

AUTOMATION FOR ON-LINE REMOTE-CONTROL *IN SITU* ELECTRON MICROSCOPY

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Abstract

We are developing and testing a multimedia system for remote operation of transmission electron microscopes and using it to control the Kratos 1500 keV microscope in Berkeley during *in situ* experiments. Tests, including heating and cooling of specimens on-line under control of the remote operator, have been conducted from Washington (D.C.) and Kansas City. Such *in situ* experiments subject the specimen under observation to external stimuli (such as heating, cooling, and straining). Full operational control requires adjustments of external stimuli, adjustment of specimen position and orientation, and manipulation of microscope controls such as illumination, magnification, and focus. In conventional (non-remote) operation, a local operator makes adjustments in response to the image from the microscope; in remote mode, current wide area networks cannot offer the real-time delivery guarantees required for the adjustments necessary for dynamic *in situ* studies. We have designed a system that minimizes the real-time delivery requirement by incorporating local automation of stage control and microscope focus. The system corrects for specimen drift (often severe during rapid heating and cooling) by controlling stage movement with optical flow fields; it provides automatic focus using wavelet coefficients with Daubechies kernels. Wavelet transforms are also used for image compression. During remote operation, we obtained 640x480-pixel images at a rate of 0.6 fps, providing effective operator control of the microscope and the experiment.

Key Words: Telemicroscopy, teleoperation, telepresence, on-line, remote control, collaborative, *in situ*, high voltage electron microscopy, Internet.

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Introduction

National scientific user facilities are established with the aim of providing users with advanced instrumentation for scientific projects. One such user facility is the National Center for Electron Microscopy (NCEM) located at the Lawrence Berkeley National Laboratory (LBNL). This facility's instrumentation includes two unique transmission electron microscopes, the JEOL (Tokyo, Japan) atomic-resolution microscope (ARM) with a resolution of 1.5 Å and the Kratos (Manchester, UK) EM-1500 high-voltage electron microscope (HVEM) with an electron energy of 1.5 MeV. Over the past thirteen years, instrumentation at the NCEM has provided hundreds of scientists with structural information from their sample materials, including metals, semiconductors and ceramics: information about the structures of new phases, defects, interfaces, and nanocrystalline precipitates. At the atomic level, information is provided from thin specimens examined in the ARM. Information at lower resolutions, often under *in situ* conditions, comes from specimens examined in the Kratos EM-1500 HVEM.

For the Kratos EM-1500 electron microscope, "*in situ*" means that samples may be thicker (more representative of bulk material) and may be subjected to various experimental conditions while under dynamic observation at 5 Å resolution. These experimental conditions can include heating, oxidation and reduction in appropriate gaseous environments, embrittlement with hydrogen, compression, and straining with a piezo-electric strain stage. Typical *in situ* HVEM sessions are likely to be much more experimental than with other more-standard microscopes, and users are typically more likely to travel to Berkeley to operate the microscope (or observe and direct while it is operated for them). To minimize travel, to better utilize NCEM facilities, and to speed the progress of typical user projects, we have established a program to provide our external users with remote on-line access to many of the NCEM electron microscopes. Initially, we have designed, and commenced to implement, a remote user interface to control both the Kratos EM-1500 and its *in situ* capabilities. Experience with this interface has shown that any viable interface for remote operation requires automation of several microscope functions.

Remote Operation

Remote on-line control of scientific instrumentation is rapidly becoming more practicable as improvements in the software and hardware of interfaces and networks continue [7, 8, 11, 18, 20]. At the NCEM, our program for full remote operation of electron microscopes [17] evolved in a project originally designed to establish use of on-line image processing as an aid to electron microscope operation [12]. We are developing and implementing a set of tools, protocols, and interfaces to provide for on-line control by remote users.

Because our present project has focused on the 1.5 MeV Kratos EM-1500 transmission electron microscope, we have designed the control interface to include full on-line remote control of the currently-active *in situ* experiment in addition to implementing on-line remote control of the microscope itself.

The computational platform that implements control in the local environment (including automatic control of selected functions) must be able to acquire images, process them at the required bandwidth, and manipulate a large number of HVEM operating functions. We have used a system based on three computers, and partitioned the control architecture over these computers in order to separate microscope control into two areas. The low frequency servo loop functions that require direct human interaction are performed over the wide area network, whereas those functions that require low latency control are performed locally using automated techniques. This approach hides the latencies in the wide area network and permits effective remote operation. The result is a teleoperation that provides the illusion of close geographical proximity for remote users of the Kratos.

Figure 1 shows the computer hardware and data paths. The video stream from the HVEM is digitized and routed to the remote user by a Sun SparcStation (Sun Microsystems, Palo Alto, CA) via a local network to the wide-area network. Teleoperation of the microscope by the remote user is achieved by routing commands to a personal computer (PC) that controls the appropriate microscope functions via three incorporated digital-to-analog converter boards and one stepper-motor board. Any image processing or analysis necessary for microscope control is performed by the DEC AlphaStation (Digital Equipment Corporation, now Compaq, Maynard, MA), and the results passed to the Sun via a fast (100Mb/s) local Fiber Distributed-Data Interface (FDDI) link.

Not all of the locally-available HVEM controls are necessary or appropriate for remote operation. We have placed only those functions that allow safe remote operation of the instrument under computer control. Control of some

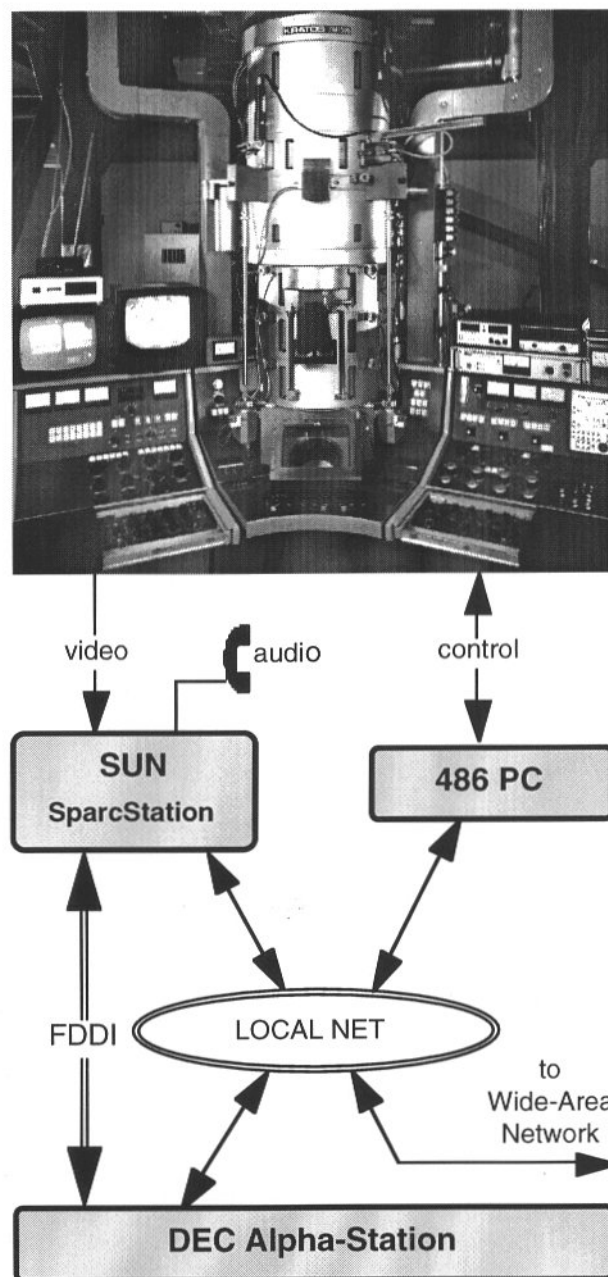


Figure 1. The operator area of the 1.5 MeV Kratos HVEM with system architecture for video and servo loops. Video from the HVEM is digitized in the Sun and transmitted to the remote user and to the DEC for processing for auto control. Commands from the DEC or the user are routed to the PC to control microscope functions.

other more-sensitive functions is not available to the remote user in order to safeguard the microscope. For example, control of the filament current is not offered because a

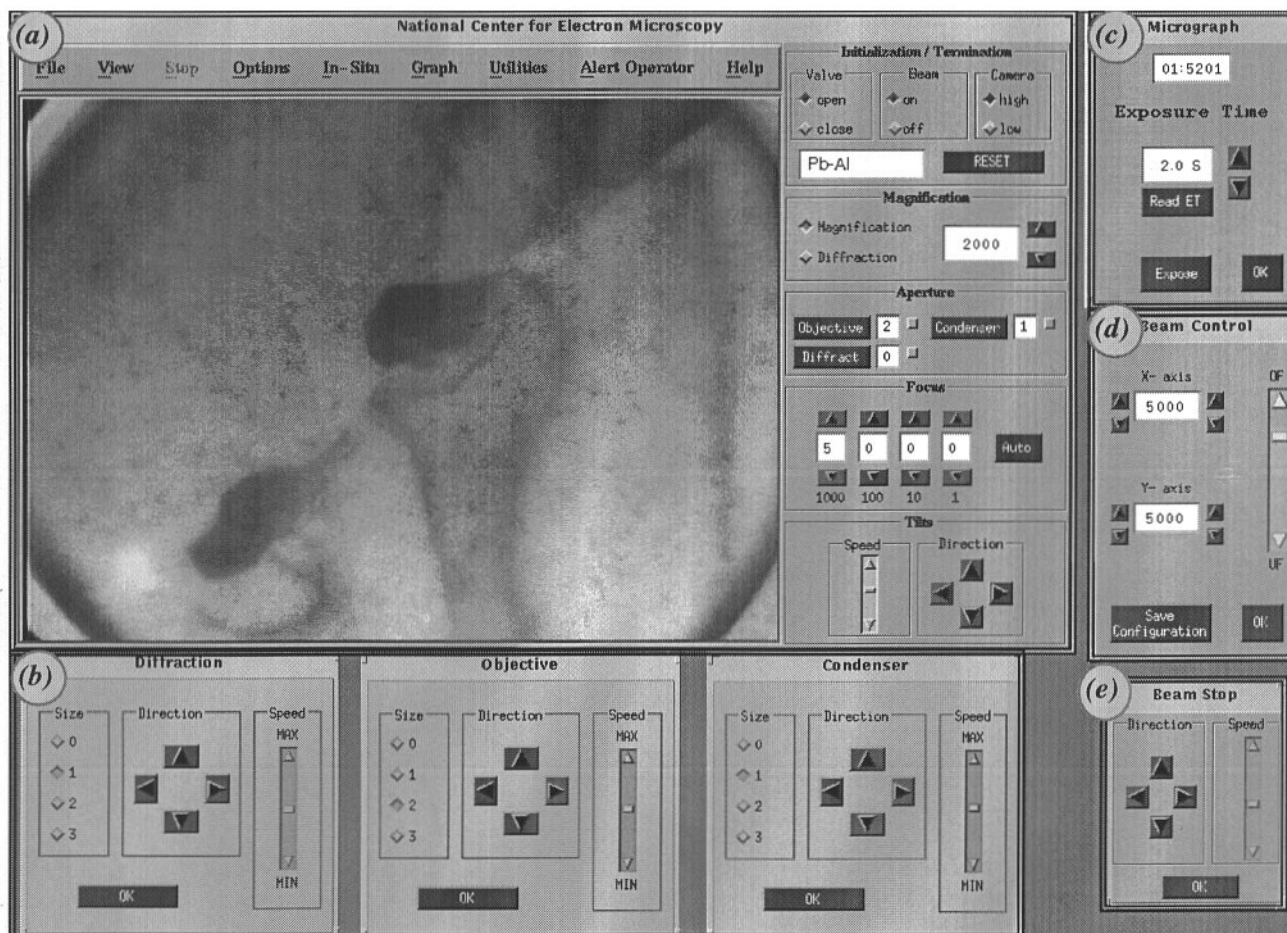


Figure 2. Standard remote operator interface configured for the Kratos EM-1500 HVEM. The main interface window (a) contains the live 640x480 NTSC (National Television Standards Committee) image and controls for magnification, aperture selection, focus and specimen tilt. Other pop-up windows control aperture positions (b), plate camera (c), beam position (d) and beam-stop position (e).

novice operator (or intruder) could accidentally damage the filament despite the microscope's safety features. All remotely operated functions have limit switches to prevent remote users from going beyond safe bounds.

Remote functions currently implemented on the Kratos include specimen translation in two orthogonal (x and y) directions, specimen tilt around two axes, objective lens focus control, beam control (position and size), and control of the specimen temperature. Magnification control, diffraction control, full aperture control (selection and positioning), and control of the standard microscope plate camera will be added in the near future.

Remote operation of the Kratos EM-1500 HVEM, with concomitant remote control of an *in situ* heating experiment, was successfully demonstrated from Kansas City in 1995 over existing wide area networks [17]. This facility is currently accessible from any single remote

location, and will soon become available to dispersed teams; in the case of collaborative teams, the software will be modified to allow transfer of control from one operator to another in order to provide simultaneous on-line microscopy to several collaborators at different geographical locations.

Graphical User Interface

Our remote control interface for the Kratos is presented to the remote operator in the form of a point-and-click graphical user interface (GUI). As implementations of on-line transmission electron microscopy become more widespread, it is desirable that suitable standards for operating interfaces be developed and adhered to. As a step in this direction, we have proposed [13] a basic standardized transmission electron microscope (TEM) user interface based on experience gained with on-line remote-

control using this GUI with the 1.5 MeV Kratos EM-1500 HVEM.

Our standardized user interface (Fig. 2) is designed to present the remote operator with the most frequently-used controls, those for image magnification, aperture placement, objective lens focus, and specimen tilt and translation. Since many TEMs already use a television (TV) camera to display the working image to the operator, the main interface window (Fig. 2a) also contains a smaller 640x480 window with a standard NTSC video image of the specimen, to provide the remote operator with the feedback necessary to position and tilt the specimen and select the requisite focus, aperture and magnification for recording the desired image. Subsidiary windows can be used for adjustments of apertures (Fig. 2b), for use of the plate camera (Fig. 2c), and for positioning the beam and beam-stop (Figs. 2d,e). *In situ* functions are accessed by pulling down the IN-SITU header in the main menu bar.

Using the main window controls (Fig. 2a), the remote operator can step magnification through the full set available on the electron microscope (29 steps for the Kratos) by using the up and down arrows in the MAGNIFICATION box. By selecting the DIFFRACTION button, the operator is able to step through the various diffraction camera lengths (6 for the Kratos).

Objective lens focus control is continuous with four up-down arrows and decade counters mapping the HVEM's controls for coarse, medium, fine, and vernier focus (as for most microscopes).

An auto-focus setting is provided to enable the remote operator to automatically establish or retain focus by using an autofocus routine running on the local computers. This routine is able to compensate for focus changes caused by specimen tilt or by any temperature-induced buckling of the foil specimen.

The specimen is tilted by using the appropriate arrow buttons in the TILTS box, adjusting the rate of change of tilt with the SPEED slide bar.

There are no arrow controls for specimen movement. Instead, the specimen stage is moved by simply clicking on any part of the image with the mouse and dragging it to the desired position within the 640x480 pixel image window. Correct scaling between the mouse movement and stage movement is maintained with auto-scaling of the stage-motion system.

The main window control allows three sets of apertures (OBJECTIVE, DIFFRACTION, and CONDENSER) to be withdrawn and inserted. Clicking on the small button adjacent to each label in the APERTURE section of the main menu (Fig. 2a) inserts the corresponding aperture (aperture size is indicated by the displayed number), or withdraws it (0 is then displayed). The size of the aperture to be inserted and withdrawn is selected initially by clicking on the

appropriate button (OBJECTIVE, DIFFRACTION, or CONDENSER) to open the corresponding pop-up window (Fig. 2b) wherein the aperture size can be selected with the SIZE buttons and the aperture centered with the arrow buttons. The step size of the centering buttons is controlled by the Speed slide bar.

Usually the apertures are selected and centered at the beginning of a session; the selection/adjustment windows are then closed and the main window aperture controls used to insert and withdraw the apertures without the need for additional positioning; however, the pop-up windows can be opened at any time in order to change the aperture size or position if desired.

Because the remote interface is based around a standard NTSC TV signal, the resolution of the interactive image is limited to 640x480 pixels. Although digitized frames from this TV image can be stored at the remote location, or indeed the whole session recorded on video tape at the microscope location, higher-quality images can be obtained by using the microscope plate camera remotely, as for a conventional (local) session on the microscope.

By opening the MICROGRAPH window (Fig. 2c), the remote operator is able to view the plate number (both overall, and the number used so far in this session), read the estimated exposure time (READ ET), adjust the exposure time with the up-down arrows, and then initiate the microscope camera exposure sequence (EXPOSE). During the exposure, the control program makes an entry in the session logbook (kept on both the local and remote computers), recording the associated parameters such as the operator and specimen names, date and time of day, magnification or camera length (depending on whether the exposure is for an image or a diffraction pattern), plate number and exposure time, and specimen temperature, tilt and position.

The illumination condition, or beam position (Fig. 2d), is controlled from a pop-up window (BEAM CONTROL) with arrow controls for positioning the beam in x and y, and a focus control for spreading it by adjusting the condenser lens (C2) current. Values of the x and y position are displayed in the pop-up window, and may be stored (together with the C2 focus value), by using the SAVE CONFIGURATION button. Saving the current values of the illumination condition writes them into the microscope magnification look-up table. As well as containing values for all the lens currents at each magnification setting, this table also maintains entries for the beam illumination conditions (x,y positions and C2 current) at each magnification. When magnification is changed, illumination also changes automatically to the last condition stored at that magnification.

Controls for additional options (e.g., objective stigmators, or a high-definition charge-coupled device (CCD) camera) can be added to this GUI (as suitable pull-

down options in the menu bar of the main window) to create appropriate subsidiary pop-up windows as required.

Automation

Conventional (non-remote) *in situ* experiments in an electron microscope require the local operator to make routine continuous adjustment of such microscope parameters as specimen orientation (tilt) and position, illumination condition, microscope focus, and occasionally magnification - all based upon the video signal coming from the imaging system. Because the specimen under observation is often subjected to external stimuli such as heating (with or without an imposed gaseous environment), cooling, or straining, *in situ* experiments often require quite dynamic adjustment of microscope controls.

In the context of remote in-situ microscopy, the system must provide the remote operator with the look and feel that is normally available to the local operator, and hide the latency inherent in the wide area network. Usually, the local operator has no difficulty in making control adjustments in response to the microscope image; however, in remote mode, current wide area networks cannot offer the real-time response required. Raw requirements for *in situ* studies cannot be met over existing wide area networks due to bandwidth limitations - for example, heating a specimen produces specimen drift at a rate that exceeds the remote operator's network response time - in fact, an image would have moved out of the field of view before the remote operator's correction signal could reach the microscope specimen stage, the stage be moved, and a new frame dispatched to (and received by) the remote operator.

This bandwidth limitation can be circumvented by using local automated corrections, based on advanced computer vision algorithms, that eliminate the requirement for real-time delivery over the wide area network. Given that we have computer control over microscope operations such as incident-beam illumination, objective lens focus and stage movement, it is possible, by partitioning the operating tasks into operator-remote and automatic-local, to ensure that only those operating functions that require man-machine interaction are performed over the (slow) wide area network. A list of operating tasks (Table 1) shows how some remote requests generate a local response that requires automation to ensure that microscope control is seen as adequate from the remote location. Functions that can be automated are performed using a local computer, in our case a DEC AlphaStation (Fig. 1), acting over a fast local area network.

For successful remote control, we require computer routines that will provide image compression, auto-scaling (to link image and stage movements), auto-recall of illumination condition (on magnification change), auto-focus (to compensate for changes in specimen height due

Table 1. Local responses to remote requests.

RemoteRequest	LocalResponse	AutoAction
TV frame	Send TV frame	compression
Change focus	Change obj current	————
Translate image	Translate specimen	auto-scale
Change magnif.	Change currents	illumination
Tilt specimen	Contrast Change	————
	Focus Change	auto-focus
	Image Translation	auto-translate
Heat specimen	Thermal Drift	auto-translate
/cool specimen	Buckling (tilt)	————
	Buckling (focus)	auto-focus
	Shape Change	auto-tracking

to tilt, translation, or temperature), auto-eucentricity (compensation for tilt-induced translation of the image), stage drift compensation, and object tracking.

Image compression

Image compression is required to ensure that the remote image window is updated at a rate sufficient for viable control of microscope functions. Our image compression routine is based on the wavelet transform, and uses Daubechies kernels that are simple, orthogonal, and separable for two dimensional processing [6]. During image compression, low order and low magnitude wavelet coefficients are ignored and the remaining ones are encoded in blocks of 16-by-16 pixels. The remote user has full control over the percentage of the wavelet coefficients that is used for compression. Typically, transmission speeds of 0.6 fps to 1.0 fps are achieved over high-speed lines at compression ratios of 50% or more.

Autofocus

Our autofocus routine also uses the wavelet transform with Daubechies kernels. In this case the sum of the wavelet coefficients is used as a measure of the goodness of focus.

In the absence of automation, the focusing procedure for the Kratos microscope is performed manually in two stages. Initially a coarse focus is found by removing the objective aperture and focusing to an imprecise Gaussian position using large steps of focus. Once an approximate focus is found (and any necessary astigmatism correction

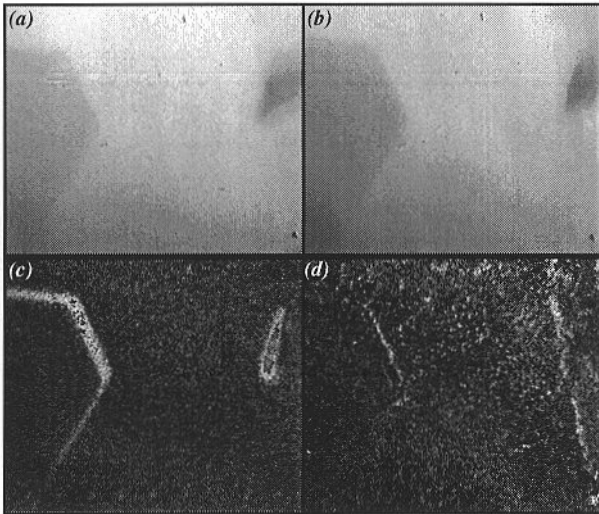


Figure 3. Estimation of drift by optical flow field. The raw difference between two successive frames (**a** and **b**) shows the image motion (**c**) that is compensated (**d**) by applying the optical flow field solution.

made), an objective aperture is positioned around the central diffracted beam and small focus steps are used to find an exact focus condition. As the experiment proceeds, the operator maintains focus by making intermittent small adjustments. The autofocus routine follows a similar two-step procedure. During initialization, with the aperture withdrawn, the objective lens current is stepped, in relatively coarse intervals, over a large current range in order to find a value that minimizes the sum of the wavelet coefficients, corresponding to a search for a global contrast minimum. Once this approximate focus is identified, the microscope operator is required to switch to diffraction mode, insert and center an objective aperture, then switch back to image mode. With the aperture inserted, the autofocus routine uses small increments in lens current, over a restricted focus range, to search for a value that maximizes the sum of the wavelet coefficients, corresponding to a local maximum in contrast.

During normal remote operation, with the aperture inserted, the autofocus routine is able to maintain focus by making small adjustments in objective lens current to compensate for small changes in specimen height (normally produced by changes in specimen tilt, translation, or temperature).

Drift control

Two methods are available to track motion in the image and to provide drift control in the microscope. One way is to track a specific image feature, but a more-general method uses optical flow fields. In our case, both drift

control and stage/image auto-scaling are implemented with an optical flow field estimation of image motion. With this method, two successive video frames are stored locally and the motion between them is estimated.

The first step in drift control, as well as in user control of specimen translation, is to calibrate the image pixel size to an equivalent number of steps in the xy-stepper motor controller of the specimen stage. Since the calibration will vary (in both x and y) for any change in specimen tilt or height, as well as for any changes in magnification and its associated image rotation, the calibration method is automated as much as possible. For stage/image-movement auto-scaling, the calibration is carried out by having the stage controller make a designated movement (depending on magnification) in x and y, and using two successive video frames (before and after the step), to measure the amount of induced image movement from the computed flow field. Once the scale between image and stage movement is stored, the remote operator's request for image movement (clicking on any part of the displayed image and dragging it to any location within the image window) will cause a corresponding movement in the specimen stage, and hence provide an updated remote image translated by the requested amount.

Drift in the HVEM is usually constant over the short term, so drift compensation can be implemented as a series of occasional adjustments to a pseudo-constant velocity. Adjustments are computed and supplied by the local motion-server software to the PC digital-to-analog converter (DAC)-server to drive the specimen stage and compensate for the stage drift motion. In the optical flow field approach, drift velocity is measured from two successive video frames by using the constraint that image motion be affine, and constructing a least-squares solution to the resulting optical flow field equations.

We define the optical flow in the video image as the instantaneous velocity of each pixel in the image [2, 3]. Then the image at time $t + \Delta t$ can be written as:

$$f(x + \Delta x, y + \Delta y, t + \Delta t) = f(x, y, t) + \frac{\partial f}{\partial x} \Delta x + \frac{\partial f}{\partial y} \Delta y + \frac{\partial f}{\partial t} \Delta t \quad (1)$$

Assuming constant brightness, we can express the image velocity in terms of the velocity components along two axes multiplied by the spatial image gradients in the x and y directions:

$$\frac{\partial f}{\partial t} = f_x U + f_y V \quad (2)$$

where U and V are velocity components and f_x and f_y are the spatial image gradients in the x and y directions.

Applying the affine constraint to the image motion, we can express the velocity components in the form:

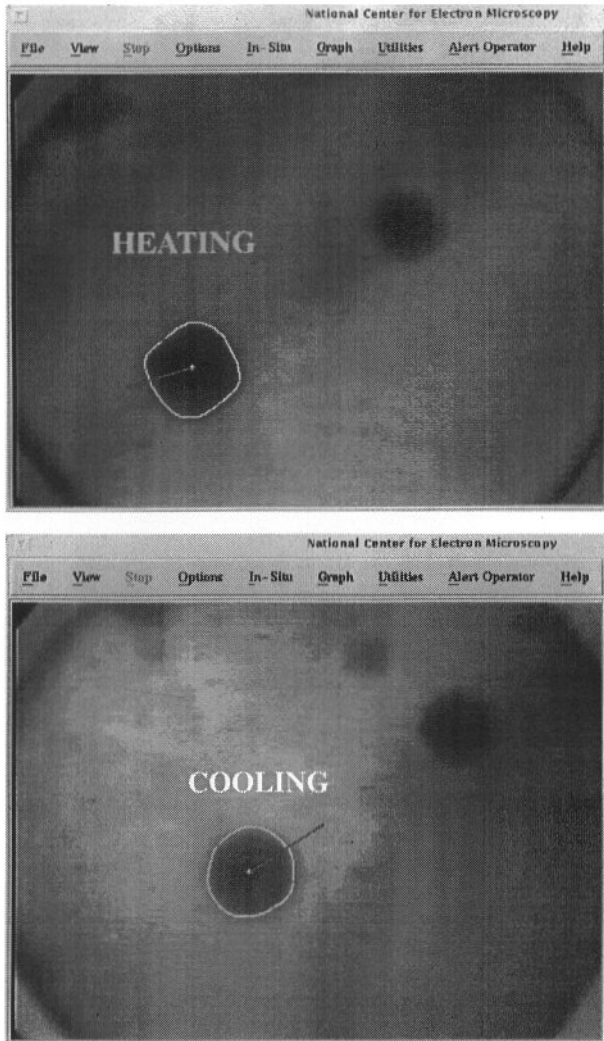


Figure 4. Precipitate tracking and stage drift compensation during one heating/cooling cycle of the specimen. A contour line traces the shape of the precipitate, and a vector from the centroid of the contour shows the correction for drift.

$$\begin{aligned}
 U(x,y) &= a_1 + a_2x + a_3y \\
 V(x,y) &= a_4 + a_5x + a_6y
 \end{aligned}
 \tag{3}$$

and use the method of gradient descent to solve the resulting system of equations. Our implementation of the optical flow field method uses a pyramid representation of the data for coarse-to-fine motion estimation. The main advantages of this representation include fast estimation of large shifts in the image plane, coupled with higher computational throughput. Our current algorithm is able to run at 4 Hz on our DEC multiprocessor AlphaStation, and is able to control drift to maintain sufficient image stability for effective remote

control of the Kratos. Figure 3 shows an example of motion estimation and compensation using optical flow fields.

Image feature tracking

Stage movement control not only enables us to set up a routine to control drift using optical flow fields, but also a shape-tracking routine that can control drift by tracking well-defined image features.

One class of experiments carried out *in situ* on the Kratos microscope involves observations of the shape changes of precipitates as the temperature is cyclically increased and decreased. Parvin *et al.* have developed techniques for detecting [15] and tracking shape changes in these precipitates [16, 17]. The results of precipitate shape tracking can also be used to correct the microscope stage for thermal drift.

Our tracking routine relies on the user to select and mark an image feature such as a precipitate. It then automatically marks (and tracks changes in) the shape of the particle with a contour line, controls the drift of the microscope stage by applying an appropriate correction, and thus hides the network latencies from the remote user. In this case, drift control is based on tracking (and then compensating any movement of) the centroid of the area defined by the contour line placed around the precipitate by the detection routine.

Precipitates have convex geometrical shapes that can be identified in the video image (Fig. 4). The technique for detecting convex objects from the image is based on perceptual grouping principles [10, 14, 15]. The implementation relies on grouping line segments, which are obtained using Canny's edge detector [5], to form convex sets through a global convexity test on groups of line segments in conjunction with a dynamic programming search strategy [16].

The precipitate detection routine provides a first approximation to the shape of the precipitate as a set of bounding polygons. This approximation is refined and tracked in subsequent video frames [15]. The contour refinement algorithm is optimized through dynamic programming to encode the desirable properties of the refined contour in terms of local edge magnitude and direction. Restraints, derived from the bounding polygon, constrain the contour refinement by placing limits on precipitate geometry and the scope of the search for the current precipitate shape. Before we impose these limits, we smooth the initial polygon with a Gaussian kernel and bound the refined contour to lie in a small neighborhood as defined by the normal lines to the smooth curve. Without Gaussian smoothing the bounded polygon may not be smooth and the normal lines may not intersect the actual boundary of the precipitate. However, given a smoothly-bounded polygon, the normal lines are forced to scan the precipitate along its real boundary.

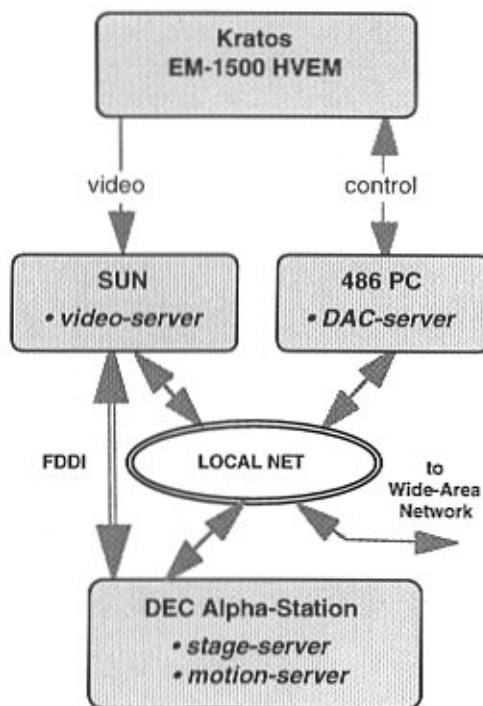


Figure 5. Distribution of the four software servers.

We use a multigrid implementation of the above algorithm for maximum speed and high tolerance for large motion. The algorithm is able to perform well in the presence of microscope artefacts such as image shading, image noise, low contrast, and non-uniform illumination.

Using the tracking routine, rather than the optical flow field method, to control stage drift is an advantage when rapid heating and cooling of the specimen is desirable, especially when shape-tracking of precipitates is already called for by the experiment. However, it obviously lacks the generality of the optical flow field method, wherein the image field need not contain a convex shape that can be readily identified and tracked.

To ensure that the stage motion is smooth, we use a Kalman filter model to predict motion parameters from noisy measurements; Kalman filtering has been used extensively for smoothing and prediction [4, 19]. In general, our control model predicts the trajectory of the motion and provides smooth compensation for drift with good tolerance for high speed. Instead of making corrections to the microscope stage position, we run the stepper controller at a constant velocity in the direction opposite to the measured thermal drift (as with the optical flow field method). We then refine the correction velocity (in both x and y) at the sampling interval of the tracker.

Figure 4 shows a tracking example taken from the remote GUI screen. While being heated from room

temperature, the precipitate has a faceted crystallographic shape (top screen). At high temperature the precipitate is more rounded, and it retains this shape while being cooled (lower screen). The centroid of the particle contour drawn by the shape-tracking routine is marked. The drift-tracking routine aims to hold this centroid motionless, and indicates the direction and magnitude of each restoring correction by a vector (shown extending from the centroid to well outside the contour).

Because the drift-tracking routine runs at 5Hz to 8Hz on the DEC AlphaStation, whereas the remote image is updated at slightly less than 1Hz, the perceived motion at the remote station (in the absence of drift correction) would be almost an order of magnitude greater than the vector length shown (since the drift is corrected more than five to eight times as often as a frame is received at the remote site). Uncorrected heating/cooling tests show that, without drift correction, the precipitate disappears from the image window in one (remote) frame time. In Fig. 4, the correction vector also shows that the thermal drift reverses its direction as the sense of the temperature change is reversed, going from an eight o'clock direction on heating to a direction lying between one and two o'clock on cooling.

Distribution of control

Signals to operate the microscope controls are allowed to come from either the remote user or from the automation routines running on local computers. The stage-server running on the DEC AlphaStation (Fig. 5) acts as a switch to arbitrate between the local and remote requests. An additional advantage of limiting the remote client interaction to only one computer, is that the user identification and authentication procedure need reside on that platform only.

The software architecture is constructed to follow a distributed client-server model for scalability, performance, and modularity. The four servers are distributed over the three computers as shown in Fig. 5. Briefly, the actions of the servers are:

- Video-server on the Sun: digitizes video frames from the Kratos as images with 640x480 pixels and transfers them via FDDI to the motion-server on the DEC for image analysis (for automatic control), or for compression and transfer to the remote user via the local and wide-area networks.
- DAC-server on the PC: communicates directly with the microscope to control its various functions, and read microscope parameters, as directed by the stage-server.
- Stage-server on the DEC: handles all manual interactions between the remote user and the electron microscope, such as changing magnification and focus, tilting and translating the specimen stage, and moving the electron beam and apertures.
- Motion-server on the DEC: runs all image analysis and servoing routines for automatic control of microscope

functions. The motion-server consists of several modules that are executed asynchronously; they use a threads programming paradigm, and communicate with data streams through sockets for minimum delay.

Communication amongst the servers is also by data streams through sockets, except that the DAC-server utilizes remote procedure calls.

A good example of the communication required is for the self-calibration of the scaling between specimen stage movement and remote image movement.

When the remote client makes a request for self calibration, the request is transferred to the motion-server (running on the DEC); the motion-server requests a video frame from the video-server (on the Sun), and the video-server sends an image to the motion-server (DEC); the motion-server makes a request for a translation from the stage-server (DEC) and a request for another frame from the video-server (Sun). After receiving the second frame, the motion-server computes the optical flow and solves the resulting linear system of equations to provide a mapping between the pixel size and the corresponding number of stage steps. This value is retained for subsequent drift corrections.

The motion-server has four threads that run asynchronously and can exchange data as required. The focus thread uses wavelet coefficients to keep the image in focus, the compression-thread uses them for image compression. The stage-thread handles all interactions with the stage-server, and the tracking thread provides drift correction for the microscope stage.

Conclusions

Remote operation of the Kratos has revealed some limitations in recording and storing final images. The 640x480 video stream makes an ideal working image for adjusting the microscope and positioning the specimen, but individual images are of too low a quality to use as final data sets. Plate camera images are much better quality, but are not immediately available to the remote user. A charge-coupled device camera would present the user with a high-quality downloadable image. In fact, a video/CCD camera combination would be ideal for remote operation. The video camera could simply be replaced with a 1024x1024 pixel CCD camera and smaller binned images used as the video stream [18].

The work described here is only a first step towards a new kind of availability for unique scientific instruments located at central facilities. It will result in increased utilization of these sophisticated instruments by providing much greater accessibility to them. It will reduce costs associated with conducting individual experiments and will improve user collaborations, allowing multiple collaborators

at scattered remote locations to monitor their on-line experiment, discuss results while it is in progress, and transfer control of the microscope from one to another as necessary - a true collaboratory.

The present project has provided us with tools essential for real-time collaborative activities, including generic techniques for manipulation of real-time video, and servo techniques for dynamic handling of video sequences. One serendipitous side-effect of our work is that our new automated methods of controlling the Kratos electron microscope have already so improved the standard man-machine interface that they have resulted in a significant easing of the users' tasks - even operating in local mode - freeing the users to concentrate on the science of the project rather than on the mechanics of running the microscope.

Our next step is to extend these techniques, and the lessons learned from the Kratos, to help us build standard systems that can interface to more of the transmission electron microscopes at the NCEM. Such an extension of the present work will require the addition of more functionality to the user interface (e.g., high-resolution electron microscopy will require the system to be able to present on-line diffractograms, and to acquire and store through-focal series of images) and including many more automation routines (e.g., high-resolution electron microscopy will require auto-alignment and auto-stigmation capabilities — and possibly an auto-focus-to-Scherzer-defocus routine, with auto-through-focal-series acquisition, and automatic on-line comparison with simulated images).

A major challenge will be to produce a user interface that will present a standard "look and feel" to the user, and yet accommodate the individual differences in the various models of microscope available from different manufacturers. At the other end of the command chain, a related challenge is to produce suitable software and hardware computer interfaces that will operate with the microscope software interface (including the manufacturers' proprietary protocols) and with the various hardware interfaces (both electronic and mechanical) provided by the manufacturers and by suppliers of microscope accessories for energy-loss spectroscopy, x-ray analysis, and high-resolution image capture.

At this time, on-line access to scientific instrumentation is generating wide-spread interest within the scientific community [1, 9]. It will soon become a required option for all central facilities that supply scientists with instrumentation for their research.

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Discussion with Reviewers

M.H. Ellisman: Of the many functions described, it is not clear which are currently implemented and which are merely planned. Your section on Remote Operation indicates that magnification control has not yet been implemented.

Authors: For completeness, we have included descriptions of all the controls required for a remote user to carry out an *in situ* experiment during a microscope session. So far, the controls that have been implemented and utilized during remote sessions are specimen translation and tilt, beam position and focus, and objective lens focus. We started with these basic controls and are adding others to our graphical user interface in such a way as to permit it to be extended logically, hopefully leading to a model for a standardized interface for on-line TEMs. Magnification is the next function we plan to implement.

M.H. Ellisman: It seems premature to propose a standardized interface, since your system is only in the beginning stages and focuses on a specific class of microscope use required for certain types of specimens, data acquisition, and *in situ* experiments.

Authors: We are hoping to establish a basic interface that can form the basis of a standardized interface. The standardized interface should be modular and allow for user-selectable control panels to be present depending on the type of microscope being controlled, its ancillary equipment, and the experiment underway. We feel that the basic interface should display the working image and offer controls for specimen translation and tilt, beam controls, focus, and a means of recording a final image. In our case, the working image is a 640x480 video stream, and the final image must be recorded using a plate camera. Ideally, when the interface is used with a microscope that is equipped with a CCD camera, the plate camera controls on the remote graphical user interface (GUI) should automatically be replaced by CCD camera controls to allow the final image to be “recorded” by downloading it to the user’s computer. If a CCD-equipped microscope happened to have no video camera, the 640x480 window for the working image should automatically be replaced by a window to display working images constructed by binning the output of the CCD camera down to 512x512, 512x256, or 256x256, depending on available bandwidth. Völkl *et al.* [18] routinely use a 256x256 working image binned from a 1024x1024 CCD camera to control a Hitachi HF2000 electron microscope remotely. Incidentally, we plan to replace the video camera on the Kratos with a CCD to facilitate the use of diffraction mode. At the moment, it is too easy for the user to damage the video camera by switching to diffraction mode under strong illumination. When the remote GUI is opened, it will interrogate the Kratos control computer, receive the reply that the Kratos now uses a CCD instead of a video camera, and will open the correct working image window.

M.H. Ellisman: It appears that the video interface is employed by the remote user primarily for specimen positioning and centering of apertures. It is not clear what other functions it is intended to provide the remote user.

Authors: The graphical user interface is designed to provide the remote user with a working image and the means to modify basic microscope conditions according to the information coming from this image. The user can “roam” the specimen, adjust its tilt, change the focus and illumination, and generally set up conditions to record a good final image containing the desired information about the specimen.

M.H. Ellisman: It is unclear why the autoscaling function is needed. Why does the stage require repeated recalibration? How accurate and repeatable is stage positioning? It would be surprising if mechanical stage motion is sufficiently accurate on your microscope, especially at high magnification.

Authors: The autoscaling function maps the stage to the current image orientation. Generally, there will be a rotational misorientation between the image and the specimen, such that a change in x (or y) in the image position will require changes in both x and y for the specimen stage. The coefficients in the matrix that maps the stage to the image will change with any change in magnification, specimen tilt and change in height (sometimes caused by buckling under heating). Stage positioning accuracy is now adequate. Initially, we fitted the microscope translation controls with geared-down stepper motors. Experience with this system has led us to increase the gear ratio by another factor of ten, in order to reduce the step size. We also re-machined all mechanical links to minimize backlash.