MRC Laboratory of Molecular Biology

Setting the stage: development of sample holders for electron cryomicroscopy

Realisation of the full potential of electron microscopy for determination of biological structures at high resolution, depends critically on technical advances – of many different components. Important factors are the microscope electron optics, the detectors, sample preparation and computer programs, as well as a host of smaller components.

Once such component is the microscope sample holder or stage

The development of sample holders was particularly important when moving from electron microscopy to electron cryo-microscopy (cryo-EM). Cryo-EM of amorphous (non-crystalline) ice embedded specimens is a method which allows the visualisation of biological molecules in a near-native state, requiring the preservation of the specimen at a temperature below -180°C (100 Kelvin) during microscopy.

Another advantage of keeping the specimen cold was the increased electron dose which can be used to record micrographs of organic and biological material at low temperatures. With the specimens at low temperature, the micrographs contain more information about the structures.

Initially, most commercial or laboratory prototype holders were limited in resolution to between 5 and 10 Å, whereas a holder which could work at 3 Å resolution and which would fit into a normal side-entry goniometer in a microscope, was required.

"In principle, the construction of such a cryo-holder should not be difficult. In practice, however, all of our experiences with holders of this type have been fraught with resolution losses of several kinds." Henderson, Raeburn & Vigers, 1991

The resolution losses were caused by vibrations and thermal expansion and contraction of the stage. The vibrations arose from both the sensitivity of the holders to ambient, airborne vibrations (sound waves) or from vibrations from the pumps providing cooling water to the microscope, and from internal vibrations in the cold holder itself, due to boiling of the liquid nitrogen coolant or turbulence in the gas boiling off from the surface of the liquid. There is also a problem of slow drifting of the image, caused by a part of the holder or the microscope cooling so slowly that it never reaches equilibrium, or steadily warming after that point is reached. Also, parts can move during specimen translation, making a new contact with small temperature differences between the contacting parts. These drifts can take several minutes to die out and may occur after each specimen translation.

Finding where the problem was in the design of holders and eliminating it was crucial for improving images and determining structures of molecules; without these developments, cryo-EM would not have achieved what it has. Since the 1980s the LMB has been actively involved in the continued development of improved holders and stages. They aim to make Cryo-EM better, faster and cheaper for the entire biology community.

Timeline of development of Cryo-EM holders

1962	Commercially available “Styrofoam” holder for electron microscopy. Nigel Unwin (LMB) used this stage in a Philips 301 microscope but once it reaches -100°C it rapidly got completely covered in ice.
1980-84	Jacques Dubochet (EMBL) used this 1962 holder with one of the first of the new Phillips 400 microscope series, which had a much, improved vacuum, for his pioneering work in developing the plunge-freezing method that marked the beginning of cryo-EM.
1981	LMB purchased a Phillips 400 microscope, but quickly identify a need to improve the 1962 holder, particularly when used with nitrogen gas cooling, to eliminate vibrations.
1983	The LMB start developing their own holders.
1985	Nigel Unwin, whilst working at Stanford, helped to develop a liquid-nitrogen cold-stage with Peter Swann at Gatan Inc.
1991	First LMB developed holder: a prototype side-entry cold holder for cryo-EM was constructed to be used with no loss of image resolution or contrast at 3.4 Å resolution compared to the normal room-temperature holder. The design was commercialised by Oxford Instruments and became the Gatan CT3500.
1986-2015	Hexland in the mid-1980s, was acquired by Oxford Instruments in 1988, and subsequently by Gatan Inc, develop improved holders.
1995	Octagonal-block cold-stage developed at LMB for integration with the microscope column. It had very simple manual controls using a micrometer screw and a 5:1 reduction lever to move sample grid.
1996	Bob Morrison of Gatan Inc., designed a holder for a new Phillips prototype to be used with liquid helium. Was eventually commercialised as the Polara microscope, with more than 30 installed in cryo-EM laboratories worldwide.
2020	Current commercial holders developed for use with the JEOL 1400HR columns from: Gatan Inc; Simple Origin; Fischione.
2021	Chris Russo (LMB) is leading the project to develop a new cryostage/cryotransfer for the JEOL UK 1400 platform including Bob Morrison as chief designer for the project.