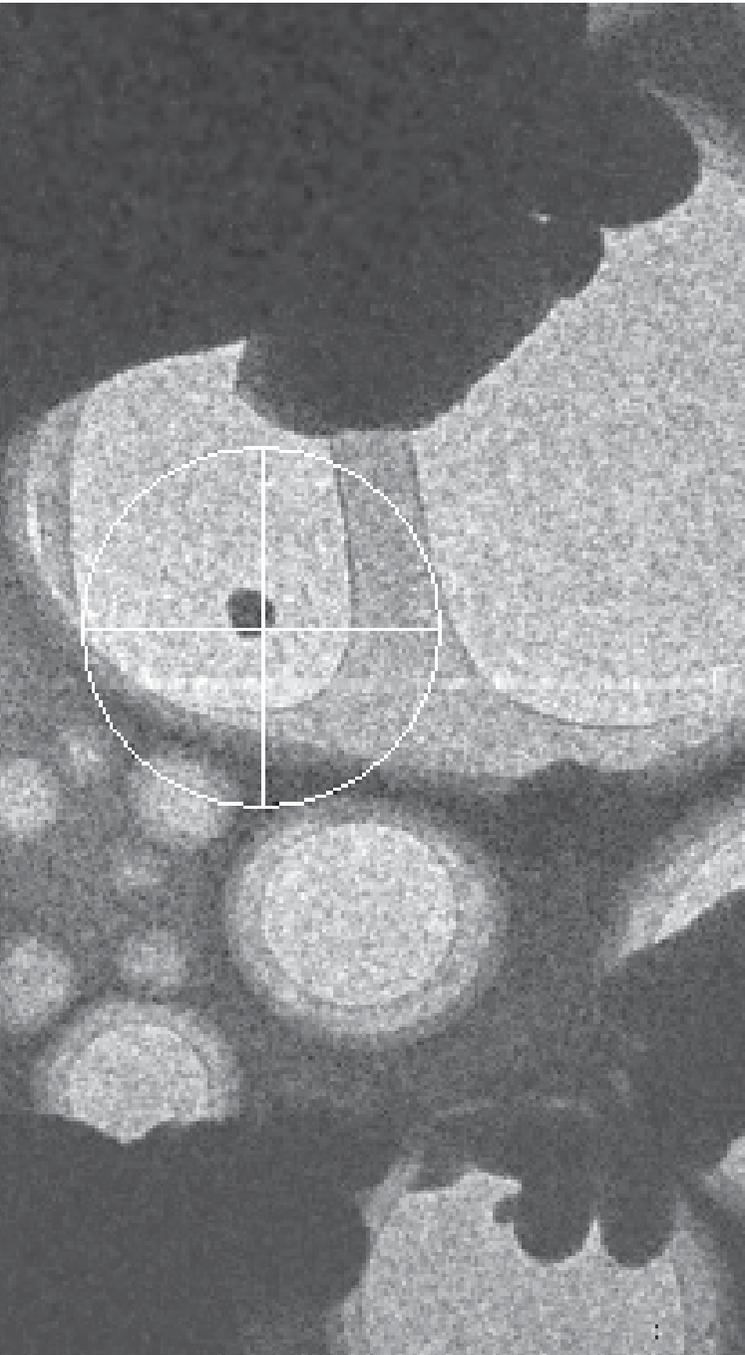


# XtaLAB Synergy-ED

AN ELECTRON DIFFRACTOMETER DESIGNED FOR THE CRYSTALLOGRAPHER

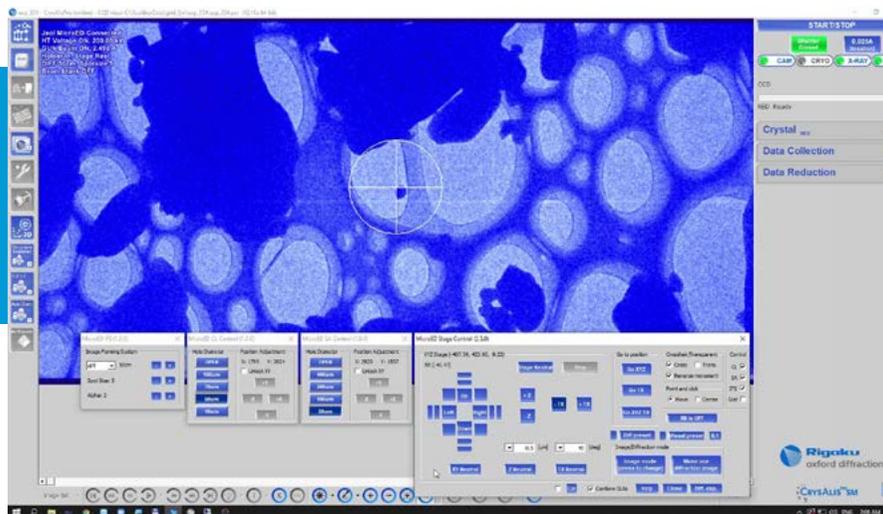


Single Crystal Electron Diffractometer



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## MicroED: A REVOLUTIONARY TECHNIQUE FOR OBTAINING 3D STRUCTURES FROM NANOCRYSTALLINE MATERIALS



The groundbreaking publication<sup>1</sup> concerning 3D structures from nanocrystalline lysozyme using electron diffraction in 2013 led to a surge in research around the world in applying the MicroED technique to other nanocrystalline materials.

Instead of competing with X-ray crystallography, electron crystallography extends the ability of researchers to obtain structural information from crystals that are too small to measure by X-ray diffraction. Conversely, due to the stronger interaction of electrons with atoms compared to X-rays, there is an upper limit on the size of a crystal that can be measured with electron diffraction, and that threshold is lower than the lowest threshold for X-ray diffraction.

There are many materials that only form nanosized crystals. Before the development of the MicroED technique, synthetic chemists were forced to rely on other techniques, such as NMR, to postulate 3D structure. Unfortunately, for complicated molecules such as natural products, the NMR results can be difficult to interpret.

MicroED has thus become a revolutionary technique for the advancement of structural science.

<sup>1</sup> DOI:

## RIGAKU AND JEOL

Recognizing the potential of MicroED, Rigaku and JEOL announced a collaboration in 2020 to develop a new product designed in a fashion that will make it easy for any crystallographer to use.

The resulting product is the XtaLAB Synergy-ED, a new and fully integrated electron diffractometer, that creates a seamless workflow from data collection to structure determination of three-dimensional molecular structures. The XtaLAB Synergy-ED combines core technologies from the two companies: Rigaku's high-speed, high-sensitivity detector (HyPix-ED), and instrument control and single crystal analysis software platform (CrysAlis<sup>Pro</sup> ED), and JEOL's expertise in generation and control of electron beams.

The XtaLAB Synergy-ED is an instrument that any X-ray crystallographer will find intuitive to operate without having to become an expert in electron microscopy.

## MicroED CHALLENGES

MicroED presents experimental challenges that are different than X-ray crystallography.

Electrons interact very strongly with matter, imposing an upper limit on the size of samples that can be studied.

- **Absorption:** It typically takes only a very small crystal to completely stop the beam.
- **Dynamical Diffraction:** The electron beam is diffracted multiple times on its path through the crystal. The thicker the crystal, the more dynamical effects can cause problems for your structure solution and refinement.
- **Sample Decay:** Exposing a crystal to a high-power, highly charged beam tends to cause rapid crystal decay. This can be mitigated by utilizing low-temperature data collection as well as properly selecting the beam intensity and the scan speed.
- **Environment:** Diffraction experiments must be performed in a vacuum. Gas in the path of the electron beam will absorb and diffract it. It doesn't take much air to stop the beam or ruin your experiment.

## HARDWARE

A JEOL 200 kV electron source, column and beam optics optimized for electron diffraction purposes.



A Rigaku HyPix-ED detector optimized for operation in the MicroED experimental setup.



A sample stage allowing x, y, z sample alignment and rotation (tilt) about a single axis. A cryo option is available.

## A MicroED EXPERIMENT

### MicroED PLACES SOME REQUIREMENTS ON THE DIFFRACTION EXPERIMENT

- **High vacuum** is needed to allow electrons to pass. Samples must therefore be vacuum stable (and/or frozen).
- **A single-rotation axis.** The need to operate in vacuum and at such high precision makes a complicated multi-axis stage difficult to achieve.
- **Data from multiple grains must be merged** to overcome low completeness.
- **Electrons damage** the samples as they diffract or are used for viewing. Since sample selection must be performed using electrons, this requires users to minimize exposure time.
- **Small samples** are an absolute requirement ( $<1 \mu\text{m}$ ), and the smaller the better with 350 nm being the ideal thickness for organic materials.



## OUR SECRET SAUCE

To date, researchers have been forced to use a combination of electron microscopy software and public domain crystallography software to measure and process electron diffraction data from modified electron microscopes.

Instead, we have combined our market-leading X-ray instrument control and data processing software pipeline ( ) with the XtaLAB Synergy-ED.

The software for selecting samples and measuring electron diffraction data on the XtaLAB Synergy-ED is all incorporated into CrysAlis<sup>Pro</sup> ED, giving the user an effortless workflow from selecting the crystal all the way to solving and refining the structure.

Any researcher with experience in measuring X-ray diffraction data and solving single crystal structures should be able to sit down on the first day and perform a MicroED experiment.

## OPERATING THE XtaLAB Synergy-ED

### SAMPLE SELECTION

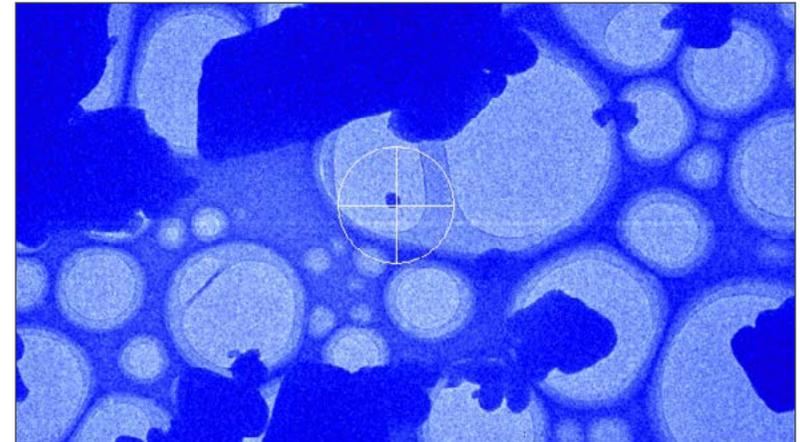
Crystallites of the appropriate size are loaded on a sample grid. Try either sprinkling some of the sample over the grid then removing the excess or put the grid into an Eppendorf tube with some of your sample and shake it up. The grains are so small they will stick to the grid, which can then be loaded onto the sample holder and inserted into the instrument.

The sample grid is visualized in CrysAlis<sup>Pro</sup> ED using electron imaging and the HyPix-ED detector. As electrons are used to visualize, it is necessary to be careful to keep the dose as low as possible when looking at a sample. CrysAlis<sup>Pro</sup> ED automatically changes the diffractometer settings to the lowest dose, although the user is free to override these settings if preferred.

CrysAlis<sup>Pro</sup> ED allows point-and-click navigation across the sample grid; click on a sample and it is brought to the center of the crosshair. If you prefer, or need to finetune, there are also up, down, left and right buttons you can use to make movements.

The minimap feature allows fast navigation across the grid. A low-magnification image of the grid is taken and you can then select an area of the grid to investigate. CrysAlis<sup>Pro</sup> ED keeps track of visited regions with markers that are placed automatically.

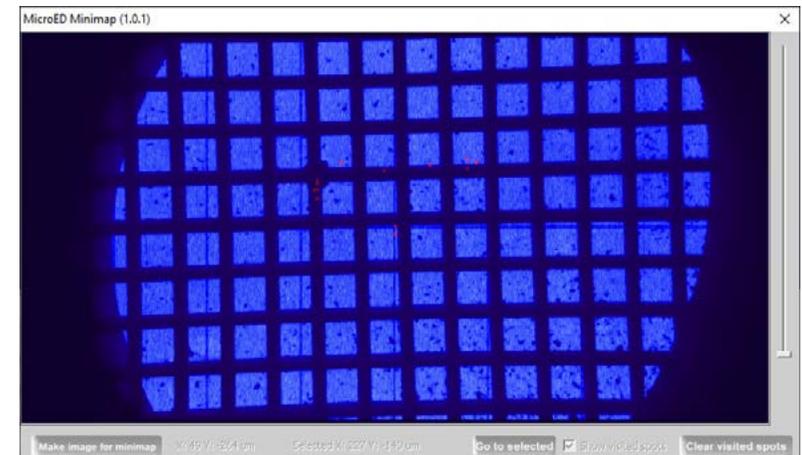
For navigation, simply click on the image where you want to go. The crosshairs on the image show you where the beam is. The goal is to move a crystal grain onto the crosshair.



Fine tuning can be accomplished with up, down, left and right buttons. You can make small movements, medium movements or large movements.



The minimap feature allows fast navigation and automatically marks regions that have been visited.



## OPERATING THE XtaLAB Synergy-ED

### DIFFRACTION EXPERIMENT

If a promising grain is found, crystal quality can be checked by taking one still diffraction image. Click the button and enter the exposure time you want; CrysAlis<sup>Pro</sup> ED will switch modes and record the image and then blank or shutter the beam to prevent damage to the sample.

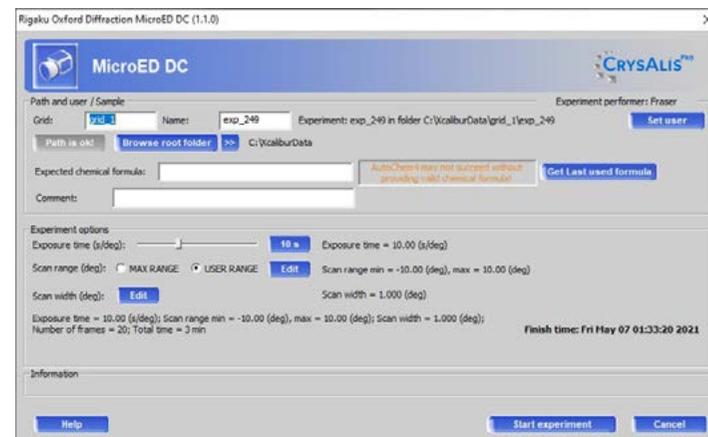
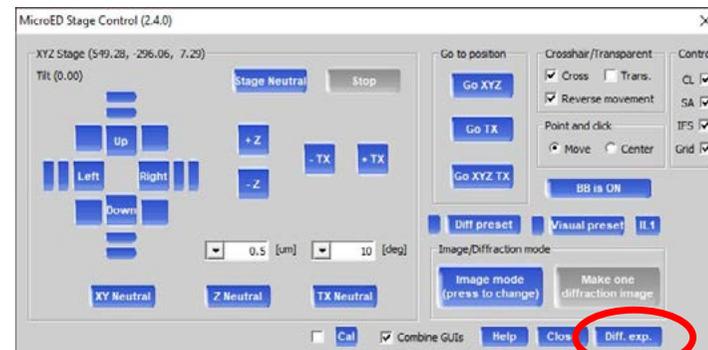
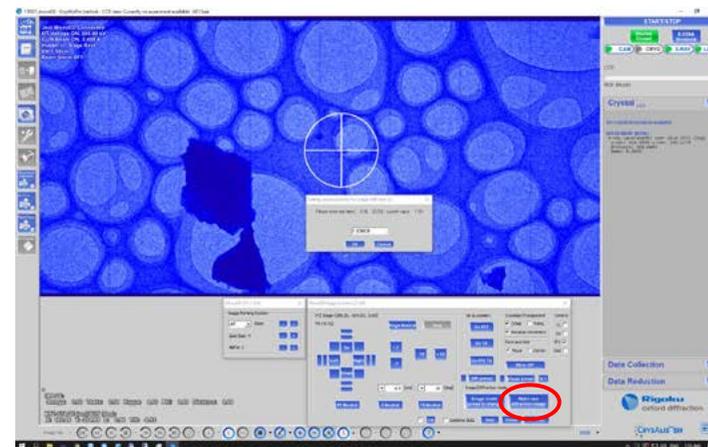
When you have a nice sample, all that remains is to run your diffraction experiment. You can click the “diff exp” button from the visual mode and CrysAlis<sup>Pro</sup> ED will switch the diffractometer into diffraction mode and give you the experiment setup menu.

For MicroED, experiments are simple rotations, so you only need to give the exposure time, scan width and the scanning range along with information about the sample and a name. CrysAlis<sup>Pro</sup> ED also uses the concept of sample grids, so you can group together the experiments by grid, which will typically be grains of the same material.

To take a single diffraction image, center the sample and then click the “Make one diffraction image” button and enter exposure time. If the sample diffracts, move to the next step.

To run a diffraction experiment you only need to click “diff exp” on the GUI. Proper setup and switching from visual mode is automatic.

Experiments are simple rotations, so you do not need a complex strategy calculator. Experiments are assigned to a sample grid, which may be named by the user.



## OPERATING THE XtaLAB Synergy-ED

### DIFFRACTION EXPERIMENT (Continued)

The diffraction experiment proceeds just like an X-ray experiment. This means that, during data acquisition, concurrent automatic data processing occurs, with even the possibility of *AutoChem* being run on your MicroED data.

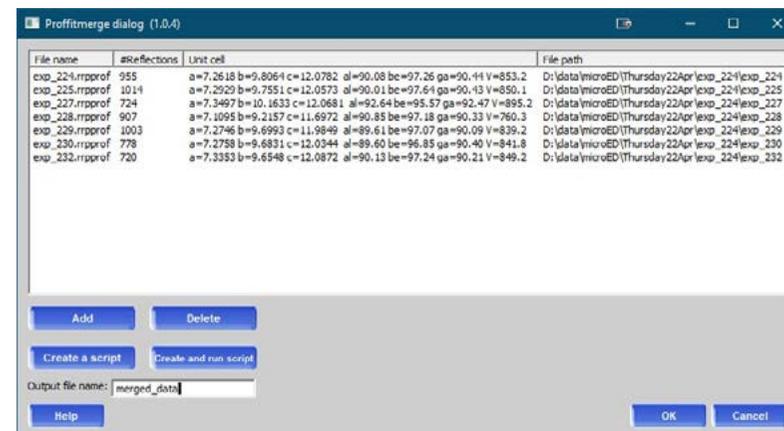
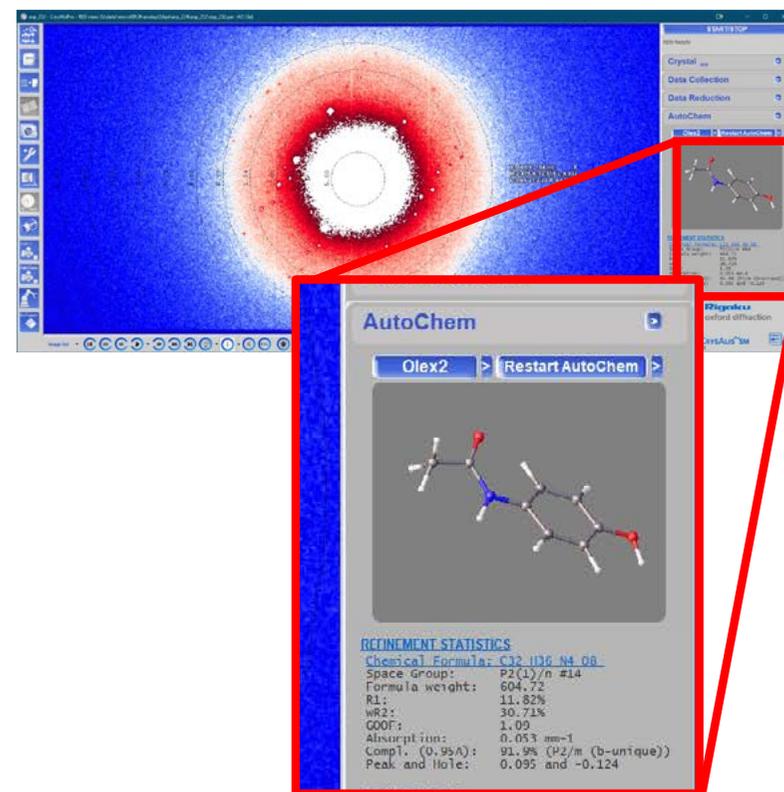
*AutoChem* is the ultimate productivity tool for small molecule chemists, offering fast, fully automatic structure solution and refinement during data collection. *AutoChem* is seamlessly integrated within CrysAlis<sup>Pro</sup> ED and forms an integral part of our "What is this?" feature. The "What is this?" feature gives you structures in seconds and ensures you are not wasting time collecting full datasets on known samples or starting materials.

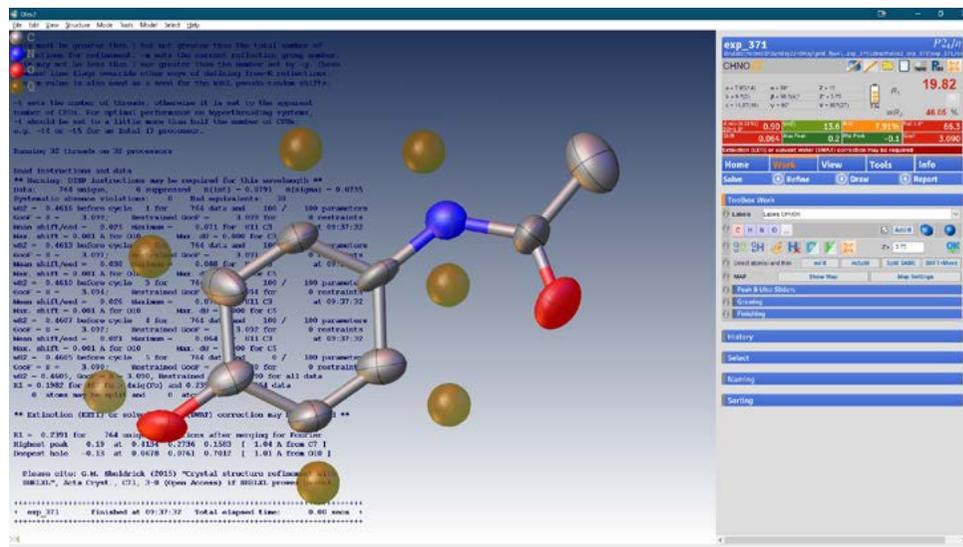
One difference in MicroED compared to X-ray diffraction is that due, to the limited sample access, it is often necessary to merge the data from multiple grains to get the completeness to an acceptable level. Dataset merging is easy in CrysAlis<sup>Pro</sup> ED with a GUI where multiple datasets can be selected to merge. The merged data is then refinalized to apply all corrections and scaling to get your final *hkl* reflection file.

As with X-ray experiments, CrysAlis<sup>Pro</sup> ED automatically and concurrently processes the data as it is collected.

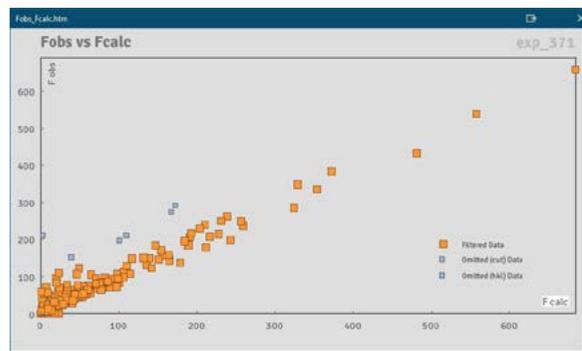
Even *AutoChem* can be used, sometimes with success, although it generally depends on completeness.

To boost completeness and redundancy, dataset merging may be required. A simple-to-use GUI allows quick multiple dataset addition and output of a merged pre-correction file.

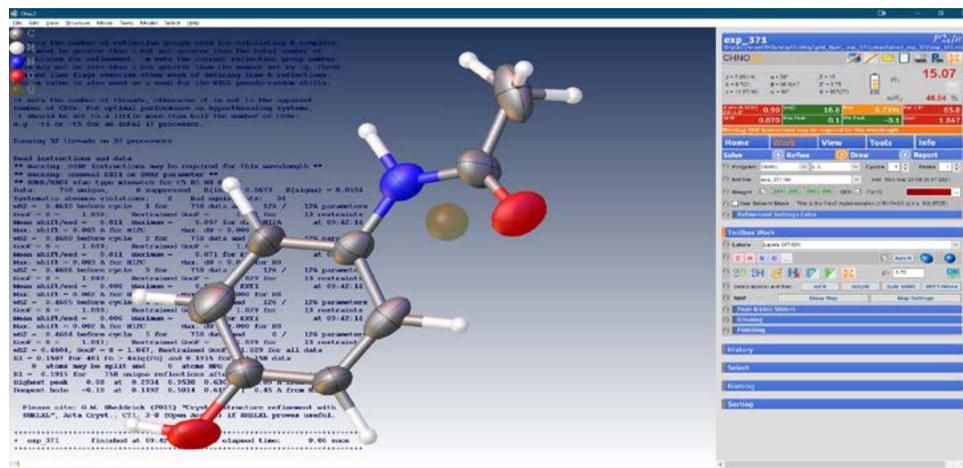




Locating hydrogen atoms in difference Fourier map



$F_{obs}$  vs.  $F_{calc}$  plot



Hydrogen atoms refined (position and  $U_{eq}$ )

