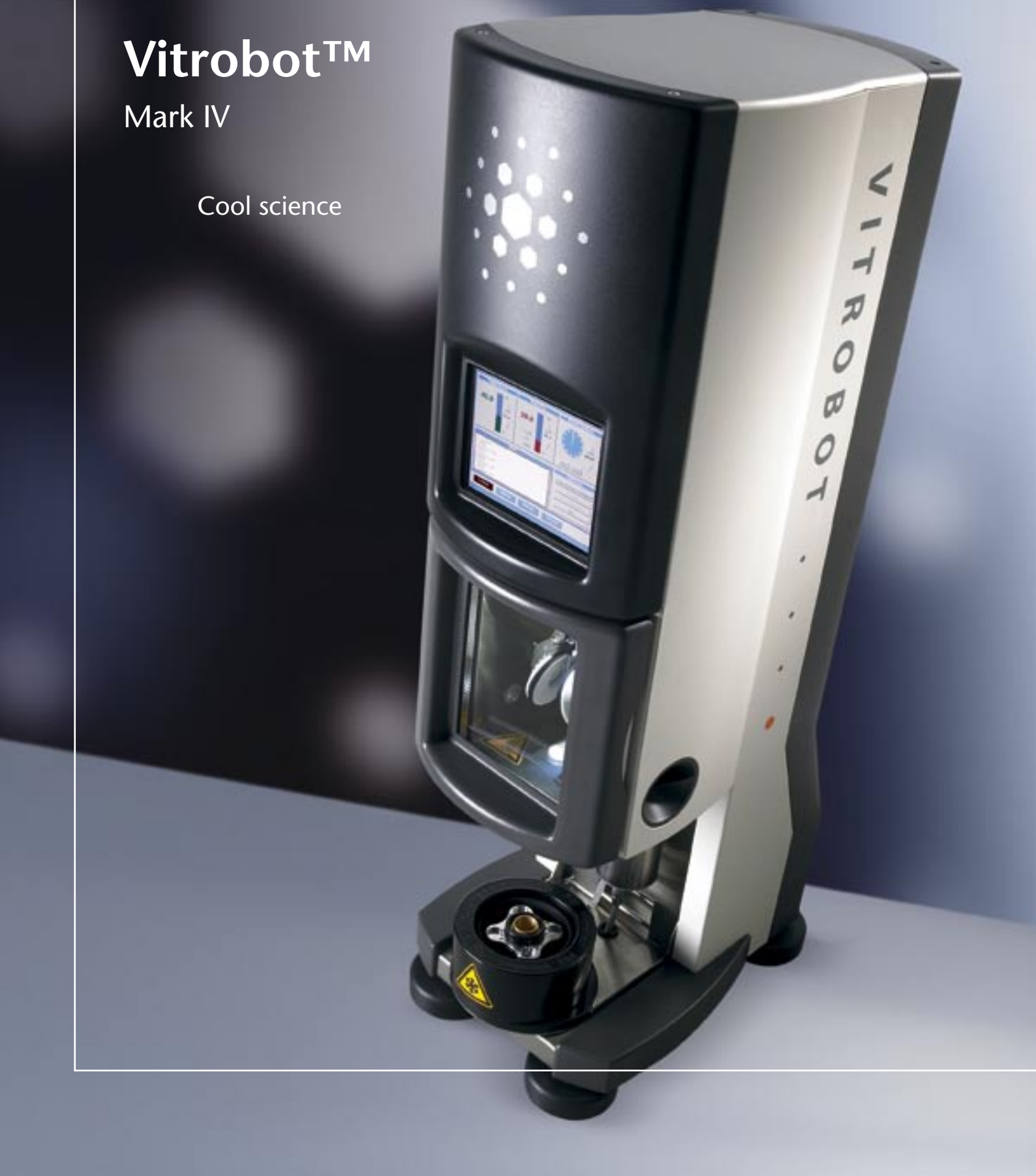
FEI COMPANY™
TOOLS FOR NANOTECH

Vitrobot™

Mark IV

Cool science



The benefits

- Fully automated, reproducible vitrification of suspensions
- High vitrification quality through controlled environment
- High sample throughput
- Easy and flexible instrument control
- Semi-automated grid transfer

Explore the real structure

Fundamental research within the scope of cell, structural biology and nanotechnology is increasingly focusing on 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 this. FEI, Tools for Nanotech™, understands the importance of reliable and reproducible sample preparation and process management as a primary step towards high quality results.

With this aim FEI Company introduces – five years after its first appearance – the next generation Vitrobot™ Mark IV, a fully automated vitrification device for plunge-freezing of aqueous (colloidal) suspensions that meets the demands of modern science.

The Vitrobot with its newly designed touch screen user interface that runs under the Linux Operating System 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.

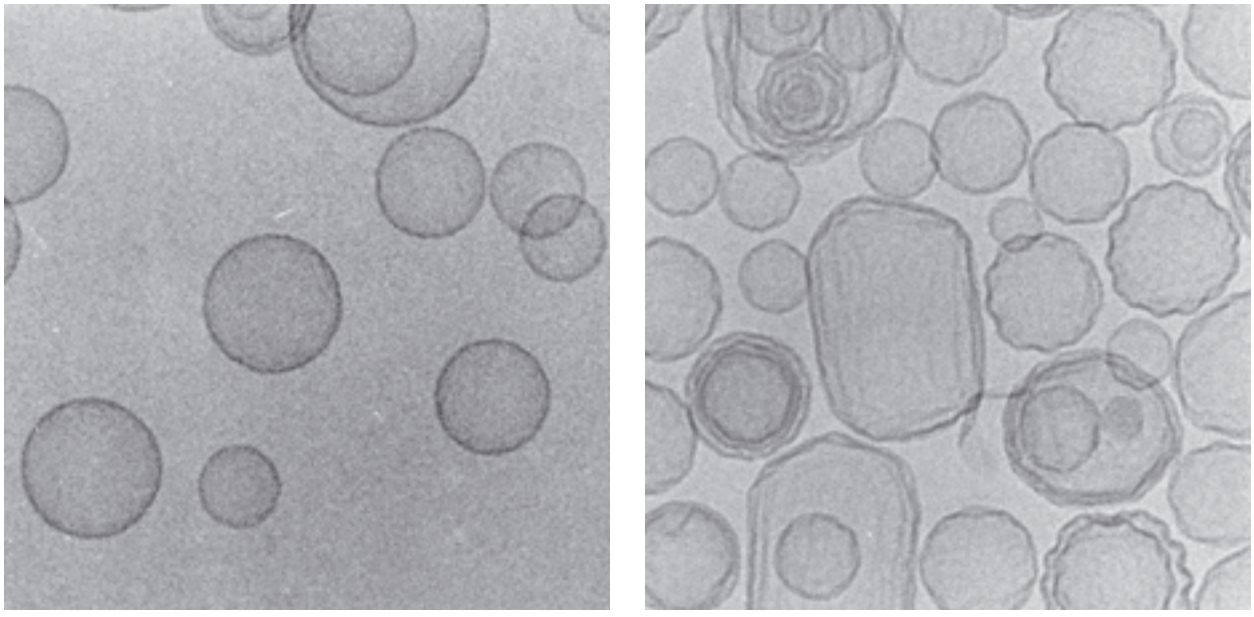
The transfer from the vitrification medium into the liquid nitrogen atmosphere has been automated, thereby ensuring an even more consistent and high yield sample output.

The new Mk IV is a state-of-the-art specimen preparation unit that offers great value to the demanding scientific areas of cell biology and molecular imaging as well as being very suitable for food, industrial, pharmaceutical and nanotechnological applications – where the true colloidal structure needs to be viewed.

Proteomics, pharmaceutics, polymers and personal pare

Cryofixation 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 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. In addition, for optimal control of developmental and manufacturing processes of polymers, food and personal care products exploration of product components or hybrids and their in vivo behavior at the macromolecular scale is indispensable.

In relation with the latter the new Vitrobot allows for time-resolved (chemical) experiments between components prior to vitrification. 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 cryofixation process at constant and user-definable physical and mechanical conditions (e.g. temperature, relative humidity, blotting conditions and freezing velocity). This ensures high quality cryofixation results and a high sample preparation throughput prior to cryo-TEM observation.



The value of the Vitrobot "environmental key technology". At 100% humidity artifact free sample preparation can be ensured (left image). Clear image artifacts – membrane aberrations - appear at 50% relative humidity.

Image courtesy of Dr. P. Frederik and Mr. P. Bomans, FEI Company, The Netherlands

Reliable vitrification is now as easy as one, two, three...

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 Mk IV ensures that both occasional and regular users can obtain the best cryofixation results without going through unnecessary training sessions and failures. All essential vitrification parameters such as temperature, relative humidity, the number of 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. In the next generation Vitrobot, the "after-freezing" handling trajectory has been further optimized. The liquid coolant container – with anti-contamination ring - minimizes the contamination risk during the grid transfer. The optimally isolated container ensures better temperature conditions and a lower consumption rate of the liquid coolant.
In addition, the grid transfer from the vitrification medium towards the grid storage box has been largely automated in order to further facilitate the sample throughput and vitrification quality.
Using the newly designed and software controlled Vitrobot user interface – that is touch screen controlled and running under the Linux Operating System - cryofixation is now easier than ever.

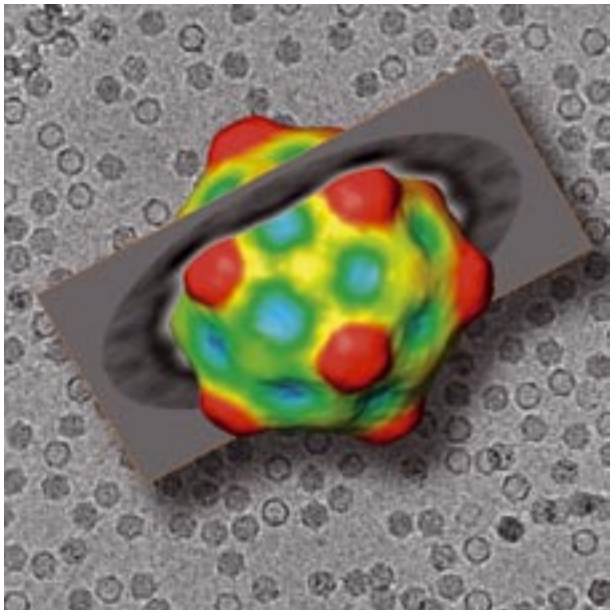
The graphical Vitrobot user interface consists of two pages, the so-called "Console" page and the "Options" page. In the "Console" page the temperature and relative humidity in the climate chamber can be set and read-out. To keep track of all experimental operations a time-linked event log is available 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. A USB memory stick allows for external communication between various Vitrobots or PCs.

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 plunging 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 it is advisable to work in a spark free fume hood given the inflammability of the propane or ethane. 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.

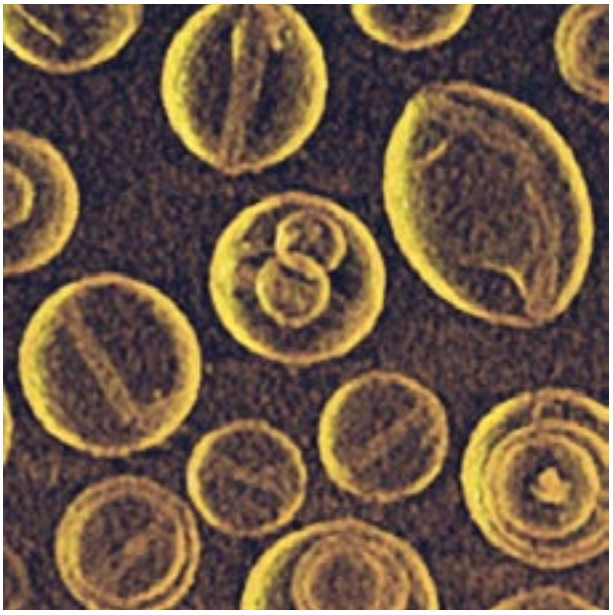
Applications data

The following illustrations show some applications examples of suspensions that can and have been vitrified with the Vitrobot.



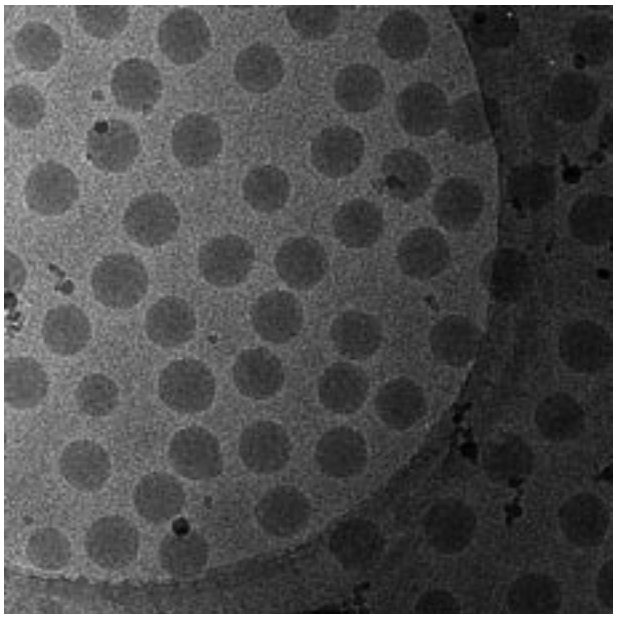
A suspension of Cowpea Mosaic Virus (CPMV) was vitrified and subjected to a single particle reconstruction procedure. The radial color distribution represents the topology of the viral capsid.

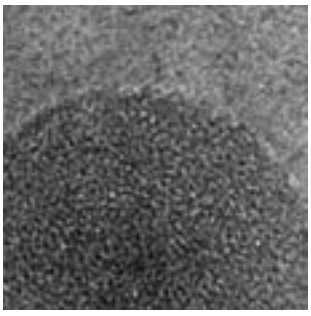
Image courtesy of Dr. F. de Haas, FEI Company, The Netherlands



Cryo-tomogram of Vitrobot frozen liposomes containing the anti-cancer drug Doxil (Nature Medicine, 2003)

Image courtesy of Dr. P. Frederik and Mr. P. Bomans, University of Maastricht, The Netherlands





Cryo-TEM image of Vitrobot frozen shampoo/hairconditioner.
To validate the various production phases of personal care products cryo-TEM may be a viable tool. Image shows the homogeneous dispersion of liposome particles.

Image courtesy of Dr. F. de Haas, FEI Company, The Netherlands

Technical specifications

Weight
31 kg

Dimensions
l/w/h	413/260/890 mm

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 – 60° 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
- The Vitrobot is fully controlled by Linux as the main operating system
- Specific instrument control and operational parameters set-up are touch screen definable/controllable. It will still be possible to connect a mouse for GUI control and/or to run the workflow with a mechanical foot-pedal switch

Sample application
- Small sample volumes can be applied manually through a small side port on the left and the right hand side of the climate chamber as to allow 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
- Repetitive sample application and blotting prior to vitrification can be set. This enables the option to do time resolved analysis i.e. triggering of (chemical) reactions between components prior to vitrification

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 interface
- 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
- New coolant container with anti-contamination ring for optimal grid transfer
- Semi-automated grid transfer from the liquid ethane/ propane towards the grid box placed in the liquid nitrogen environment

About FEI

FEI Company is a global leader in providing innovative instruments for nanoscale imaging, analysis and prototyping. FEI focuses on delivering solutions that provide groundbreaking results and accelerate research, development and manufacturing cycles for its customers in life sciences, semiconductor and data storage, academic and industrial R&D markets. With R&D centers in North America, Europe, and India, and sales and service operations in more than 50 countries around the world, FEI's Tools for Nanotech™ are bringing the nanoscale within the grasp of leading researchers and manufacturers.

See more at fei.com

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TÜV Certification for design, manufacture, installation and support of focused ion- and electron-beam microscopes for the NanoElectronics, NanoBiology, NanoResearch and Industry markets.

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