Molecular weight (Da)	155.16
Measurement temperature (K)	96
Space group	$P2_12_12_1$
a, b, c (Å)	5.27, 7.44, 18.99
# of reflections	417
R factor (%)	19.81

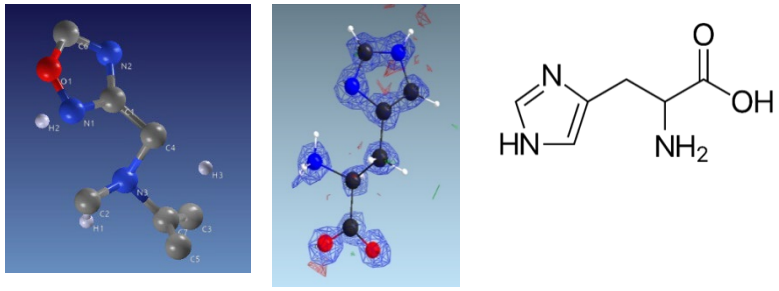

Fig 3: Structure of L-histidine after initial direct method calculation using SIR2019 (left) and after refinement using SHELXL (middle) with the structure formula shown right.

Conclusion: Micro electron diffraction data can be readily acquired using a JEOL cryo-electron microscope using relatively shallow tilt series. The resulting data can immediately be processed using available pipe lines for X-ray diffraction data enabling rapid structure determination of small molecules by electron diffraction.

Contact your local JEOL representative to learn more about JEOL TEMs and microED using SIF Recorder or SerialEM.

References:

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2. D.N. Mastronarde, J. Struct. Biol. 152 (2005) 36.
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5. Guzmán-Afonso et al., Nature Comm 10 (2019) 3537.