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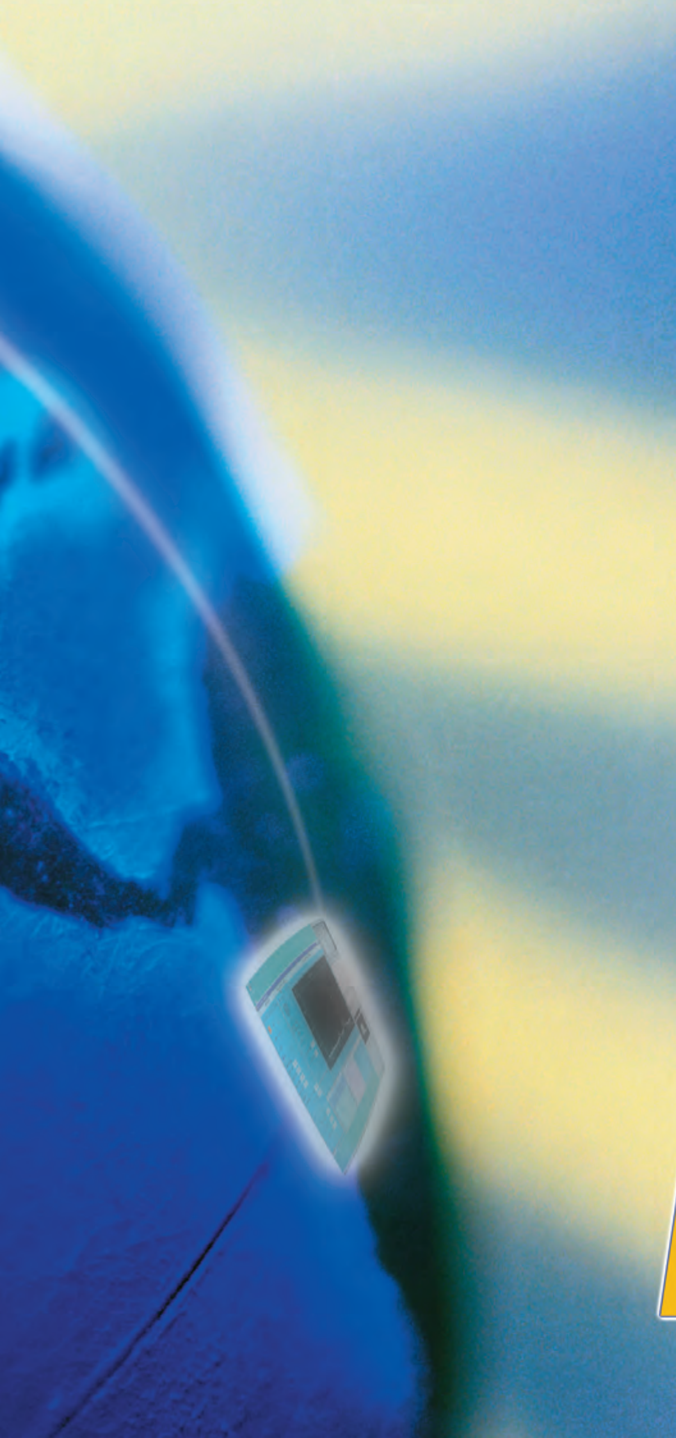
Remote Experimentation over the Net: Our First Year with MALDI



When you absolutely, positively don't have to be there.

As analytical chemists, we see evidence of a networked community all around us. We search the Web for citations and other reference material, use e-mail to communicate with friends and colleagues, evaluate data, and co-author papers through the exchange of files. In our offices, homes, and hotel rooms, we are well connected to the outside world. But can the same be said when we enter the laboratory?

The concept of a collaboratory—a laboratory that does not require all researchers to be physically present to participate in an experiment—is not new (*1–4*). An effective collaboratory provides the opportunity to both remotely access instrumentation and communicate in a variety of ways (*1–3*). Although a handful of laboratories have embraced this concept and developed innovative approaches to make it work, widespread use remains a goal rather than reality.

Researchers interested in establishing a collaboratory are initially faced with two sets of questions. First, how hard is it to implement a collaboratory? Will it require dedicated software that must be written or specific hardware that must be purchased? If it is going to require significant setup time, competing work-related demands are likely to take precedence. Second, once the capability becomes available, will it really prove useful in research? Is remote operation of an instrument really as reliable and convenient as working in the laboratory? Or will instrument and experimental capability be compromised? In this article, we will address these questions through our experience with remote operation of a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer. Our interest in remote operation was stimulated by a grant from the National Science Foundation, which provided funds for an instrument that could be used by researchers at several institutions.

Life in a collaboratory

The goal of a collaboratory is to provide access to instrumentation that is not available locally. The paradigm that usually comes to mind is a single researcher performing an experiment through remote operation of an instrument. Remote operation is relatively straightforward because many workstations allow users to log on from a separate terminal. In most cases, a researcher logged on at one terminal cannot view what another researcher is doing at a second terminal. Although the one-instrument, one-researcher paradigm is common, it is not the only way in which research is done. Many experiments are interactive. For example, researchers skilled in the instrumental method may work side by side with other researchers who are knowledgeable about the sample. Together, they evaluate the results in real time and revise the course of action as needed to solve the problem.

Troubleshooting instrument and sample problems may also require the collective input of several researchers. In a conventional laboratory, these individuals are nearby and can physically come to the instrument. In a collaboratory, these researchers may be at several locations. They must simultaneously access, control, and view the instrument workstation, yet they have different computing hardware. This type of remote experiment is much harder to implement.

How do these two experimental approaches relate to a MALDI experiment? Over the past decade, MALDI has developed into a powerful method for the analysis of high-molecular-mass materials. In the past four years, MALDI has been the subject of six A-page articles in this journal alone (*5–10*). Samples are prepared by mixing microliter amounts of matrix and analyte solutions on a probe surface. When the solvent evaporates, the analyte becomes embedded in a solid layer of matrix. The probe is inserted into a mass spectrometer and irradiated with a pulsed laser beam. The matrix absorbs the laser radiation and vaporizes in a manner that produces gas-phase analyte molecular ions.

The sample probe could be prepared at the remote laboratory and shipped overnight to the instrumentation laboratory. The next day, the probe would be inserted into the mass spectrometer by local personnel and then analyzed in conjunction with personnel at the remote laboratory. Alternatively, the sample could be shipped to the instrumentation laboratory where local personnel would spot it onto the probe. The approach chosen depends on sample stability, the need for special sample preparation procedures, and the availability of appropriate personnel at each location.

MALDI over the Internet

In our work at the home laboratory at the University of Delaware, we use a MALDI instrument supplied with *X Windows* application software on an attached Sun SPARCstation operating under Solaris 7. The *X* client programs restart and stop the laser, control its energy, control the movement of the sample probe, adjust the high-voltage settings of the electrodes, and acquire and analyze data. An external, secondary video monitor displays an internal charge-coupled device (CCD) video camera image of a single sample spot on the plate. For remote operation, the CCD output is captured with a video card and displayed in a separate window.

Once the probe is placed in the mass spectrometer, a single user can activate most other functions through a single *X* display server. On the *X* desktop display, controls in one window are used to select the sample spot on the probe to be analyzed; controls in a second window allow the probe to be positioned precisely. Other windows control the laser energy, spectral acquisition parameters (electrode potentials, timing electronics, number of laser shots averaged), and spectral analysis. Some of these windows become icons at different stages of operation, and the operator may move among windows to complete the entire acquisition and analysis sequence.

Although an investigator at a remote *X* display server could theoretically control an instrument, not all *X* servers worked well with all of the *X* clients. For example, some of the clients depended on fonts that were not on every *X* server. Or the data acquisition software's plotting client would not clear the plotting window on all the *X* servers. The *X* clients worked well, however, when they were run locally on the attached Sun workstation. Finally, it should be noted that several *X* display servers did not allow the remote and local investigators to simultaneously view and perform the same experiment.

Several software approaches were implemented by the information technologies group at the University of Delaware and tested for remote, multi-investigator operation. Although no single approach was ideal, the AT&T Research Laboratory's *Virtual Network Computing* (*VNC*) software provided the most robust solution. This product allowed researchers in multiple locations to take mouse and keyboard control of the mass spectrometer at will (*11*; details can also be found at http://www.udel.edu/topics/internet2/proj/maldi/). The platforms tested were Sun (Solaris), SGI (IRIX), PC (MS Windows), and Mac (MacOS). All performed well in remote applications.

VNC is free, General Public License software that uses a non-platform-specific remote frame buffer (RFB) protocol, thereby making it potentially useful to all operating systems, windowing systems, and applications (*11*). The RFB protocol allows several geographically distant researchers to concurrently share the view and control of the mass spectrometer *X* desktop and a separate video stream of sample from a CCD camera.

Installing *VNC* software is straightforward, although problems may be encountered in some cases. We downloaded and installed the *X Windows* version, including *vncviewer*, *vncserver*, *vncpasswd*, and *Xvnc*. *VNC* can be run in user mode, which avoids the need for the MALDI instrument technician to know the superuser password. The *Perl* programming language, freely downloadable from http://www.perl.com, is required to use the *VNC* server script. The *VNC* server script may require trivial modification if components such as *Perl*, *X*, or the *VNC* files are in nonstandard locations on the computer. In our installation, we encountered only one problem when initially starting a *VNC* server and found the solution at the FAQ page on the *VNC* Web site (*11*). Because our permissions did not allow us to write to a required directory, we could not establish the necessary Unix listening socket. Setting the permissions in the local *X* start-up script fixed this problem.

To view the *X* desktop and CCD video stream at a remote workstation, a *VNC* viewer client must be run. Three alternatives currently exist for the viewer: the native *VNC* viewer client from AT&T Research [available for Windows 9x, Sun (Solaris), MacOS, and generic *X Windows* used for the SGI (IRIX) platform], a Java appletviewer, or a Java-enabled Web browser (Netscape 4.x, Internet Explorer 4.x). In practice, we have found the *VNC* viewer client to be the most robust. Glitches were more frequently encountered with Web browsers, and some browsers did not have the correct version of Java. Because the *VNC* viewer program is stand-alone executable, it only needs to be run after downloading.

Figure 1 shows the setup for an actual demonstration performed at the Internet2 Spring 2000 Joint Technical Meeting in Washington, DC, in March 2000. Three researchers were involved—the first at the University of Delaware MS laboratory using the Sun workstation (Solaris 7) from the vendor, the second at George Washington University using an SGI Indy (IRIX 6.5), and the third at the conference using a remote PC (Windows 98). A *VNC* server was run on the local workstation to provide a virtual display. The MS workstation was used for convenience, although the server could have been run anywhere.

The *X Windows* client applications supplied by the vendor to operate the mass spectrometer were run in the same manner as for local operation. The *VNC* server acted as an *X* server to the mass spectrometer's *X* clients and drew each client's application output window in the RFB. A *VNC* viewer client was run on each remote workstation and on the MS workstation. Each *VNC* viewer pulled its updates from the RFB, constantly refreshing the *VNC* window on each workstation's monitor.

Running the *VNC* server on the MS workstation starts the sharing session. The remote participants start their *VNC* viewer clients after learning what port and password the investigator in the MS laboratory has chosen. Collaborators can join in and drop out of the sharing sessions as needed. Everyone sees the same monitor output and has equal access to instrument operation.

Figure 2 shows the monitor screen of the remote PC (Windows 98) during operation. The larger *VNC* viewer window displays the *X Windows* desktop. The smaller window (upper-right corner) is a second *VNC* viewer window that displays the CCD camera image. The CCD camera output is sent at 10 frames/s to a second *VNC* server's RFB. Because of the MS workstation's resource limitations, a separate computer is used as the server for the camera image even though the video card is located in the MS workstation. (As stated previously, the *VNC* server need not be on the same workstation.) Microsoft *NetMeeting* was used to manage a chat session in the lower-right corner. *NetMeeting* was also being used to provide a point-to-point audio link between this PC and a laboratory laptop PC.

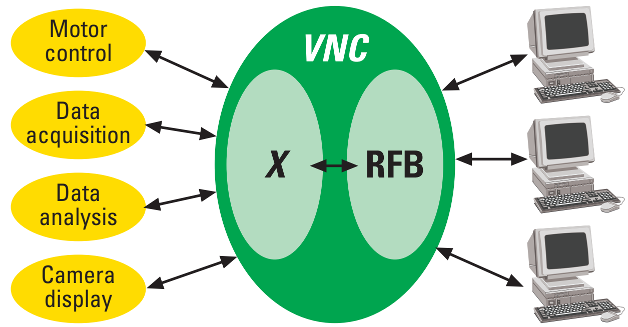


FIGURE 1. Setup for simultaneous, multi-investigator operation of a MALDI-TOF mass spectrometer.

Works and plays well with others

Once an acceptable software approach is found, a new piece of equipment can be adapted for Internet use quickly by downloading and installing the appropriate server/viewer software. However, there are always nuances to be learned. For example, the instrument used in this work requires proper opening and closing of *X* client windows before and after an Internet session to avoid resource conflicts. These problems aside, new collaborators can access an Internet-ready instrument almost instantaneously by downloading the viewing software and logging on properly.

How does working in a collaboratory compare with a conventional laboratory? The *VNC* software provides the same user interface as the instrument's workstation. In practice, our workstations required a desktop area a bit smaller than the instrument's workstation. This is, at most, a minor distraction when viewing multiple windows. The greater problem is network traffic, which can slow down response time. This is particularly important for the CCD output, which requires ~1 megabit/s throughput (10 frames/s; 8-bit digitization). In addition, procedural problems exist that need special attention. For example, we have configured *VNC* software so that any of the remote researchers can seize control by moving the mouse or pressing the keyboard. Anarchy is avoided by establishing operational ground rules among the collaborators. It should be noted that *VNC* software can be configured to give remote users either "view-only" or "full-control" privileges.

The MS workstation is connected to the Internet2's Abilene network. (Information about the Internet2 initiative can be found at http://www.internet2.edu/) Remote operation is rarely subject to network slowdown when all the collaborators are connected. Collaborators with other network connectivities may experience difficulty. An extreme example is connection through a modem. In one test, we connected to the system using a 56-Kbs modem to dial into an Internet service provider in another part of the country. Not surprisingly, the CCD camera display lagged severely, and other instrument functions exhibited lag times of ~1 s. Although this performance is not acceptable over the long run, it is sufficient for a remote collaborator with limited Internet access to "watch" an experiment in progress and comment on the results.

A remote experiment

At George Washington University, four modified oligonucleotide samples were deposited on a 384-well sample holder at various matrix-to-analyte ratios using four different matrices. The loaded sample holder was packaged and shipped overnight to the University of Delaware. The following day, a collaborator at the home laboratory introduced the sample plate into the MALDI MS system and provided the *VNC* port and password information to the remote operator. Except for the eventual removal of the sample plate from the instrument at the end of the analysis, no further participation of home laboratory personnel was needed.

Excellent quality spectra of the four samples were collected through remote operation in a time duration comparable with what would be required on-site. Clearly, this effort was facilitated by previous experience with similar samples. This prior knowledge helped reduce the number of matrix–analyte combinations deposited on the plate and ensure an adequate sample preparation. As in a conventional experiment, the effectiveness of a remote experiment greatly depends on the researcher's skill and knowledge. Remote operation opens up new possibilities because this experience can be gained in a virtual laboratory setting without compromising locally available instrument functions.

Of course, remote experimentation is never the same as being there. Samples must be brought to the home laboratory, prepared, and inserted into the instrument, and routine maintenance must be performed. Nonetheless, with the help of on-site personnel in the home laboratory, experiments can be performed even if it is impossible for all investigators to be physically present. Most of our work has involved collaborations with investigators who are highly skilled with MALDI. There has been relatively little need for direct interaction during an experiment. Even so, the ability to simultaneously view an experiment from multiple locations has proven to be an indispensable tool for troubleshooting the mechanical and software problems that inevitably arise as an experiment proceeds. For the same reason, an audio link, either through a telephone or the Internet, is also quite helpful.

Instructional benefits and pedagogical impact

Educators must continually find new ways to introduce their students to the uses of new analytical instrumentation. Unfortunately, the size, weight, and configuration can make these instruments poorly suited for the classroom. This problem can be addressed through remote operation by bringing the imagery of real instruments into the classroom. Students can gain experience with sophisticated instrumentation and conduct real experiments. Remote instrumentation can help students understand real problems such as contaminated samples or inadequately tuned instruments. Students that take the lecture course without the corresponding laboratory course can still gain a deep appreciation of analytical tools. Finally, potential safety hazards (high voltage, radioactivity) are avoided.

> **Students gained the experience necessary to advance to independent research.**

On a small scale, we have explored the instructional use of remote operation, using DNA analysis as an example. Several methods of taking successful MALDI spectra were demonstrated remotely. The effect of laser energy and the presence of a threshold laser-irradiance were shown by systematically changing the laser intensity. The impact of selecting the right matrix was demonstrated by taking spectra of the same DNA with different matrices (these samples were preloaded onto the sample holder). Detection limits were determined by taking measurements from standards preloaded at different concentrations. The students optimized instrument parameters such as electrode potentials and discussed their influence with regard to instrument design and function. From the training session, each student gained the experience necessary to advance to independent research. Classroom use on a larger scale is now being planned.

Moore's law and other indicators tell us that processing speed, network bandwidth, and software options will increase with time. Indeed, some technical aspects of our discussion may be obsolete by the time this article appears in print. Our brief experience with MALDI shows that, even with current technology, experimentation in a collaboratory is relatively easy to establish and is effective in practice. These capabilities will only improve in the future.

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Murray Johnston is a professor and Frederick Cox is a graduate student at the University of Delaware. Johnston's current research inter-

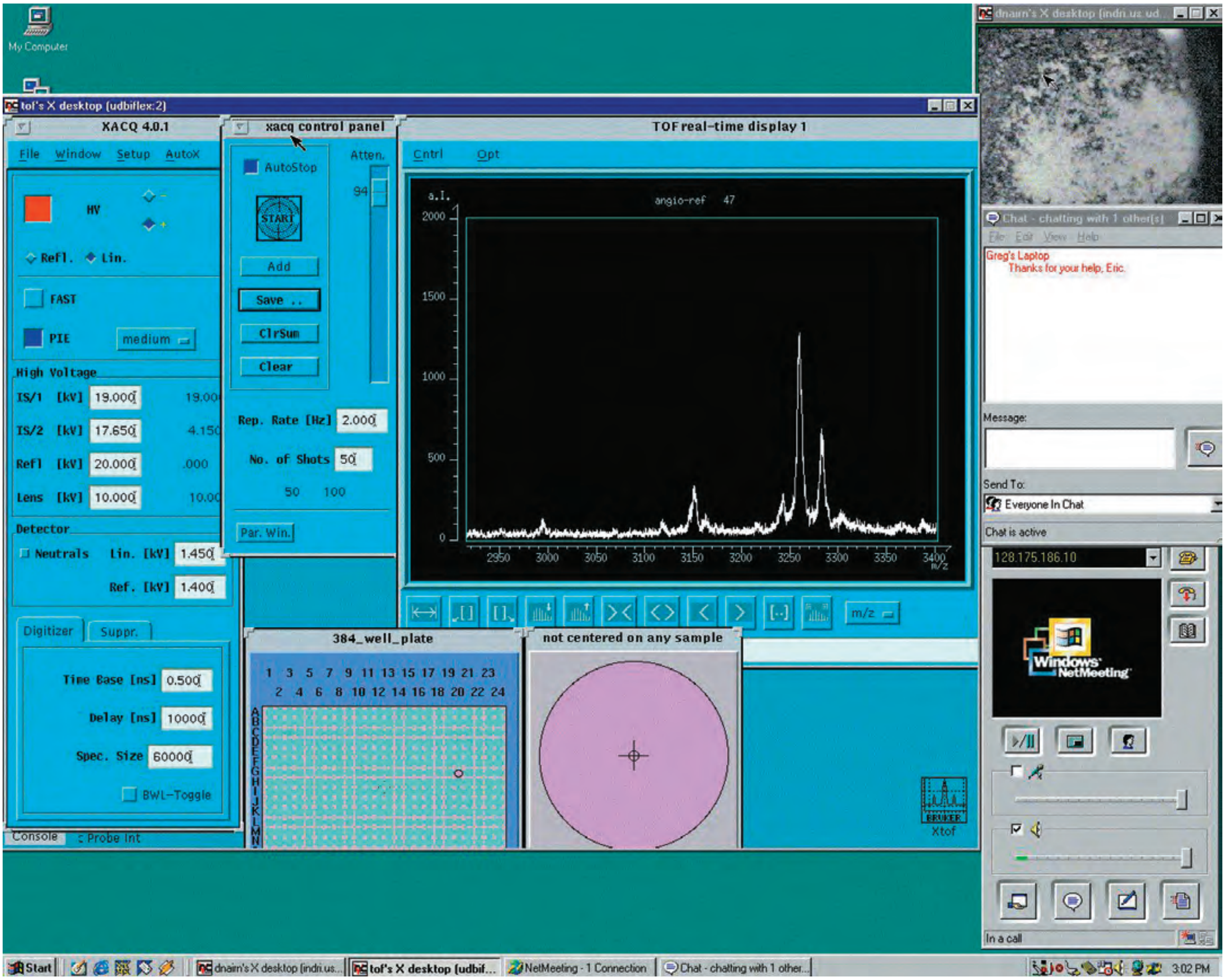

FIGURE 2. Monitor screen of a PC operating the MALDI-TOF mass spectrometer from a remote site.

ests include applying MS to large molecules, molecular complexes, and airborne particulate matter. Cox's research interests include instrument development and applying MALDI and photoionization MS to synthetic polymers. He is also a participant in the Multisite Research Program in MS. Greg Forte, Dean Nairn, Richard Sacher, and Anita Schwartz are members of the University of Delaware's Research Data Management Services (RDMS) staff. RDMS provides campus-wide information technologies and consulting support for researchers, facilitating the use of emerging technologies such as Internet2, as well as computational methods in mathematics, statistics, GIS, and database development. Akos Vertes is a professor at George Washington University. His current research interests include laser desorption and ablation in biomaterial analysis and synthesis, and the analysis and synthesis of nanoscale materials using electrospray.

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