DIFFRACTION DATA COLLECTION ON XRD2 BEAMLINE OF ELETTRA

1. Loops containing previously-mounted lysozyme crystals are retrieved from the storage dewar and are transferred in the liquid nitrogen dewar of the automatic mounting system at the XRD2 beamline of the Elettra Synchrotron (similar to Figure 1).
   (Automatic mounting systems of Elettra beamlines XRD1 and XRD2 were designed and realized by research and technical personnel of the Synchrotron Elettra. In general, macromolecular crystallography beamlines of synchrotrons possess different mounting systems, but with similar purposes.)

Figure 1: Automatic mounting system of the beamline MASSIF-1 of the ESRF (European Synchrotron Radiation Facility) synchrotron in Grenoble.

   The automatic mounting system retrieves one sample at a time, keeping the loop at cold temperature to avoid its thawing. Crystals are quickly mounted on the goniometer of the beamline diffractometer using the magnetic base. After positioning the loop on the diffractometer, a stream of nitrogen gas at low temperature (usually 100 K) keeps the sample frozen during the whole data collection.
   The robot is remotely controlled using a computer outside the Experimental Hutch of the beamline, or even by a user at home through a remote control software. Mounting and unmounting procedures for a single sample (crystal) take about 2 minutes.

2. Crystal centering: The crystal is centered on the diffractometer so that it remains in the same position, under the incident X-ray beam, during the full rotation required for data collection. The user can position the crystal using the optical camera mounted on beamline. The system is set up so that the position of the incident X-ray radiation is marked on the camera. The XRD2 Automatic Mounting System allows for the software-aided centering of the crystal, the user is only required to identify his sample and click on it 3 times, in different orientations of the loop (Figure 2). At the end of the centering procedure, the crystal should lie in the eucentric point of the goniometer.

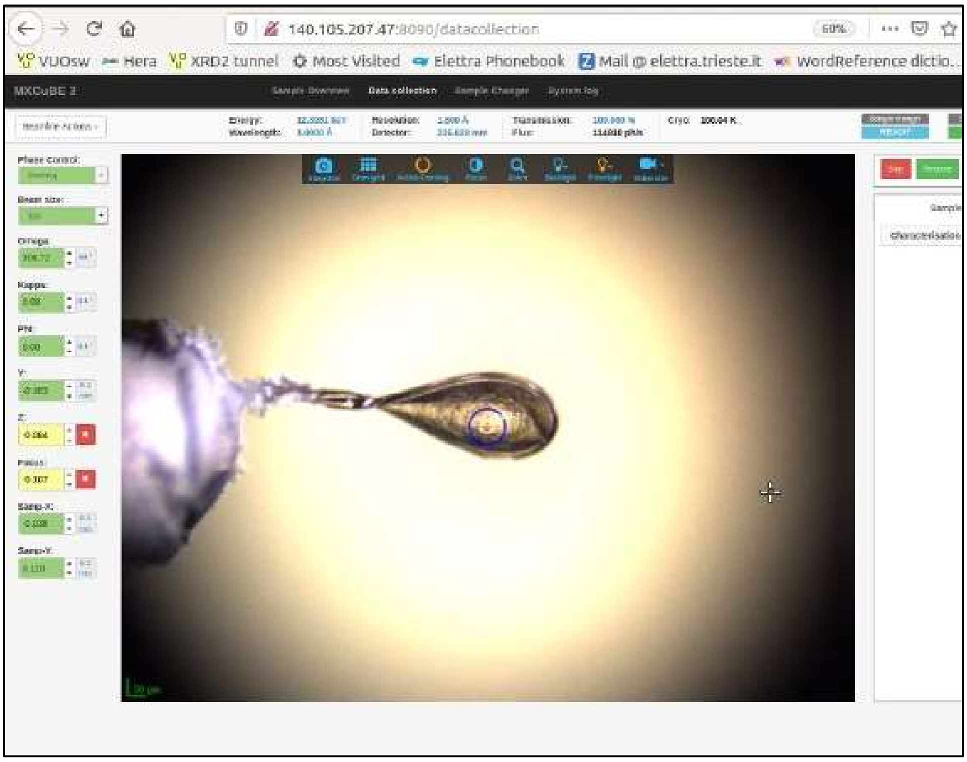
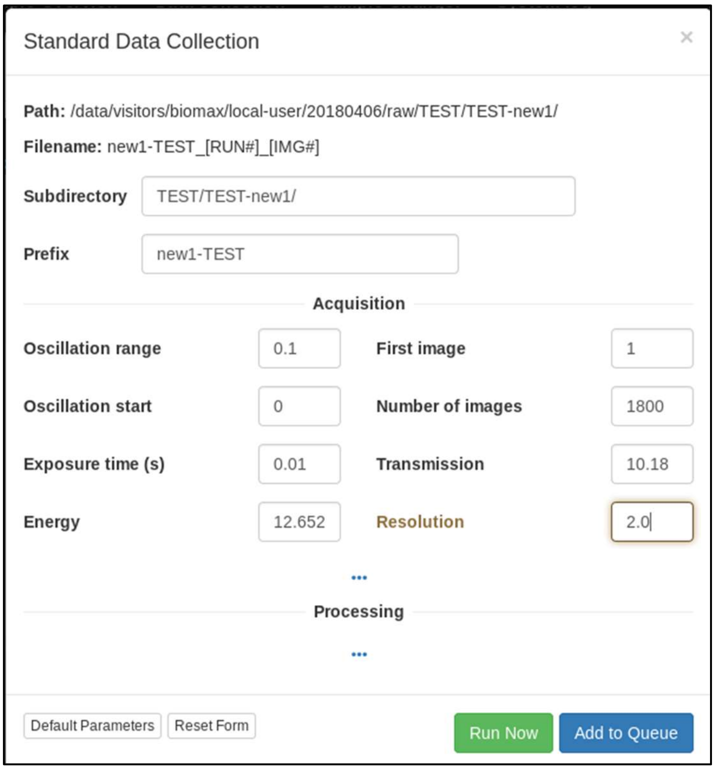


Figure 2: Automatic centering software used at the XRD2 beamline of Elettra.

3. Before data collection, few images are collected to assess crystal quality, resolution limit and anisotropy of the diffraction. On the beamline software, the user can specify crystal-to-detector distance, exposure time, and rotation range per image (Δφ) and data are automatically collected at 0° and 90° of rotation (φ). The difference between the two is indicative of the anisotropy of the crystal, i.e. the different degree of crystallinity in perpendicular directions. Initial images allow the user to evaluate optimal parameters for the following data collection.

4. Once initial images have been analyzed, the user decides whether the diffraction power of the crystal is sufficient. If this is not the case, the crystal can be quickly unmounted and data collection proceeds with the following sample. If the crystal quality is good, the user sets up the parameters of data collection on the beamline software (Figure 3): crystal-to-detector distance, rotation range, exposure time, oscillation start, and number of images to be collected (determined considering the symmetry of the crystal).


Standard Data Collection

Path: /data/visitors/biomax/local-user/20180406/raw/TEST/TEST-new1/

Filename: new1-TEST_[RUN#]_[IMG#]

Subdirectory TEST/TEST-new1/

Prefix new1-TEST

Acquisition

Oscillation range	0.1	**First image**	1
Oscillation start	0	**Number of images**	1800
Exposure time (s)	0.01	**Transmission**	10.18
Energy	12.652	**Resolution**	2.0

Processing

Figure 3: Window of the remote control software of the XRD2 beamline of Elettra. On this window the user can specify parameters of the data collection. In this software, crystal-to-detector distance is not directly set up, but the user can specify the resolution at the detector edge. Edge resolution depends not only on the crystal-to-detector distance but also on the wavelength used.