Gatan KnowHow

GATAN'S HOW-TO NEWSLETTER FOR ELECTRON MICROSCOPY

VOLUME 3, ISSUE 1 – JULY 96

Digital Imaging

Desktop Imaging for TEM

Is your transmission electron microscope a lean, digital data machine? More and more laboratories are turning away from the drudgery of darkroom work and equipping their microscopes for digital image capture. Digital imaging for SEM has been the standard for years, with developments in both hardware and software that have opened up a world of new possibilities for data acquisition and display for transmission microscopes in the film-free laboratory. How can digital imaging change the way you use a microscope? Here are some key points to consider when thinking of digital imaging for TEM.

Digital cameras simplify your work. The original "slow-scan" cameras were primarily used in a static sense, recording the image as an alternative to film after search and focusing the traditional way on the microscope. Today, MultiScan™ cameras with variable and fast frame rates allow you to do everything on the computer monitor. Low magnification sample scanning is simplified with "live time" fast frame rates, a subarea is used for focusing and slower frame rates immediately produce a high quality image. Imagine being able to survey your sample viewing images of comparable quality to film.

Digital data is fast and flexible.

When you grab a digital image, there is no guesswork about the image you have just taken; it is immediately displayed on the monitor for your evaluation. The degree of order in a crystal can immediately be assessed with live Fast Fourier Transforms. Automatic contrast enhancement brings out the detail in low contrast or unstained samples. All of the tools of the darkroom are elec-

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Secondary lysosome in a human hepacyote imaged with a Gatan 794 MultiScan CCD camera. Note the membrane definition captured with the Model 794.

In this Issue

Variable Angle PIPS

Gatan designed the new Model 691 Variable Angle PIPS™ (Precision Ion Polishing System) unit to produce high-quality TEM specimens with large, clean, electron-transparent areas. Major features of this new system include high thinning rates, variable-angle milling, and double-sided milling—all of which lead to improved specimen quality.

In this new unit, variable angle ion guns replace the fixed angle guns in the previous PIPS. The new gun assembly contains a standard Penning Ion Gun (PIG) mounted into the longitudinal axis of a rotating cylinder. The gun centerline is

The Model 691 Variable Angle PIPS

offset from the cylinder's center of rotation at an angle of 10°. The gun angle can easily be changed by rotating the cylinder externally about its longitudinal axis without breaking the vacuum or having to interrupt the high voltage.

A full range of 0° – ±10° milling angles provides true double-sided milling, whether it is one gun from the top and the other from the bottom: both guns from the top: or both guns from the bottom. Double-sided milling (top and bottom) has the advantage of continuously and simultaneously removing material from both sides of the specimen. The results are cleaner specimens by avoiding the redeposition of sputtered materials

To select the milling angle, you use a scale engraved around the circumference of each gun knob. The ion gun assembly incorporates two spring loaded x, y micrometer drives to center the beams on the specimen. When the guns are properly aligned at 10°, there is no further need to realign the guns when used at any other angle.

You can change the milling angle of each gun independently, continuously, and reproducibly while they are in operation, which means you can thin the specimens under numerous conditions to obtain both quality and high thinning rates.

The new variable angle-ion guns are particularly good for cross-sections due to:

- larger thin areas with better surface finish
- lower differential milling rates
- fewer ion beam related damages
- less specimen contamination
- higher milling rates at angles >4°
- improved beam alignment
- variety of milling conditions

The Model 691 PIPS is a fully self-contained, bench top unit with a clean, oil-free vacuum. Good thermal contact between the ion gun and the specimen chamber allows full function of the gun without water cooling.

The variable angle feature is standard on all new PIPS and any of the older fixed angle PIPS can be upgraded to the variable angle milling version by an easy-to-install upgrade package. ■

XTEM micrograph of a zirconium oxide layer on pure zirconium. Dimple ground to a thickness of ~15 µm and thinned in the PIPS with liquid N_2 cold trap and ion beam modulator, at 5 keV with a milling angle of 4° for the dimpled side and 3° for the flat side.

New DuoPost

The new DuoPost offers the following advantages:

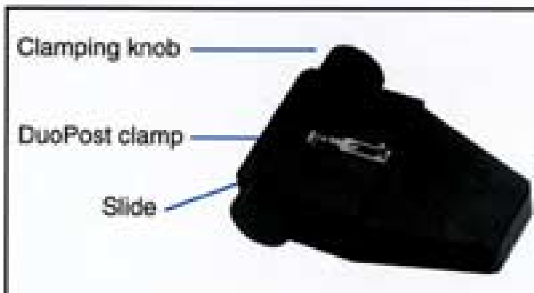
- Double sided milling down to 0°
- Milling rate increase
- One-step specimen mounting by gluing or clamping
- Minimizing/eliminating specimen contamination
- Long life expectancy

Two new posts, glue type and clamping type, permit simultaneous double-sided milling of a specimen at 0°– ±10°. The posts consist of a pedestal with two extending specimen support arms adapted to engage the edge of a specimen. The support arms have a narrow profile, so the majority of the specimen's edge remains unrestrained and spaced from the pedestal. The distance provides for line-of-sight clearance for the ion gun arrangement and permits the ion beams to impinge upon both sides of the specimen.

The specimen is secured to the post by seating it in a recess in the support arms. The depth of the recess is less than the height of the specimen.

In the glue type post, the securing means can be a low melting point wax. The wax is disposed in the support arms' recess and the specimen is secured by melting the wax, seating the specimen, and then cooling the wax. The wax also provides the advantage of a heat transfer medium from the specimen to the support arms, reducing ion-beam-induced heat damage.

In the clamping type post, spring-loaded arms engage an edge of the specimen to secure it. Use this post when securing the specimen with wax is undesirable.



DuoPost Loading Dock

Both of the posts continue the tradition of allowing through-transmission illumination and use of the PIPS AutoTerminator.

When the specimen is affixed to the posts, the ion guns may be positioned in relation to the specimen without striking either the pedestal or the support arms. As a result, the posts may conveniently and efficiently be used in conjunction with the ion-beam modulation system of the PIPS.

When the DuoPost is rotated to a position where there is line-of-sight clearance to the specimen surface, the beam is 'on' and whenever the support arms enter the path of the ion beam, the gun is 'off'. This effect reduces specimen contamination from sputtering of material from either the support arms or the pedestal and provides high quality specimens as well as increasing the post's life expectancy. ■

Loading the Specimen

1. Rotate the clamping knob back to release the clamp.
2. Insert the DuoPost into the DuoPost slot.
3. Rotate the knob forward to lock the DuoPost into the Dock and open the clamping arm.
4. Place the specimen on the slide and move the slide towards the DuoPost.
5. Gently rotate the clamping knob to close the clamping arm.
6. Retract the slide and rotate the knob fully to release the DuoPost.
7. Remove the DuoPost from the Dock and insert into the PIPS.

Improving Cryoholder Performance for Cryomicroscopy

Methods to monitor and improve the performance of specimen holders for TEM cryomicroscopy

One continuing goal at Gatan is the improvement of the design and performance of our products to keep pace with design improvements in electron microscopes. A case in point is the development of the 626 system to quickly and easily transfer cryospecimens into the TEM. Gatan first developed the 626 cryotransfer system in 1980, and subsequently has included the following design concepts: the cryoshield and external shutter control, insulating antivibration mounts for the conductor, the tapered clipring, zeolite pump, molded integral heater and silicon diode temperature sensor, and springloaded antidrift rod. A tool kit and workstation were also designed for fast sample loading while keeping good thermal stability of specimens at low temperature.

Cryomicroscopy at high resolution still presents many challenges. Although most cryoholders can easily achieve 3.4Å resolution, information perpendicular to the holder axis may be degraded due to vibration. Absolute drift after mechanical and thermal equilibrium as well as drift after movement of a holder may cause loss of resolution in any direction. Methodology to examine the response of side entry cryoholders to external vibration has been described by Henderson and Faruqi (Ultramicroscopy 60:375-383, 1995). Their study of six cryoholders allowed identification of vibration modes due to the combination of the microscope and goniometer, the holders and goniometer, and modes specific to the holders. Whereas the photomultiplier used by Henderson and Faruqi can be adapted to a microscope to study vibration response, a CCD camera and appropriate software which we have developed at Gatan can provide information on the vibration and drift performance of a specimen holder.

The drift performance of a Gatan Model 626-DH cryoholder at ambient and low temperatures can be measured by means of a routine scripted into DigitalMicrograph™ software. Standard graphite-holey carbon and oriented gold calibration grids are good test specimens and are readily available from EM supply companies. The digital images upon which the cross-correlation analysis are based are recorded on a Gatan Model 794 MultiScan camera bottom-mounted to the microscope column. The drift measurement script run in DigitalMicrograph™ sequentially numbers the sampling points and displays them on the original image for easy reference to the orientation of the sample in the microscope.

A representative result from running the drift measurement script is shown in Figure 1. Sequential measurements of relative and total drift are displayed in a results window of the software, allowing the user to determine whether drift rates are increasing, decreasing, or constant. The display of sampling points on the original image tells the user if the direction of drift is along or at an angle to the axis of the goniometer, and whether the holder has achieved thermal equilibrium. The user can choose a number of options, including the number of measurements taken by the CCD camera, exposure time of the camera, the time delay between measurements, image binning and image size. A copy of this script is available to our customers by contacting Gatan.

The possibility to judge specimen quality and cryoholder stability online gives feedback that allows the user to optimize specimen holder performance as an experiment is in progress. High resolution images such as Figure 2 of the 2Å periodicity in the oriented gold lattice are achievable with today's microscopes, cryoholders and digital cameras. Several practical suggestions to achieve best cryoholder performance include 1) remove any particulates and excess vacuum grease from the o-ring, 2) remove any ice nucleation points on the inner surface of the cryoholder dewar with a cotton applicator, 3) disconnect the temperature control box cable from the holder dewar when imaging the specimen, or clamp the cable to the microscope column to minimize transmission of room noise and vibration to the holder, 4) minimize air drafts around the cryoholder, and 5) eject any ice crystals from the liquid nitrogen in the dewar with lengths of Teflon tubing.

Figure 1 showing a drift performance of less than 20Å/min is demonstrated by the overlapping markers on the image of graphitized carbon at a cryoholder temperature of -185°C. Drift mea-

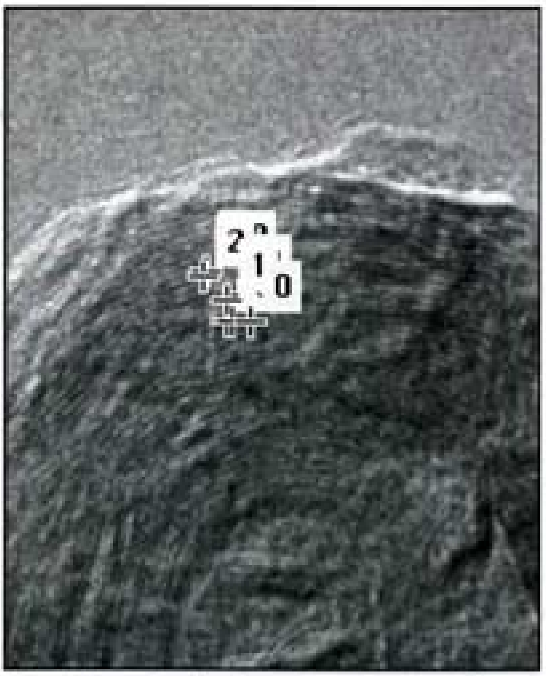


Figure 1 Overlapping markers showing drift


Figure 2 After optimization, the performance of a a cryoholder reflects the resolution of the TEM

surement is based on the cross correlation of successive digital images taken with a CCD camera. The position of a central feature is automatically tracked and indicated by a numbered cross on the initial image. The number 0 and associated cross at the lower left of the annotation indicate the first measurement point of the series. Measurements 1-9 are represented by the cluster of crosses and annotations underneath the initial three readings.

When all conditions are optimized, the performance of a cryoholder can reflect the resolution of the TEM with which it is paired. The 2Å spacing of an oriented gold crystal calibration standard was imaged in a model 626 cryoholder at -186°C. The result is shown in Figure 2. ■

Digital Imaging

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tronically available for image enhancement, dodging, and image montage as well as filters to sharpen or uncover "hidden" information. The archiving system can keep pace with high image generation rates in a multi-user facility and simplify record keeping. Annotate images and store all specimen information with the industry standard data formats.

Digital images are electronically portable.

Online microscopy gets results fast to the production line or to the pathology lab, supplying decision-making data ahead of schedule. Wherever you are, you can rapidly share your images with colleagues across the room or around the world in a cost-effective, efficient manner. Innovations such as telepresence microscopy and on-line teaching bring the resources of the microscopy lab to a broader field of potential users. Many scientists save time and enhance the visual impact of their data by presenting it at conferences and meetings directly from the desktop of a small personal computer.

Let software do the work.

The ability of a computer to quantitatively analyze data on-line makes automated microscopy a practical reality. Stigmation and alignment can be done faster, more accurately and with less specimen damage by our AutoTuning Software than by the most skilled human operator. In addition, DIFPACK, the Diffraction Analysis Package automates the analysis of diffractograms and Holoworks automates the acquisition and analysis of holograms. The powerful scripting language in our DigitalMicrograph software lets you tailor programs to your needs.

What's the bottom line? Contact Gatan for innovative imaging solutions. ■

Gatan Launches New Online Services

Gatan is committed to customer service by expanding its use of online information to respond to its customers needs. Recently, the Gatan web site has been expanded to include product information on most of our current products. Product literature can be requested directly from the web via a convenient literature request form. An extensive image library from Gatan imaging products including, camera images, GIF application examples, as well as SEM images acquired with DigiScan and DigitalMicrograph is posted online in low resolution format for quick loading via modem. This library is being constantly added to to give you the latest Gatan imaging examples. A special section is devoted to David Scharf, photographer and microscopist specializing in color SEM images in both biological and materials sciences. Past and current version of KnowHow will be posted as they become available.

We have also expanded our email addresses to better direct your questions: General and technical information about our products can be requested at info@gatan.com. For sales or service questions, email help@gatan.com and a sales or service representative will contact you directly regarding your question. To ask a software question email software@gatan.com. For bug reporting, use the bug report form on the software home page.

Gatan Email and Web addresses

Gatan, Inc.	www.gatan.com
General email	info@gatan.com
Sales/Service	help@gatan.com
Software	software@gatan.com
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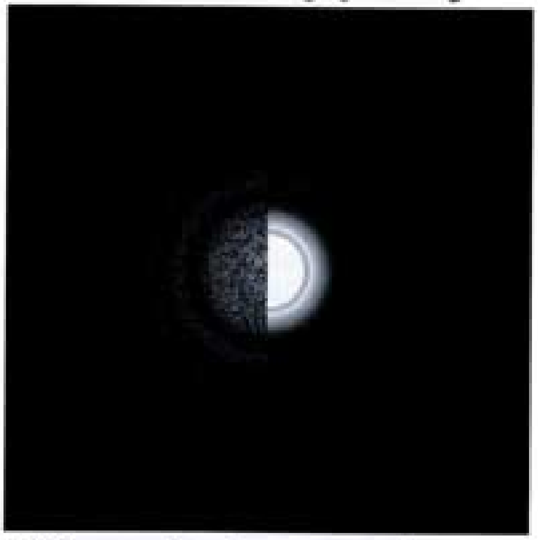
Automating a Task with DM Scripting

DigitalMicrograph comes with a built-in scripting language (DMS) that simplifies many aspects of customized image processing. With



DMS, you can implement custom image processing algorithms and procedures specific to your application. A script can be written within DM and saved in the menu for easy access to your custom automated feature.

This script performs rotational average of a diffractogram. A front image (packed complex as a result of FFT) is read in, and data type is changed to complex and real by means of modulus extraction. Then the image dimension (height and width) is found from the diffractogram. This information is used to declare several intermediate images such as "dst", "line_projection", and "rotational_average".

The front image is displayed in cardinal coordinates, and needs to be transformed into polar coordinates. Variable "sample" defines the number of segments of the 360 degree angular range. The transformation of coordinates ("temp" in cardinal/"dst" in polar) is realized by bilinear interpolation (warp function). Then line projection is easily calculated by adding up all the columns in "dst" (polar coordinates). The averaged line intensity is also normalized by the number of segment ("sample"). Finally this (1D) line profile is used to generate a (2D) image —"rotational_average" by using again the bilinear interpolation technique (the warp function). The last line is to display the rotational averaged image.

To use this script, enter it into DigitalMicrograph, save it to a disk file, and install it in the menu using the "Install Plug-In" under the "File" menu in DigitalMicrograph.

```
number samples = 256, xscale, yscale, xsize, ysize
number centerx, centery, k, halfMinor

image temp, rotational_average, dst, line_projection
compleximage frontImage

frontImage := GetFrontImage()
ConvertToComplex(frontImage)
temp := modulus(frontImage)

GetSize( frontImage, xsize, ysize )
halfMinor = min( xsize, ysize )/2

centerx = xsize / 2
centery = ysize / 2

dst := CreateFloatImage( "dst", halfMinor, samples )

k = 2 * pi() / samples

dst = warp( temp, icol*sin(irow*k) + centerx, icol*cos(irow*k) + centery )

line_projection := CreateFloatImage( "line projection", halfMinor, 1 )
line_projection = 0
line_projection[icol,0] += dst
line_projection /= samples

rotational_average := CreateFloatImage( "rotational average", halfMinor * 2,
             halfMinor * 2 )
rotational_average = warp( line_projection, iradius, 0 )

ShowImage( rotational_average )
```

Where Gatan will be

MSA St. Paul, MN	Aug 12-15
EUREM Dublin, Ireland	Aug 26-30
MRS Boston	Dec 2-6
ASCB San Francisco	Dec 7-11

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Gatan Inc.
6678 Owens Drive
Pleasanton, CA 94588
Tel 510 463 0200
Fax 510 463 0204

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

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

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

Gatan Online
www site: http://www.gatan.com
General Info: info@gatan.com
Sales/Service help@gatan.com

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STEM EELS Imaging

A new look for Gatan's Imaging Filter

Digital Image Montage

Tools of the Trade: Specimen Preparation

Digital Image Enhancement: Useful Tips

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