

# Gatan *KnowHow*

GATAN'S HOW-TO NEWSLETTER FOR ELECTRON MICROSCOPY

VOLUME 5, ISSUE 1 – JANUARY 1998

## TEM AutoTune™

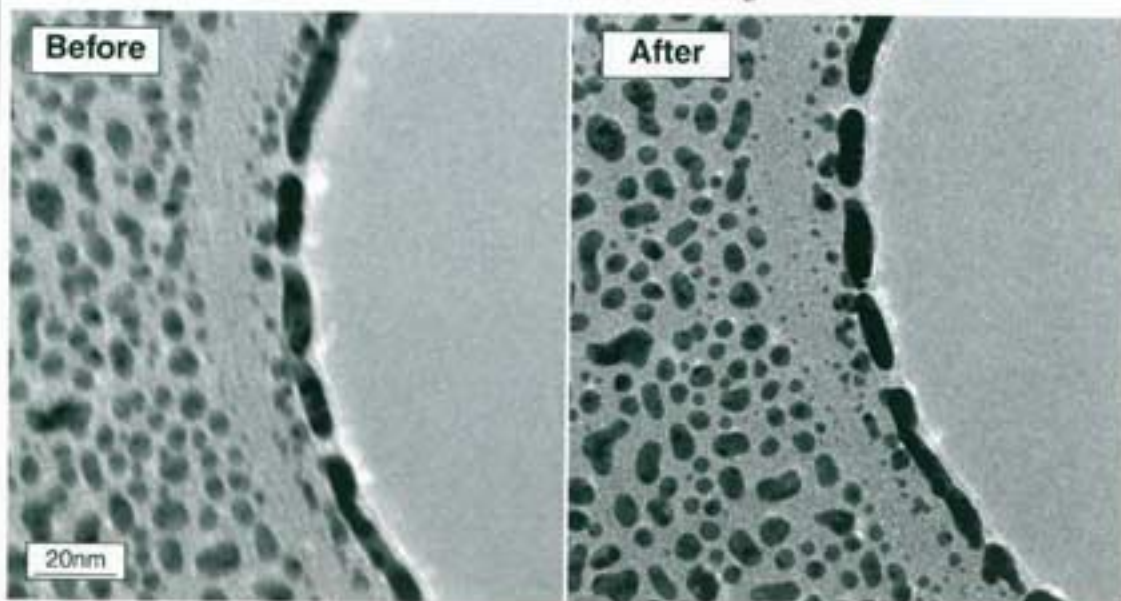
With the ability of accessing image data online, CCD-based digital-imaging techniques make TEM autotuning possible and practical. TEM AutoTune operates by tilting the electron beam and measuring the corresponding image-shift vector. By analyzing several image-shift vectors, values of defocus and astigmatism can be determined. Similarly, misalignment to the image-rotation center can be determined from the correspondence between image shift and change in defocus.

The autotuning algorithm is based on the well-known "image wobbler" technique routinely used in TEM setup. For instance, when the image wobbler is turned on, a double image is observed if the image is out of focus. This occurs when the objective lens is defocused. The defocus causes the "object plane" to deviate from the "specimen plane" by a defocus value. When the incident beam is tilted back and forth about its initial position (wobbler on), a specimen feature (in the specimen plane) will be projected onto two different positions on the object plane. As a result, a double image is observed. When the objective lens is focused, the specimen plane and the object plane coincide and a single image is observed.

Because astigmatism is defined by two parameters, magnitude and rotation angle, astigmatism can be measured from image-shift vectors when the beam is tilted along both the x and y axes. Misalignment to the image-rotation center can be determined by measuring image shift as focus value is changed. When properly aligned, changing image focus will not result in image shift.

Since all the tuning parameters are essentially derived from image-shift measurements, the most critical step in the entire autotuning procedure is the ability to measure image shift with sub-pixel accuracy. Image shift is thus measured by cross-correlation technique and to meet the accuracy requirement, a sophisticated procedure to measure peak positions in the cross-correlation function was developed.

TEM AutoTune has performed well in practice; it can tolerate large amounts of image noise; is effective for a wide range of magnifications, and has minimal requirements on initial microscope conditions. Furthermore, it is shown to be successful in focusing energy-filtered TEM images. TEM AutoTune will definitely be an asset to TEM operators in both the materials and the biological sciences. ■



Images of Au particles before and after astigmatism correction.

## DualView for the PC

When composing a wish list for the ideal digital camera, at the top of everyone's list is fast TV framerate output and great image quality. The unique DualView™ CCD camera combines the sensitivity and performance of a cooled scientific camera with the conventional plug-and-play video output (either RS-170 or CCIR) of a TV camera. You can display virtually real-time frame-rates -15 frames per second on a video monitor for feature location and focusing, then interface with a Windows environment to capture a high quality 1030 x 1300 pixel image for archiving, printing, and network transfer.

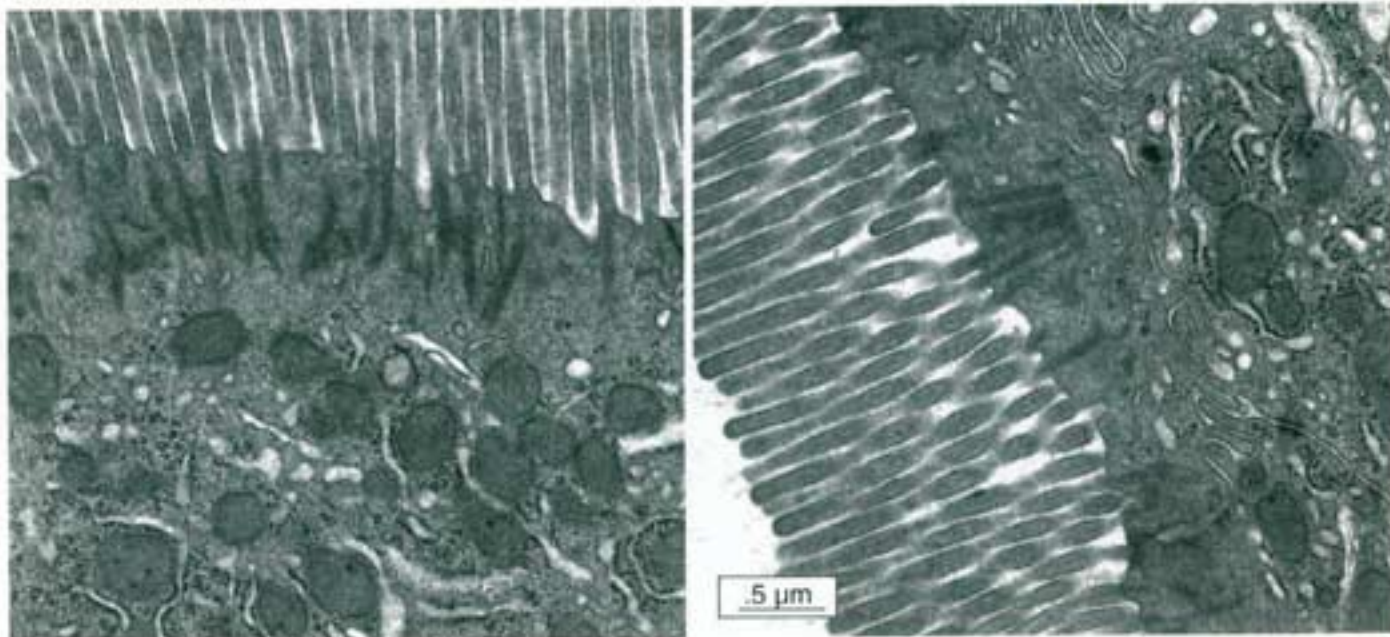
This low-cost camera was designed with the biomedical and teaching laboratory in mind—a fast and sensitive solution to digital imaging. In the 35-mm port, the lens-coupled camera and high-resolution scintillator sample a field-of-view larger than that of photographic sheet film. The camera rotates 360° to orient features in the image window for best presentation. The electronic shutter of the camera and pneumatic action of the imaging prism translate into quiet and precise camera operation. Pneumatic operation ensures sub-pixel positioning accuracy of the retractable phosphor scintillator. The compact design of the camera and prism interface does not alter the standard operation of the microscope; simply flip a switch to return to standard imaging mode. High-resolution image display is optimized on a single large monitor—an entire group can view the same sample in a brightly lit micro-

scope room or network the system to a classroom to facilitate teaching and discussion.

The air-cooled camera is simple to install and occupies the wide-angle ports of the TEM to capture a large field-of-view. A complete system includes the camera, a pneumatically activated phosphor prism and optical coupler, the MicroMax CCD camera from Princeton Instruments, an interface board to the PC, and DigitalMicrograph software, the TEM industry leader for image acquisition and automated microscope solutions. Images acquired with the DualView are immediately available with live background correction in the Windows environment, where they can be examined, processed, analyzed, annotated, printed, and archived using the latest desktop image processing and file transfer techniques. Live imaging capabilities save time for the critical diagnosis, reduce costs because film, darkrooms, and processing chemicals are not needed, and truly bring the microscopy lab into the classroom.

The DualView represents Gatan's first product exclusively on DM version 3.2, which offers more features that continue to make DigitalMicrograph the preferred software choice for the microscopist. Multiple display types, including spreadsheet, raster, and surface plot, can be dragged and dropped between DigitalMicrograph windows or from other applications to create professional page layout copies. The zoom feature is extended to support continuous image-size alteration; stretch or shrink an image to fit the printing area available and the scale bar changes as well. Please check the Gatan website at [www.gatan.com](http://www.gatan.com) for more details about the DualView and the full range of imaging products we provide for electron microscopy. ■

Pancreas brush border captured on a Model 780 DualView at 120 kV.



## Gatan LowDose for Cryo-Reconstruction

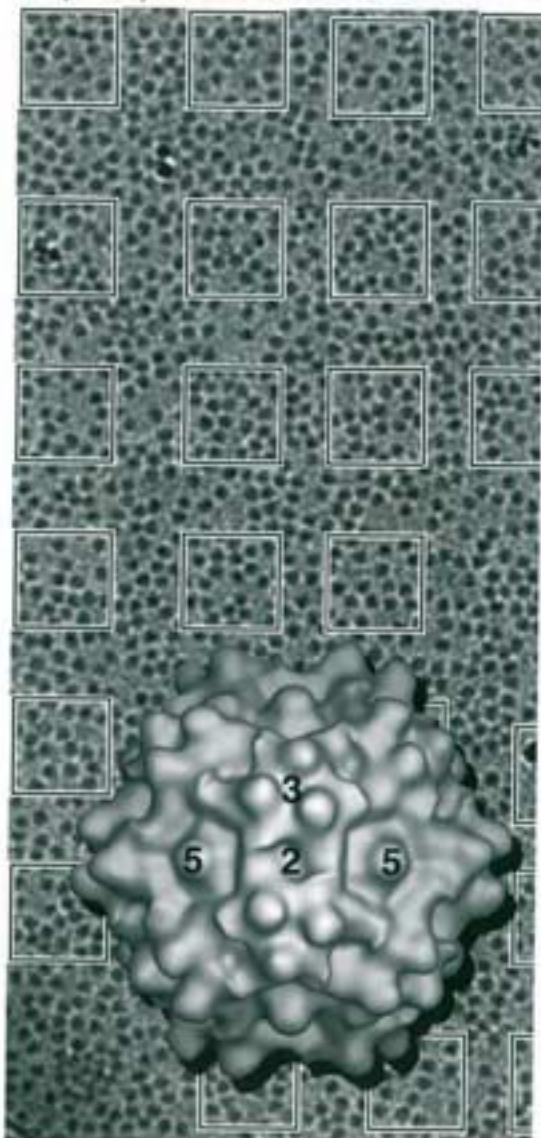
**G**atan's automated low-dose software was described in the January, 1997, issue of *KnowHow*, but how does it work in practice? Norm Olson in the laboratory of Tim Baker at Purdue University has been collecting data to construct a three-dimensional model of Aleutian mink disease parvovirus (ADV). This 28.5-nm diameter virus causes severe financial losses to the mink industry. ADV is a member of the Parvoviridae family, which also contains human, canine, and feline infective agents. In addition, it is interesting because the virus and virus-antibody complexes are both infectious, thus the virus cannot be neutralized with antibodies.

A big advantage the CCD camera brings for examining frozen-hydrated virus suspensions is the ability to prescreen the sample live without recording images on photographic film. Time is of the essence because the virus samples are often very labile and they must be frozen and suspended over holes in a perforated carbon film in a timely manner. Electron doses in the range of 14 electrons per  $\text{\AA}^2$  are used for image capture on the Model 794 MSC camera; higher doses destroy the sample. At these microscope settings, the low intensity and contrast make it difficult to determine the quality of the sample on the microscope viewing screen or with a TV-rate camera. The 794 MSC camera produces high-contrast images that allow the user to quickly determine if the sample is suitable for data collection. Images can be taken closer to focus since enhancing the contrast by defocusing the objective lens is not as necessary due to the high contrast of the CCD images.

The data collection procedure where the CCD camera is only RAM-limited in terms of the number of images it can take of a sample field mimics low-dose protocols for photographic image capture. Typically grids are examined at low magnification (3,800x) for suitable areas and a survey image of an area is recorded. The software brings up box annotations that are dragged and dropped with the mouse over good particle dispersions in the low magnification field. The selected areas represent the area to be sampled at the final magnification, in this case 50,000x. These areas are then automatically recorded by the camera and saved to the hard drive. One area over the supporting carbon film is imaged in order to calculate and set the defocus value of the specimen. The quality of the ice and par-

ticle dispersion can immediately be judged by examining the digital images, the guesswork has been eliminated as has time in the dark-room.

Eighty-seven images of ADV particles from 11 CCD images were needed to calculate an ADV reconstruction to approximately 21 $\text{\AA}$  resolution. The images were transferred from the PowerMac to VMS DEC Alpha machines where view orientations were determined to generate the reconstruction. In comparing the TEM results of ADV to the x-ray structure of canine parvovirus (CPV), a closely related member of the family, it was determined that the major antigenic sites of ADV are most likely located on the three-fold axes of the protein shell as they are in CPV, but these regions have more prominent projections in ADV. Protein sequencing studies have shown that ADV has 30 more amino acids in loops on the three-fold axis than CPV. Both the x-ray and TEM results are being used to model the primary structure of ADV. ■



Background: Partial view of Aleutian mink disease parvovirus (ADV) showing spot scan markers used in the reconstruction. Foreground: Reconstruction of ADV virus. The location of the icosahedral 5, 3, and 2-fold axes are indicated.

## Gatan Brings Asia Into Focus

Like many companies in high-tech industries, Gatan, Inc. has experienced a period of strong growth during the last two years. Japan and the Far East have proven to be two key areas fueling that growth. In response, Gatan has expanded its efforts in Japan and the Asia-Pacific Region by opening offices in Tokyo and Hong Kong.

### Tokyo

In November, 1997, Nippon Gatan was formed, a division of Nippon Roper Industries, replacing the former Gatan Service Corporation with a structure more suitable to



our current business activities in Japan. The General Manager, Yasuji Miyamori, is a veteran of the SEM and TEM business world having worked for Seki Technotron and Noran Instruments Japan prior to joining Gatan. Mr. Miyamori is backed

by a team of three service/applications engineers who provide installation, training, and post-installation support for a broad range of customers. Two of these engineers are long time Gatan employees and are among the most senior engineers in our company.

Dr. Colin Trevor trained as a microscopist at the University of Bristol in the UK, where he developed his expertise in energy-filtered microscopy techniques. He has undergraduate and graduate training in Physics and this enables him to fully understand the problems customers wish to solve. His capabilities insure that each user gets the most out of his Gatan equipment. Colin has been in Japan since February 1997 and is learning his way around the country. Since his ability to communicate in "American" is limited, there is little hope that he will progress very far in speaking Japanese. He is trying hard, but his Japanese sentences are usually met with polite grins from the locals.

Mr. Daniel Ray, a 12-year veteran with Gatan, uses his very broad and deep knowledge of Gatan's products to insure proper matching of instruments to applications and microscopes. Daniel has spent more than two years in Japan

organizing our service efforts there and has had a major positive impact on customer satisfaction with Gatan and its products. A recognized workaholic, Daniel spends what free time he has exploring the video-games frontiers and is the envy of all the "techies" in California because he has access to a number of games that have not yet surfaced in the US.

Mr. K. Joh, a newcomer to Gatan, has many years as a Field Service Engineer in the EDS applications arena and is rapidly learning the details of Gatan products to better support our Japanese customers. We look forward to seeing him expand his capabilities and to having a local engineer who is able to train customers in their native language.

Nippon Gatan will expand the customer support and promotional activities that have been a primary focus for Gatan in Japan in the past years. Through an intensive workshop and seminar program we generate interest in current products, while continuing to educate our installed customer base on the best utilization of Gatan instruments.

### Hong Kong

In August of 1997, Dr. Michael Felsmann, based in our new representative office in Hong Kong, joined Gatan as Area Manager for the Asia-Pacific Region. Dr. Felsmann came to Gatan after seven years with Philips Electron Optics as an applications specialist and two years in Hong Kong as TEM specialist in the Asia-Pacific Competency Center there. Michael was trained in Physics at the University of Münster in Germany, has a broad scientific background and an excellent foundation in transmission electron microscopy. Michael will focus very intently on supporting our distribution network in the Asia-Pacific Region and will pay particular attention to getting to know our customer base in that region. During the month of October, Michael traveled throughout the Region meeting distributors and OEM sales partners and renewing acquaintances with customers in his new role at Gatan.



Already very busy in his new role, Michael is organizing the first ever meeting of Gatan Distributors in the Asia-Pacific Region to be held in Singapore in February, 1998. With all this activity, it is difficult for him to find time to continue the sailing lessons he started for his daughter last summer. By the time he gets back to his boat, she will be a better sailor than he.

### **A New and Local Focus**

For the first time in Gatan's history, our Asia-Pacific customers will have technical and other support from highly-skilled Gatan employees located in a local time zone and not half-way round the world. We look forward to a major impact of this new model on customer satisfaction in the Asia-Pacific Region.

As a company we are very proud to be able to offer such a highly qualified group of colleagues in support of our valued customers in Japan and the Asia-Pacific Region. Please feel free to contact them via phone or e-mail with any questions you may have concerning Gatan products or their applications. ■

### **How to contact our Asian representatives**

Nippon Gatan Japan	Mr. Yasuji Miyamori Dr. Colin Trevor Mr. Daniel Ray Mr. K. Joh	miyamori@gatan.com ctrevor@gatan.com dray@gatan.com	General manager Field Applications Specialist Field Applications Specialist Field Applications Specialist
Eastern Pacific Hong Kong	Dr. Michael Felsmann	mfels@gatan.com	Sales manager

The new building serves as Gatan's primary R&D facility and corporate headquarters.

In addition, we have given special consideration to our customers by including a new larger training and demonstration center:

We hope that you have the opportunity to visit our new facility in the near future.



## New Partnerships

# Gatan and EMiSPEC Join Forces for DigiPEELS Users

ES Vision software brings enhanced EDX and PEELS Analysis to DigiPEELS

**G**atan, Inc. of Pleasanton, California, and EMiSPEC Systems, Inc. of Tempe, Arizona, have reached an agreement for Gatan to become a worldwide distributor of EMiSPEC products. The ease of use and integrated multi-technique approach EMiSPEC has developed enhance Gatan's ever-expanding imaging and analytical product lines. While the initial focus of future joint development will be to simplify and extend EELS as an analytical tool, Gatan will market, sell, and support all of EMiSPEC products.

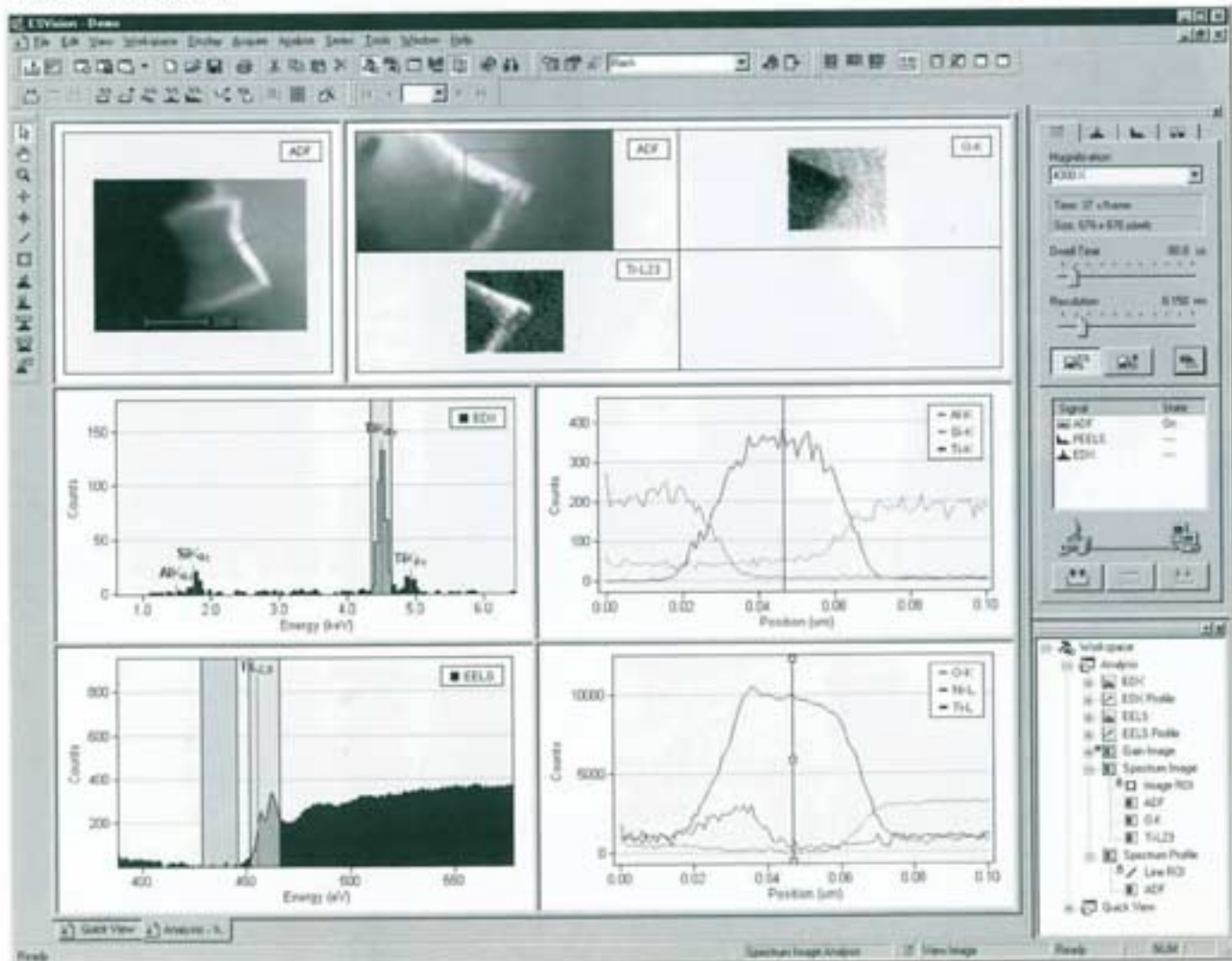
*Stay tuned for more developments!*

This illustration shows a typical example of simultaneous EDX and PEELS spectrum images and profiles from a semiconductor.

ES Vision is a system designed to control electron microscopes and to acquire data from all of the detectors on the microscope. The key word is integration. All signals and all control features are available through a single application accessed through a single mouse, keyboard and computer screen. What used to require up to three separate computers can now be done with a single ES Vision system.

The two most obvious advantages of a single-control computer are improved ease-of-use and new capabilities. Sophisticated experiments are easy to perform because all control is done through a single application with a user interface that is consistent between even very different acquisition modes and detectors. New capabilities are available because the single computer has direct control of both the microscope and its detectors. These capabilities include spectrum imaging and spectrum surveying.

In conventional elemental mapping, spectroscopic information is acquired while the beam is scanned across the sample, but only the processed information from pre-selected elements is saved. The original data is lost, and



with it, possibly important information about other elements, or other essential information about the sample.

In a spectrum image, an entire spectrum, or any selected portion of a spectrum, is acquired and stored from every point on the specimen. From that single set of data, a user can create as many elemental maps as exist in the data and the maps can be created by simple built-in post-processing. In addition, the original spectrum from any position can be displayed at any time simply by pointing to that position.

Many specimens do not require full spectrum images. Due to its tightly integrated imaging and spectroscopy, ES Vision allows the user to accurately position the beam interactively while watching both EDX and PEELS spectra acquire in real time. This spectrum surveying is indispensable in quickly evaluating what is in a specimen, and in finding an area of the specimen suitable for elemental mapping.

ES Vision dramatically enhances the microscope capabilities of TEMs equipped with a Gatan PEELS or DigiPEELS by tightly integrating PEELS with scanned imaging. These microscopes can now combine the broad range of detectable elements offered by EDX with the exceptional low-Z sensitivity and high spatial resolution of EELS. Fields as diverse as electronics, ceramics, and biology will benefit greatly from the ability to combine more con-

ventional EDX mapping with PEELS spectrum imaging.

In ES Vision, data is not only presented to the user in a completely integrated fashion, the data is also stored such that all relevant information for an experiment is saved in a single file. Images, spectrum images, line profiles, and elemental maps can all be recalled by opening a single document.

Interactive control is taken to a new level in ES Vision. From a control panel with sliders for adjusting acquisition parameters, to dragging axis in order to rescale displays, to interactive analytical background subtraction, the user interface has been optimized to provide true real-time control over all aspects of an experiment.

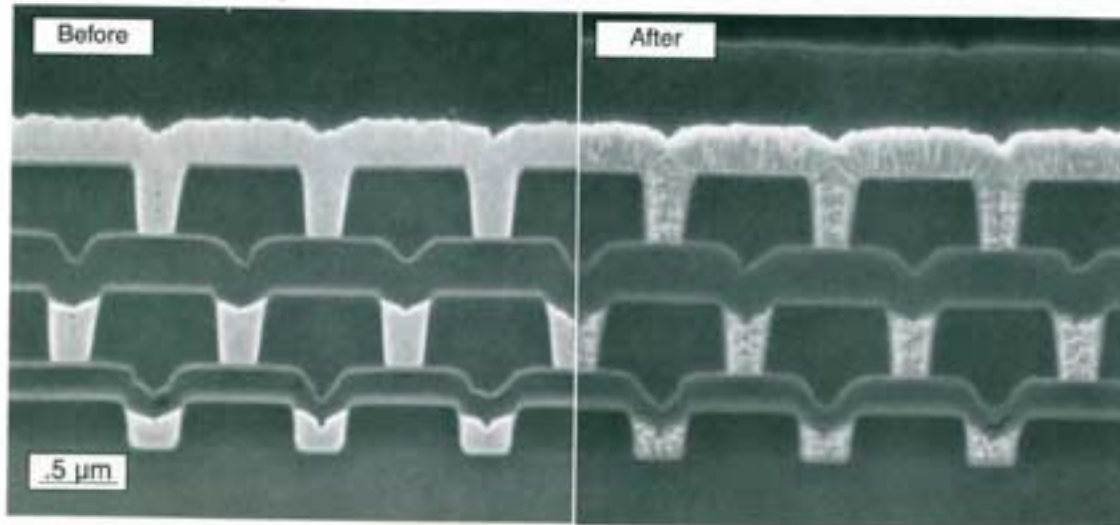
An essential aspect of keeping these sophisticated capabilities accessible to users of all levels is the use of the Windows NT 4.0 computer platform. Many features of ES Vision will look familiar to anyone who has used a modern word processor or drawing program. Users can take advantage of the huge variety of other applications available for the Windows NT platform, from word processing and presentation applications, to scientific and numerical processing packages, to database and archiving software. In addition ES Vision users benefit from Windows NT's unsurpassed support for networks, printers, and storage media. ■

## Correction

### Precision Etching Coating System (PECS)

The September issue of the KnowHow contained an incorrect image pair for the article on Gatan's new Precision Etching Coating System (PECS). The correct image pair is shown below with the correct caption.

Before and after SEM images showing the mechanically polished cross-sectional view of a typical semiconductor device. The sample was etched in the PECS for 5 minutes, with a perpendicular Ar ion beam at 6 kV and coated with 15 Å of Au/Pd. In the etched image, the detailed grain structure of the tungsten plug and tungsten metallization layer are clearly visible.



Gatan Inc.  
5933 Coronado Lane  
Pleasanton, CA 94588  
Tel 510 463 0200  
Fax 510 463 0204

Gatan Online  
<http://www.gatan.com>  
[info@gatan.com](mailto:info@gatan.com)  
[help@gatan.com](mailto:help@gatan.com)

Gatan Inc.  
780 Commonwealth Drive  
Warrendale, PA 15086  
Tel 412 776 5260  
Fax 412 776 3360

Gatan GmbH  
Ingolstädter Straße 40  
D-80807 München Germany  
Tel (089) 352 374  
Fax (089) 359 1642

Gatan KnowHow is published three times a year by Gatan, Inc. BioScan, DualView, DigitalMicrograph, DigitalMontage, DuoPost, GIF, MultiScan, PECS, PIPS, and TEM AutoTune are trademarks of Gatan, Inc. All rights reserved.

Gatan Ltd.  
17 Medicott Close  
Oakley Hay, Corby  
NN18 9NF, UK  
Tel 01536 743150  
Fax 01536 743154

Gatan Inc.  
Hong Kong branch  
No. 55, 5th street,  
Hong Lok Yuen, Tai Po  
New Territories, Hong Kong  
Fax: +852 2658 6002  
Tel: +852 2658 6003  
E-mail: [mfels@gatan.com](mailto:mfels@gatan.com)

Nippon Gatan  
Casa Esperanza Rm#208  
5-8-3 Sekimachi-kita  
Nerima-ku, Tokyo 177  
Japan  
Tel: 03-5387-3585  
Fax: 03-5387-3585

## In the Next Issue

### Spectrum Imaging

**Custom imaging feature:  
Measurement for quality  
control with DigiScan**

**Gatan installs first MSC for  
1.5 MeV TEM**

**New Scripting example**

