PRODUCT DATA

FEI COMPANY™

Vitrobot™

New sample preparation technology for Cryo microscopy

- *Fully automated, reproducible vitrification process*
- *High vitrification quality through controlled environment*
- *High Sample Preparation Throughput*
- *Easy and flexible instrument control*
- *Best value-for-money*

Fundamental research within the scope of cell and structural biology is increasingly focusing towards unraveling interactive biological and biochemical processes and pathways at the macromolecular level. For this high resolution transmission electron microscopy (TEM) is indispensable. Of paramount importance is the three-dimensional visualization of macromolecular structures and molecular machines in their native hydrated state. Their physical fixation within ultra-thin vitrified ice layers is the crucial starting point for advanced study. FEI, The Structural Process Management Company, understands the importance of reliable and reproducible sample preparation and process management as a primary step towards high quality results.

FEI Company introduces the Vitrobot™, a fully automated vitrification device for plunge-freezing of aqueous suspensions that meets the demands of modern science. The Vitrobot™ with its automated sample (suspension) application to the TEM grid, blotting and vitrification is robust and easy to use. Moreover, its robotics guarantee high quality, reproducible sample freezing and a high sample throughput. Its controlled environment key technology prevents cooling and concentration artifacts that are inevitable for other “open space” freezing methods. It is a state-of-the-art specimen preparation unit that offers great value to the demanding scientific areas of cell and structural biology as well as being very suitable for proteomics and nanotechnological applications.

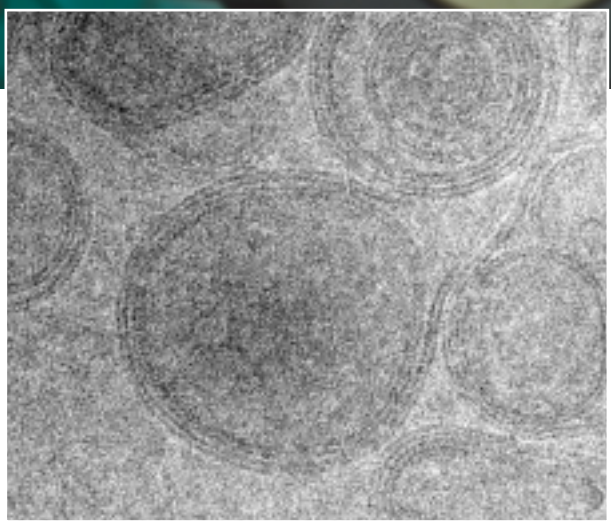
Structural Biology and Pharmaceutics

Cryo fixation and subsequent imaging of tissues and suspensions at cryo temperatures in a TEM is gaining in importance. The unraveling of the human genome and the subsequent demand for the exploration of genomic functionality, as part of the development towards new medical interventions, has been a tremendous stimulus to reveal the numerous mutual macromolecular interactions between genetic material, proteins, membranes and organelles.

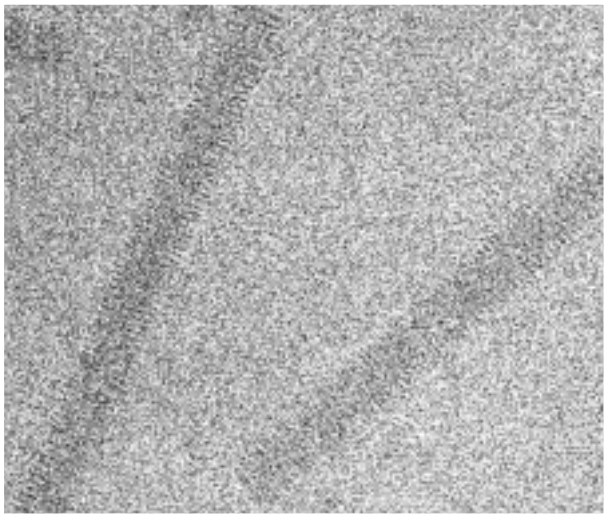
High-resolution cryo-TEM allows studying of these macromolecular structures and interactions albeit that the natural state of these structures is well preserved. The Vitrobot™ offers the unique opportunity to automate the cryo-fixation process at constant and user-definable physical and mechanical conditions (e.g. temperature, relative humidity, blotting conditions and freezing velocity). This ensures high quality cryo fixation results and a high sample preparation throughput prior to cryo observation in a TEM.
The easy, straightforward and reproducible control of the vitrification process makes the Vitrobot™ a very attractive tool for multi-user scientific environments, where many people with various expertise levels and a large sample diversity work. The ease-of-use concept opens the way to cryo-EM for both regular users and the cryo-EM experts in spé.

Vitrification is now easier than ever

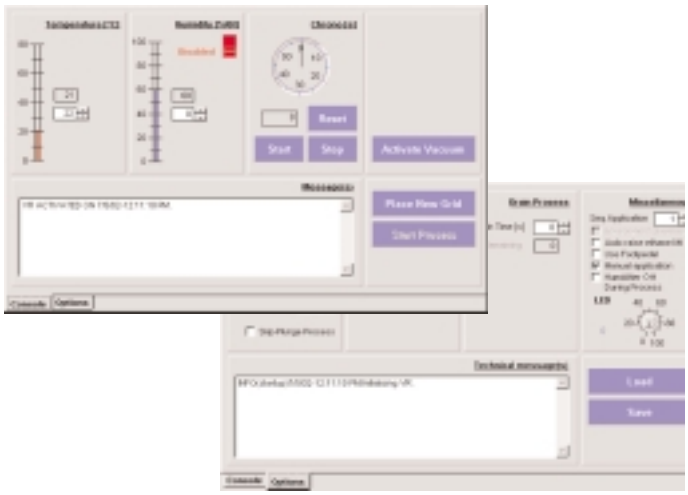
Although vitrification seems easy to achieve it can be quite demanding to realize it, considering the various sample properties and the different expertise levels of the users. The Vitrobot™ ensures that both occasional and regular users can obtain the best cryo-fixation results without going through unnecessary training sessions and failures. All essential vitrification parameters such as temperature, relative humidity, the number of



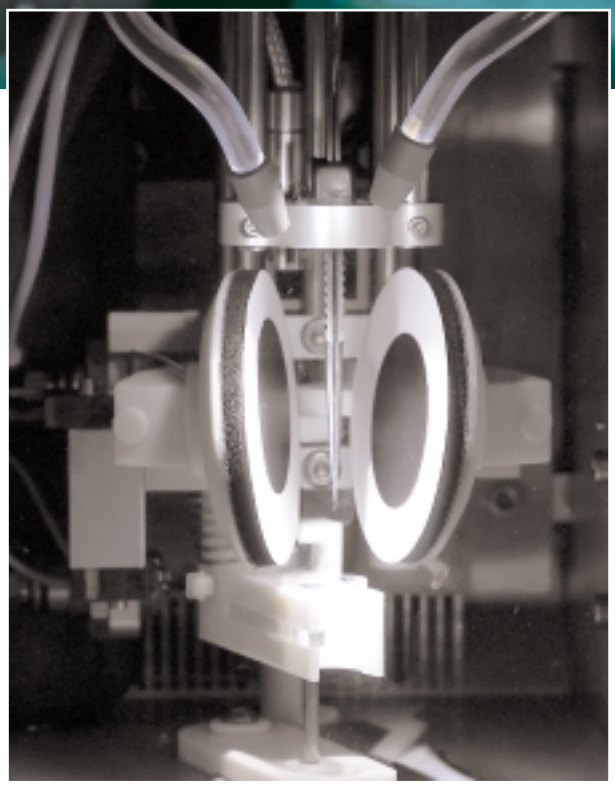
Vitrobot frozen suspension of DOPC/Cholestorol vesicles on a lacey carbon film grid. Image taken at -900 underfocus on a CM12 TWIN TEM.
Image courtesy of Mr. Paul Bomans, University of Maastricht, The Netherlands



Vitrobot frozen suspension of Tobacco Mosaic Virus (TMV) on a lacey carbon film grid on a CM12 TWIN TEM.
Image courtesy of Mr. Paul Bomans, University of Maastricht, The Netherlands



The graphical Vitrobot User Interface



Vitrobot climate chamber

blottings, blotting pressure and drain time can be programmed for each individual application and set for automatic retrieval. In fact, once the proper - specimen specific - freezing parameters are set, the plunge freezing session can be performed almost fully automatic and reproducible. Using the newly designed and software controlled Vitrobot™ User Interface, cryo fixation is now easier than ever.

The graphical Vitrobot User Interface consists of two pages, the so-called “Console” page and the “Options” page. In the “Console” page the temperature and relative humidity in the climate chamber can be set and read-out. To keep track of all experimental operations a time-linked event log is available for data registration. In the “Options” page, additional parameters such as the application time of suspensions onto the grid, blotting parameters (e.g. number of blots, blot position, wait and drain time) can be defined. In the event specific experimental conditions are needed, they can easily be set, saved and/or subsequently loaded.

Safety First

The Vitrobot™ is designed with the focus on security and safety for the users and their environment. Each step of the vitrification process has safety restrictions that are defined by software, hardware and healthcare requirements. Safety rules do not allow to plunge freeze if e.g. the door of the climate chamber is not properly closed, the freezing parameters are set out of range or the container with the liquid coolant is not properly positioned. No need to say that the inflammability of the propane or ethane still requires working in a spark free fume hood. For healthcare reasons, the water container of the Vitrobot™ humidifier is removable and can be refilled with fresh double-distilled water at the start of each experiment.

Technical Specifications

Weight		29 kg
Dimensions	L/W/H	423/183/894 mm
Air (pneumatics)	Clean dried air	6 -16 bar through 6 mm hose inlet
Power supply	Voltage	110 – 230 V 50 –60 Hz
	Fuse	4 AT (110 V USA) 2 AT (230 V Europe)
	Power cable	90-250 V

Operating parameters

Working Temperature

- 4 – 70° C
  (at an ambient temperature range between 18 – 25° C)
- Peltier controlled heating/cooling

Relative Humidity

- ambient humidity – 100 %
  (no condensation at an RH < 85%)
- Ultrasonic controlled humidification

Instrument Control

- Specific instrument control and operational parameters set-up are done through a mouse controlled User Interface and/or with a mechanical foot-pedal switch.

Sample Application

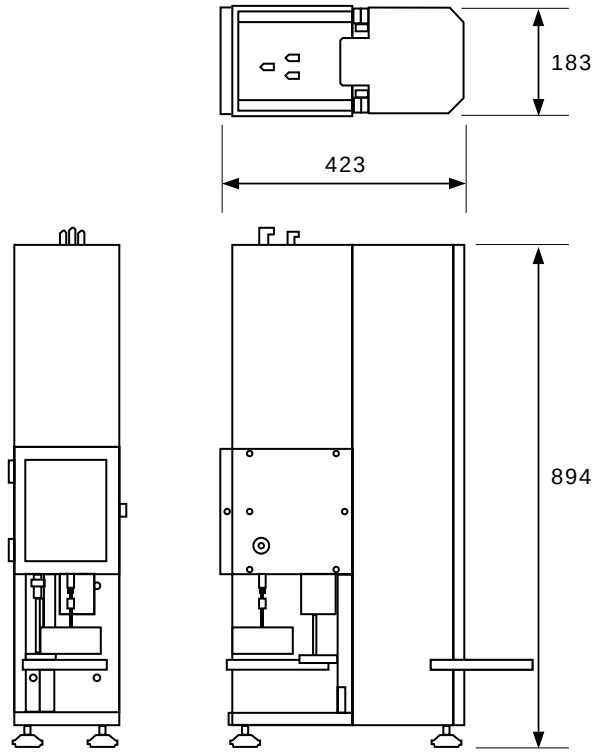
- Software controlled dipping/withdrawing of the grid from a vial. Small sample volumes can also be applied manually through a small side port allowing access to a pipette.
- Both application time and wait time (time between application and blotting) are software controlled and can be set in the User Interface.

Blotting Device

- Access of fluids is removed from the grid by (repeatedly) blotting with filter paper on rotating foam pads.
- Number of blotting actions (max. 16 times for one grid) and the duration of blotting (blot time) is software controlled and can be set in the User Inteface.
- Longitudinal grid positioning ('blot offset') as well as the wait time between blotting and vitrification ('drain time') is user definable.

Vitrification Process

- Automated shutter control allows for an instant and smooth injection of the sample grid into the container with liquid ethane/propane. A lift for the container brings the coolant as close as possible to the shutter to ensure optimal vitrification.
- Parallel coupling between lowering of the coolant container and position of the frozen grid remaining inside the coolant. This minimizes any possible contamination prior to the sample transfer into a storage box or cryo holder.



Vitrobot dimensions

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