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NEW HOLDER FOR DUAL-AXIS CRYO SOFT X-RAY TOMOGRAPHY OF CELLS AT THE MISTRAL BEAMLINE

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Abstract

A new dual-axis sample holder has been designed and built for the Transmission soft X-ray Microscope (TXM) at the MISTRAL beamline (ALBA Synchrotron) [1, 2] to perform cryo-soft X-ray tomography of cells with dual tilt configuration to reduce the missing wedge.

The design, with restricted dimensions Ø7x30mm, enables using commercial Auto-Grid support rings that give rigidity to the sample grid handling. It consists of a guided miniature handle with a spring system that allows sample rotation by 90º around the beam axis inside vacuum and in cryogenic conditions by using the TXM sample loading robot keeping a rotation of ±65˚ at the sample stage. Two magnets fix the positions at 0˚ and 90˚. The two tilt series can be collected consecutively and the use of Au fiducials permits combining both improving the final quality of the 3D reconstructions. In particular, cellular features hidden due to their orientation with respect to the axis of rotation become visible. The main frame is made in aluminium bronze to enhance the thermal conductivity and in addition, all the pieces have undergone an ion implantation treatment in order to reduce friction and improve the anti-seizure property of the parts.

INTRODUCTION

Structural Cell Biology demands detailed structural and functional descriptions of the different cellular components which must be correlated with a topological 3D map of these components at the whole cellular level. In this frame, an emerging technique such as cryo-SXT can provide structural information at the level of a whole cell without further sample preparation except for the cryo-fixation required to prevent radiation damage while collecting the data. The penetration power of soft X-rays in the so-called water window spectral range, between the inner-shell absorption edges of carbon and oxygen (from 284 eV to 543 eV), allows penetrating water layers of up to 10 µm thickness while carbon-rich structures are visualized with good absorption contrast. Thus, frozen-hydrated specimens can be imaged close to their native state providing significant complementary information to existing biological imaging techniques at a spatial resolution of 30 nm. Cryo-SXT generates, as in electron tomography, 3D absorption maps of the specimen from a series of 2D soft X-ray microscopy projections recorded with a CCD [3]. In the current TXM design at Mistral we use TEM grids as sample support on which the cells are grown. Due to the choice of the flat sample support and the reduced distance from the sample to the objective lens, the available tilt range is ± 70º. This angular restriction results in a region empty of information in the Fourier space, the so-called "missing wedge" (see Fig. 1), which in real space produces an elongation of every point of the reconstructed volume along the beam direction. In addition to this well-known effect, in the reconstructions some cellular features will not be visible due to their orientation with respect to the axis of rotation. A way to reduce the missing-wedge effect in the reconstructed volumes is therefore by doing dual-tilt tomography (see Fig. 1)

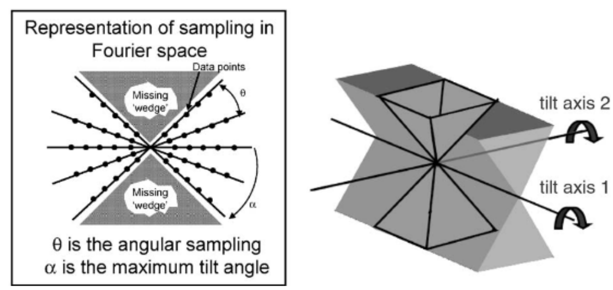

Figure 1: Representation of the sampling in Fourier space showing the limited tilt range (left) leading to a missing wedge 3D reconstruction in a single tilt tomography (right) and the sampling achieved by dual axis tomography which is reduced to a missing pyramid (right). [4]

TECHNICAL SPECIFICATIONS

The dual-axis holder must comply with the following specifications:

- Two orthogonal tilt axis.
- High vacuum compatible (10^{-7}mbar).
- Good thermal conductivity to get 100K at the sample.
- Motions inside TXM.
- Compatibility with AutoGrid sample support.
- Sample visibility in the range ± 65º degrees for the existing tilt.
- Second tilt with two fixed positions (0º- 90º).

DESIGN

The design consists of tiny mechanism of metal parts that have been treated with an ion implantation process to improve the anti-seizure property and reduce the friction in cryogenic temperatures. The holder body is produced in aluminium bronze to achieve a good thermal conductivity and it is manufactured with precision to fit perfectly in the motorized sample stage of the TXM. The

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system facilitates the usage of the AutoGrid which securely holds the specimen into the Dual-axis holder. At the same time it provides precise, in-plane specimen rotation perpendicular to the beam. The design has been optimized to maximise sample visibility, even at high-tilt angles. The main advantage is that the rotation can be done inside the TXM (maintaining the cryogenic temperature and the HV). Initially, the specimen can be rotated around a range ± 65º to acquire the first tilt series. Additionally thanks to the movement of the TXM robot gripper which operates a spring system the holder can be rotated by a fixed 90º angle by means of a simple lever fitted to the top of the holder, which rotates the mobile part while two magnets fix the two-positions at 0º and 90º. These features greatly facilitate the acquisition of a dual-axis tilt series. The whole assembly can be shown as Fig. 2.

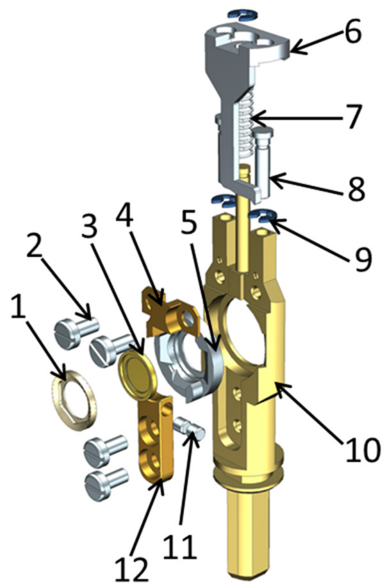

Figure 2: (1) AutoGrid holder, (2) Screw M1x2, (3) AutoGrid, (4) Bushing retainer, (5) Rotating bushing, (6) Holder handle, (7) Spring, (8) Rod, (9) C-clip, (10) Holder base, (11) Magnet, (12) Bushing holder.

MOVEMENTS DESCRIPTION

The dual-axis holder is rotated with the motorized sample stage of the TXM. The first tilt is orthogonal to the beam direction with a maximum tilt angle of ± 65º without any visual or mechanical interference. This is the main and accurate motorized movement to obtain the tomography images (see Fig. 3).

Figure 3: First tilt with motorized movement.

Then, for the second tilt series, the specimen needs to be rotated inside the dual-axis holder using the gripper robot which will activate the mechanism that achieves the rotation in the axis parallel to the beam. With this sample rotation the second sample position is obtained to perform the second series (see Fig. 4).

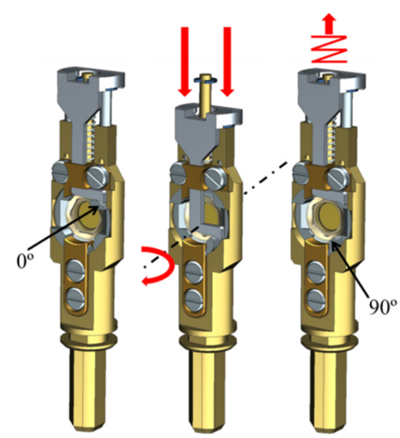

Figure 4: Second tilt with robot help.

At the same time, due to the new dimensions of the dual-axis holders a new sample shuttle that accommodates up to 4 dual-tilt holders has been designed and built. New covers have also been built to protect the sample from contamination during all sample transfers from atmospheric pressure to HV (see Fig. 5).

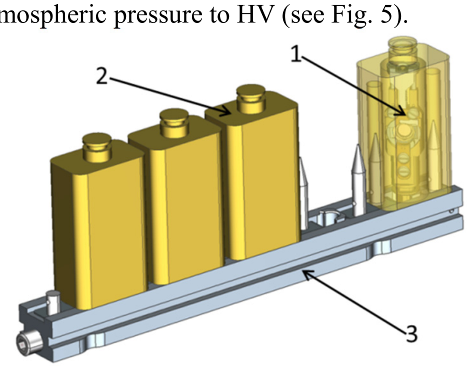

Figure 5: New cartridge. Sample load system. (1) dual-axis holder, (2) hood, (3) shuttle.

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ASSEMBLY & TESTS

The sample environment shows the dimensional constrains that exist with the optical components and the shielding system to maintain the cryogenics conditions (see Fig. 6).

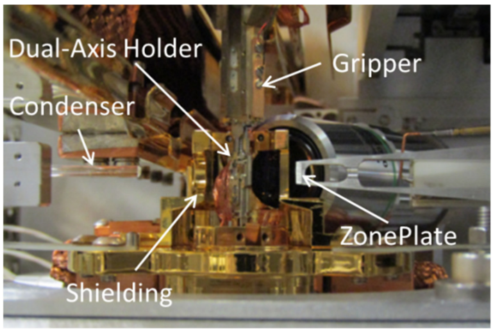

Figure 6: Inside TXM the sample environment.

In order to test the dual-axis holder different types of tests were performed:

Test of the dimensions, using a dummy to check the possible interferences with the rest of the components in the TXM.

Test of all rotation movements, using the TXM itself, first in air conditions and after in HV and cryogenic conditions.

Finally the dual-axis holder was tested with a real sample and the specific conditions of the Mistral TXM.

RESULTS

Figure 7 shows an example of two biological samples with the single tomographies in the two fixed positions (0º-90º) reconstructed independently and the improved reconstruction obtained by combining both (DT, dual tilt tomography). The 3D DT reconstruction clearly shows an improvement in the visibility of the features marked with arrows such as for instance the mitochondria cristae (bottom row) [5].

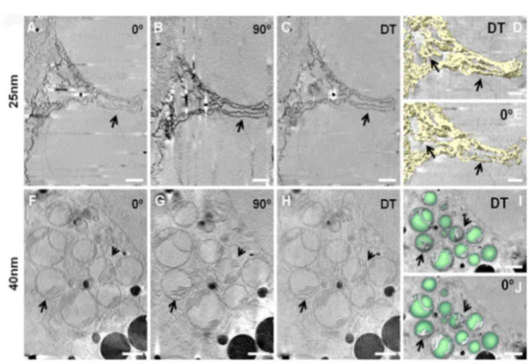

Figure 7: Two 3D DT reconstructions. For the ZP25 is a polyribosome consist of large numbers of ribosomes. For the ZP40 regions with large mitochondria were selected.

CONCLUSION

In summary, the objective of this DT new capability is to improve the final quality of the 3D reconstructions of all cells imaged at the Mistral beamline by reducing the missing-wedge artefacts occurring in single tomography.

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