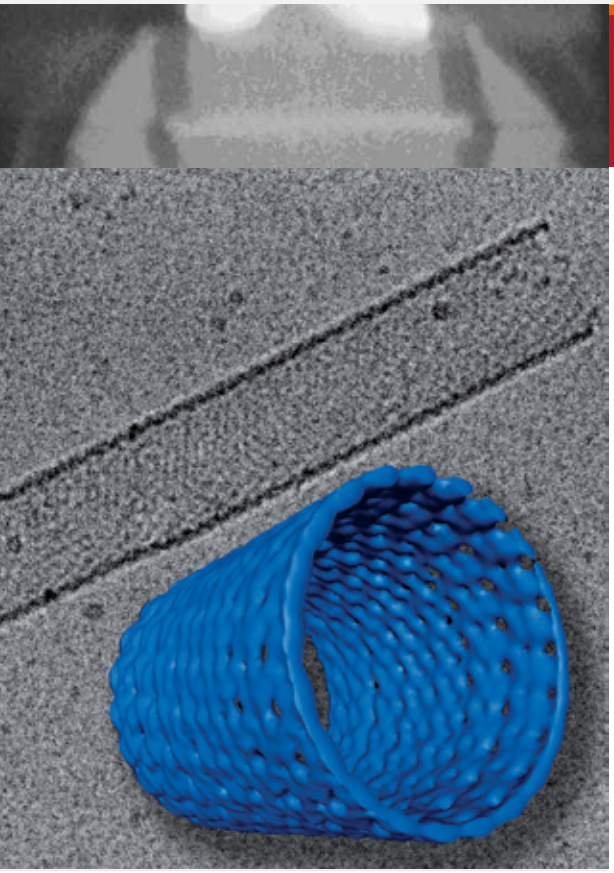


Xplore*3D*™

The total solution for electron tomography on Titan™ and Tecnai™

Electron tomography has become an essential technique available to transmission electron microscope (TEM) users at all levels and in all fields. The technique, well established in life science, quickly gained ground in materials science and industrial applications driven by the need for high resolution 3D information. This growing interest in electron tomography was stimulated initially by digital imaging and computer controlled TEM. More recently advances in automation, computing hardware, and the advent of high angle annular dark field scanning TEM (HAADF-STEM) tomography boosted performance, ease of use and opened new paths in materials 3D characterization.

FEI Company provides a total solution for electron tomography on a Tecnai™ or Titan™ TEM: the software suite ``. Xplore*3D* integrates pioneering work on electron tomography of several scientific institutes, and leverages FEI's intimate knowledge and expertise on TEM technology to combine all the phases of the tomography process into a streamlined and efficient workflow. The package consists of three software modules: the Automated Tomography Data Acquisition Software (ATDASW) for acquisition of tomography data series, Inspect*3D*™ for data reconstruction and an FEI edition of the commercial available software package ResolveRT for visualization of the result.

Xplore*3D* is suitable for a broad application range where volume information on a TEM sample is required. Typical applications include the distribution of pores and active sites for catalysis, 3D organization of membranes and organelles in cells, 3D spatial organization of polymer blends or 3D continuity of barrier layers in a semiconductor device, etc.

Key Benefits

- Total solution:
  - a coherent acquisition-reconstruction-vizualistion workflow optimized for ease of use and time to result
- Advanced, accurate, and interactive alignment routines:
  - cross-correlation (no markers needed), bead tracking and general feature tracking
- Advanced and swift reconstruction techniques:
  - weighted back-projection, ART, and SIRT
- Advanced features:
  - TEM and STEM, energy filtering, batch scheduler, low dose, dual-axes
- Extensive documentation and tailored training

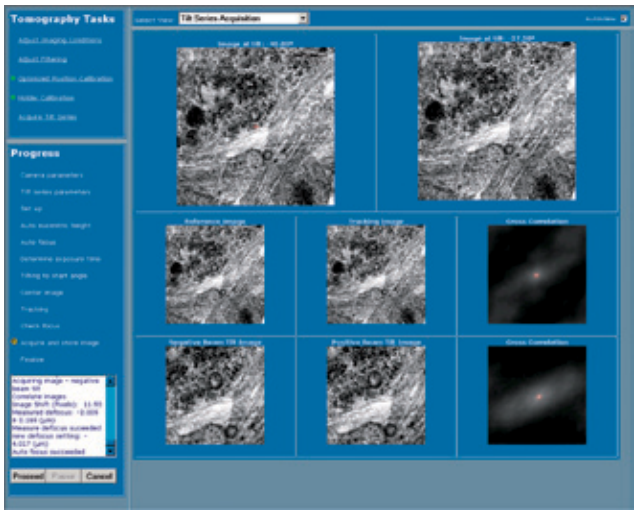
Xplore*3D* software suite

Automated tomography data acquisition module

The automated tomography data acquisition software module is the first step in FEI's total solution for tomography. It provides a comprehensible, fast and reliable way to acquire tilt series.

The quality of a tomogram depends directly on the consistency of the acquisition process, which in turn demands strict control over:

- Calibration: internally consistent calibration routines which are stable, fast and reproducible. Xplore*3D* uses its own set of calibrations which determine the image pixel size and link it to image shift, stage shift and focus shift.
- Alignment: to minimize image shifts, focus and magnification changes, the tilt axis must be intersecting the optical axis of the imaging system. Xplore*3D* fulfils this requirement with the 'optimized position' concept.
- Mechanical tolerance: measuring and correcting the mechanical behaviour of the stage (for fast and accurate acquisition) is possible owing to the highly reproducible CompuStage tilting behaviour and optical alignments of the Tecnai and Titan.
- Drift: at higher magnifications, parameters such as image shift, focus or zero-loss peak position (when using energy filtering), can be periodically checked and corrected on the fly during acquisition.



Data acquisition view showing acquired images at the top followed by tracking and focusing images with corresponding cross correlations (only when tracking and check focus are selected).

Basic calibrations, optimized position and holder calibration are performed once, and usable for all subsequent acquisitions.

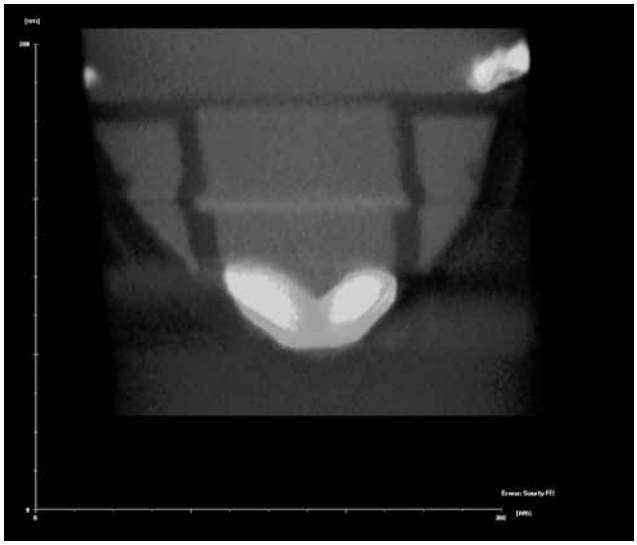
General software feature

- Easy and interactive automated tomography data acquisition software embedded in the Tecnai / Titan user interface.
- The software guides the user through the complete data acquisition process by means of a self-explanatory and intuitive workflow approach. During data collection, all relevant images are shown and the acquisition can be paused to manually adjust conditions before continuing with the automatic collection.
- User interface designed to make acquisition and set-up intuitive and straightforward: click-to-centre feature, click-and-drag contrast and brightness adjustment, mouse scroll-wheel zooming, etc.
- The tilt series can be acquired at tilt intervals either constant linear scheme or decreasing with the tilt angle Saxton scheme.

STEM tomography

HAADF-STEM is an established technique in materials science for the following reasons.

- The contrast forming the image is relatively strong. It is proportional to density, thickness and (to a good approximation) the squared atomic number of elements in the specimen.
- Electron tomography rests on the criterion that contrast in the images is a monotonic function of the mass thickness of the specimen; diffraction contrast in crystalline materials breaks this criterion. HAADF-STEM is an incoherent imaging technique therefore diffraction contrast does not contribute significantly to the image.
- Xplore*3D* includes an effective STEM auto-focus routine. It uses through-focus series measurement which works up to magnification as high as 500 kx, that is a pixel size as low as ca. 0.2 nm.
- Dynamic focusing keeps the probe focused on the tilted specimen during scanning. This allows perfect focus over large fields of views at high tilt thus keeping the resolution optimal.



STEM tomography of a memory cell obtained from a needle shaped FIB cut. Courtesy of H. Bender, IMEC, Belgium.

Inspect*3D*, post-alignment and reconstruction module

Inspect*3D* carries out automated alignment of the data based on image cross-correlations or feature tracking.

- Cross-correlation sequentially throughout the entire tilt series is assisted by a set of filters and image processing tools: Hanning window, taper, Sobel, band-pass, non-linear filters, morphological operation, threshold ... projections can be stretched to compensate for compression perpendicular to the tilt axis. Addition of gold markers is unnecessary.
- Recognizable features (e.g. markers) can be selected and automatically tracked in all images of the tilt series. The user can also guide the tracking procedure. The software will calculate the 3D positions, enabling a precise alignment, magnification changes, as well as the rotation of the tilt axis in each image.
- Inspect*3D* is also capable of locating and tracking general features (no markers needed) for accurate alignment, magnification and tilt axis adjustments.
- Bead cloaking: use image processing to remove gold beads from the data after they have been used for alignment so they do not show in the reconstruction.

For reconstruction of the 3D volume, three different methods are available:

- Weighted back projection (WBP) is essentially the inverse of the projection process: it 'smears' a filtered version of the two dimensional projections back into a 3D model space. WBP is simple and fast, but spatial frequency information is lost.
- In both algebraic reconstruction technique (ART) and simultaneous iterative reconstruction technique (SIRT), the projections of the reconstructed cube is compared to the original images and corrected to minimize differences. Besides reconstructing the full spectrum of spatial frequencies, SIRT greatly enhanced the signal to noise ratio: information possibly lost in the noise on original projections may very well come out in a SIRT reconstruction.
- Amongst the strong points of Inspect*3D*'s approach to reconstruction is the full graphical control the user has over all steps in both the alignment and the reconstruction, with many visual feedback mechanisms. At all points the user is in full control.

Dual-axis reconstruction, whereby a two-data set are taken of the same specimen area, one rotated about 90° from the other is also available. It reduces the missing wedge problem to a missing pyramid:

- Any reconstruction algorithm
- No markers needed
- Powerful algorithm require little user interaction

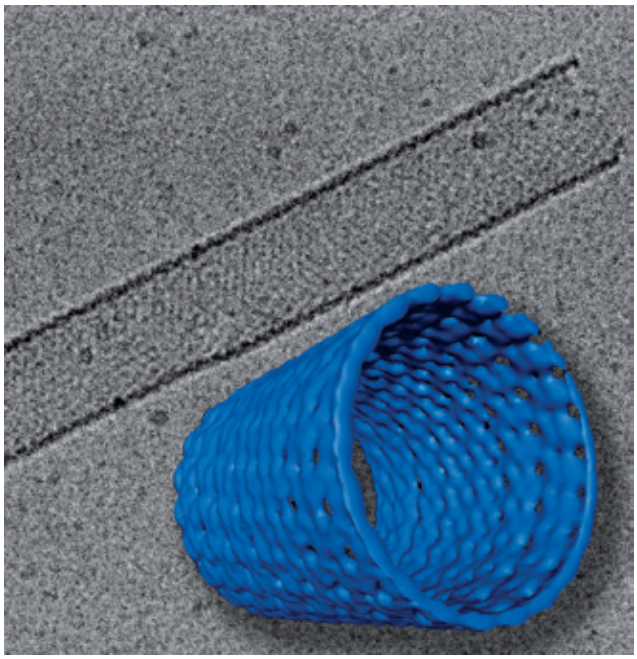
Features and benefits

Automated data acquisition

- Coherent acquisition reconstruction, ease of use and time to result.
- 'Optimized position' and 'holder calibration' concepts: reduce the number of software corrections during tilting for higher quality data, faster acquisition and lower total dose on the sample.
- Continuous, automatic and interactive drift/shift tracking and focusing during acquisition.
- Linear and Saxton tilt schemes.
- TEM, STEM, low dose, and EFTEM supported.

Advanced alignment and reconstruction routines

- Three complementary alignment routines: cross-correlation (no markers needed), bead tracking and general feature tracking
- Most advanced reconstruction techniques implemented for sub-pixel accuracy with interactive feedback and close monitoring.
- Iterative and GPU-based (100 times faster than CPU-based) reconstruction SIRT routines: preserve full frequency information, minimize artefacts, and enhance signal to noise ratio.
- Semi-automatic determination of the tilt axis position and orientation.



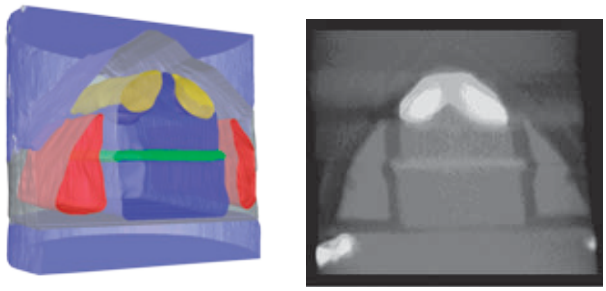
Tubes of gp23 from bacteriophage T4 imaged with low dose cryo tomography. Courtesy of B. Kükrer Kaletas.

Advanced features

- EM techniques supported: TEM and STEM, energy filtering, and low dose.
- Batch tomography: several positions stored for sequential tilt series acquisition.
- Dual-axis tomography supported.
- Job scheduler: multi-user remote reconstruction on an Inspect*3D* Xpress server.

Documentation and training

- Xplore*3D* user interface guides users through the entire 3D imaging process.
- Extensive help file in line with the documentation, which has contributed to the success of Tecnai and Titan TEMs.
- Interactive tutorial animations guiding users through every step.
- Full support guaranteed by FEI and dedicated customer training and workshops provided by FEI Academy.



STEM tomogram of a memory cell obtained from a needle shaped FIB cut. Courtesy of H. Bender, IMEC, Belgium.

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DS0017 01-2008