Breaking the 1-μm barrier with the electron diffractometer XtaLAB Synergy-ED

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Abstract

3D electron diffraction (3D ED)/Micro electron diffraction (MicroED) is a technique that can provide measurers with three-dimensional molecular structures from crystals of submicron order. However, 3D ED/Micro ED requires expertise in both electron microscopy and crystallography. Here, we introduce the newly developed electron diffractometer XtaLAB Synergy-ED specialized for 3D ED/MicroED experiments, its instrument configuration, measurement flow, and measurement examples.

Configuration of the XtaLAB Synergy-ED

Historically, 3D ED/microED has been performed with a high-resolution cryo-electron microscope, which is used mainly for protein structure determination by single particle analysis. However, high-resolution cryo-electron microscopes require intensive adjustments of lens conditions and other factors when switching to diffraction mode to perform 3D ED/microED experiments properly, and only experts familiar with electron microscopes can use them. To enable non-experts to perform 3D ED/microED experiments easily, the XtaLAB Synergy-ED has been designed with the following configuration[1] (See also Figure 1); the XtaLAB Synergy-ED is dedicated to electron diffraction; therefore, the ability to acquire high-resolution transmission electron microscope (hereafter "TEM") images is not necessary, and a magnification of about 10,000× (i.e., the ability to observe crystals of a few nm) is sufficient to search for crystals on a grid. The diffraction experiment requires a parallel beam and minimal changes in lens values when switching between image and diffraction modes. To achieve this, the electron optics were optimized by excluding the condenser mini-lens from the lens system and replacing the strong objective lenses with weak ones. These changes elevated the parallelism of the incident beam and minimized the change in lens values when switching between image and diffraction modes.

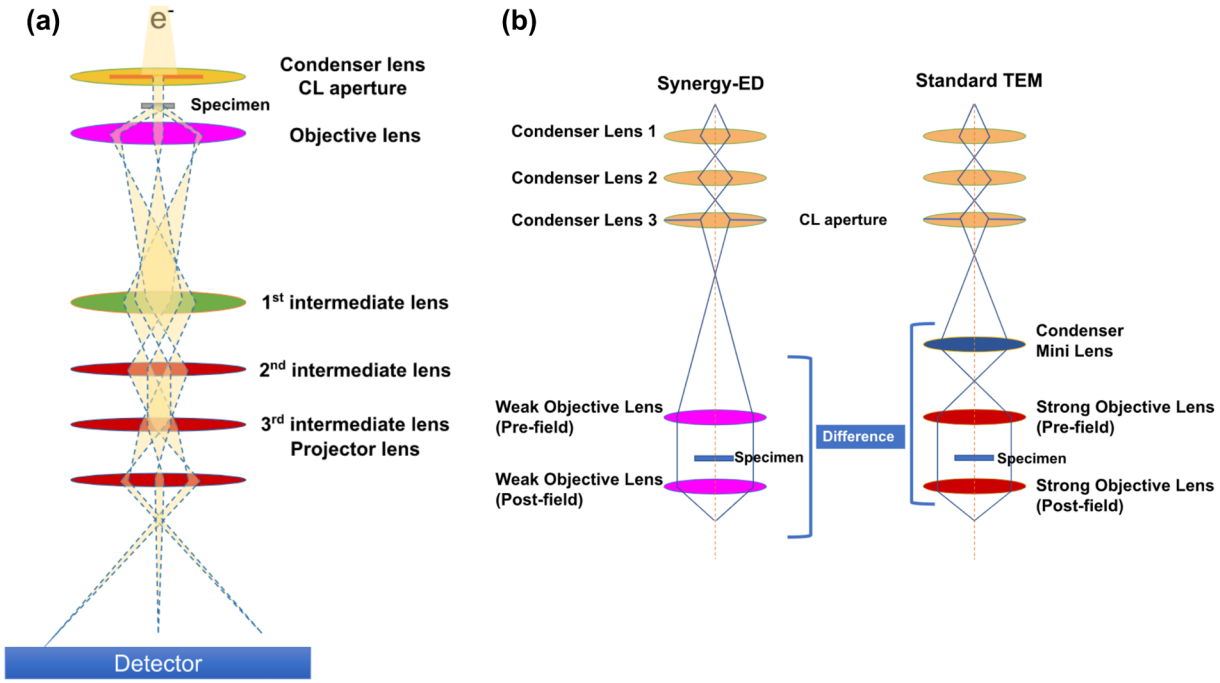

Fig. 1. The XtaLAB Synergy-ED electron diffractometer, specifically designed for 3D ED/microED experiments (a) Overall view of the lens system of the XtaLAB Synergy-ED. Although simplified in this figure, there are three condenser lenses and two objective lenses installed, as shown in (b). (b) Differences in lens systems between this study and a standard TEM

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This allows us to switch between modes by clicking a button in the integrated software package CrysAlis^{Pro} for ED. This simplification of the lens system also allows the XtaLAB Synergy-ED to use a fixed setting for the intermediate lens in the imaging system, which greatly reduces the amount of work for the measurer, such as manual adjustment of each lens value and the selection of appropriate apertures. Finally, a detector for electron diffraction, the HyPix-ED, has also been developed and incorporated into the XtaLAB Synergy-ED. The HyPix-ED is a direct detection detector based on the HyPix detector used for X-ray diffraction. This robust detector is resistant to the incident beam and does not require a direct beam stop. In addition, HyPix-ED is capable of detecting a single electron, which allows high-quality measurements.

Experimental Section: From Sample Preparation to Structure Determination

We have developed a number of X-ray diffractometers, and the latest flagship model, the XtaLAB Synergy Custom equipped with an FR-X generator, can measure crystals as small as roughly one micron in size. However, crystals smaller than 1 μm are difficult to measure with X-rays, and we call this the "1-μm barrier". To overcome this, the XtaLAB Synergy-ED is used to enable crystal structure analysis of nanocrystals. The measurement process can be briefly described as follows (see Figure 2): 1. Spread fine grains of the compound on a grid. A pair of microscope glass slides was used to crush the samples to reduce the crystal size when the average crystal size was larger than 1 μm. The thickness—which means here the size measured along the beam direction—of the crystal is more important than its other dimensions. Even if the crystal size itself is large, it can be measured as long as its thickness is less than 1 μm. It is important to note that the optimal sample thickness depends on the type of compound and the acceleration voltage of the electron beam; for organic molecules, less than 1 μm, for organometallic complexes, less than 500 nm, and for inorganic materials, less than 100 nm with a 200 kV electron beam. However, nearly any sample can be assessed if the sample thickness is less than 1 μm. 2. Install the grid into the XtaLAB Synergy-ED. 3. Locate the crystals in imaging mode and find the eucentric position (a step known to X-ray crystallographers as "centering"). 4. Irradiate the crystal with an electron beam and measure the diffraction intensity[(2)]. Importantly, once the specimen is loaded into the XtaLAB Synergy-ED, CrysAlis^{Pro} for ED performs all operations: instrument control, switching between imaging and diffraction modes, data processing, and automated structural analysis. CrysAlis^{Pro} for ED

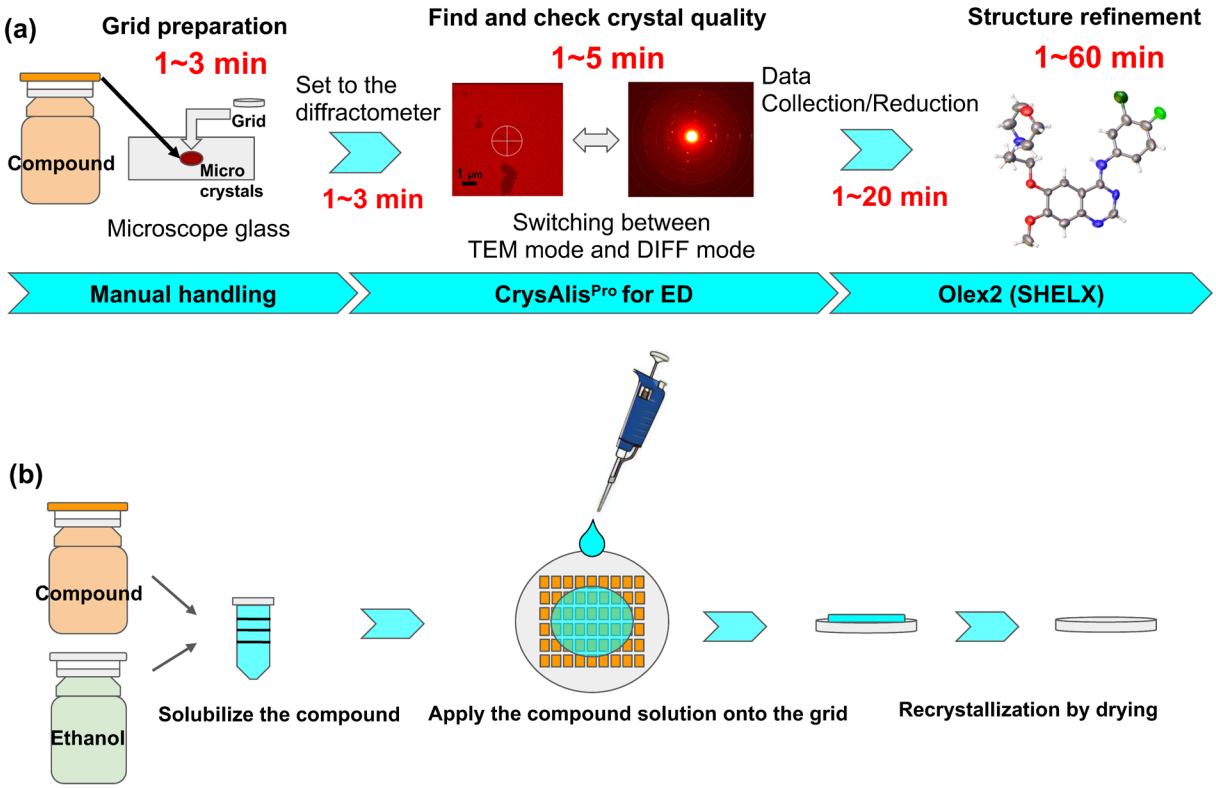

Fig. 2. Measurement flow (a) from sample preparation to structure determination. Highly crystalline samples can be loaded directly on the grid. The amount of sample required is typically less than 1 mg. The crystals are scattered on the grid by simply placing the grid on the crystals placed on a microscope glass slide. Instrument control, data acquisition, and data reduction are all performed by CrysAlis^{Pro} for ED. Phasing and refinement are performed in the conventional way used in X-ray crystallography. (b) Schematic representation of on-grid re-crystallization for samples with poor crystallinity. This process sometimes improves crystallinity. Dissolve the compound in some solvent, load a few microliters of the solution onto the grid, and dry the solution.

may also be used in off-line mode for more detailed data processing, followed by analysis with Olex2 and SHELX[3]–[5].

Crystal Structures Determined by the XtaLAB Synergy-ED

The authors have analyzed various samples sent by potential customers using the XtaLAB Synergy-ED at the Rigaku Application Laboratory and have successfully determined the structures of more than 100 compounds so far. The statistics for these compounds, shown in Figure 3, are presented to demonstrate the diversity of the sample types and typical analysis quality.

For the compounds for which structures have been determined to date, the data sets almost all have a completeness greater than 70%. This value may be considered as a threshold for successful structure determination. Figure 3 also shows compounds with completeness below 70%. All of these are structures of impurities introduced during the measurement. As for the types of compounds, organic compounds account for more than 60% of the total, but this bias might be caused by the fact that we measured compounds requested by our potential users. However, organometallic and inorganic compounds have also been measured.

Crystals belonging to highly symmetric crystal systems, such as cubic, are easier to measure because they require a narrower tilt range due to their high diffraction symmetry, while those belonging to the triclinic or monoclinic systems are more difficult because they require a wider tilt range to obtain sufficient completeness. However, 70% or higher completeness can be achieved with 1–2 crystals for monoclinic and 1–3 crystals for triclinic crystal systems with the XtaLAB Synergy-ED, which can tilt the stage by ±90°. CrysAlis^{Pro} for ED helps achieve high completeness by easily merging data sets obtained from multiple crystals. The mean and median values of R1 in refinement were both 16.41%. These values are higher than those obtained by X-rays because the effect of multiple scattering is considerably larger in electron diffraction. Still, the authors regard these values as much better than expected because these analyses were performed by kinematical refinement, which does not consider the multiple scattering effect.

We also compared the R1 values found in the CIFs of electron diffraction experiments already deposited in the CSD (mean 21.70%, median 18.91%) with the R1 values from the CIFs determined by the XtaLAB Synergy-ED, and found that the R1 of the XtaLAB Synergy-ED was better.

Application Examples Using the XtaLAB Synergy-ED

The XtaLAB Synergy-ED can be used not only for the structure determination of nanocrystals but also for various other applications. Here, two examples are presented: impurity identification and polymorphism detection. The crystal size of these samples was about 100–500 nm, which was difficult to measure by single crystal X-ray crystallography.

When the contents of a commercial pharmaceutical

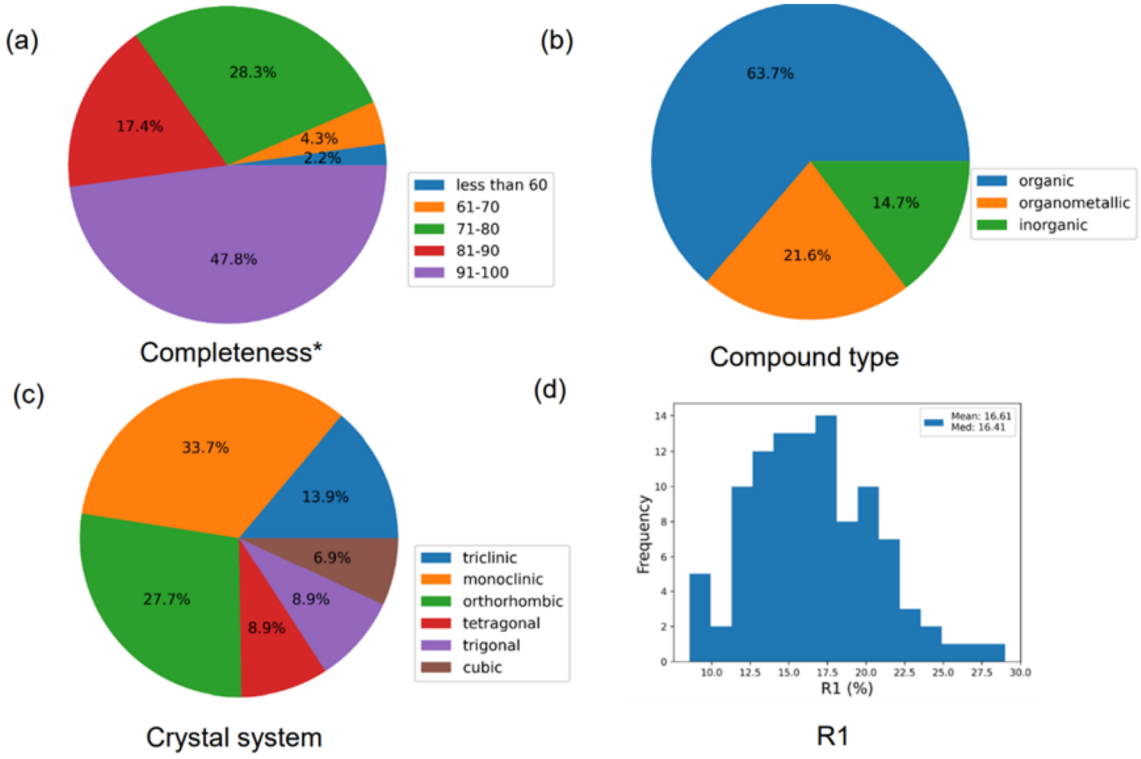

Fig. 3. Statistics of 102 compounds whose structures have been determined using the XtaLAB Synergy-ED as of Dec. 20th, 2021. (a) completeness, (b) compound type, (c) crystal system, and (d) R1 value are shown. *All compounds with less than 70% completeness were not the target compounds but rather contaminants.

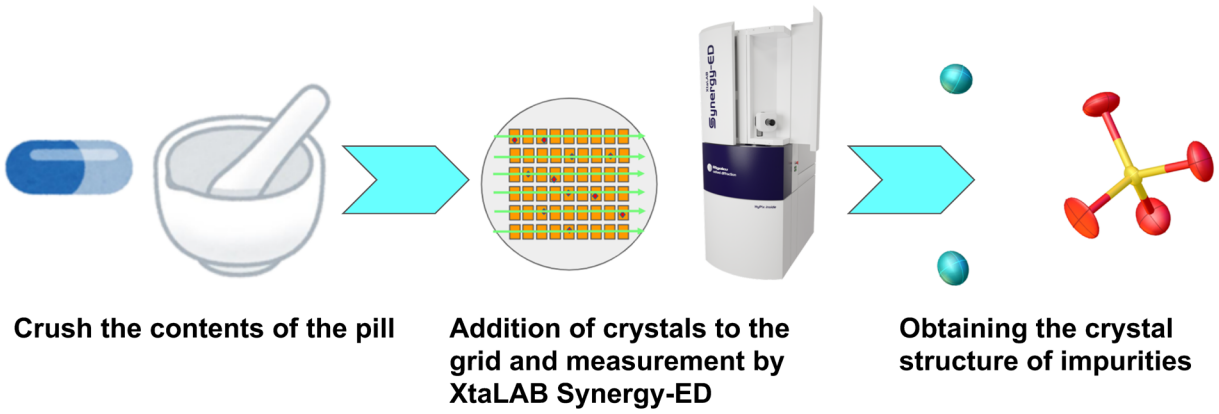

Fig. 4. Identification of impurities. Polymorphism detection can also be performed in a similar manner

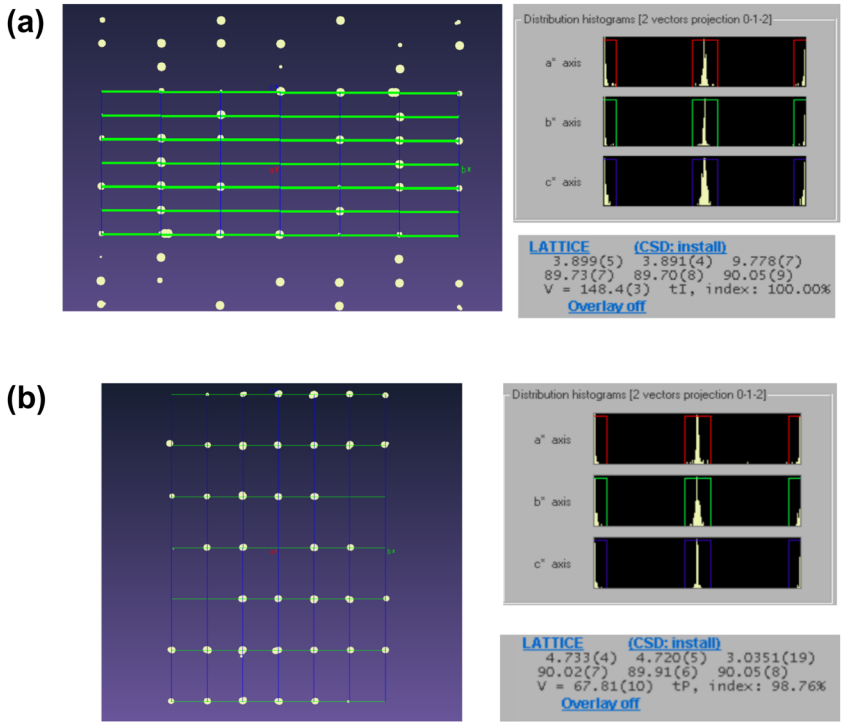

Fig. 5. Detection of polymorphism in titanium oxide. Display of reciprocal space by Ewald explorer in CrysAlis^{Pro} for ED. a-axis projections are shown. Based on the values of unit cells, polymorphs of (a) anatase and (b) rutile were identified.

capsule were removed and a number of its crystals were measured, an impurity, sodium sulfate, was identified (Figure 4). This impurity was not listed in the ingredients list of the drug, but sodium dodecyl sulfate was. Since both of these compounds have a sulfo group and sodium dodecyl sulfate can be degraded, it is possible that this sodium sulfate is a degradation product of dodecyl sulfate, which is used as an additive in pharmaceuticals. Thus, it can be seen that the XtaLAB Synergy-ED can contribute to the identification of small amounts of components present in a sample.

For polymorphism detection, titanium oxide was used as a test sample. This compound is known to have two major polymorphic phases, rutile and anatase. The XtaLAB Synergy-ED easily identified the two phases in the sample (Figure 5). Importantly, the amount of sample used in this measurement was much less than 1 mg, and the measurement time for a crystal was about 1 minute, indicating that the XtaLAB Synergy-ED is useful for rapid measurement of samples that can only be prepared in small quantities. Since polymorphism has a significant influence on the physical properties of compounds, the simple and rapid detection of polymorphic phases using the XtaLAB Synergy-ED is expected to be used in various fields.

Conclusion and Future Plans

In this application note, the configuration of the XtaLAB Synergy-ED, statistics of the determined structures, and examples of applications with the XtaLAB Synergy-ED were presented. There are numerous crystal structures of many important compounds that have not been determined by X-ray diffraction due to the small size of available crystals. The XtaLAB Synergy-ED

can be a powerful tool to analyze the structures of such nanocrystals. In addition, the identification of impurities and polymorphs in the field of formulation and material development by the XtaLAB Synergy-ED may become more popular in the future. The XtaLAB Synergy-ED is undergoing further improvements, specifically automation of measurements and various functions for data processing and structural analysis considering multiple scattering. In the near future, electron diffraction will become one of the major methods for structure determination complementary to X-ray diffraction, and the XtaLAB Synergy-ED will contribute to many structure determinations.

References

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