

Tecnai G² Spirit Highlights

- High contrast, high resolution for 20 –120 kV operation
- Optimized for 2D & 3D imaging of cells, cell organelles and soft matter
- High level of automation: Auto-Gun and automatic tuning
- Smart Tracking Position System for sample navigation
- Customized protocols for diverse applications
- Sample observation at liquid nitrogen temperature
- Technology for sharp imaging of thicker samples
- Ergonomic design for operational comfort



Tecnai™ G² Spirit Next Generation Microscopy for Life Sciences



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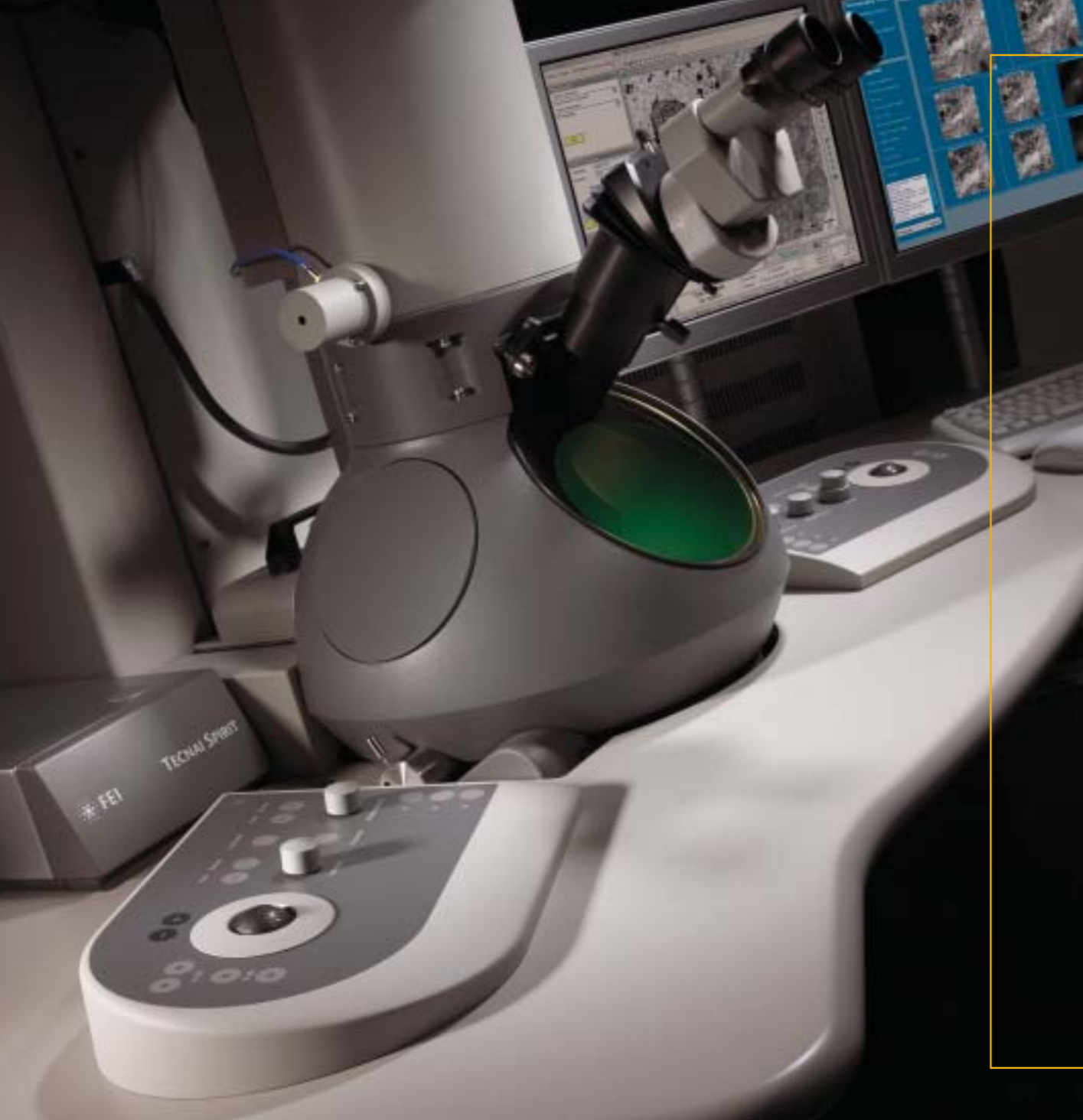
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Meeting the needs of the scientific community

Fundamental research within the scope of cell biology, structural biology, soft matter and nano-technology is increasingly focusing on understanding interactive biological processes, pathways and structures at the macromolecular level. Transmission electron microscopy (TEM) is of paramount importance to obtain high-magnification and high-resolution 2D and 3D information of cells and organelles or even smaller cell constituents. A pre-requisite for optimal 2D and 3D-tomographic imaging is a high level of microscope automation and intelligence. The new Tecnai™ G² Spirit – built on more than 50 years' experience in Life Science – combines modern day technology to satisfy the stringent demands of both today's and tomorrow's scientific community.

The Tecnai G² Spirit ensures high-quality images, is easy to use and is designed to facilitate the work of the microscope user. It has a high level of automation, yet doesn't limit full control for experienced expert users. The microscope is ready for novel applications like 3D-imaging using tomography and/or cryo-electron microscopy. The Spirit's design philosophy ensures application flexibility for today and tomorrow, it holds a ticket to the future: functionality can be added or upgraded by uploading current and new application software. In the world of Life Sciences, developments occur at a high pace. FEI Company has ongoing collaborations with the relevant scientific communities to better understand their needs and co-develop dedicated tools.

The Tecnai G² Spirit is building a further and more detailed understanding in the world of decreasing dimensions: today and in the future.

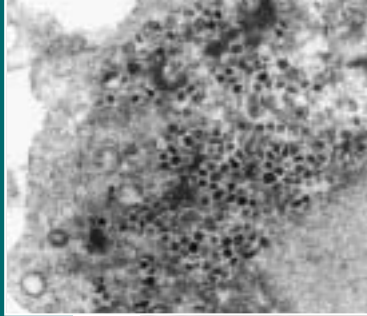
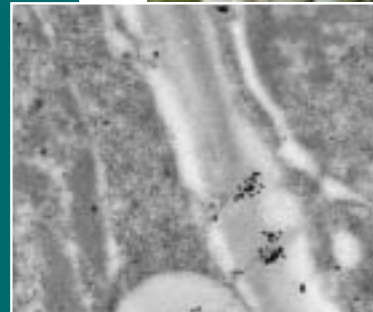


Figure 1:
Diagnostic Research
Lung biopsy from a patient
suffering from SARS.
Image courtesy of: Queen
Elisabeth II Hospital
Hong Kong.

Figure 2:
Cell Biology Research
Immunogold labeling of
plasmodesmata in genetically
modified plants



Diagnostic research

As a consequence of the growing and aging world population, the number of patients needing advanced medical care and treatment inevitably increases. This stresses the urgency for fast, reliable screening of disease patterns. As a mainstream diagnostic modality in medical centers, the Tecnai G² Spirit is a ready-to-use system that ensures a high level of automation and a high sample throughput to cope with the need for increasing the speed of diagnostics. Furthermore, the full digital Tecnai G² Spirit allows for on-line consultation with colleagues and experts in the field to enable a fast and correct diagnosis.

Cell Biology research

Achieving breakthroughs that can improve human health or increase the quality or yield of agricultural products requires research to the underlying fundamental processes. For studying the functionality of ongoing physiological and pathological pathways, the Tecnai G² Spirit is perfectly suited. For retrieval of cell biological data, the Spirit is set for fast and (semi-)automated 2D and 3D high-quality image acquisition and analysis. Its ease-of-use makes the instrument very attractive for multi-user scientific environments like universities and institutes.

Pharmaceutics and Chemistry

The Tecnai G² Spirit is an effective and efficient tool to gain insight into release mechanisms of drugs in the body. In addition, the effects of medicines on the morphology and functioning of tissues and organs need to be investigated. The Spirit, optimized for 2D and 3D imaging of cells, cell organelles and soft matter combined with a high level of automation can play an essential role in this and is of significant importance for continued advances in healthcare and chemistry.



Figure 3:
Pharmaceutics and Chemistry
Cryo TEM image of cis-Pt particles packaged
in PS liposomes. The liposomes are used as
novel drug targeting entities in anti-cancer
research.
Image courtesy of Dr. P. Frederik, University of
Maastricht, The Netherlands.

High Image quality – throughout the entire magnification range
All biological TEM specimens, ranging from ultra-thin sections to negative stained samples, have in common that for high-quality visualization maximized contrast and resolution are required. In general, the imaging magnifications are in the intermediate range, roughly from about 500 to 100 000 times covering 95% of the used applications.

The optics of the Tecnai G² Spirit – with the well proven and patented BioTWIN and TWIN concept – provides sharp, high-contrast and high-resolution images at all magnifications. The electron source in combination with the excellent vacuum ensures a high brightness and a long life-time of both the tungsten and LaB₆ filaments. To facilitate the practical work, one can store and re-load optical parameters like magnification

and illumination settings, in conjunction with stage coordinates to re-allocate interesting areas of the specimen, at will. Moreover, alignments or operations parameters can be stored easily and retrieved at the push of a button. Each user has his or her own secure and customized microscope environment, and works with own or supervised settings and alignments. Microscope alignment efforts are reduced to a minimum so one can concentrate on getting the best out of the specimen.

In addition, the user is given the flexibility to choose different imaging modes, e.g. rotation free magnification or isotropic chromatic aberration corrected magnification. In other words, the optical system can be adapted to the needs of biological research: easy zoom in or maximized sharpness, even for thicker samples.

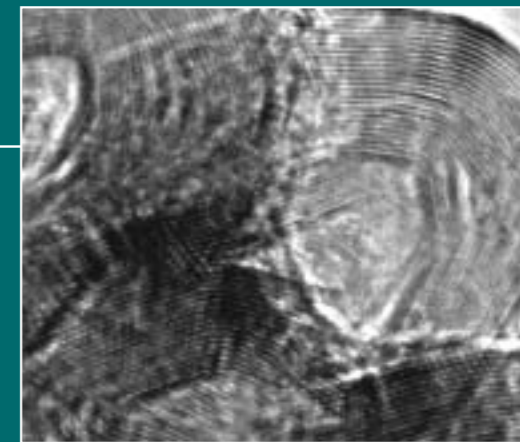
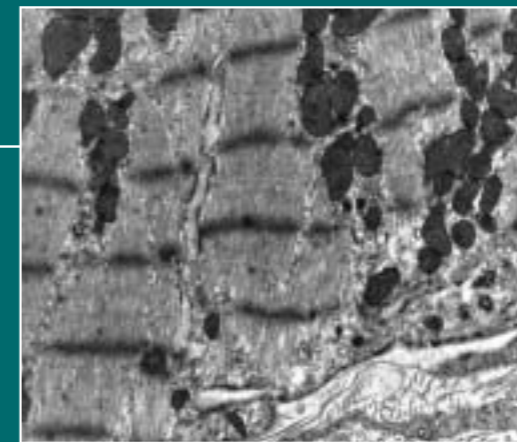


Figure 4
Left-hand shows a typical image at a low magnification on the Tecnai G² Spirit: it demonstrates the value of the patented high contrast performance. The right-hand image shows the high-resolution performance. For reasons of reference a graphite sample was used.

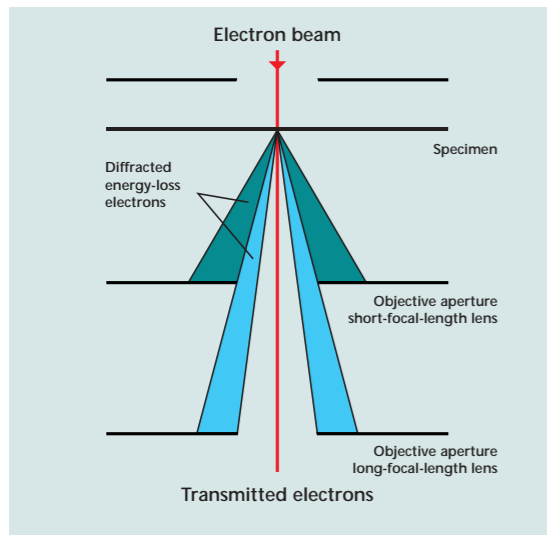


Figure 5. In a long focal length lens system, the objective aperture stops many more diffracted electrons and energy loss electrons – and thereby provides higher contrast – than in a short focal length lens system. The Tecnai G² Spirit BioTWIN has the longest focal length worldwide.

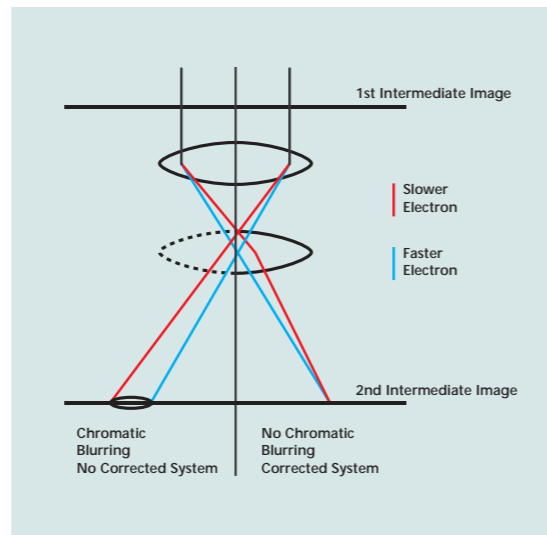


Figure 6. The chromatic error correction system of the Tecnai G² Spirit re-unites electrons of different energies (shown in red and blue), thereby giving sharp, high-quality images even for thick specimens. On microscopes without chromatic error correction, these electrons separate and cause blurring of the image.

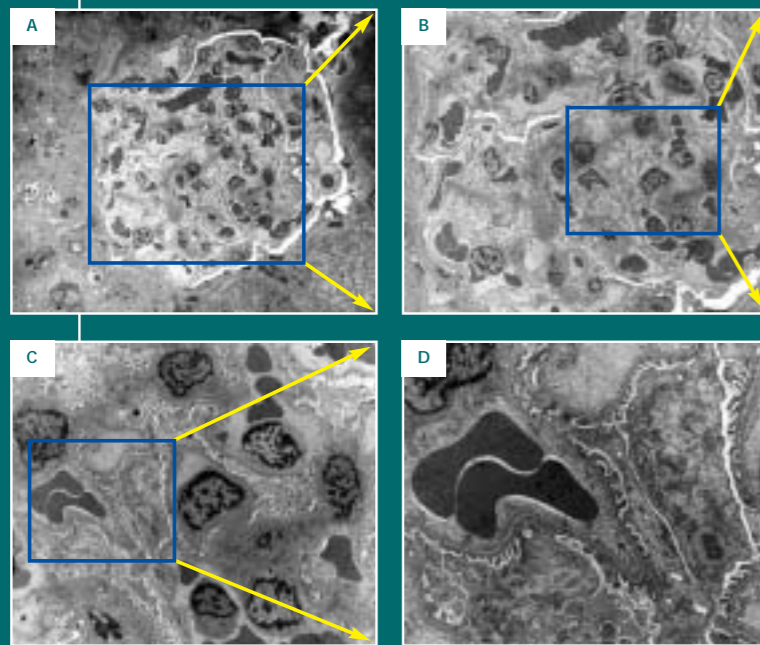


Figure 7. Displayed images are from an ultra-thin section of a kidney glomerulus post stained with lead citrate and uranyl acetate. The electron optics magnification of the images shown are taken at 610x, 1250x, 2550x and 6000x (A,B,C,D). There is no limitation of the field of view in these images, not even at the lowest magnification (610x) and with the smallest objective aperture.

High Image quality – at various section thicknesses

Typically, energy loss of transmitted electrons due to thicker samples will lead to unsharp, blurred images. The projection system of the Tecnai G² Spirit limits this effect since the objective aperture – due to the long focal length of the objective lens – blocks the majority of the electrons with multiple energy levels.

Furthermore, the projection system can be used in such a way that both energy loss and zero-loss electrons are focused on the same point as the zero-loss electrons. This approach ensures sharp, high contrasted images even for thick specimens. This is in particular valuable for 3D tomography applications where tilting can lead to tripling of thickness at high tilt. Image brightness remains high due to the Tecnai G² Spirit high coherence, high brightness electron gun.

Operational comfort

The Tecnai G² Spirit is a fully digital microscope allowing a high level of automation to facilitate its use for the various experience levels in the user community. Flexibility is paramount. The user can control the microscope manually or automatically at will. Besides via the software user interface – displayed on the LCD screen(s) – the microscope operator is also communicating with the system via the newly designed control panels. In addition to those buttons representing the standard microscope functions (i.e. magnification, focus, intensity), each user has the flexibility to assign various application dedicated functions to the programmable buttons. Moreover, the optical trackballs guarantee a smooth control of sample and beam shift. Needless to say that the trackballs can also be assigned to almost any user-defined microscope function.

Automation – of essential microscope functions

The Tecnai G² Spirit stands for an unprecedented automation of essential and most frequently applied microscope functions. With the Auto-Gun function, retrieving an optimal screen brightness is literally one mouse click away. Its intelligence thinks for you and corrects all gun alignments and conditions and saturates the filament fully automatically. Moreover, the image focus and objective astigmatism can be routinely corrected using the Auto Tune function, always giving the best possible result with the lowest effort. Needless to say that experienced users can always take full manual control of all micro-



Figure 8. Hand Panel Controls

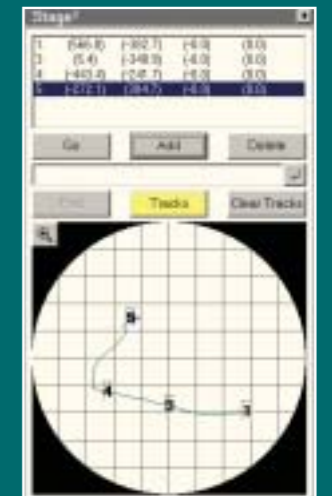


Figure 9a: Smart Tracking Position System module User Interface.

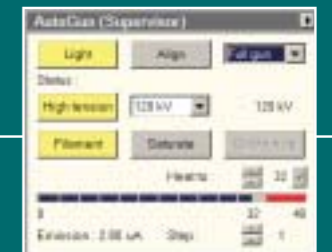


Figure 9b: Auto Gun User Interface.

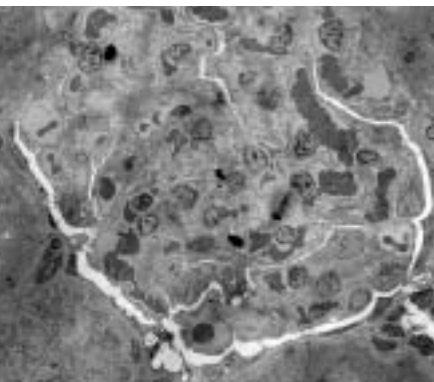
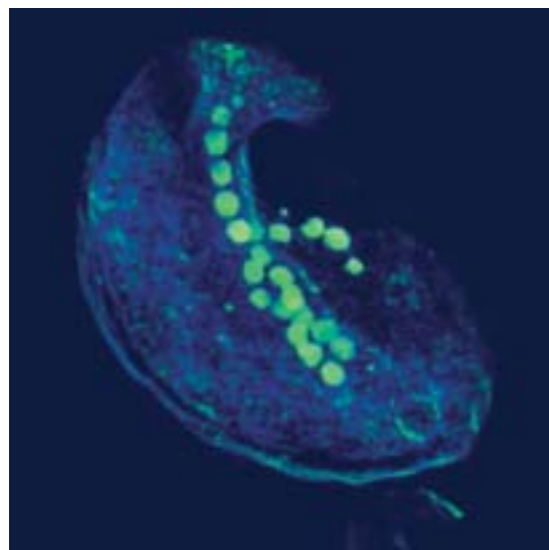


Figure 10.
Illustrates an automatically stitched 3k x 3k image composed out of 9 individual 1k x 1k images. The result is a high-quality low-magnification image.

Automation – for optimal image acquisition and quality
The functional combination of hardware, optics, stage and digital detectors like CCD cameras in an embedded software environment has opened the door for advanced, (semi-) automated microscope applications. One of the most frequently performed applications is image resolution enhancement by stitching multiple digital images together. In the Tecnai G² Spirit, stitching occurs fully automatically. Upon selection of the final resolution goal, all images are recorded and are stitched into one image within a few seconds time.

Even though microscopy has entered the digital era, the microscope still features the option for conventional image recording on film plates. In line with this, software is available to allow photomontage in a guided manner.

Figure 11.
Shows a 3D-image of magnetosome chains in magnetotactic bacteria. Data automatically acquired on a 200 nm section on a slow scan CCD camera and processed using the FEI Xplore3D software.
Courtesy: Dr. Kobayashi, National Institute of Advanced Industrial Science and Technology, Osaka, Japan



3D-imaging – “Confocal TEM”
For the last 50 years, the typical result of a transmission electron microscopy session was a 2D-image of a 3D-structure. It takes a lot of experience to understand and interpret the information in the correct way. Confocal Laser Scanning Microscopy (CLSM) was the trigger to master the third dimension. A lot of cellular processes are easier to understand when 3D information of cells and cell structures can be obtained. The automation of the Tecnai G² Spirit microscope in combination with FEI’s proprietary intelligent tomography solution enables the user to achieve 3D TEM-results fully automatically and in a limited time, thus revealing high-resolution confocal TEM images ~ in analogy with confocal light microscopy (down to nanometer resolution scale).

The Tecnai G² Spirit can completely and automatically acquire a tomographic data set. The Spirit features a comprehensive tomography data acquisition and processing software package. To acquire a series of images, samples are tilted in the electron microscope from + 70 to -70 degrees at user defined intervals to ensure the highest possible resolution and reconstruction quality.

FEI’s Xplore3D automatically determines changes in image movement and focus after tilting. The software has a high degree of flexibility, allowing individual users to optimize the procedure for obtaining their best results. Image data are collected using a CCD camera and stored on hard disk or DVD.

Cryo electron microscopy
Cryo-fixation by rapid freezing has been accepted as a key-technology to study the natural state of a biological specimen. In this way, the integrity of the specimen is maintained during transfer and data collection sessions in the microscope. The results obtained at high resolution contain the information to understand the processes and related structural changes during the many biological processes within a cell. The goal is ultimate specimen protection to achieve ultra-structural details down at the Ångstrom level.

Many techniques have been designed to maintain specimen integrity dur-

ing preparation, transfer and observation. For the vitrification process as such, the parameters to control are humidity and temperature. The quality of vitrification is reproducibly improved since the commercial availability of the Vitrobot™.

Once the vitrified sample is in the microscope, the clean vacuum ensures a contamination-free observation for long working sessions. The observation at liquid nitrogen temperature in combination with the low dose software gives the best protection for beam-sensitive specimens. Important other improvements are the optimization of the low-magnification high-resolution performance.

The microscope is designed to obtain the ultimate resolution at low magnifications. Finally, in recent years a significant change has taken place in image recording. Highly sensitive CCD cameras are used to record cryo-results. The major advantage of this approach is the further automation of the cryo-observation process. Automation will inevitably lead to optimal, statistically validated results with minimal user effort – a prerequisite for single particle analysis. The system reliability ensures researchers have a high sample throughput.

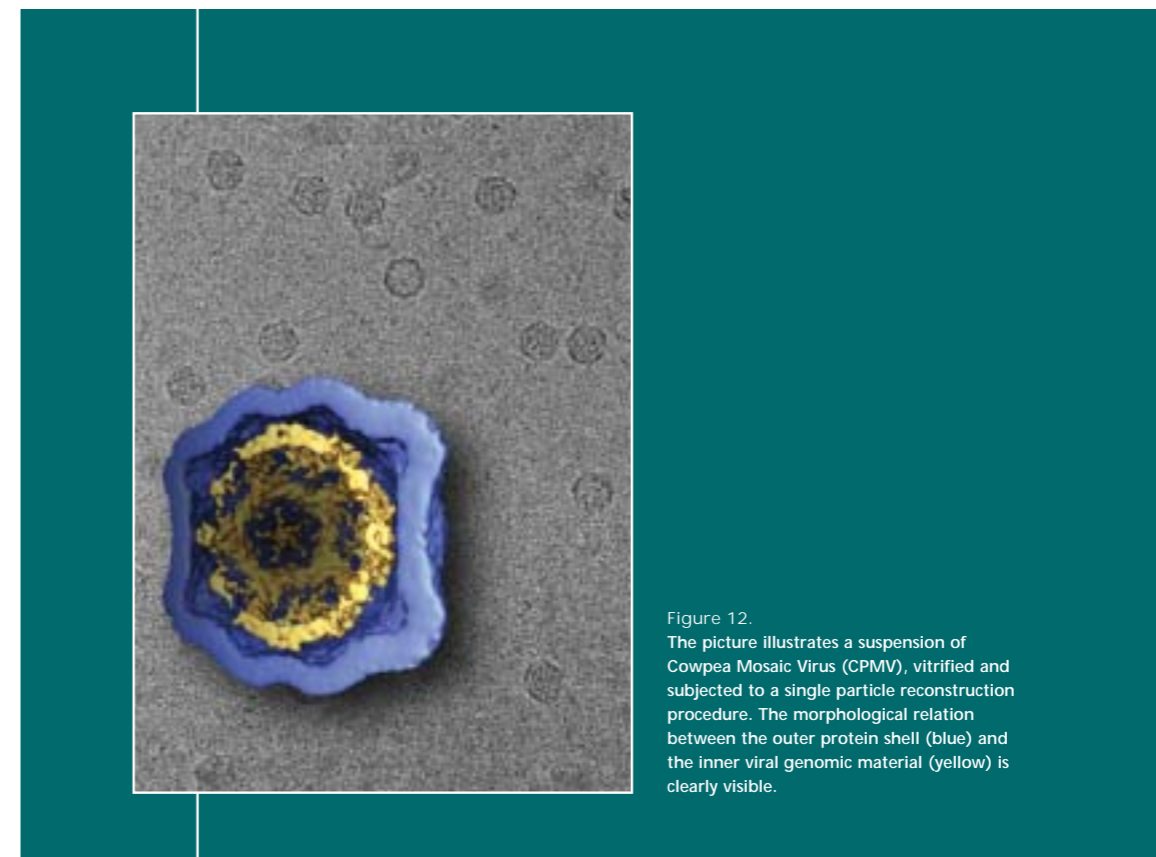


Figure 12.
The picture illustrates a suspension of Cowpea Mosaic Virus (CPMV), vitrified and subjected to a single particle reconstruction procedure. The morphological relation between the outer protein shell (blue) and the inner viral genomic material (yellow) is clearly visible.

Tecnai G² Spirit Bio(TWIN) – Specifications

Embedded control of cameras and analytical technology. Optimal information acquisition at maximum ease-of-use is a continuous challenge for further improvement of FEI's instrumentation. A big step in realizing this is the embedded control of frequently used microscope functions (e.g. CCD image acquisition) and additional, analytical functions (e.g. STEM and EDX). Through the embedding concept, the Tecnai G² software interface basically has full control over these functions resulting in increased ease-of-use.

Service support. Customer surveys show that FEI instruments have an excellent reputation for reliability and robustness, even after being in operation for more than a decade. Each microscope comes with detailed and complete digital service manuals as well as built-in self-diagnostics and on-line

help manuals. Unsurpassed instrument up time is ensured by high-quality, first-line service support, available throughout the world. Highly skilled service, development and factory engineers from the product divisions establish back up.

Our philosophy of support. Dedicated training is pivotal in FEI's customer support philosophy. Training aims to share and transfer operational skills and to show the versatility and possibilities of various electron microscopy techniques. Above all, it strives to show you how to reach your best result for different applications. In other words: FEI Academy – a mature competence center- helps you maximize the value of your installed equipment by optimizing the potential of the person controlling it. Please visit our website www.feiacademy.com to learn more about FEI Academy and the latest courses and workshops.

About FEI: Tools for Nanotech™. FEI Company provides advanced Tools for Nanotech to a range nanotechnology markets including materials science, life science, nanotechnology, semiconductors, and data storage. Its ion beam and electron beam products, market-leading DualBeam™ systems, and productivity-enhancing automation software packages enable researchers and manufacturers to fabricate and modify nanoscale structures and to view and characterize them in three dimensions down to the atomic level. The company has been paving the way for nanoscale exploration and discovery since 1946. FEI Company currently has more than \$360 million in revenue, enjoys consistent profitability, has manufacturing and development facilities in North America and Europe, and service operations in more than 40 countries worldwide.

For more information visit: www.feicompany.com or www.tecna.com for more technical info.



| | BioTWIN | (TWIN) |
|--------------------------|---------------------------------|----------------|
| Electron Optics | | |
| Line resolution | 0.34 nm | (0.20 nm) |
| Information limit | < 0.20 nm | |
| High tension (min step) | 20 - 120 kV, [continuous- 10 V] | |
| HT stability | ≤ 2 ppm/min | |
| Illumination modes | Micro/nanoprobe | |
| Number of spot size | 11 | |
| Number of C1 apertures | 1 fixed | |
| Number of C2 apertures | 4 exchangeable | |
| Number of obj. apertures | 4 exchangeable | |
| Focal length | 6.1 mm | (2.8) mm |
| Cs | 6.3 mm | (2.2) mm |
| Cc | 5.0 mm | (2.2) mm |
| Minimum focal step | 9.0 mm | (3.0) nm |
| Magnification | 22 - 300 kx | (18 - 650 kx) |
| SA Camera Length | 0.05 - 8.9 m | (0.02 - 4.2 m) |

| Operating System | |
|------------------------------|--|
| Controller | PC [Windows 2000] |
| User interface | User defined |
| Control Panels | New ergonomics, with optical and removable trackball |
| User levels | Three |
| Scripting facilities | Via Tecnai Scripting [optional] |
| Remote Operation | Taro software [optional] |
| Accessories (STEM, EDX, CCD) | Embedded |

| Vacuum | |
|--------------------------------|----------------------------------|
| Gun vacuum | 1 x 10 ⁻⁶ Pa |
| Column vacuum | 1 x 10 ⁻⁵ Pa |
| Filament lifetime | ≥ 1 year |
| Filament exchange time | < 5 min |
| Air lock pumping | Oil-free |
| Differential aperture | 200 μm |
| Pumping time for sample holder | 10 -180 secs |
| Pumping time for (film)chamber | 5 min |
| Cold trap | Optional [for cryo applications] |

| Specimen Manipulation | | |
|------------------------------|---|-------|
| spec. alfa tilt angle | ± 80° | (70°) |
| x,y direction | 2.0 / 2.0 mm | |
| z direction | 0.75 mm | |
| Recalling accuracy | < 0.1 μm | |
| Number of stored positions | Infinite, including optics setting i.e. intensity, magnification, spotsizes | |

| | BioTWIN | (TWIN) |
|----------------------|---|--------|
| Sample exchange time | < 30 sec [without switching off High Tension and Filament] | |
| STPS | Smart Tracking Position System Tracking interface for visualization of searching pathway incl. stored positions | |
| Intelligent Recall | Comment mediated stage position recall | |
| Specimen drift | ≤ 1.0 nm/min | |

| Accessories | |
|--|---|
| CCD cameras | Embedded control for all supporting products: Gatan, SIS, Tietz |
| Magnification calibration for double CCD cameras | Automatic |
| EDX detector resolution | ≤ 135 eV |
| EDX detector window | SUTW |
| EDX solid angle | 0.13 str. (0.21) |
| STEM resolution | 1.0 nm |
| STEM magnification | 150 - 3.1 Mx |

| Tomography | |
|--|--|
| Calibrations, tilt series acquisition, reconstruction and volume rendering | Fully automatic acquisition Xplore.3D intelligent solution |

| Cryo TEM | |
|-----------------|----------------------------------|
| Transfer | Standard Gatan holders [60°/70°] |
| Sample analysis | Low Dose supported |

| Automation | |
|-------------------|--|
| AutoGun module | Enables automated gun conditioning, saturation, alignments. Including a one push button for direct light |
| AutoTune module | Enables automatic focus and astigmatism correction |
| Tomography | Automated calibrations and image series acquisition. Reconstruction and rendering through Xplore.3D |
| STPS | Smart Tracking Position System for interactive and navigational stage control |

| Applications support | |
|-------------------------------|--|
| On-line help files | Yes - English, Japanese, Chinese |
| Application instructions | Available |
| Customer FEI Academy training | Basic, Advance Materials, Advanced Life-sciences, Advanced Cryo and workshops in English |