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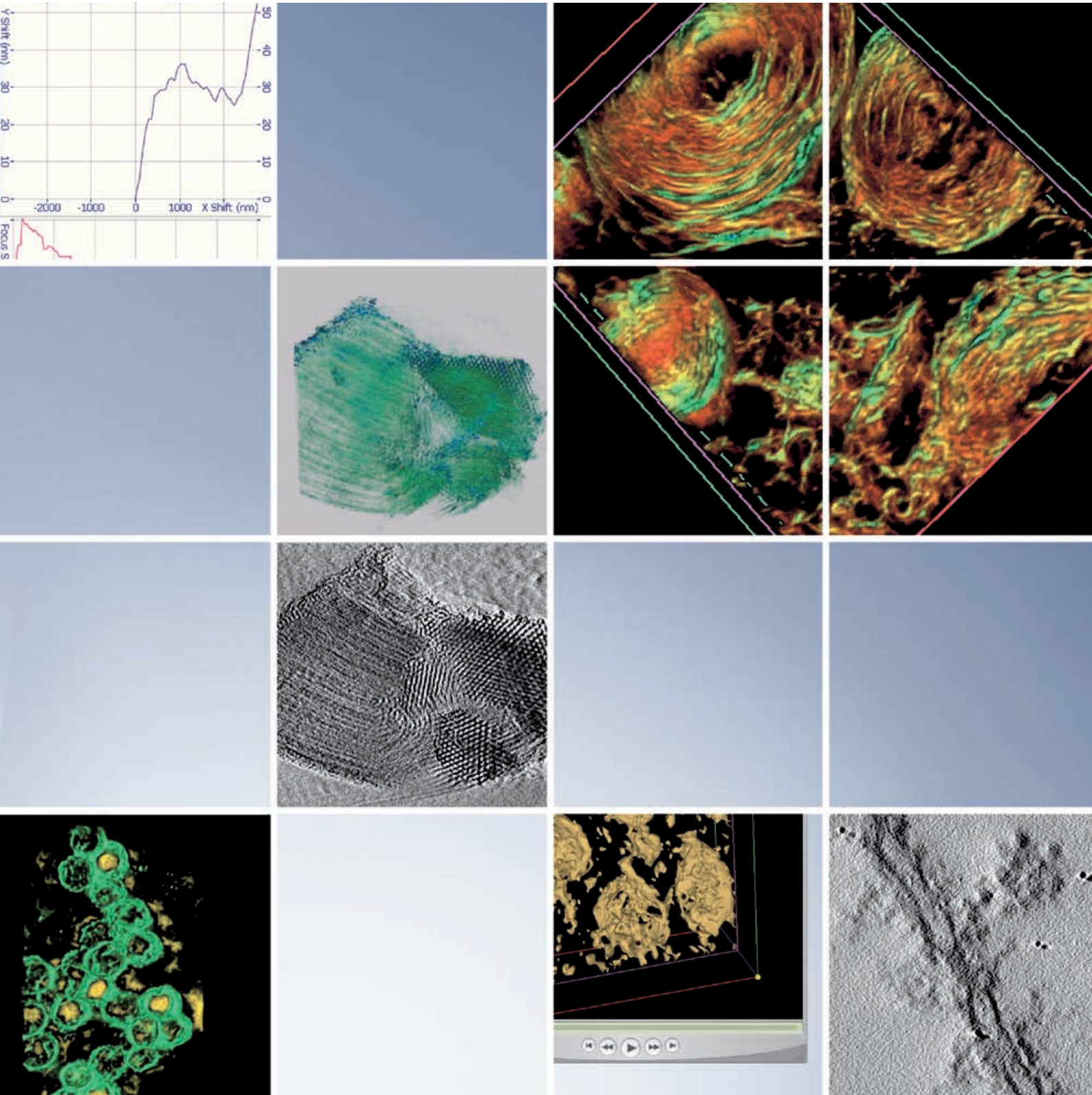
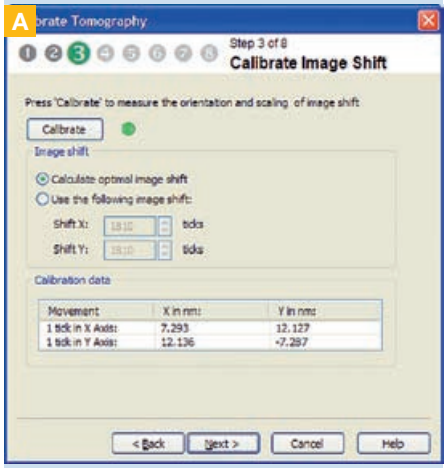
Digital TEM Imaging Solutions

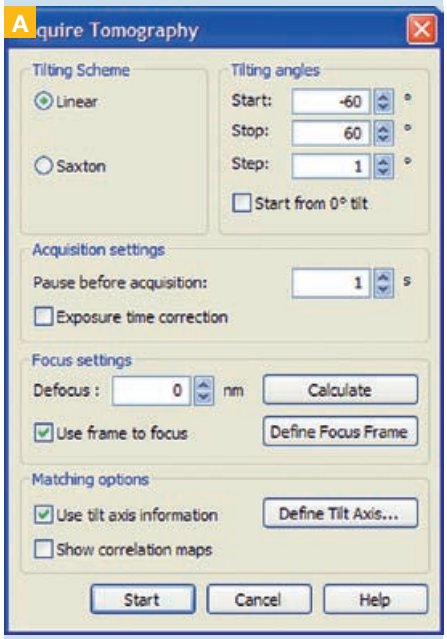
iTEM Solution Tomography

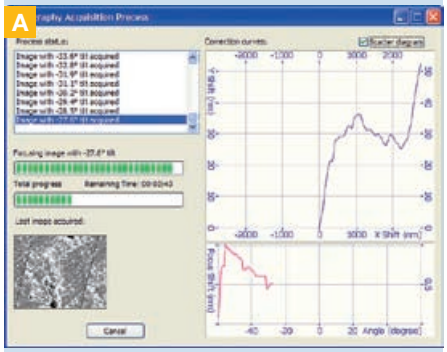
iTEM

# Acquisition, Reconstruction and Vizualisation of Electron Tomography Tilt Series






# TILT SERIES ACQUISITION, RECONSTRUCTION AND 3-D VISUALIZATION

**Tomography using Transmission Electron Microscopes, or Electron Tomography, is developing into a more widely known and versatile method for obtaining TEM sample information from the third dimension. This 3-D information is of critical importance for understanding biological systems such as proteins, protein-nucleic acid complexes and macromolecular machines. The iTEM Solution Tomography enables the user to obtain the desired results with the highest accuracy and reliability – with minimal user intervention. The user-friendly workflow including the automatic acquisition, alignment, reconstruction and visualization makes it very easy to achieve the best results from a tomographic sample – even for inexperienced users. As with all other extensions, the iTEM Solution Tomography is fully integrated with the iTEM platform, the Olympus Soft Imaging Solutions platform for image analysis in the transmission electron microscopy field.**

## Basics of Electron Tomography

The theoretical resolution of a transmission electron microscope @ 100 kV is around 0.002 nm for 3-D data. However, the practical resolving power of most modern electron microscope is, at best, 0.1 nm (1Å), and the projected picture of thick specimens is superimposed increasing blur and lowering resolution. The resolution of structures is improved in stereo-pair images. But in an approach that is even more modern, a complete 3-dimensional view of the actual structures of microscopic matter is now available via electron tomography. It is a reconstruction imaging technique, that is optimal for investigating all three dimensions of biological specimens such as cell organelles and tissue sections. It can also be used for materials science and semiconductor devices.

3-D reconstructions are obtained by processing a series of TEM images of a sample tilted through a certain range (usually from -70° to +70° at a resolution of 1°, for example). The images of the tilt series acquired are combined to compute a 3-D projection of the specimen density.

Due to specimen and/or goniometer movement, lateral shifts as well as horizontal shifts (obvious in focus changes) are likely in need of correction. If these movements are reproducible in their absolute movements (and not only with the relative resulting vectors), correction values obtained from pre-calibration series can be used. In practice, however, these corrections are only valid for a short period of time. This is why it is advisable to conduct calibrations at certain intervals, depending on stage accuracy. A calibration method that is easy and reproducible should be used.

Some acquisition scenarios require keeping the electron dose to a minimum – eg, low dose and cryo-applications. This means there needs to be an intelligent way of ensuring that these specimens are focused and tracked.

The advent of computer-controlled TEMs, large-scale digital TEM cameras, special imaging software and hardware solutions make automated procedures for acquiring tilt series (with hundreds of images) recorded under many different conditions possible.

## The iTEM Solution Tomography Workflow

The iTEM Solution Tomography has the easiest workflow for achieving perfectly reproducible and reliable results from a tomography-suitable sample. Automated tilt series can be acquired on almost every modern TEM system – the list of supported TEMs is expanding constantly. The iTEM Solution Tomography comprises three main parts: acquisition, alignment and visualization.

### Acquisition of Tilt Series

**A** The iTEM Solution Tomography allows the user to acquire a tilt series while automatically correcting focus changes, illumination conditions, specimen drift and mechanical instabilities of the goniometer by using live image tracking. The goniometer of any particular TEM does not necessarily use real world coordinates. The image shift movements used for the autofocus, for example, might result in beam shift movements. This makes calibration and subsequent correction required. Normally, this is a time consuming and complicated task. The Solution Tomography, however, gives you a hands-on wizard guiding you through the calibration process in just a few minutes – reliable, reproducible and easy.

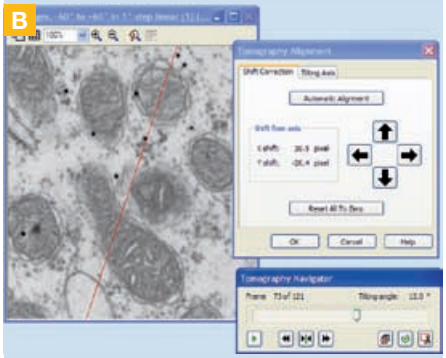
The acquisition that follows involves acquiring images while tilting the goniometer from -X° to +X° at a certain step width. The main goal is to keep the specimen centered, visible and focused. This is easy to accomplish by repeatedly tilting, tracking, focusing and acquiring. The automatic focus adjustment is fast and reliable. Automatic iTEM exposure control ensures acquisition conditions are optimal. This makes for fast image acquisition and low sample damage. iTEM Solution Tomography acquisition supports the standard linear tilting mode (equal distance tilting steps). It also offers the Saxton scheme – a sinusoidal approach with finer steps while acquiring the images at the higher tilt angles giving increased resolution in the reconstructed tomogram. The acquired tilt series can be stored as a standard multiple page TIFF file with all necessary image information and meta data (tilt axis, angle, shift, goniometer data, etc). This data is then used for alignment and construction in the iTEM Solution Tomography. In addition, stacks can be stored as an AVI file and played on any third party software.

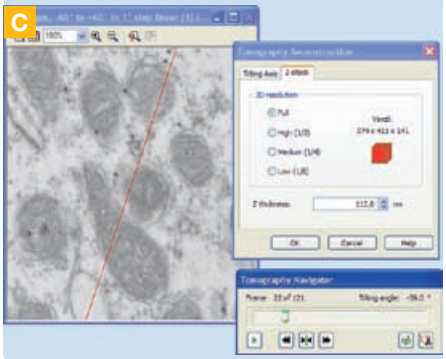
### Tilt Series Alignment

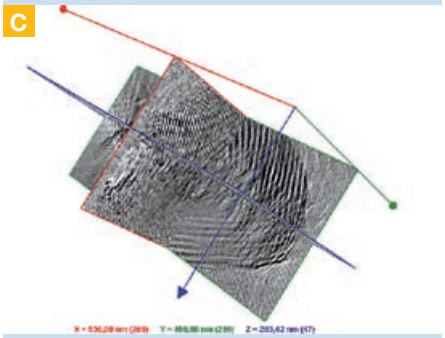
**B** Tilt axis alignment is necessary after acquisition due to thermal effect, stage inaccuracies (backlash), specimen movement and so forth. There is observable drift between the individual stack images. A comprehensive alignment routine aligns the images reliably along the tilt axis without requiring fiducial markers. Any necessary filters and morphing algorithms are applied automatically. Additionally, any tilt series acquired using other tomography acquisition software, or manually with non-automated TEMs, can be imported and processed further. Supported file formats for stacks are TIFF, MTIFF, MRC, AVI and others.

### Reconstruction and Visualization

**C** A near perfect alignment is the primary prerequisite for perfect reconstruction. To reduce the waiting time, it is easy to choose one of the various reconstruction resolutions. The software selects the required settings automatically. The final visualization step is done using SliceViewer and VoxelViewer, the fully integrated 3-D visualization tools. These tools offer display and navigation throughout all kinds of image stacks. Viewed with the SliceViewer, the reconstruction result (eg, FFT-based weighted back projection – WBP) enables the user to immediately assess the quality of the reconstructed tomogram. The VoxelViewer offers various methods for 3-D object display: various projections (minimum, maximum, sum), isosurface (with triangulation) and a blending surface lets you easily generate useful images. Moreover, spatial positions and distances can be measured within the 3-D object. The images generated using the VoxelViewer can be copied to the clipboard, exported as an animated AVI movie file or saved as multi-page TIF files.

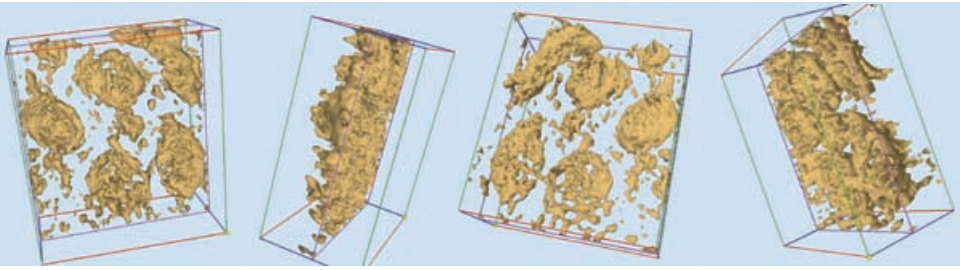






**C** **3-D visualization**

The 3-D reconstruction can be exported as an animated AVI movie file or saved as multi-page TIF files



## Specifications

**Acquisition**

Calibration Wizard (6 steps) for easy adaption of goniometer/holder vs. software

Auto-correction of focus changes, illumination conditions and specimen movement

Supported tilting schemes: Linear and Saxton

Preadjustment of tilt axis for higher precision

Cryo- and Low-dose acquisition – under development

**Alignment**

Automatic offline alignment

Automatic location of tilt axis (no fiducial markers needed)

**Reconstruction**

Automatic application of image filters

Supported reconstruction methods:
- Weighted back-projection (WBP)
- Iterative reconstruction (SIRT, ART,SART) – under development

**Visualization**

SliceViewer: display of orthogonal section planes

VoxelViewer:
- Intensity projections
- Isosurface projection (with triangulation)
- Blending projection with userdefinable LUTs

Variable clipping plane for easy insight views

Measurements within 3-D model (positions, distances, polygons)

**Supported image file formats**

Import: TIFF (also multi page), MRC, etc.

Export: TIFF, AVI (eg, from 3-D model), Stereo RGB, etc.

**Notes**

There are two versions available of iTEM Solution Tomography (only upon request):
- Acquisition add-in: acquisition only
- Reconstruction add-in: alignment, reconstruction and visualization

The TEM must be equipped with a computer controlled (motorized) sample stage (goniometer) as well as an illumination and imaging system. Modifications necessary for establishing remote communications to the TEM are not included in the regular purchase price.

# ADDITIONAL iTEM SOLUTIONS

**iTEM can be further expanded according to your individual needs via a wide range of specially developed solutions. Users can thus put together a personalized software solution for their particular application. All solutions work together seamlessly. The list of the available solutions is growing continually.**

**iTEM Solution ASAC –** The iTEM Solution ASAC automatically determines the strain tensor in semiconductor devices using image analysis on CBED image series.

**iTEM Solution Detection –** The iTEM Solution detection offers simple, fast and flexible particle detection and classification.

**iTEM Solution EMarker –** The iTEM Solution EMarker provides you the decisive assistance for counting and analyzing your colloidal gold markers automatically.

**iTEM Solution EFTEM –** The iTEM Solution EFTEM is comprehensive software for acquisition, analysis, management and display of energy loss image series.

**iTEM Solution Diffraction –** The iTEM Solution Diffraction offers diffraction pattern analysis including calibration, indexing and measuring of single or polycrystalline diffraction images.

**iTEM Solution telePresence –** The iTEM Solution telePresence enables the user to conveniently operate transmission electron microscopes, TEM cameras and motorized stages online unrestricted by time or place.

**TEM camera solutions for iTEM –** Various bottom and side-mounted scientific-grade CCD TEM cameras are fully integrated in iTEM.



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www.soft-imaging.net

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OLYMPUS

**OLYMPUS SOFT IMAGING SOLUTIONS GMBH**
Johann-Krane-Weg 39
48149 Münster
Germany
Phone: +49 (251) 7 98 00-0
Fax: +49 (251) 7 98 00-6060
info.osis@olympus-sis.com

**OLYMPUS SOFT IMAGING SOLUTIONS CORP.**
12596 West Bayaud Ave., Suite 300
Lakewood, CO 80228
USA
Phone: +1 (303) 234-9270
Fax: +1 (303) 234-9271
info.osis@olympus-sis.com

**OLYMPUS SOFT IMAGING SOLUTIONS PTE.LTD.**
No. 2 Jurong East Street 21 #05-30,
IMM Building, Singapore 609601
Singapore
Phone: +65 (6777) 7898
Fax: +65 (6777) 7218
info.osis@olympus-sis.com