

Operator's Manual

NEN[®] Model 4300 DNA Analyzer



LI-COR[®]
Biosciences



Biosciences

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Declaration of Conformity

Manufacturer's Name: LI-COR, Inc.

Manufacturer's Address: 4308 Progressive Ave.
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declares that the product

Product Name: NEN® DNA Analyzer

Model Number(s): 4300S, 4300L

Product Options: All

conforms to the following Product Specifications:

Safety: UL 3101-1 : 1993
CSA 22.2 #1010.1 : 1992
EN61010-1 : 1993
EN61010-1 : Amendment 1 : 1995
21 CFR Chapter 1 Subchapter J : Class 1 Laser Product
IEC 825-1 : 1994 : Class 1 Laser Product

EMC: EN61326 : 1998 - Class A
EN61000-4-2 : 1995 : 8kV CD / 15kV AD
EN61000-4-3 : 1995 : 3V/m
EN61000-3-3 : 1995
EN61000-3-2 : 1995

Supplementary Information:

The product herewith complies with the requirements of the Low Voltage Directive 73/23/EEC and the EMC Directive 89/336/EEC.

May 1, 2003
Document #53-07045

Dave Dilley
Director of Engineering

Note on Safety

LI-COR products have been designed to be safe when operated in the manner described in this manual. The safety of this product can not be guaranteed if the product is used in any other way than is specified in this manual.

Equipment markings:



The product is marked with this symbol when it is necessary for you to refer to the manual or accompanying documents in order to protect against damage to the product.



The product is marked with this symbol when a hazardous voltage may be present.



The product is marked with this symbol for a Chassis Ground connection.

WARNING **Warnings** must be followed carefully to avoid bodily injury.

CAUTION **Cautions** must be observed to avoid damage to your equipment.

Manual markings:

WARNING **Warnings** must be followed carefully to avoid bodily injury.

CAUTION **Cautions** must be observed to avoid damage to your equipment.

NOTE **Notes** contain important information and useful tips on the operation of your equipment.

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Patents granted as of April 1, 2003: United States: 5,346,603; 5,230,781; D0,368,143; 5,360,523; 5,571,388; 5,366,603; 5,549,805; 5,534,125; 5,863,403; 5,755,943; 5,723,298; 4,729,947; 5,207,880; 6,004,446; 6,143,151; 6,143,153; 6,495,812. Australia: 588229; 662273; 670622. Canada: 1,465,405; 1,388,840; 1,481,989; 1,230,161; 1,480,850; 1,230,161. Europe: 0157280B1; 0533302B1; 0670374B1; 0753584A1; 0753584A1; 0754765A2. Japan: 2007209. Other U.S. and foreign patents pending, University of Nebraska and LI-COR Inc.

**Federal Communications Commission
Radio Frequency Interference Statement**

WARNING: This equipment generates, uses, and can radiate radio frequency energy and if not installed in accordance with the instruction manual, may cause interference to radio communications. It has been tested and found to comply with the limits for a Class A computing device pursuant to Subpart J of Part 15 of FCC rules, which are designed to provide a reasonable protection against such interference when operated in a commercial environment. Operation of this equipment in a residential area is likely to cause interference in which case the user, at his own expense, will be required to take whatever measures may be required to correct the interference.

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Chapter 1: Safety and Operational Information

In This Manual...

The following topics are discussed in this manual:

- Safety considerations.
- Instrument placement and power considerations.
- Description of detection optics and signal processing.
- Instrument operation and internal server software.
- Use and maintenance of the electrophoresis apparatus.
- Maintenance recommendations.
- Troubleshooting

All references in this manual to the NEN[®] Model 4300 DNA Analyzer refer to either the Model 4300S or 4300L except where noted.

Safety Considerations

Laser Safety

The Center for Devices and Radiological Health (CDRH) was established in October, 1982, by the U.S. Food and Drug Administration (FDA) to protect the public health in the fields of medical devices and radiological health.

Manufacturers of products subject to performance standards under the Radiation Control for Health and Safety Act of 1968 are required to furnish various reports to the CDRH.

The NEN[®] Model 4300 DNA Analyzer is certified as a Class I laser product, containing a Class IIb laser. This means that hazardous laser radiation is *not* emitted outside the instrument. Laser radiation emitted inside the instrument is confined within protective housings and external covers. A series of safety interlocks ensures that the laser beam cannot escape during any phase of user operation. In addition, the laser beam is focused through the aperture onto the gel apparatus, after which it disperses and is blocked by a shield on the lower buffer tank cover.

The CDRH implemented regulations for laser products on August 1, 1976 (CDRH radiation performance standard 21, Code of Federal Regulations Chapter 1, Subchapter J). Compliance for products marketed in the United States is mandatory. The label that must be attached to laser products marketed in the United States is shown in Figure 1-1 and is located on the rear of the sequencer, indicating compliance with CDRH regulations.

MODEL 4300 DNA SEQUENCER

A PRODUCT OF NEN LIFE SCIENCE PRODUCTS, INC.
MANUFACTURED IN THE USA BY LI-COR, INC.
FOR NEN LIFE SCIENCE PRODUCTS, INC.

NEN LIFE SCIENCE PRODUCTS, INC. 549 ALBANY STREET BOSTON, MA 02118 USA	LI-COR, INC. 4421 SUPERIOR STREET LINCOLN, NE 68504 USA
--	---

THIS DEVICE COMPLIES WITH PART 15 OF THE FCC RULES. OPERATION IS SUBJECT TO THE FOLLOWING TWO CONDITIONS: (1) THIS DEVICE MAY NOT CAUSE HARMFUL INTERFERENCE; AND (2) THIS DEVICE MUST ACCEPT ANY INTERFERENCE RECEIVED, INCLUDING INTERFERENCE THAT MAY CAUSE UNDESIRED OPERATION.

CERTIFIED TO BE IN COMPLIANCE WITH 21 CFR 1040.10
PATENTS GRANTED AS OF FEBRUARY 1, 2002 **UNITED STATES** 5,346,603 5,230,781
D0,368,143 5,360,523 5,571,388 5,366,603 5,549,805 5,639,874 5,534,125 5,863,403
5,355,943 5,800,995 5,723,298 4,729,947 5,207,880 6,004,446 6,143,151 6,143,153
6,207,421 **AUSTRALIA** 588229 662275 670622 **CANADA** 1,465,405 1,388,840 1,481,989
1,480,850 1,230,161 **EUROPE** 0157280B1 0533302B1 0670374B1 0753584A1
0754765A2 **JAPAN** 2,007,209
OTHER U.S. AND FOREIGN PATENTS PENDING UNIVERSITY OF NEBRASKA AND LI-COR, INC

MODEL NUMBER	<input type="text"/>
SERIAL NUMBER	<input type="text" value="DNA43-"/>

Figure 1-1.

CAUTION: Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

The NEN[®] Model 4300 DNA Analyzer contains two lasers; one emitting at 785 nm, and one at 685 nm. Each has a peak power rating of 30 milliwatts. The 685 nm laser emits visible laser radiation – ***direct exposure to either beam may cause eye damage.***

The label shown below is affixed to the rear door of the NEN[®] 4300 instrument case.

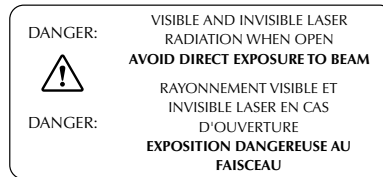


Figure 1-2.

High Voltage Power Supply

The Model 4300 uses a high voltage power supply for gel electrophoresis. ***Do not attempt to disassemble or service the power supply at any time!*** There are no user serviceable parts inside the Model 4300.

Safety Interlocks

The Model 4300 has two safety interlocks that prevent access to the laser and high voltage power supply when the instrument case is opened during operation. ***Do not attempt to defeat these interlocks.*** The interlocks are located on the front and rear doors of the instrument.

If the front door is opened during operation, three things happen:

- A tone sounds to alert the user that a safety interlock has been violated.
- The front panel display indicates that operation is paused and the front door is open.
- The **High Voltage** front panel light on the instrument turns off, indicating that the laser and high voltage power supply are turned off.

If the door is opened during the pre-run, the pre-run starts over when the door is closed. When the door is closed again, you must press the **Start (Y)** key to select the **Continue** option.

Laser Safety Shield

The lower buffer tank cover is fitted with a black shield (Figure 1-3). When the cover is in place, the shield blocks the laser aperture, and is the primary barrier against laser radiation. Do not operate the Model 4300 if this shield is damaged or missing!

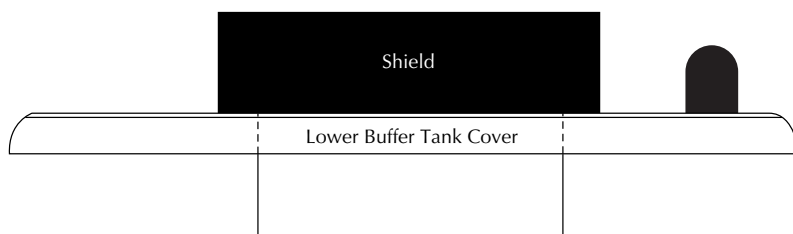


Figure 1-3. Lower buffer tank cover, with laser safety shield.

Chemical Safety

LI-COR recommends that all biochemicals be handled carefully, and that safe laboratory procedures be followed at all times. Be aware of the hazards associated with any chemical before you begin work.

Placement in the Laboratory

A Model 4300S weighs approximately 29 kg (64 lb) and a Model 4300L weighs 36 kg (80 lb). The instrument should be placed on a level laboratory bench that is capable of bearing its weight and does not sway during operation.

Ambient Laboratory Conditions

Place the Model 4300 away from external heat sources (furnaces, radiators, distillation devices, etc.). Additional heating can cause high temperatures within the enclosure, resulting in instrument shut down. Place the instrument away from sinks or other sources of water that pose a shock hazard. Recommended operating conditions are 15-35 °C and a dew point not greater than 20 °C (non-condensing). Complete operating specifications can be found in Chapter 10.

Ventilation

Fans located on both left and right sides ventilate the instrument. To prevent overheating and ensure reliable operation, do not block or cover these openings (see *Routine Maintenance* later in this chapter).

Space Requirements

The Model 4300S and 4300L are both 47 cm (18.5") deep and 51 cm (20") wide. When the front door is fully open, 102 cm (40") of width is required. The Model 4300S is approximately 59 cm (23") tall and the Model 4300L is 84 cm (33") tall.

Moving the Model 4300

Be cautious when moving the instrument. Always be sure to lock the scanning system by moving the motor lock switch on the instrument back panel to the **Transport** (right) position before moving the instrument. Use the tip of a pen or a small screwdriver to move the

motor lock switch. After moving the instrument, return the motor lock switch to the **Operate** (left) position. The following label is located below the motor lock switch:

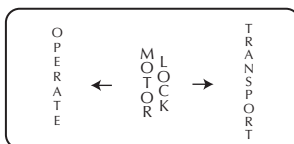


Figure 1-4. Motor lock switch label.

Remove the gel apparatus and buffer tanks before moving the instrument. Use a minimum of two people when moving the instrument. Place your hands under the metal enclosure on each side of the instrument, and keep the instrument vertical as you lift. Gently set the sequencer at its new position.

The instrument should be leveled after it is moved. A small bubble level is included in the spare parts kit. Center the level on the platform below the heater plate. Each of the 4 plastic feet on the bottom of the instrument case has a threaded metal insert that can be turned up or down to adjust its height. Adjust the feet as necessary, until the instrument is level. After the instrument is level, make sure all four feet contact the bench top to prevent the instrument from rocking.

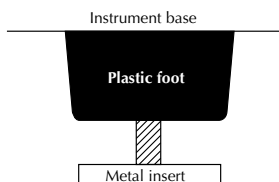


Figure 1-5. Turn the metal inserts on the feet to adjust the height.

Electrical Considerations

The ground pin of the power cord should be connected to earth ground in order to meet U.S. Underwriters Laboratory (UL) safety code compliance.

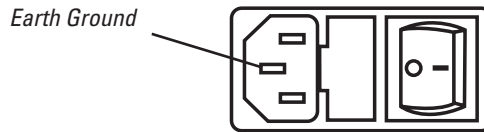


Figure 1-6. Model 4300 power entry module power cord connection.

Power Cords

The Model 4300 is equipped with a 3-wire grounding-type plug. This plug will only fit into a grounding-type outlet. This is a necessary safety feature. If you are unable to insert the plug into the outlet, you will need to replace the outlet. Do not defeat the purpose of the grounding-type plug.

Do not locate the Model 4300 where the power cord will be walked on or exposed to water or chemical spills.

The Model 4300 draws approximately 4 amperes current at 120V. If an extension cord is used, make sure the total of the ampere ratings on the instruments plugged into the extension cord does not exceed the extension cord ampere rating. Also make sure the total amperage of instruments plugged into the wall outlet does not exceed the amperage capacity for the outlet. In the United States, this is usually 15 or 20 amperes.

Fuse Replacement

Important: Replace fuses only with fuses of the specified type and current ratings.

The mains fuses are accessible from the rear of the instrument. Disconnect the power cord and pry open the cover of the fuse holder on the power entry module using a screwdriver, as shown below.



Figure 1-7. Insert screwdriver as shown to pry open the cover of the fuse holder on the power entry module.

Replacement fuse specifications are listed below:

<u>Function</u>	<u>Current Rating</u>	<u>Type</u>	<u>Size</u>	<u>LI-COR Part#</u>
Mains @ 100/120 VAC	8A	Fast (F)	5 × 20 mm	439-04640
Mains @ 220/240 VAC	4A	Time-Delay (T)	5 × 20 mm	439-04482

Important: There are no other fuses or user-serviceable parts inside the Model 4300.

Uninterruptible Power Supplies

Even momentary power interruptions can cause data collection to stop in the middle of electrophoresis. All Model 4300's shipped in the United States include an Uninterruptible Power Supply (UPS) that provides approximately 20 minutes of operation. A UPS is strongly recommended to ensure consistent operation.

Routine Maintenance

The Model 4300 requires only minimal maintenance. However, as with any equipment utilizing high voltages, there is a danger of fire or electrical shock if the equipment is not properly maintained. LI-COR recommends that you routinely inspect the Model 4300 and the electrophoresis apparatus. The following are some general maintenance guidelines:

- Wipe all chemical spills from the case and/or heater plate to prevent damage to the surface coating. Wipe away any caked-on chemical residue before starting a run.
- Clean the two fan filters once a month to prevent the enclosure from overheating.
- Inspect all cables and power cords for evidence of fraying, exposed wire, or loose connections.
- Check for potential leaks that may occur due to crazing or cracking of the buffer tanks. Buffer leaks can lead to electrical arcing and the potential for fire.
- Keep the electrophoresis area free of organic solvents and other combustibles.
- Check the platinum electrode on the upper and lower buffer tank lids for signs of breakage. Replace the lid if necessary.
- Clean the exterior case parts with warm water and a damp cloth. The exterior case parts are painted with a durable urethane coating that is resistant to chemical spills. Do not use scouring compounds, solvents such as acetone, benzene, carbon tetrachlorides, lacquer thinner, or alcohol to clean the case.

What's Next?

If you purchased e-Seq™ or Saga™, a good way to learn how to operate the Model 4300 is to complete the tutorial exercises in the *e-Seq* or *Saga Tutorial Manual*. If you did not purchase application software, Chapters 5-9 of this manual describe the operation of the Model 4300 server software, which is accessed via an Internet browser.

Chapter 2: System Description

Overview

The NEN[®] Model 4300 is a network server device. The operating system provides network communications (TCP/IP), security protocols, instrument operation, and temporary data storage.

As a network server device, the Model 4300 can be operated in a network or stand-alone configuration (see *NEN[®] Model 4300 Installation Manual*). In a network configuration, computers anywhere on the network can be used to start runs, retrieve and analyze data. If the network is connected to the Internet and remote access is permitted, any computer on the Internet can be used to operate the Model 4300.

In a stand-alone configuration, the computer and instrument are connected to each other with no connection to an external network. Only the computer connected to the Model 4300 can be used to operate the instrument in a stand-alone configuration.

Runs can be initiated using either e-Seq[™] DNA Sequencing Software or Saga[™] Software for microsatellite or AFLP[®] analysis. These programs provide both instrument operation and data analysis functions. Instrument operation and DNA sequencing can also be performed using an Internet browser to log on to the Model 4300 server and initiate electrophoresis. Runs can also be started directly from the instrument front panel without using any computer software (Chapter 4).

After a run is initiated using a browser or LI-COR application software, the server software in the Model 4300 controls electrophoresis and data storage until the run is complete. The computer is not used during the run and can be used for other tasks.

Data are stored on an internal hard disk in the Model 4300. The number of runs that can be stored is highly dependent on the application. DNA sequencing images are the largest and require as much as 20 MB per image file (two per run). **Note that the Model 4300 should be used for temporary storage only; when the hard disk is full, the oldest files are overwritten automatically to make space.**

When using LI-COR application software, data are automatically transferred to the computer after the run is complete. The User Guide for each application program has complete descriptions of starting runs, retrieving data, and analyzing data. When using an Internet browser, runs can be opened and saved to hard disk as described in Chapter 7.

The server software in the Model 4300 also performs a variety of security, account management, and troubleshooting functions as described in Chapters 7, 8, and 9. For DNA sequencing applications, the internal server software can sequence images in real time and generate SCF curve files compatible with widely used analysis software. These additional functions are all accessed using Internet browser software to log on to the Model 4300 server.

Laser/Microscope Description

The fluorescence peaks of the two IRDyes™ used with the Model 4300 are separated by over 110 nm. Two independent excitation/detection channels are used to eliminate errors due to fluorescence overlap. Each channel is optically tuned to measure fluorescence from only one of the IRDyes. A separate image is collected for each IRDye.

Two solid state diode lasers simultaneously provide light excitation at 685 and 785 nm. Peak power rating is 30 mW. Collimating lenses, optical bandpass filters, and a focusing lens focus and tune the laser beams to produce an excitation spot in the gel. The small spot size allows better resolution of closely spaced bands as they pass the detection window.

The microscope electronics modulate the laser beams to discriminate the IRDye signal fluorescence from background fluorescence. The microscope detection optics focus on the excitation spot and collect light from the fluorescing IRDyes.

After collection by the microscope objective, the light is passed to a dichroic mirror. The mirror splits the light and essentially sorts the fluorescent signals by transmitting the light above 810 nm and reflecting light below 750 nm. Transmitted and reflected light travels two independent paths through optics designed to remove scattered and stray light. The light is ultimately focused onto one of two avalanche photodiodes that converts the light to an electrical signal for processing by the microscope detection electronics.

In the microscope detection electronics, the signal is amplified, filtered, and finally converted to a digital value by an analog-to-digital converter. The digital signal is demodulated, filtered again, and coordinated with the microscope position by a Digital Signal Processor (DSP) to produce the image file.

16-bit image data (>65,000 grayscales per pixel) are stored as the laser/microscope scans back and forth across the gel during electrophoresis. If a computer running LI-COR application software is connected during electrophoresis, image data can be viewed on the display in real time as they are collected. The image can also be displayed as it is collected using the browser interface.

Scanning Platform

Diode laser technology provides high reliability, low power consumption (<0.2 W), low noise, low cost, light weight, and small size. Because of their small size, the lasers are mounted directly to the scanning platform. The entire compact laser/microscope assembly travels on a platform that moves from side to side across the entire width of the scanning window.

During the pre-run, the microscope is focused in the gel. A focus motor moves the microscope perpendicular to the gel apparatus in order to position the focal plane of the detection microscope precisely in the gel.

Focusing the microscope in the gel optimizes image quality and allows the Model 4300 to accommodate a variety of gel thicknesses. Scan speed is adjustable through either the browser interface or application software, adding flexibility when using alternate gel thicknesses, gel lengths, gel matrices, and electrophoresis voltages. Recommendations for scan speeds and electrophoresis conditions are given in Chapter 7.

During microscope focusing, the nominal background fluorescence of the glass plates is used to automatically focus the microscope on the gel. The fluorescence profile of the glass-gel-glass sandwich is used to adjust the focal plane of the microscope with no user interaction.

The Heater Plate

The heater plate contains thermostatic controls to maintain the gel at the desired temperature. The rear gel plate rests against the heater plate, so heat is provided by direct thermal contact. The temperature of the gel (30-50 °C) is affected both by the electrophoresis power conditions and contact with the heater plate. The heater plate can heat gels up to 50°C, though temperatures may exceed 50 °C due to gel heating when electrophoresis power is applied. The heater plate can also be turned off using the browser interface, e-Seq, or Saga for applications when it is not needed.

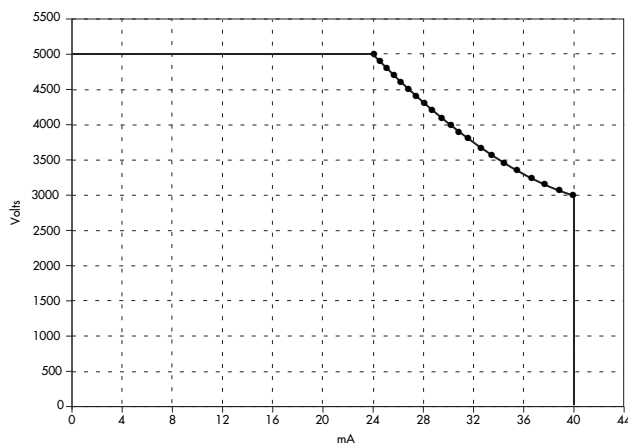
The laser microscope assembly scans through a horizontal aperture at the bottom of the heater plate.

The heater plate is anodized with a black protective coating. This coating can be etched (turned white) by high voltage leaking through spilled buffer solution, so be sure to wipe any spilled solution off the heater plate before starting gel electrophoresis. Etching of the anodized coating will not harm the heater plate, nor will it affect the operation of the instrument.

The High Voltage Power Supply

The Model 4300 uses a 5000 volt high voltage power supply for gel electrophoresis. The HIGH VOLTAGE front panel light illuminates when the high voltage is on. The current and voltage outputs for this power supply are represented in the graph below. Maximum power output is 120W.

Figure 2-1. Current and voltage output for the 5000V electrophoresis power supply.



Error Detection

The Model 4300 constantly monitors for error conditions, including three types of errors related to the electrophoresis apparatus: electrical leaks, open circuit conditions, and electrical arcing conditions. These problems generally occur because of improper assembly of the gel apparatus, buffer leaks, or improper cleaning of the gel apparatus before installation. The *Model 4300 Applications Manual* (Section 6, DNA Sequencing tab) discusses these errors and has tips for troubleshooting the electrophoresis apparatus.

If any of these error conditions are detected, the electrophoresis power supply is turned off and scanning is stopped. If application software such as e-Seq or Saga is running, the error message is immediately displayed in the application software. In e-Seq or Saga, a diagnostic report can be created to view any error messages during unattended operation. If you start a run using the browser interface, error messages can be viewed through the **View Run Log** link on the Utilities page.

Chapter 3: Electrophoresis

Apparatus Description

General Description

Figure 3-1 is an exploded diagram showing the parts of the electrophoresis apparatus. The individual parts of the apparatus are described throughout this section. Complete assembly instructions and procedures for gel pouring can be found in the Model 4300 Applications Manual.

Description

1. Upper buffer tank lid
2. Silicone tubing gasket material
3. Upper buffer tank
4. Comb
5. Spacers
6. Rectangular glass back plate
7. Notched glass front plate
8. Left rail assembly
9. Right rail assembly
10. Lower buffer tank lid
11. Lower buffer tank
12. High voltage cable

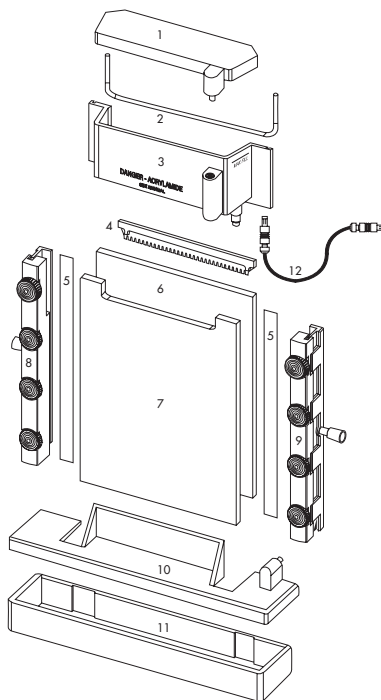


Figure 3-1. Exploded view of electrophoresis apparatus.

Gel Plates

Gel plates are 25 cm wide, 0.5 cm thick and come in standard heights of 41, 25, and 18 cm. Separation distance for each of the three plate heights is 31, 15, and 9 cm, respectively. 66 cm plates with a 56 cm separation distance are also available for the Model 4300L. The front plate is distinguished by a notch at the top that allows insertion and removal of the comb.

The gel plates used with the Model 4300 are borofloat glass, which has excellent flatness that can be used for gels as thin as 0.2 mm without grinding or polishing.

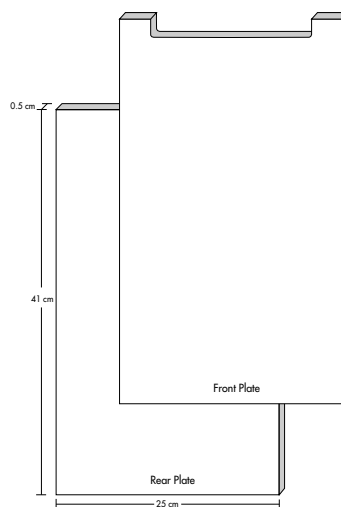


Figure 3-2. 41 cm gel plates.

Note: Prior to the Model 4300, various gel plates were used with LI-COR Model 4200 and 4000 DNA sequencers. If you have one of these older instruments in your lab, it is important to be able to distinguish older gel plates. Soda lime glass was used with the Model 4000 and one-dye versions of the Model 4200. Soda lime glass is distinguished by its blue-green color and gel plates have two corners dubbed. Two-dye Model 4200 systems originally used borofloat glass (see below) and now require Starfire® glass (a change in borofloat glass composition made focusing difficult on the Model 4200). Starfire glass has a comparatively blue color and gel plates have two corners dubbed. Borofloat plates have only one corner dubbed and have a yellow-green or yellow-white color. The borofloat plates that LI-COR currently sells are only compatible with

the Model 4300. Soda lime glass should not be used with the Model 4300 or the two-laser version of the Model 4200. If you have soda lime, Starfire, or older borofloat plates in your lab, you may want to consider marking them with a diamond pencil so they can be distinguished. Never mix plates with different glass compositions in the gel apparatus. This can result in focusing failures or image intensity problems.

Gel plates are most commonly damaged during apparatus assembly or cleanup. The following recommendations will help prevent chips and maximize lifetime:

- 1) During assembly, do not overtighten the apparatus rails. Overtightening rails can cause cracks to initiate near chips.
- 2) Always use a plastic wedge (P/N 6540-165, included) to separate gel plates during cleanup. Never use metal tools to pry plates apart. Lab spatulas, razor blades, or similar tools can chip plates.

Electrophoresis Apparatus Rails

An exploded diagram of an electrophoresis apparatus rail is shown in Figure 3-3 below. There are two clamping rails in the gel apparatus. The lower knobs are used to clamp the glass plates, and the upper knob on each rail is used to clamp the upper buffer tank. The tank clamp knob can be distinguished from the glass clamp knobs by its larger size.

Pressure is applied to hold the upper buffer tank and the glass plates together by turning the clamp knobs clockwise. Tighten the knobs lightly – just past the point of resistance. **Do not overtighten the clamp knobs.** Overtightening can damage the glass plates and also increases the possibility of leaks between the glass plates. Overtightening or uneven tightening can also result in a “smile” in the band patterns. Notice that the rail backs have a set of compression springs that provide a resistance to the clamp knobs, as shown in Figure 3-4.

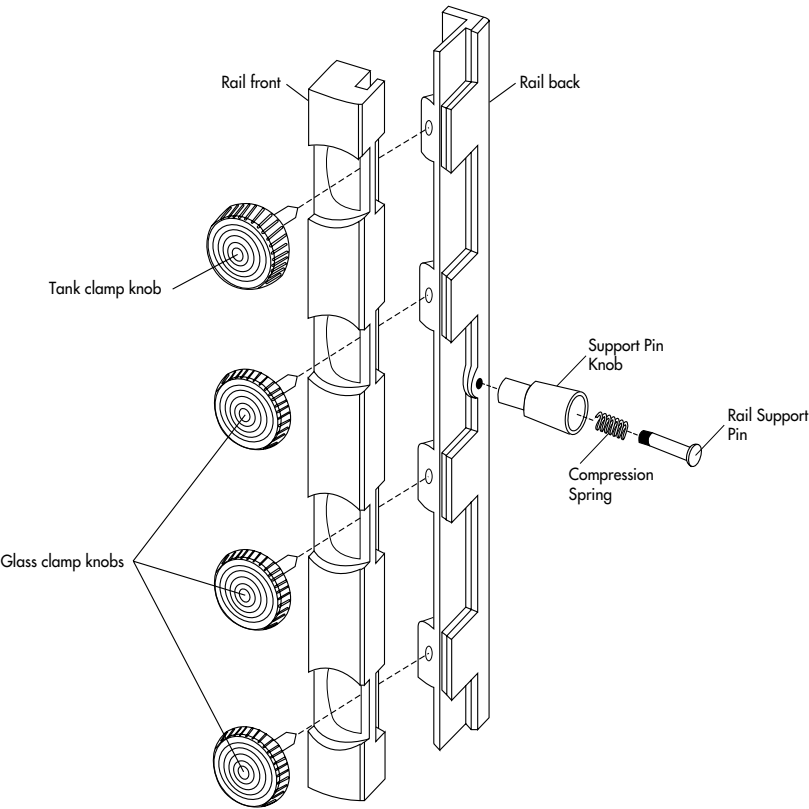


Figure 3-3. Exploded view of rail assembly (right rail shown).

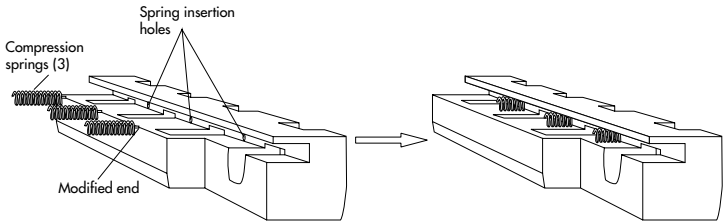


Figure 3-4. Side view of rail showing compression springs.

Support Pin Knobs

The support pins hold the gel sandwich against the heater plate when mounted on the instrument. The pins rest on the apparatus support brackets on either side of the heater plate. The support pins also allow minor adjustments to the position of the electrophoresis apparatus in the event that there is an aberration in the gel at the location of the scanning window. Aberrations in the gel such as air bubbles, dust particles, etc., can cause images to have a vertical streak.

To adjust the apparatus, pull the support pin knobs outward until the apparatus drops down (approximately 0.16 cm). The support pin knob is spring loaded and when pulled out, the pin inside is exposed which is a smaller diameter than the outer knob. This difference is usually enough to move small aberrations out of the field-of-view of the scanning microscope.

Cleaning the Rails

Rails should be cleaned with water and a mild detergent. Do not use organic solvents, alcohols, or abrasive cleansers. If it becomes necessary to disassemble the rails to clean them, simply unscrew the 4 clamp knobs all of the way, until the rail back is released.

Caution: Make sure rails are clean and completely dry before use.

Buffer Tanks

As shown in Figure 3-1, there is an upper and a lower buffer tank. Each buffer tank holds buffer solution that makes electrical contact with both a platinum electrode and the gel. The buffer in the tanks completes the electrical circuit between the top and bottom of the gel.

The upper buffer tank attaches to the front of the gel plates by sliding the tank into the upper, notched portion of the apparatus rails and tightening the tank clamp knobs. Before inserting the tank, a 30 cm piece of silicone tubing (provided) is inserted into the recessed groove on the back of the tank to prevent buffer from leaking down the front of the gel plate. Complete assembly and disassembly instructions are given in the Model 4300 Applications Manual.

The upper buffer tank is connected to the high voltage connector on the instrument chassis with the high voltage cable (Figure 3-1). The cable is terminated on both ends with a male connector that fits into the female receptacles on the tank and chassis. There is no polarity designation for the high voltage cable.

The lower buffer tank rests on the platform under the heater plate. The tank has two recessed areas where the apparatus rails rest when the assembled apparatus is installed on the apparatus supports (Figure 3-5).

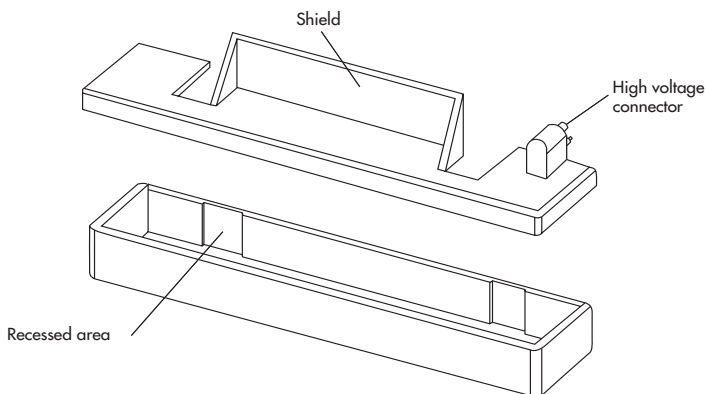


Figure 3-5. Lower buffer tank and lid.

Both the upper and lower buffer tank lids have a platinum wire electrode that contacts the buffer solution. The electrical circuit between the top and bottom of the gel is complete as long as the buffer solution in both tanks is in contact with both the gel and the electrode. If a leak develops and the buffer solution no longer contacts the electrode in the upper buffer tank, the electrophoresis power supply automatically shuts off. Likewise, the power supply shuts off if the level of buffer solution in the lower tank drops below the bottom of the gel plates.

Periodically rinse the portion of the tanks and lids that contact the buffer solution. Use water and/or a mild detergent, followed by a thorough distilled water rinse. **Do not use alcohol, organic solvents, abrasives, or strong detergents to clean the tanks.** Exposure to alcohols can result in "crazing" (fine cracks that develop over time).

Important: Water should never contact the high voltage connectors. Also be aware that the platinum electrode is very delicate.

Combs and Spacers

Spacers are available to make gels with 0.2 and 0.25 mm thickness. At the time of printing 64- and 48-well plastic combs (sharkstooth and rectangular tooth) are available for both 0.2 and 0.25 mm gel thicknesses. Browse LI-COR's web site for the most current information on the available combs.

Rectangular tooth combs have a center-to-center well spacing of 3.4 mm and 2.25 mm for 48- and 64-well combs, respectively. Sharkstooth combs have a center-to-center well spacing of 3 mm and 2.25 mm, respectively for 48- and 64-well combs. 64-well comb spacing matches that of 96-well plates, allowing the use of multi-channel pipettes.

Combs and spacers can generally be cleaned with water and/or a mild detergent, followed by a thorough distilled water rinse.

Chapter 4: Front Panel Operation

This chapter discusses starting runs using the Model 4300 front panel and several other operations that can be performed using the instrument front panel. Many of these functions are also available through either the browser interface (Chapters 5-9) or through LI-COR application software, giving you multiple methods to operate the instrument.

Front Panel Controls

The front panel display is a 2-line, 20 character Vacuum Fluorescent Display (VFD) for displaying operating parameters. The front panel keypad (Figure 4-1) is used to enter software commands and to edit operating parameters.

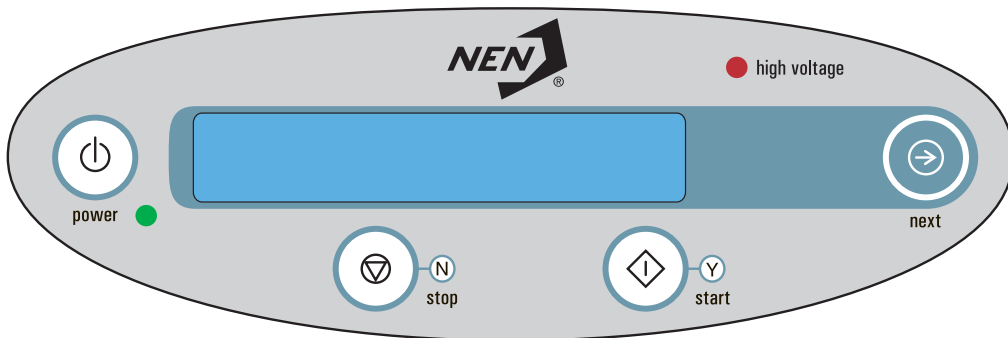


Figure 4-1. Model 4300 keypad and display.

The front panel keys perform the following operations:

Power: Powers the instrument On/Off. Also performs instrument reset if held down for 4 seconds while the instrument is on.

Stop (N): Stops or pauses a run, or cancels the option currently visible on the display.

Start (Y): Starts a run, or confirms the option currently visible on the display.

Next: Scrolls through options for a given software operation.

For clarity, the symbols on the keypad keys are often duplicated in options on the display. For example, a **No** or **Cancel** option on the display usually has a triangle next to it to match the symbol on the **Stop (N)** key. Likewise, **Start** or **Yes** options on the display have a diamond shape next to them to match the **Start (Y)** key and **Next** functions have an arrow beside them.

Powering the Instrument On and Off

Powering On the Model 4300

Press the **Power** key (Figure 4-1) on the front panel to power the instrument on. The power light will turn on immediately. The display will be blank or show messages for several minutes while the instrument is being initialized. When the instrument is ready for operation “4300 DNA Analyzer” is shown on the display.

Powering Off the Instrument

The Model 4300 and Ethernet switch (if any) can operate continuously, or be powered off when idle. If the instrument needs to be powered off, briefly press the **Power** key on the front panel keypad.

A message will be displayed asking you to confirm that you want to shut down the instrument. Press the **Start (Y)** key to shut down or **Stop (N)** to resume operation. Make sure you press the **Power** key only briefly, or you will reset the instrument as described below.

Users with *Administrator* rights can also shut down the instrument using the **Shutdown The Instrument** link on the Utilities page of the browser interface as described in Chapter 9.

Resetting the Instrument

A reset function is provided in the event that the instrument is unresponsive and you cannot connect to the instrument using an Internet browser. The instrument can be reset by holding down the **Power** key for at least 4 seconds until the instrument turns off. This reset function actually cuts power to the instrument and internal hard disk, so it should be used only when communication cannot be established by any other means. Unless there is a hardware problem, normal operation should resume after the **Power** key is pressed again to turn the instrument back on, though start up may take several minutes.

Checking Instrument Status and Addresses

When the instrument is powered on and not in some other software routine, you can press the **Next** key repeatedly to scroll through the following parameters:

<u>Prompt</u>	<u>Parameter Description</u>
MAC ADDR:	Network MAC Address
CR:	Name of the current run (not shown if a run is not in progress).
ELAPSED TIME:	Elapsed time of the current run (not shown if a run is not in progress).

USER:	Name of the user who started the current run (not shown if a run is not in progress).
IP:	Current IP address of the Model 4300.

Changing Network Addresses

The keypad can be used to choose the method by which the network IP address is assigned (Static vs. DHCP) and to enter the IP address for Static addressing. The procedures for configuring both Static and DHCP addressing are discussed below.

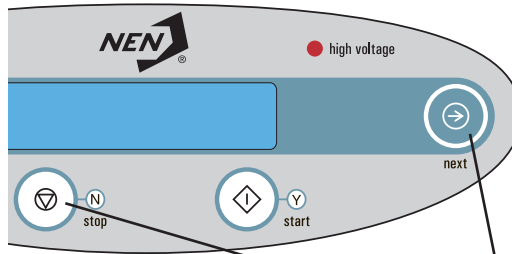
Entering a Static IP Address Using the Keypad

Whether you are using e-Seq software, Saga software, or the browser interface, the IP address is used to connect to the Model 4300 via TCP/IP. For this reason, a static IP address that does not change provides more trouble-free operation compared to dynamic addresses (DHCP) that automatically expire at periodic intervals and are re-assigned.

Static IP addresses in the Model 4300 are entered and displayed as four groups of three numbers, separated by periods. An example is 172.024.041.180. Your administrator may give you the IP address using abbreviated notation, such as 172.24.41.180. Leading zeros are commonly left off in the abbreviated form. The “long” form of the address must be entered in the Model 4300. If you have been given an abbreviated address, add zero(s) to the left of any group of numbers that has less than three digits. In the abbreviated IP address above, this means that 24 should be entered as 024 and 41 should be entered as 041.

Use the following steps to enter a static IP address:

- 1) Press the **Stop** and **Next** keys simultaneously.



Press both the **Stop (N)** and **Next** keys simultaneously to enter the IP address.

The **IP TYPE** prompt should be displayed.



If the **IP Type** prompt is not displayed, you may not have pressed the **Stop** and **Next** keys simultaneously.

- 2) If necessary, choose **Static** by pressing the **Next** key to toggle between **Static** and **DHCP** until **Static** is displayed.



- 3) Press **Start (Y)** to choose **Static** addressing.



- 4) Enter the first number of the IP address by pressing **Start (Y)** to increment the first digit from 0 through 9 until the first number of your IP address is displayed.



- 5) Press **Next** to move the cursor to the next digit. Continue to enter each digit of the IP address by using the **Start (Y)** key to increment the number and pressing **Next** to move to the next digit.
- 6) When you have finished entering the address, press **Next** again. The “Change IP Address” prompt is displayed so that you can accept the changes (**Start (Y)**) or cancel the changes (**Stop (N)**).



- 7) Press **Start (Y)** to confirm the address or **Stop (N)** to abandon changes. (Notice that the diamond shape beside **YES** on the display matches the diamond in the **Start (Y)** key and the triangle beside **NO** matches the triangle in the **Stop (N)** key.)

Correcting Mistakes While Entering the IP Address

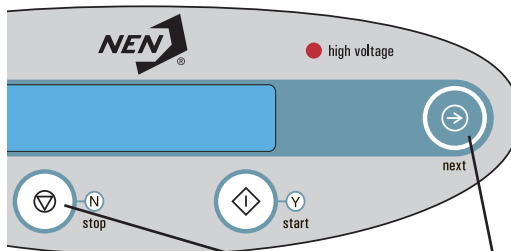
If you make a mistake entering the IP address, you cannot back up and make changes. Instead, you can press **Stop (N)** at any time to cancel address entry. The IP Address entry software can then be restarted as described above by simultaneously pressing both the **Stop (N)** and **Next** keys. (If they are not pressed simultaneously, either the **Next** or **Stop** function will be executed.)

Using Dynamic Addressing (DHCP)

When using DHCP, the Model 4300 is automatically assigned a unique IP address, making IP address management easier for

network administrators. DHCP addresses are usually set to expire automatically at some interval, after which the Model 4300 is automatically assigned a new address. When this happens, you will not be able to log on to the instrument until you determine the new IP address as described below.

- 1) To use DHCP, start by simultaneously pressing both the **Stop** and **Next** keys on the keypad.



Press both the **Stop (N)** and **Next** keys simultaneously.

The **IP TYPE** prompt is displayed.

```
IP TYPE:  STATIC
↑CANCEL  →OK  →MORE
```

*If the **IP Type** prompt is not displayed, you may not have pressed the **Stop** and **Next** keys simultaneously.*

- 2) Press the **Next** key to toggle between **Static** and **DHCP** until **DHCP** is displayed.



- 3) Press **Start (Y)** to select **DHCP**.



- 4) At the “**START DHCP NOW**” prompt, press **Start (Y)** to start DHCP or **Stop (N)** to cancel.

Determining a Dynamically Assigned IP Address

After DHCP is enabled, an address will be automatically assigned to the Model 4300 by your network (it can take as long as several minutes depending on the DHCP server). The current IP address can be determined by repeatedly pressing the **Next** key to scroll through various parameters until the **IP** label is displayed along with the current IP address.

Starting Runs From the Front Panel

The browser interface of the Model 4300 server can be used to save electrophoresis conditions files. These files specify all the electrophoresis parameters for the pre-run and run, including current, voltage, power, scan speed, etc. Chapter 7 describes how to use the **Adjust Electrophoresis Conditions** link on the Utilities page of the browser interface to edit and save electrophoresis conditions files. Once created, any electrophoresis conditions file stored on the Model 4300 can be chosen when starting a run from the front panel keypad.

Runs can be started from the front panel as follows:

- 1) Press the **Start (Y)** key to initiate the run.
- 2) Use the **Next** key to scroll through the list of electrophoresis conditions files until the desired file name is displayed.

- 3) Press **Start (Y)** to select the displayed electrophoresis conditions file.

Note: If you select an electrophoresis conditions file that has autosequencing enabled, autosequencing will not be performed because the sample loading order is unknown. If you want to use the autosequencing software in the Model 4300, you must start the run using an Internet browser as describe in Chapter 7.

- 4) At the “Pre-run?” prompt, press the **Start (Y)** key to start the pre-run for the first run on a gel.

Note: If you are reloading gels for microsatellite or AFLP® applications, you can also press **Stop (N)** to skip the pre-run (see the Model 4300 Applications Manual for details on reloading gels).

The high voltage light is illuminated to indicate that the high voltage power supply is operating. The elapsed time of the pre-run is shown on the display. The duration of the pre-run is determined by the time specified in the electrophoresis conditions file.

During the pre-run, the microscope is focused in the gel and the detection electronics are optimized for the run. The gel is brought up to the specified temperature by the heater plate and normal electrophoresis power is applied to the gel.

The pre-run can be paused at any time by pressing the **Stop (N)** key. To resume a paused run, press the **Start (Y)** key. To quit a paused run, press the **Stop (N)** key. If you pause a pre-run before the microscope focusing software has finished, the pre-run will start over from the beginning if you resume the run.

After the microscope has been focused but the pre-run is still in progress, the display shows that the **Load** function can be activated by pressing the **Start (Y)** key. The **Load** function lets you skip the remainder of the pre-run and go directly to sample loading (not recommended for normal operation).

Occasionally you may see an error message on the display like “Focus Error”, indicating that focusing could not be completed normally. A common cause for this error is that the gel is not

mounted flush against the heater plate and the misalignment is too great for the microscope to compensate. An error of this nature can be corrected by opening the door, re-aligning the gel and pressing **Start (Y)** to select the **Retry** option on the display. If the problem cannot be resolved, you can press **Stop (N)** to select the **Quit** option (aborts the run) or **Next** to select the **Default** option. The **Default** option uses preset microscope focus position that may work well depending on gel thickness and placement of the gel apparatus. Other potential errors are discussed in the troubleshooting section of the Model 4300 Applications Manual. See Chapter 7 for more information on focusing errors and other troubleshooting tips.

The pre-run ends automatically after the full pre-run time has elapsed. When the pre-run has ended, the front panel display reads “Load Samples”. While “Load Samples” is displayed, the heater plate remains turned on, but the electrophoresis power supply and lasers are turned off.

- 5) After the pre-run, load samples as described in the Model 4300 Applications Manual.
- 6) Start the run by pressing **Start (Y)**.

To abandon the run rather than starting it, press **Stop (N)**. After pressing **Stop (N)**, the display reads “Quitting” and lists options to save or delete the run files. Press **Stop (N)** to stop the run and delete the empty run files.

When the run starts, “Run In Progress” is shown on the top line of the front-panel display. The bottom line shows the run name. When runs are started using the keypad, run names are automatically assigned using the first 7 characters of the chosen electrophoresis conditions file, followed by the date and a sequential index number.

An example of a run name for a run started from the keypad is:

Microsa-12-10_2

which indicates that the *Microsatellite* electrophoresis conditions file was used for the run. The run was conducted on December 10 and this was the 2nd run of the day that was initiated from the keypad.

At the end of the run, the **High Voltage** indicator light on the instrument front panel turns off and the display reads “4300 DNA Analyzer”, indicating that the Model 4300 is idle. Image files from runs initiated from the front panel are always stored in the *public* scan group. Finished images can be downloaded using the browser software as described in Chapter 8. LI-COR’s e-Seq and Saga software can also be used to download and analyze images.

Chapter 5: Accessing the NEN 4300 With an Internet Browser

Overview

The Model 4300 has an internal server that duplicates some of the software functions found in LI-COR application programs like e-Seq or Saga. Using the internal server software you can start runs, retrieve and manipulate stored images, and even sequence images for DNA sequencing applications. Internet browsers such as Netscape Navigator (version 4.7 or above), Internet Explorer (version 5.0 or above), or equivalent can be used to access the server software.

Not all functions in the Model 4300 server software are available to all users. For example, users who are assigned *Browse* or *Control* access rights cannot perform functions like adding user accounts that require *Administrator* access rights. Functions that have restricted access are noted throughout the remainder of this manual.

Note: Browser-based access to images on the Model 4300 is possible only for stand-alone configurations and networks that permit such access.

Connecting With a Browser

Each DNA analyzer is assigned an Internet Protocol (IP) address. The IP address distinguishes each instrument from any other device on your network, or the Internet. Your network administrator may also assign a host name. When the Model 4300's IP address or host name

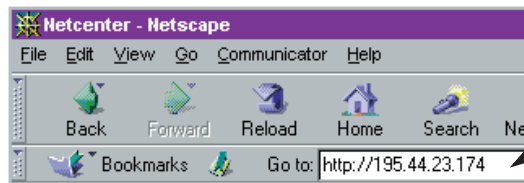
is entered into an Internet browser as a URL, the Model 4300 server software sends HTML pages that can be used to start runs, retrieve data, etc.

Follow the steps below to log on using an Internet browser.

- 1) Open an Internet browser application on your computer (i.e., Netscape Navigator 4.7 or above, or Internet Explorer 5.0 or above).

Note: When the browser opens, you may get an error message that implies a connection failure or that the browser cannot find a certain server. This message is not related to the 4300 and can be dismissed. The message is displayed because the browser is configured to automatically load a “home” page from the Internet. If the computer is not connected to the Internet, the browser preferences can be used to disable automatic loading of a home page, if you so desire.

- 2) In the **Location/URL** field of your browser, enter **http://** plus the IP address or host name assigned to the Model 4300. (Use the IP address supplied by your System Administrator, not the one shown in the graphic.)

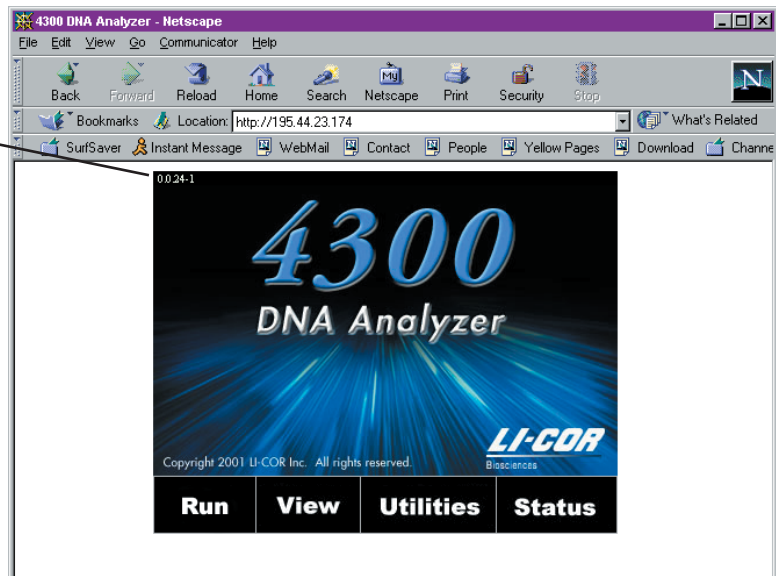


If your IP address is automatically assigned by DHCP, you can determine the current address (see Chapter 4).

Important: The IP address that should be entered in the URL field is an abbreviated form of the IP address entered in the instrument. Leading zeros within each group of three digits should not be entered. Thus, if 172.024.041.180 is the current address in the instrument, 024 becomes 24 and 041 becomes 41 when the IP address is entered in a browser. The address entered in the browser in this example should be `http://172.24.41.180`.

The Model 4300 sends a “home page” with four links that correspond to pages for starting runs, viewing and manipulating images, system utilities, and instrument status. Access beyond this page is password protected. Your access rights determine which functions you can actually use.

The current version of software in the Model 4300 is displayed in the upper left corner of the “home” page.

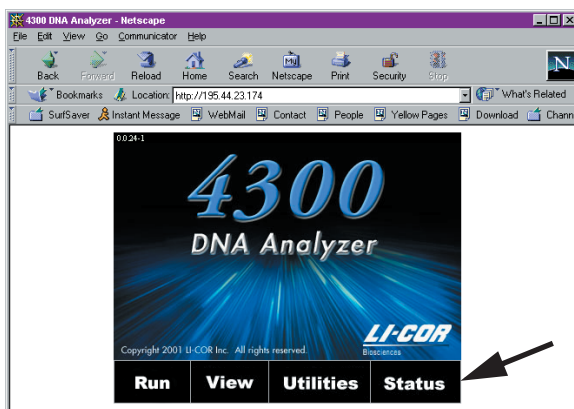


Checking Instrument Status

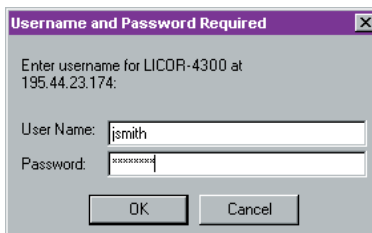
The Model 4300 is a server, allowing multiple users to be logged on at the same time. In other words, one user may be starting a new run while others are retrieving and analyzing images from finished runs stored on the internal hard disk.

Using the **Status** link on the home page, you can find out whether the instrument is in operation. If your instrument is connected to a network, any computer on the network with browser software can be used to check instrument status.

- 3) Click the **Status** link on the home page.

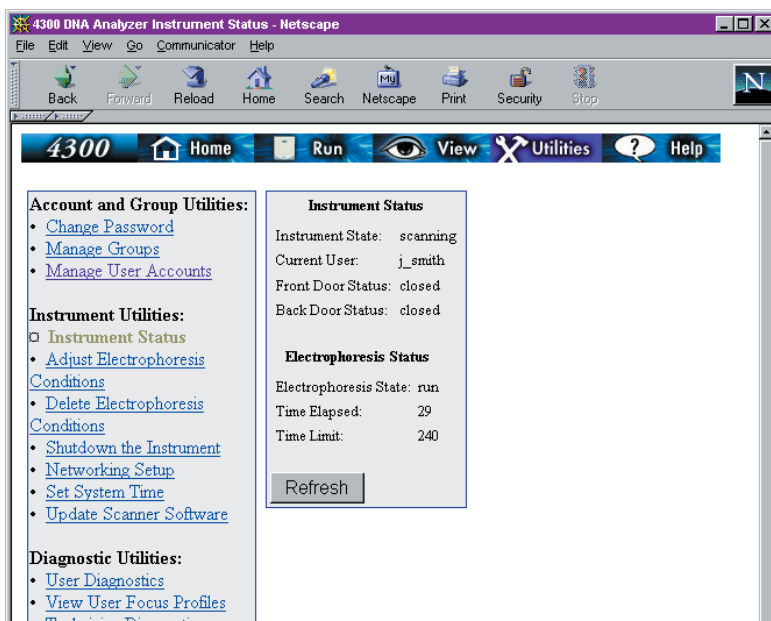


- 4) In the password window, enter the **User Name** and **Password** assigned to you by your Model 4300 Administrator and click **OK**.



User Name and Password are case sensitive.

The Status page has a navigation bar at the top of the page. Utilities is highlighted to indicate that the Status page is in the Utilities section. The left-side panel has navigation links for the various utility functions.



In addition to user functions, links for system administration and technician functions are displayed. However, only user accounts with *Administrator* or *Technician* access rights will be able to use those functions.

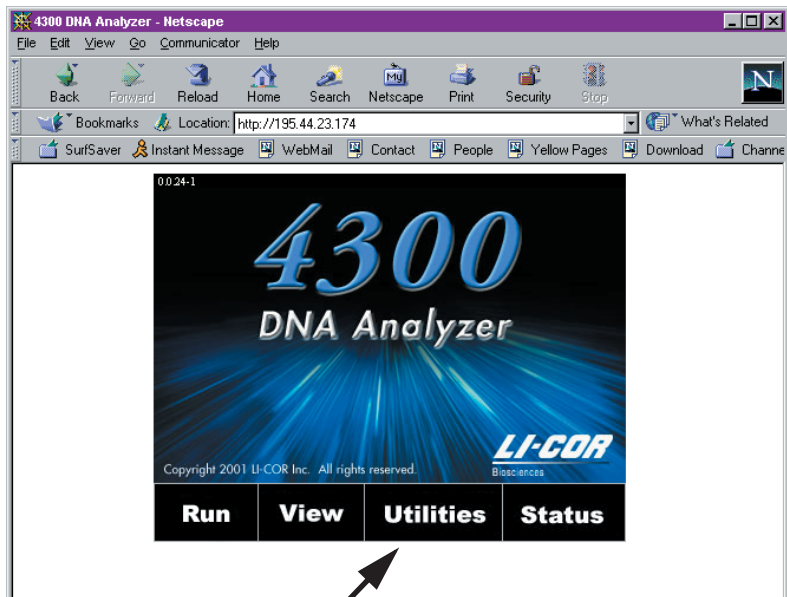
In the main body of the page is a box showing the status of the instrument. The status is typically *Idle*, *Paused*, or *Scanning*. (The status could also be some error state.) When the instrument is in operation, as shown above, the user is listed as well as the time remaining until the run is complete. Because the browser connection is not continuous, the remaining run time is not automatically updated. If you are waiting for a run to finish and the Status page has been displayed for a while, click **Refresh** to get the latest status information.

Chapter 6: User and Scan Group Administration

Adding User Accounts

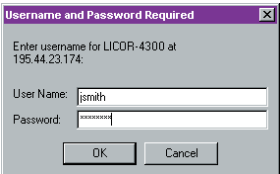
Adding user accounts requires *Administrator* access rights.

- 1) Log in to the Model 4300 server as described in Chapter 5.



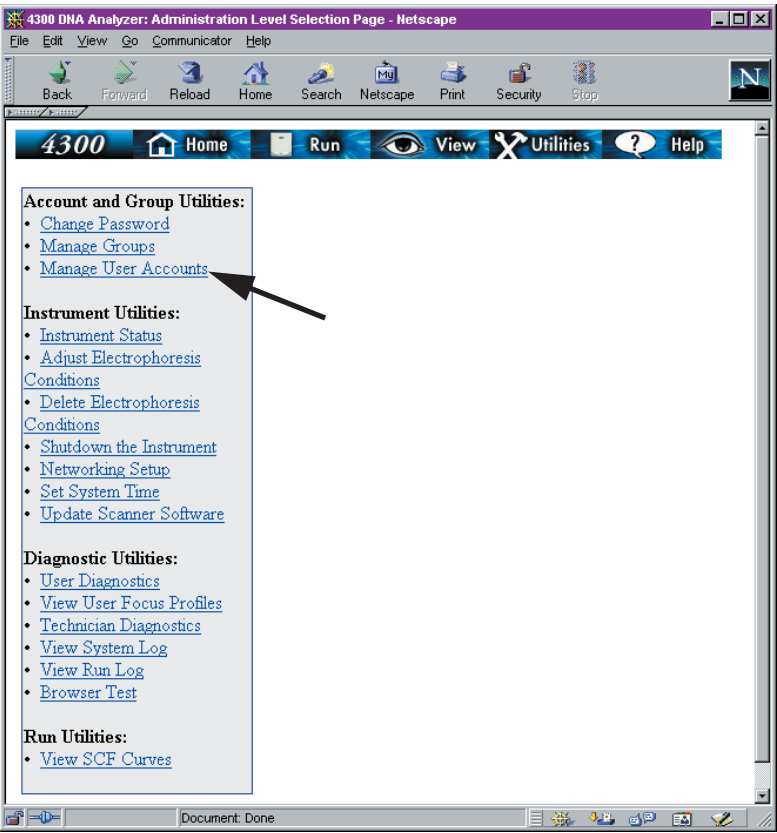
- 2) Click **Utilities** to open the Utilities page.

- 3) In the password window, enter the **User Name** and **Password** of a current account with *Administrator* rights. **User Name** and **Password** are case sensitive.



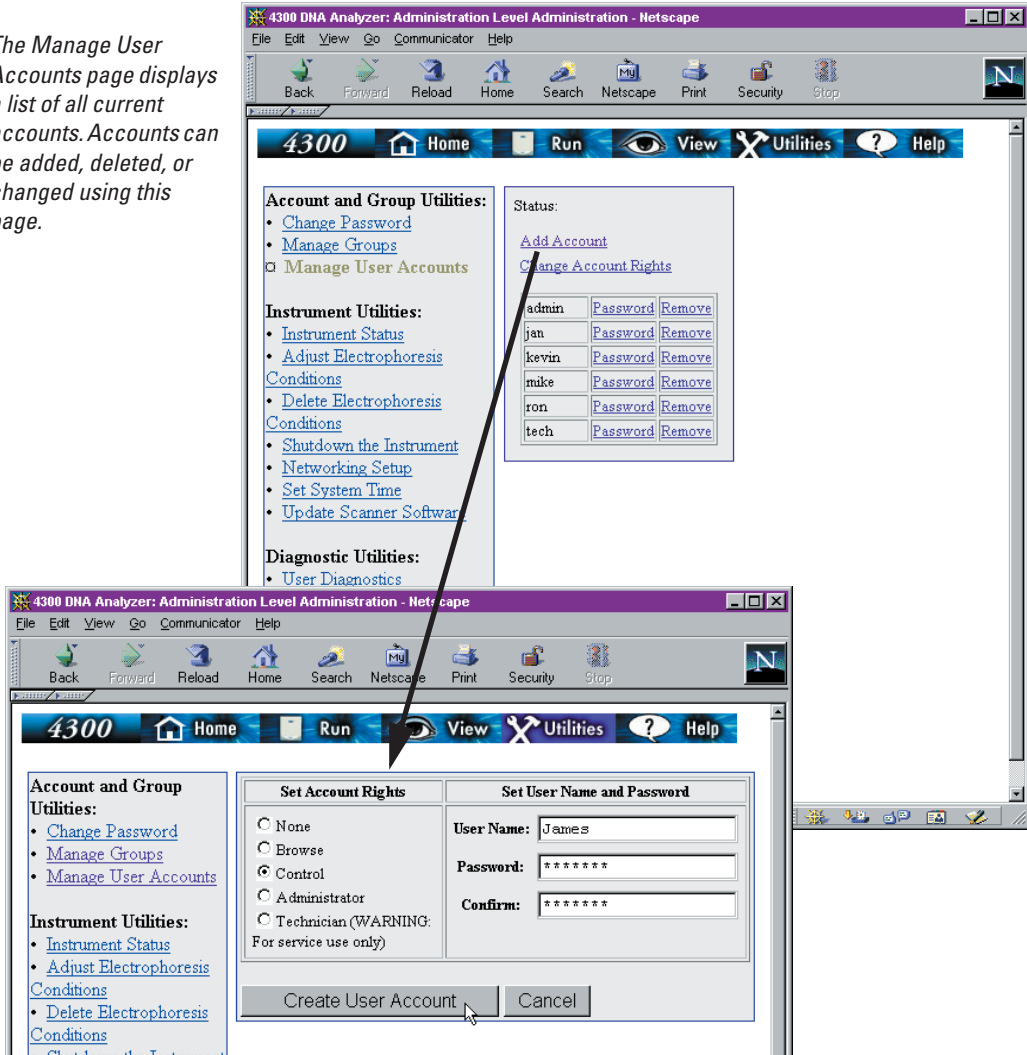
- 4) Click the **Manage User Accounts** link on the Utilities page.

The left side of the Utilities page lists all the utilities for account management, instrument maintenance, and diagnostics.



5) Click the **Add Account** link.

The Manage User Accounts page displays a list of all current accounts. Accounts can be added, deleted, or changed using this page.



6) Select the **Account Rights** for the new user according to the following definitions:

- **None:** User accounts become inactive when account rights are set to **None**. Data are not deleted, but the user cannot access data or start runs.
 - **Browse:** A user with **Browse** rights can access data in a “read only” capacity. **Browse** rights do not permit a user to start new runs, but images can be viewed, manipulated, or downloaded.
 - **Control:** Most users are given **Control** rights, which consists of **Browse** rights plus the ability to start new runs.
 - **Administrator:** Users with **Administrator** rights have **Control** rights, plus complete access to all Administrator level pages. **Administrator** rights should be restricted to as few qualified individuals as is practical.
 - **Technician:** **Technician** rights are only used by LI-COR or LI-COR Distributors in order to perform instrument diagnostic functions. **Technician** rights are not needed for any user operation or maintenance functions. Improper use of technician functions can render the instrument inoperable.
- 7) Enter the new user name (case sensitive) in the **User Name** field.
- User names and passwords can be 1 to 32 characters long. Alphabetic or numeric characters, as well as dashes and underscores, are acceptable. Do not use any other characters.*
- 8) Type the password for the new user in the **Password** field. Type the password again in the **Confirm** field for verification.
- 9) Click **Create User Account**.
- 10) Go back to the Manage User Accounts page and verify that the new account is in the account list.

Note: Creating a new account automatically creates a new scan group for the account that has the same name as the account name. Use the **Manage Groups** link to give other users access to the scan group, if desired.

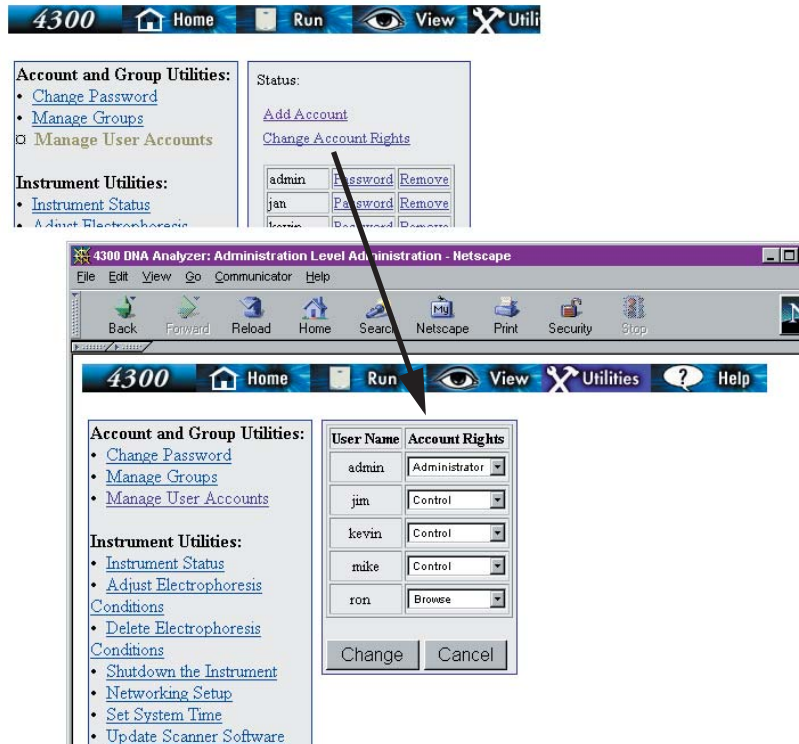
Adding User Accounts in Other Applications

User accounts also must be added to any application software such as e-Seq or Saga. Consult the User Guide for your software for a discussion of adding accounts.

Changing User Account Rights

If you are logged in using an account with *Administrator* access rights, you can change the account rights of any user as follows:

- 1) Click the **Change Account Rights** link on the Manage User Accounts page.

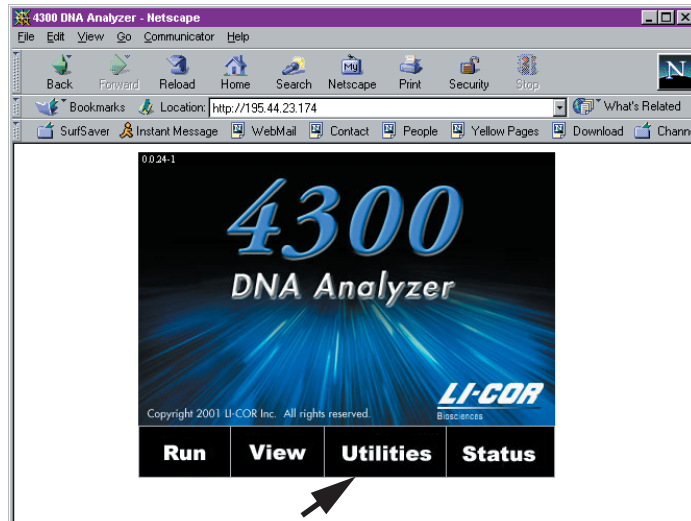


- 2) Use the **Account Rights** drop-down list next to the appropriate **User Name** to select new account rights. (Account rights are described in *Adding User Accounts* above.)
- 3) Click **Change** to save any changes.

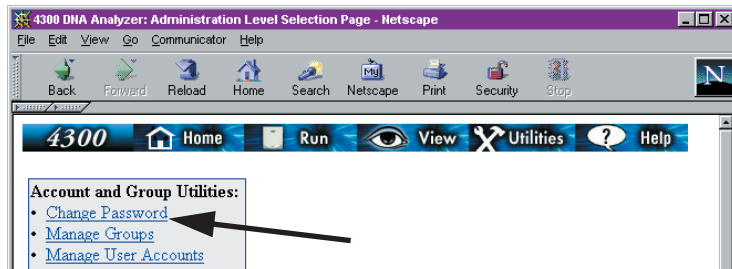
Changing Your Own Password

Any user with *Browse*, *Control*, or *Administrator* rights can change their own password as follows:

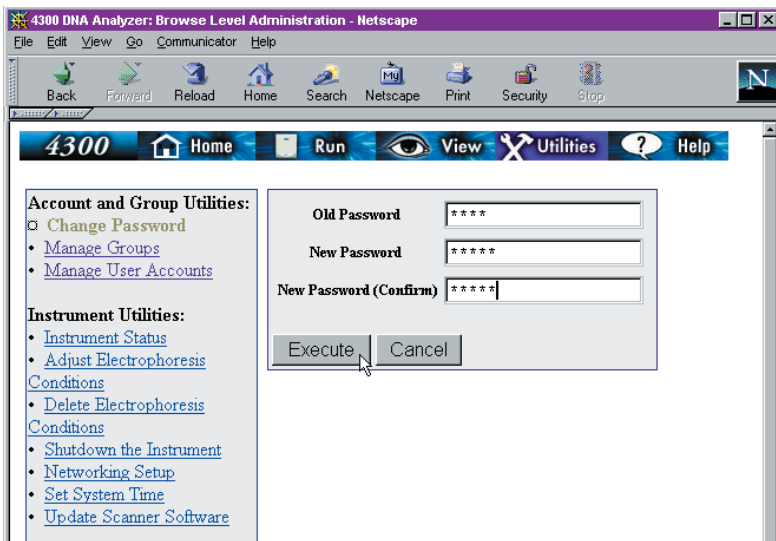
- 1) Click **Utilities** on the home page to open the Utilities page.



- 2) In the password window, enter your **User Name** and **Password**. (**User Name** and **Password** are case sensitive.)
- 3) Click the **Change Password** link.



- 4) Enter your current password in the **Old Password** field.



- 5) Enter the new password in the **New Password** field and confirm it by typing it again in the **New Password (Confirm)** field.

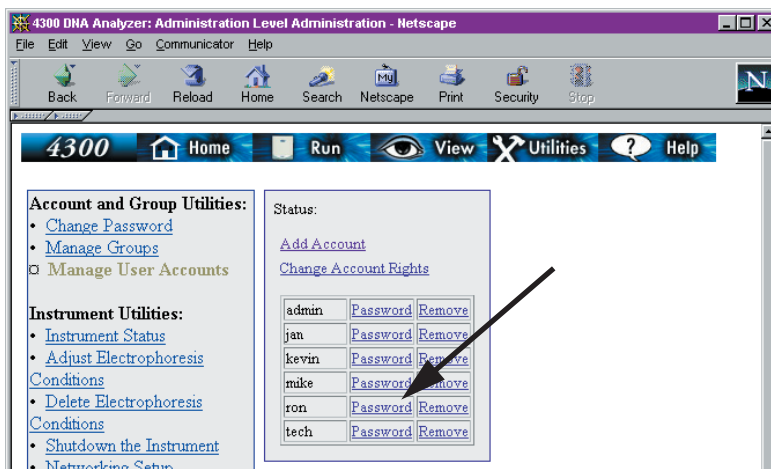
Passwords can be 1 to 32 characters long. Alphabetic or numeric characters, as well as dashes and underscores, are acceptable. Do not use any other characters.

- 6) Click **Execute** to change the password.

Changing Other User's Passwords

If you are logged in using an account with *Administrator* access rights, you can change the password for any user account as follows:

- 1) On the Manage User Accounts page, click the **Password** link next to the appropriate User Name to change that user's password.



- 2) Enter the new password in the **New Password** field, and confirm it by typing it again in the **New Password (Confirm)** field.

Selected User: ron

New Password:

New Password (Confirm):

The old password is not required, which makes it easy to assign a new password to users who have forgotten theirs.

Passwords can be 1 to 32 characters long. Alphabetic or numeric characters, as well as dashes and underscores, are acceptable. Do not use any other characters.

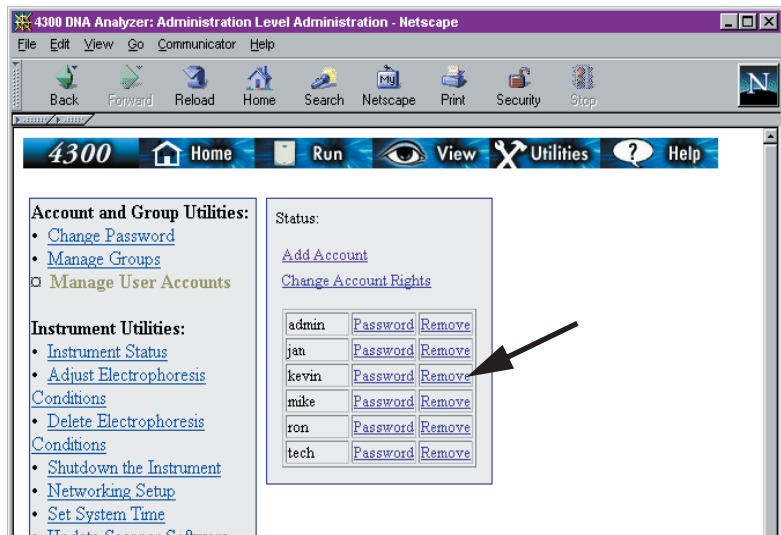
- 3) Click **Change Password**.

Note: If you need to change the account name, delete the existing account and add a new account with the correct name.

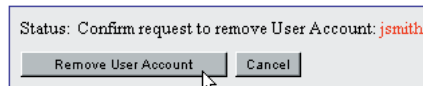
Deleting User Accounts

If you are logged in using an account with *Administrator* access rights, you can delete user accounts as follows:

- 1) On the Manage User Accounts page, click the **Remove** link next to the user name you wish to remove.



- 2) Click **Remove User Account** to confirm removal of the user account or **Cancel** to leave the account unchanged.



Note: The last Administrator account cannot be deleted. An error message will be displayed if you attempt to delete the last Administrator account.

Deleting the Original Admin Account

To prevent unauthorized access, create a new “Administrator” account and delete the *admin* account that was shipped with the Model 4300 (the *admin* account can’t be deleted if it is the last account with administrator rights).

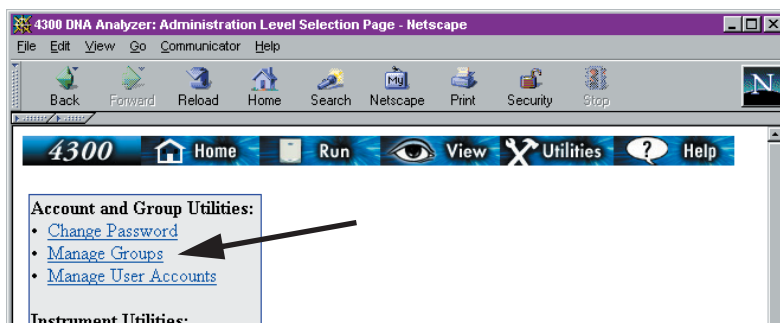
Creating and Changing Groups

Initially, most users have access to their own scan group and the *public* scan group. Additional scan groups can be created for special purposes. For example, if several people are all doing runs for a particular research project, it might be useful to keep all of the images for a project in one scan group.

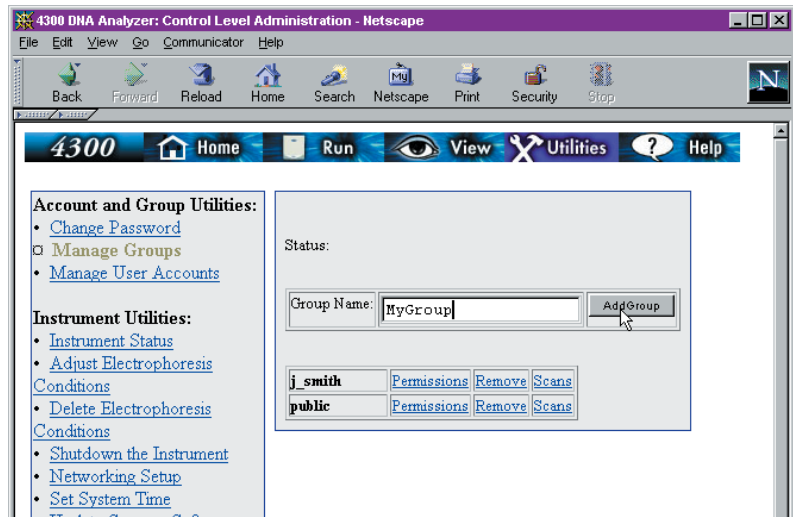
Important: The Model 4300 should be used only for temporary storage. Image files should be moved to a local or network drive after runs are completed. Both the browser interface and application software (e-Seq, Saga, etc.) can be used to download files.

Creating a New Scan Group

1) Click the **Manage Groups** link on the Utilities page.



- 2) Enter a name for the new group in the **Group Name** field.



Group names can be 1 to 32 characters long. Alphabetic or numeric characters, as well as dashes and underscores, are acceptable. Do not use any other characters.

- 3) Click **Add Group** to create the new group.

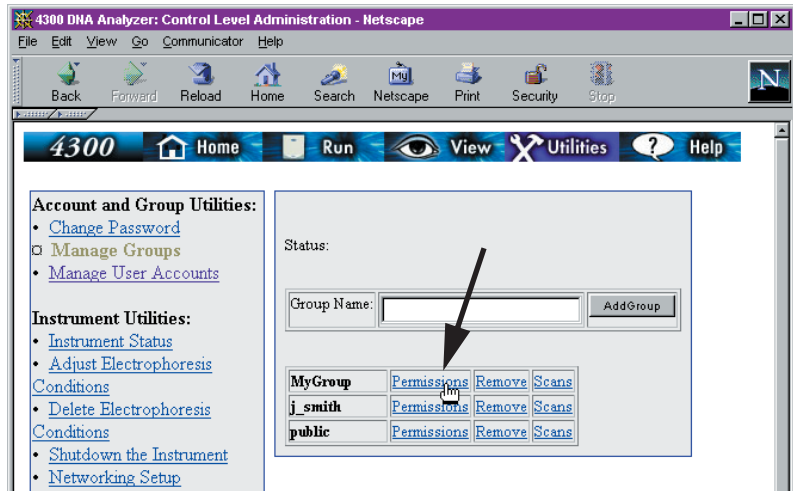
The status line will indicate that the group has been successfully created. In addition, the newly added group should now be listed in the table of group names. The user who created the group is automatically given permission to access the new group. The next section shows how to give others access to the group.

Changing Access Permission of a Scan Group

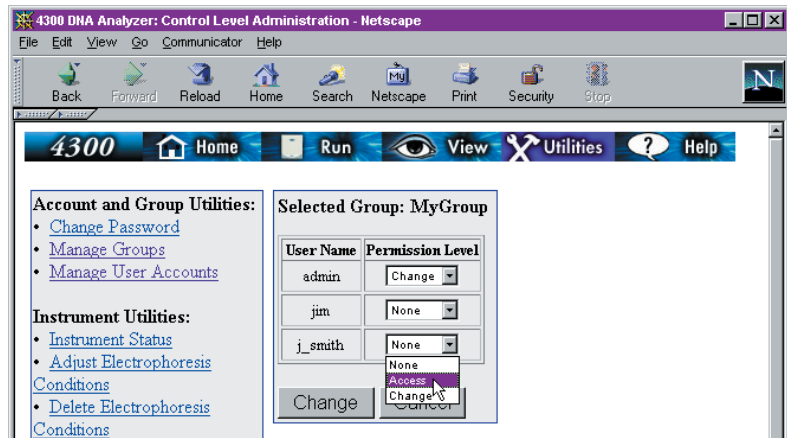
Any user with *Control* account rights and *Change* permission to a scan group can permit other users to access the scan group. Users with *Administrator* rights can change permission for any scan group.

- 1) Click the **Manage Group** link on the Utilities page.

- 2) Click the **Permissions** link next to the scan group that you want to change.



- 3) All users are listed. Use the **Permission Level** drop-down list next to the user name to change access permission of the user.



A permission level of *None* indicates no access. Users with permission level of *None* will not see the indicated group when selecting a group for new runs or while opening images.

Access permission level allows users to open completed runs, but not change them. *Change* permission allows users to start new runs and store them in the scan group, as well as editing privileges for any images in the scan group. A user with *Change* permission that belongs to a scan group can allow other users to access the scan group by changing their access permission.

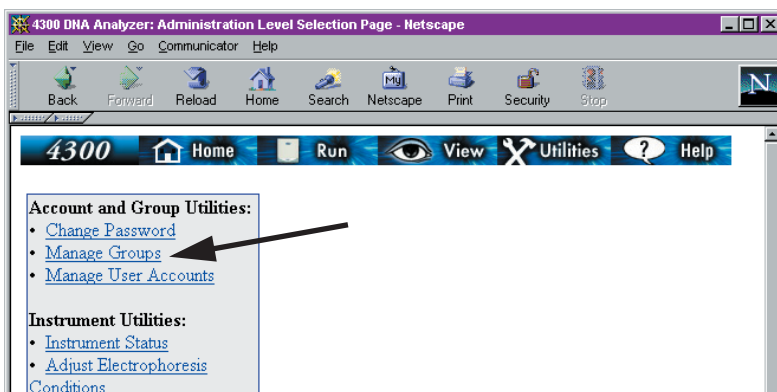
- 4) Click **Change** to implement the new permission level.

Deleting Scan Groups

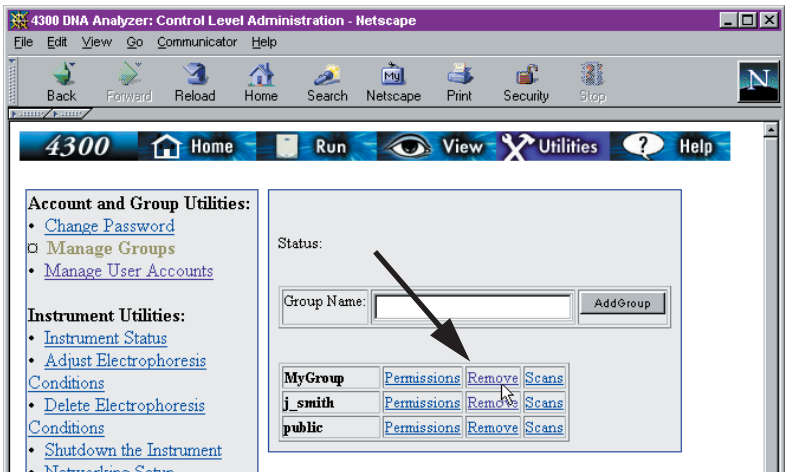
Any user with *Control* rights and *Change* permission to a scan group can delete the scan group using the **Manage Groups** link on the Utilities page. Users with *Administrator* rights can delete any scan group. Note, however, that the corresponding user account must be deleted first. For example, a scan group named *JohnSmith* cannot be deleted until the user account named *JohnSmith* has been deleted.

Important: Deleting a scan group deletes all images within the group. Once a group is deleted, it is not possible to retrieve images that were in the group.

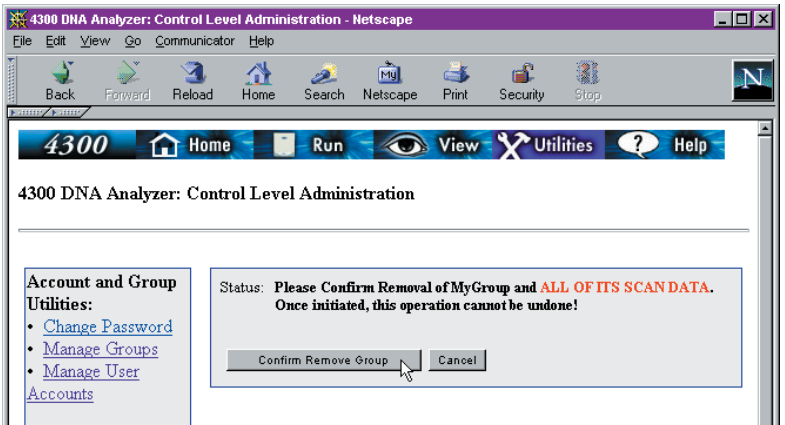
- 1) Click the **Manage Groups** link on the Utilities page.



- 2) Click the **Remove** link next to the scan group that you want to delete.



- 3) Read the warning and click **Confirm Remove Group** if you want to continue.

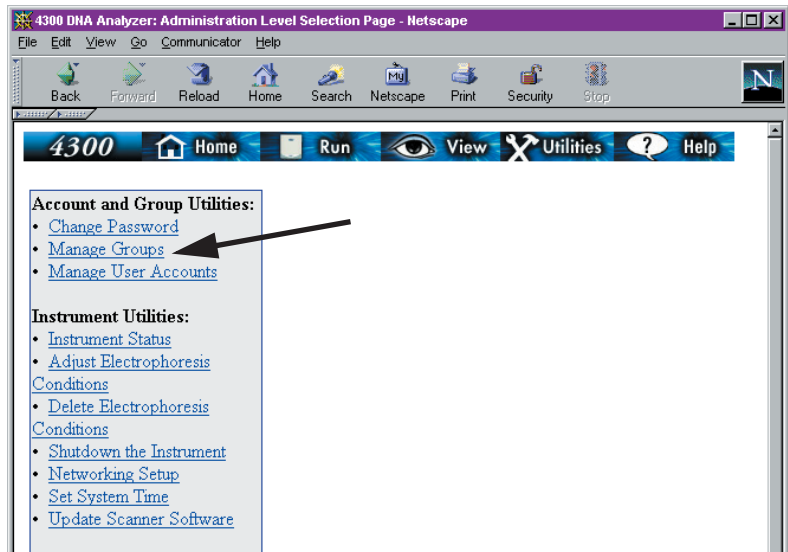


Deleting Scans From a Scan Group

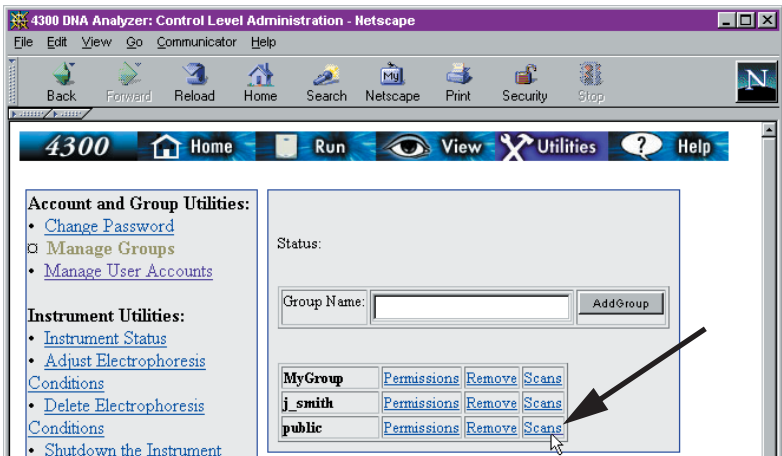
Any user with *Control* rights and *Change* permission to a scan group can use the Utilities page to display a list of images in a group and delete any of the listed images. Users with *Administrator* rights can delete scans in any scan group.

Important: Deleted images cannot be restored. Make sure you want to delete the image from the Model 4300 before using this function. This function does not delete images stored on your computer.

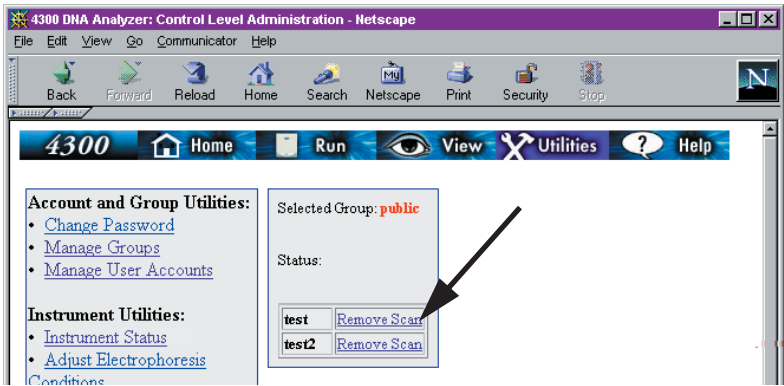
1) Click the **Manage Groups** link on the Utilities page.



- 2) Click the **Scans** link next to the scan group containing the scans you want to view or delete.



- 3) All images in the selected group are listed. To delete a run and all related files, click the **Remove Scan** link next to the run name that should be removed.



Chapter 7: Starting New Runs Using an Internet Browser

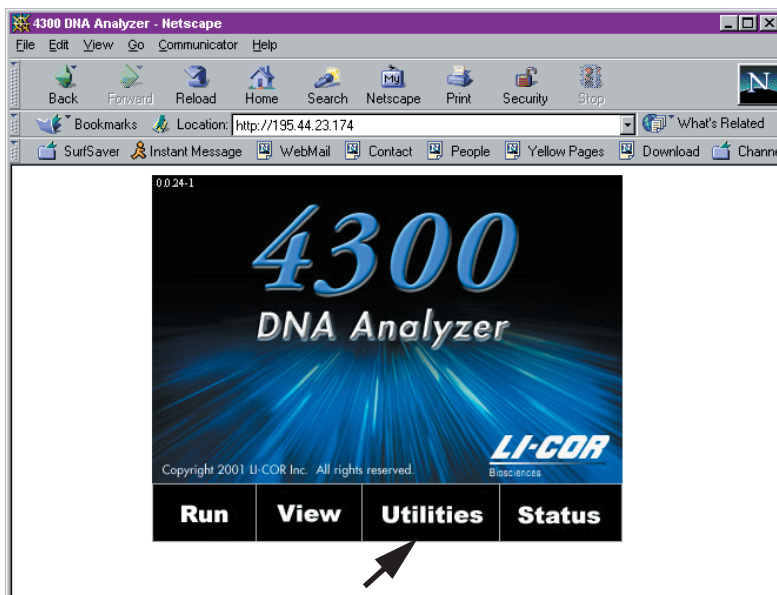
Introduction

In addition to using LI-COR e-Seq or Saga application software to start runs, runs can also be started using an Internet browser. To start a new run from a browser, all you need to do is to log in to the Model 4300 using Internet browser software (Chapter 5) and start a run using the Run page. After a run is started, the image file(s) can be viewed as they are collected during electrophoresis.

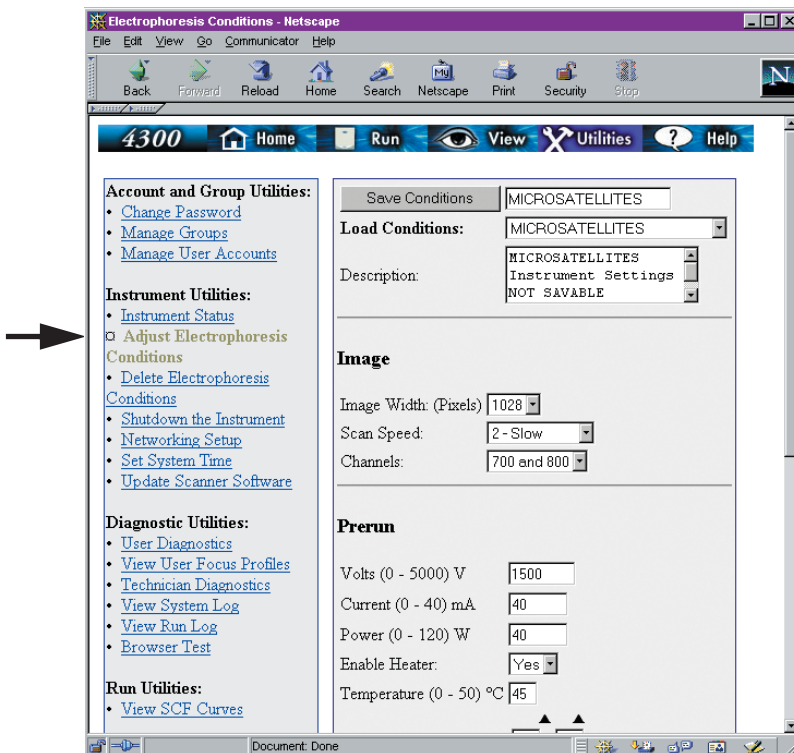
Creating Sets of Electrophoresis Conditions

When you start a run using the browser software (or the instrument keypad), one of the selections you must make is to choose the set of electrophoresis conditions that you want to use for the run. The electrophoresis conditions include the voltage, current, gel temperature, and a variety of other parameters.

- 1) To create a set of electrophoresis conditions, click **Utilities** on the home page.



2) Click the **Adjust Electrophoresis Conditions** link.



3) Enter a new name for the set of electrophoresis conditions in the field next to the **Save Conditions** button.

Names can be 1 to 32 characters long. Alphabetic or numeric characters, as well as dashes and underscores are acceptable. Do not use any other characters.

4) Enter changes to the electrophoresis parameters and click **Save Conditions**.

Recommended electrophoresis conditions for various gel sizes and applications are given in Tables 1 through 4.

Table 1. Parameters for 25 cm Sequencing Gels

Parameter	0.25 mm	0.20 mm
Image Width	1028	1028
Scan Speed	2	2
Channels	User Choice	User Choice
Pre-run Voltage	1500	1500
Pre-run Current	40	40
Pre-run Power	40	40
Pre-run Enable Heater	Yes	Yes
Pre-run Temperature	45	45
Pre-run Time	00:20	00:20
Run Voltage	1500	1500
Run Current	40	40
Run Power	40	40
Run Enable Heater	Yes	Yes
Run Temperature	45	45
Run Time	05:00	05:00
Analysis	Collect Image Only	Collect Image Only

Table 2. Parameters for 41 cm Sequencing Gels

Parameter	0.25 mm	0.20 mm	0.2 mm KB ^{Plus}
Image Width	1028	1028	1028
Scan Speed	2	2	2
Channels	User Choice	User Choice	User Choice
Pre-run Voltage	1500	2000	2000
Pre-run Current	35	25	25
Pre-run Power	31.5	50	50
Pre-run Enable Heater	Yes	Yes	Yes
Pre-run Temperature	50	45	45
Pre-run Time	00:30	00:30	00:15
Run Voltage	1500	2000	2000
Run Current	35	25	25
Run Power	31.5	50	50
Run Enable Heater	Yes	Yes	Yes
Run Temperature	50	45	45
Run Time	09:00	09:00	09:00
Analysis	Collect Image Only	Collect Image Only	Collect Image Only

Table 3. Parameters for 66 cm Sequencing Gels (4300L only)

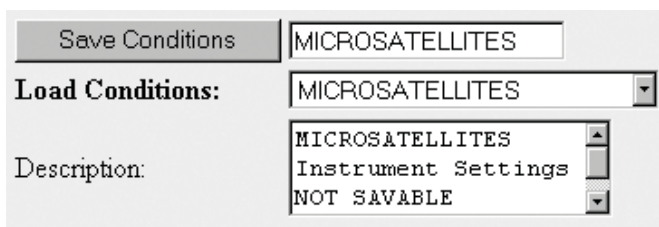
Parameter	0.25 mm	0.20 mm	0.2 mm KB^{Plus}
Image Width	1028	1028	1028
Scan Speed	2	2	2
Channels	User Choice	User Choice	User Choice
Pre-run Voltage	2000	3000	3000
Pre-run Current	25	30	30
Pre-run Power	45	75	80
Pre-run Enable Heater	Yes	Yes	Yes
Pre-run Temperature	45	45	45
Pre-run Time	00:30	00:30	00:30
Run Voltage	2000	3000	3000
Run Current	25	30	30
Run Power	45	75	80
Run Enable Heater	Yes	Yes	Yes
Run Temperature	45	45	45
Run Time	20:00	14:00	14:00
Analysis	Collect Image Only	Collect Image Only	Collect Image Only

Table 4. Parameters for Microsatellite or AFLP[®] Gels (0.25mm thickness)

Parameter	18 or 25 cm
Image Width	1028
Scan Speed	2
Channels	User Choice
Pre-run Voltage	1500
Pre-run Current	40
Pre-run Power	40
Pre-run Enable Heater	Yes
Pre-run Temperature	45
Pre-run Time	00:25
Run Voltage	1500
Run Current	40
Run Power	40
Run Enable Heater	Yes
Run Temperature	45
Run Time	01:30
Analysis	Collect Image Only

Electrophoresis Parameter Descriptions

An existing set of electrophoresis conditions can be loaded by selecting it from the **Load Conditions** drop-down list. If you want to create a new set of conditions that is similar to an existing set, it is generally easier to load the existing set, change the name, change the parameters, and save the set under the new name by clicking **Save Conditions**.



The screenshot shows a software interface for saving and loading electrophoresis conditions. It features a 'Save Conditions' button, a 'Load Conditions' dropdown menu, and a 'Description' field. The 'Load Conditions' dropdown is currently set to 'MICROSATELLITES'. The 'Description' field contains the text 'MICROSATELLITES Instrument Settings NOT SAVABLE'.

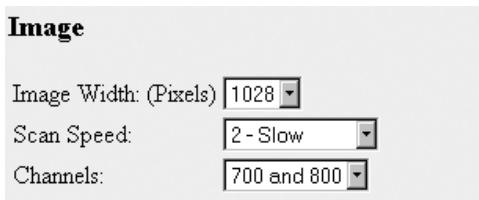
Save Conditions	MICROSATELLITES
Load Conditions:	MICROSATELLITES
Description:	MICROSATELLITES Instrument Settings NOT SAVABLE

Three sets of electrophoresis conditions that match standard conditions are included: SEQUENCING, AFLP, and MICROSATELLITES. The SEQUENCING conditions file matches the 41 cm KB^{Plus} gel with 0.2 mm thickness listed in Table 2. The AFLP and MICROSATELLITES conditions files match the 25 cm gels listed in Table 4.

The **Description** field can be any text that helps you distinguish one set of conditions from another.

Image Width: The Model 4300 always scans the full width of the gel. However, you can control how coarse or fine the image is by using the **Image Width** parameter to control how many image pixels are created as the laser/microscope scans across the gel. For normal

runs, **Image Width** should be set at 1028 pixels. Though not recommended for typical runs, **Image Width** can also be set to 2055 for higher resolution scans with larger file sizes.



The image shows a software interface titled "Image" with three settings:

- Image Width: (Pixels)** set to 1028 (dropdown menu)
- Scan Speed:** set to 2 - Slow (dropdown menu)
- Channels:** set to 700 and 800 (dropdown menu)

Scan Speed: The **Scan Speed** determines the rate at which the scanning laser microscope assembly moves across the gel during electrophoresis. Higher motor speeds are useful with a shorter gel apparatus, where the rate of migration of the DNA fragments is very fast. Scan speeds are as follows:

1. Very Slow = 2.5 cm/s
2. Slow = 5.0 cm/s
3. Moderate = 10 cm/s
4. Fast = 20 cm/s
5. Very Fast = 30 cm/s

Channels: Single-dye or two-dye runs can be started depending on how the **Channels** parameter is set. When set to **700 and 800**, both channels are scanned and two electrophoresis images are created during the run. When set to either **700** or **800**, only the 700 channel or 800 channel will be scanned, respectively.

The pre-run is an important process during which normal electrophoresis voltage is applied to the gel before samples are loaded. The pre-run generally uses the same voltage, current, power, and temperature settings as the run, but the duration is shorter.

Pre-run Volts: The voltage (in volts) specified in this field is the maximum voltage applied across the electrophoresis apparatus by the High Voltage Power Supply during the pre-runs. Range is 0 to 5000 volts.

Prerun

Volts (0 - 5000) V

1500

Current (0 - 40) mA

40

Power (0 - 120) W

40

Enable Heater:

Yes

Temperature (0 - 50) °C

45

Time (HH:MM)

00 : 25

Pre-run Current: The current, in milliamps, specified in this field is the maximum current applied through the electrophoresis apparatus during the pre-run. Range is 0 to 40 milliamps.

Pre-run Power: This value represents the maximum power, in watts, that will be applied to the electrophoresis apparatus during the pre-run. Range is 0 to 120 watts.

Pre-run Enable Heater: During electrophoresis, a heater plate in contact with the electrophoresis apparatus maintains a constant gel temperature. For normal operation, the **Prerun Enable Heater** parameter is set to **Yes** so that during the pre-run the gel is warmed to the same temperature that will be used for the run. The temperature can be set from 0 to 50 °C using the **Temperature** field.

Time: The pre-run should be specified in HH:MM format. The time can be typed in the fields, or the small arrow buttons can be used to increase or decrease the time shown in the fields.

The **Voltage**, **Current**, **Power**, **Enable Heater**, **Temperature**, and **Time** parameters for the run are exactly as described above, except that these are the set points that are maintained during electrophoresis and image collection.

Run

Volts (0 - 5000) V	<input type="text" value="1500"/>
Current (0 - 40) mA	<input type="text" value="40"/>
Power (0 - 120) W	<input type="text" value="40"/>
Enable Heater:	<input type="button" value="Yes"/>
Temperature (0 - 50) °C	<input type="text" value="45"/>
Time (HH:MM)	<input type="text" value="01"/> : <input type="text" value="30"/>

Analysis: Select the **Collect Image Only** choice for non-sequencing applications (microsatellites, AFLP[®], etc.). **Collect Image Only** is also the correct choice for if you want to just collect an image and analyze it later using e-Seq or other sequencing software. (e-Seq offers greater flexibility and ease-of-use compared to the sequencing software in the Model 4300.)

Analysis: ☐ Autosequence ☒ Collect Image Only

Choose **Autosequence** if you are sequencing DNA and want the Model 4300 to sequence the images in real time as they are collected. (The Autosequence software is discussed at the end of this chapter.)

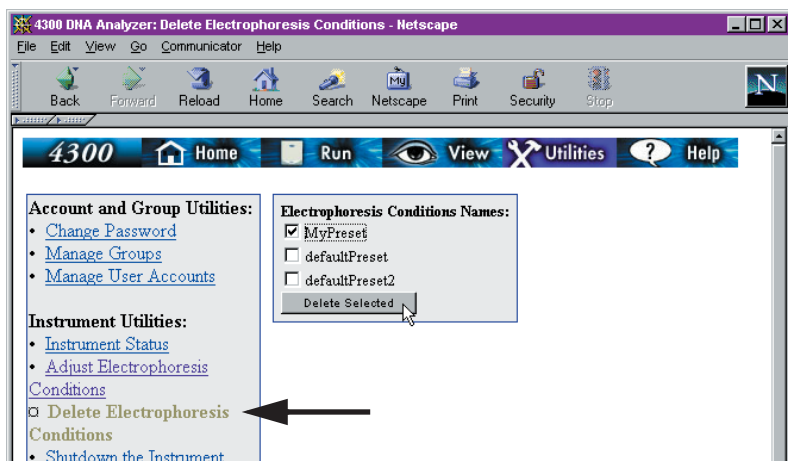
Modifying Sets of Electrophoresis Conditions

To modify an existing set of conditions, use the following procedure:

- 1) Click **Adjust Electrophoresis Conditions** on the Utilities page.
- 2) Load an existing set of electrophoresis conditions by selecting it from the **Load Conditions** drop-down list.
- 3) Change the electrophoresis parameters as needed.
- 4) Click **Save Conditions** to save the modifications.

Deleting Sets of Electrophoresis Conditions

To delete a set of electrophoresis conditions, click the **Delete Electrophoresis Conditions** link on the Utilities page. All sets of electrophoresis conditions are listed. To delete a set, select the check box next to the set and click **Delete Selected**.

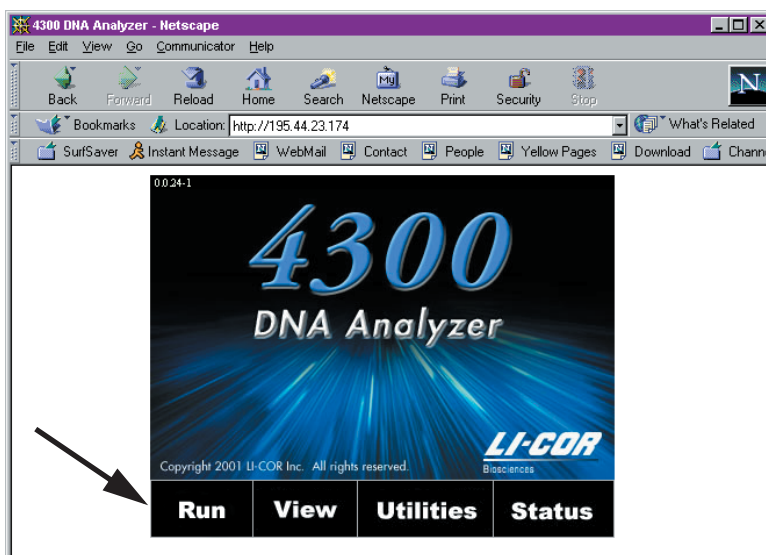


Starting a Run That Uses the Autosequence Software

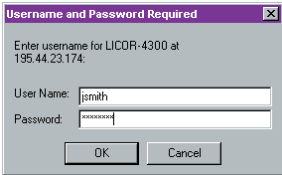
Use the instructions in this section only if you want to start a DNA sequencing run in which the sequencing software in the Model 4300 will be used to call bases from the image data. If you want to start runs for microsatellite or AFLP® applications, or just collect images for later analysis with e-Seq or other software, skip to the section titled *Starting A Run Without Autosequencing* later in this chapter.

Before you begin, make sure the electrophoresis gel apparatus is properly mounted and the instrument door is closed as described in the Model 4300 Applications Manual. Also make sure that you have created an electrophoresis conditions file that matches the gel you are using, as described earlier in this chapter. Use the steps below to start a new run using an Internet browser:

- 1) Log in to the Model 4300 using browser software (Chapter 5).
- 2) Click **Run** on the home page.



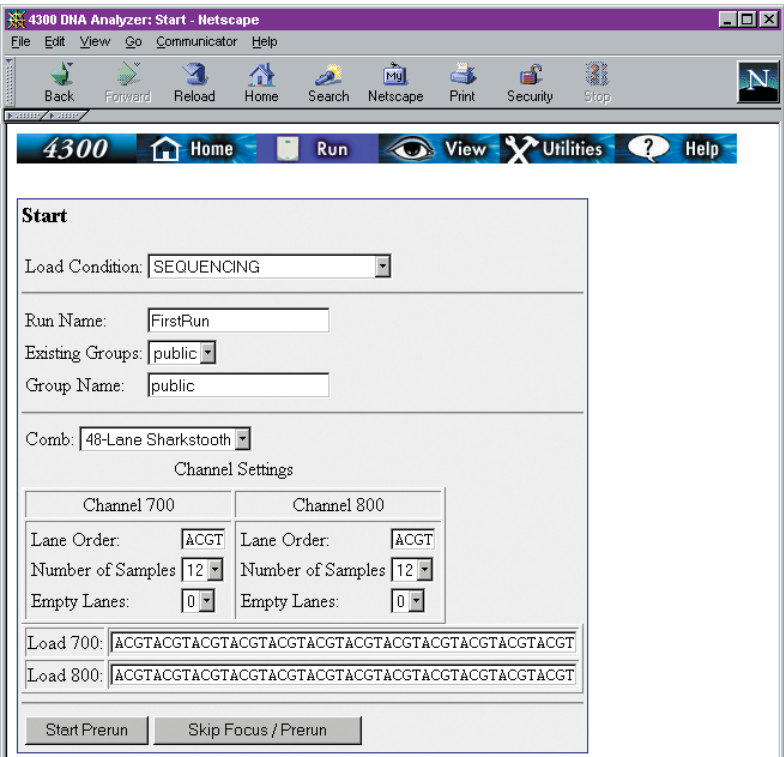
- 3) In the password window, enter the **User Name** and **Password** assigned to you by your Model 4300 Administrator and click **OK**.



User Name and Password are case sensitive.

Entering Run Information

The Start page is used to choose a set of electrophoresis conditions, name the new run, and enter lane loading information that the autosequencing software needs to sequence the image data.



***Note:** The lane loading fields do not appear when the electrophoresis conditions file has **Autosequence** deselected.*

- 4) Select a set of electrophoresis conditions from the **Load Conditions** drop-down list.

Note: In order for the autosequencing software to work, you must choose an electrophoresis conditions file, such as the default **SEQUENCING** file, that has the **Analysis** parameter set to **Autosequence**, as described earlier in this chapter.

- 5) Enter a name for the run in the **Run Name** field.

Run names can be 1 to 32 characters long. Alphabetic or numeric characters, as well as dashes and underscores, are acceptable. Do not use any other characters.

- 6) From the **Existing Groups** drop-down list, choose an existing group in which to store the run. You can also create a new group by entering a group name in the **Group Name** field.
- 7) Select the comb you are using in the gel from the **Comb** drop-down list.
- 8) Set the **Lane Order**, **Number of Samples**, and **Empty Lanes** for each image channel.

Important: All samples for a given channel must be loaded in the same lane order. In the **Lane Order** fields for each channel, enter the letters A, T, C, and G in an order that matches the actual loading order. Select the number of samples that were loaded in each channel from the **Number of Samples** drop-down lists. The **Empty Lanes** drop-down lists let you specify the number of empty lanes (if any) between samples (does not apply to any other empty lanes).

The **Load 700** and **Load 800** fields at the bottom of the window show the current sample map for each detection channel. These sample loading maps are automatically updated any time the lane order, number of lanes, or empty lanes are changed. The sample loading order can also be edited in these fields.

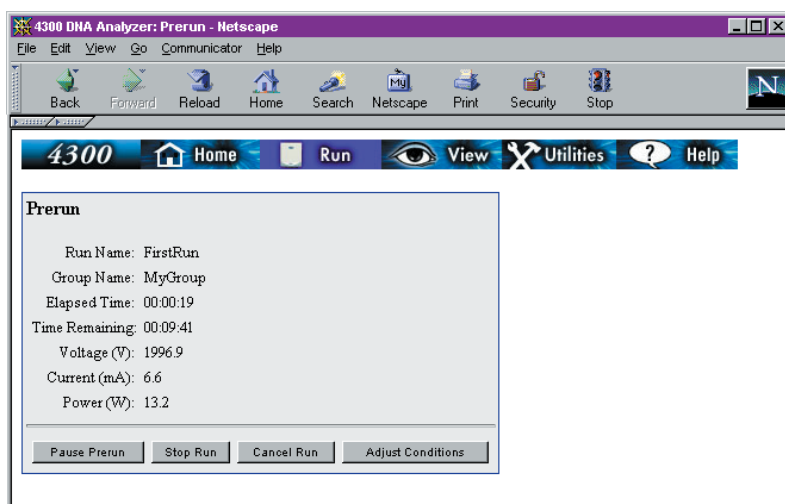
Starting the Pre-run

- 9) Click **Start Prerun** to begin the pre-electrophoresis procedure for the gel.

During the first 10 minutes of the pre-run, the laser/microscope is focused in the gel and the detection electronics are optimized for the run. For typical runs, the electrophoresis voltage and temperature used during the pre-run are the same as the voltage and temperature that will be used during the run.

Note: You should not use the **Skip Focus/Prerun** button for sequencing applications. The pre-run can be skipped only when gels are reloaded for microsatellites, AFLP[®], and other applications.

When the pre-run starts, the Prerun page is displayed. The actual electrophoresis conditions (voltage, current, and power) are displayed as well as the time remaining in the pre-run. The pre-run information is periodically updated with new values from the Model 4300.

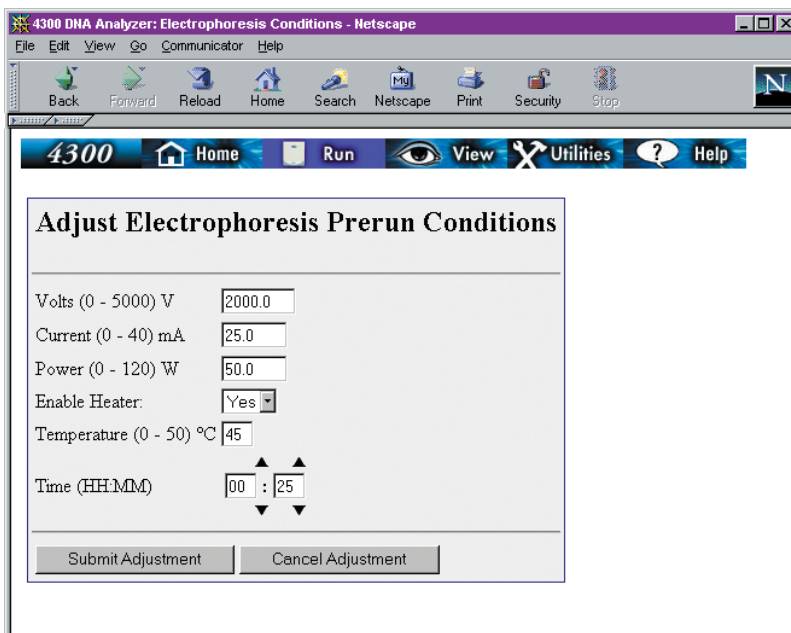


If you need to pause the pre-run for some reason, such as opening the door on the instrument, click the **Pause Prerun** button. The **Pause Prerun** button will change to **Resume Prerun**. Click **Resume Prerun** to restart the pre-run from the beginning (you cannot resume a paused pre-run). The **Start (Y)** key on the instrument keypad can also be used to restart a paused pre-run.

If you need to abort the run rather than just pausing it, click **Cancel Run**. The run file and all associated files are deleted when a run is cancelled. The **Stop Run** button also stops the pre-run but it saves all files that have been created. **Stop Run** is useful for completing a run early once electrophoresis has started, but it is not generally used during the pre-run because no image data have been stored.

Occasionally you may get a focus error if the focus routine cannot accurately determine the center of the gel. The *Troubleshooting Tips* at the end of the chapter explain how to correct focus errors.

If you need to change the duration of the pre-run or the electrophoresis conditions, click **Adjust Conditions**.



The screenshot shows a Netscape browser window titled "4300 DNA Analyzer: Electrophoresis Conditions - Netscape". The browser's address bar shows "http://www.4300.com/". The main content area displays a form titled "Adjust Electrophoresis Prerun Conditions". The form contains the following fields and controls:

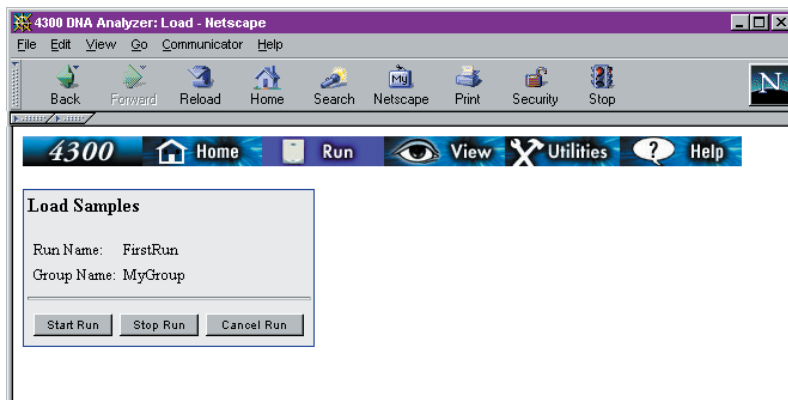
- Volts (0 - 5000) V: A text input field containing "2000.0".
- Current (0 - 40) mA: A text input field containing "25.0".
- Power (0 - 120) W: A text input field containing "50.0".
- Enable Heater: A dropdown menu with "Yes" selected.
- Temperature (0 - 50) °C: A text input field containing "45".
- Time (HH:MM): A time selection control showing "00 : 25" with up/down arrows.

At the bottom of the form are two buttons: "Submit Adjustment" and "Cancel Adjustment".

Each parameter is described earlier in this chapter. To make changes, edit the fields and click **Submit Adjustment**. The electrophoresis power supply and heater plate temperature are adjusted when the changes are submitted (takes a few seconds).

Loading Samples

When the pre-run time has elapsed, a message is displayed indicating that it is time to load the samples on the gel. While this message is displayed, the electrophoresis power supply is turned off, but the heater plate remains on so that the gel temperature is maintained.



10) Load samples as described in the Model 4300 Applications Manual.

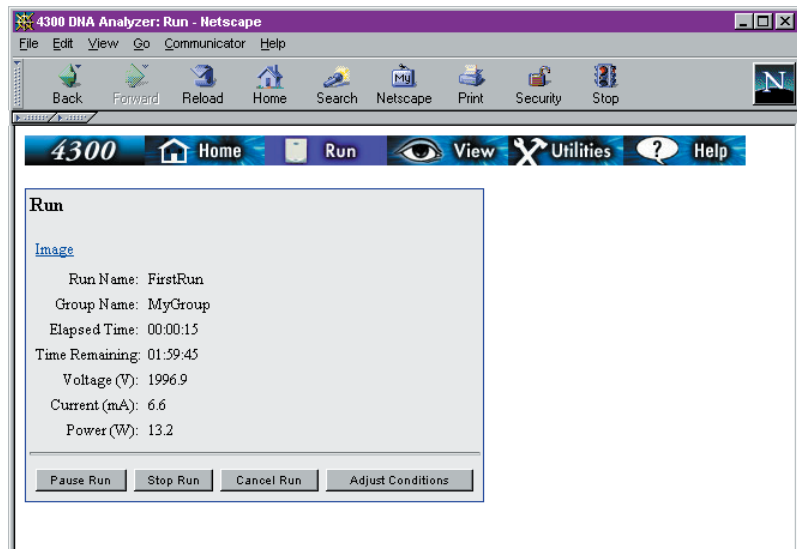
Important: The autosequencing software in the Model 4300 expects each sample to be loaded in four contiguous lanes and all samples must be loaded in the same order. The only allowable variation is to have empty lanes between samples. This does not allow for many variations or mistakes in lane loading compared to sequencing programs such as LI-COR's e-Seq DNA Sequencing Software. If you make a mistake during lane loading or damage a well, the troubleshooting tips at the end of this chapter show you how to compensate for the problem. In general, any problem samples must be excluded from autosequencing using the Verify Lanes page.

Starting the Run

11) After loading samples, click **Start Run** to begin electrophoresis.

As described above, the **Cancel Run** button aborts the run without saving files. The **Stop Run** button stops the run and saves any files that have been created.

When the run starts, the Run page is displayed. Like the Prerun page, the actual electrophoresis conditions (voltage, current, and power) are displayed as well as the time remaining in the run. The run information is periodically updated with new values from the instrument. If you need to adjust the electrophoresis conditions, click the **Adjust Conditions** button and change the conditions as described above for the pre-run.



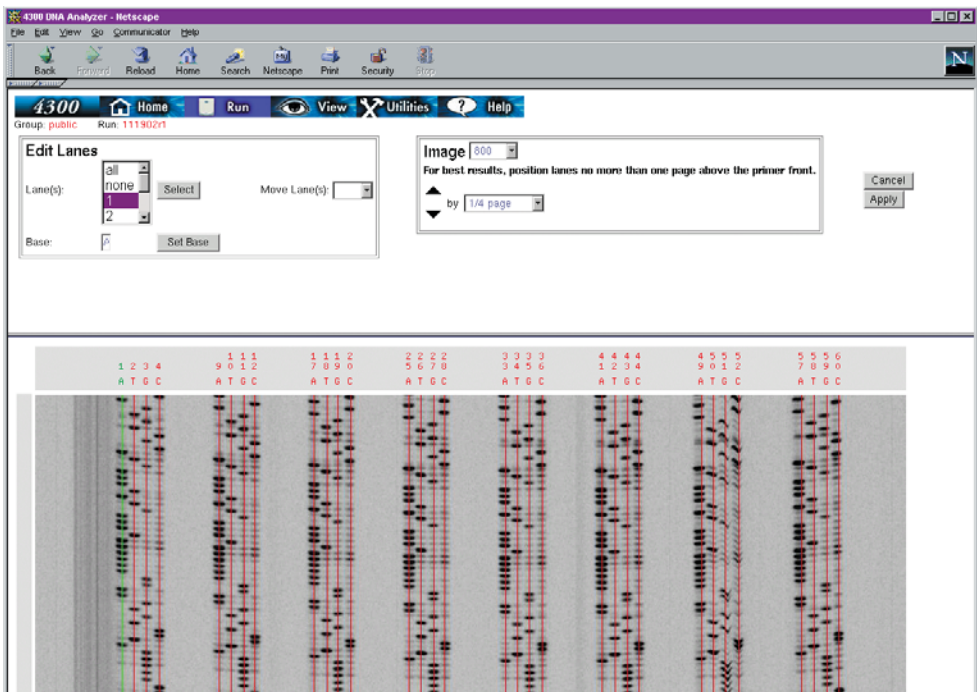
As electrophoresis progresses, you can view the image data that have accumulated by clicking the **Image** link on the Run page. Clicking **Image** opens the View page that lets you view and manipulate image data (described in Chapter 8). The 700 channel image is displayed with red bands and the 800 channel has green bands. Where the two

channels have fluorescence at the same location, the two colors combine to form some shade of yellow. After automatic lane finding, a link named **Curves** is also displayed if the electrophoresis conditions have autosequencing enabled. The **Curves** link opens the Curve Viewer page (described later in this chapter).

Automatic Lane Finding

Automatic lane finding does not begin until enough bands have accumulated in the image data to definitively determine the lanes. A link named **Verify Lanes** is displayed at the bottom of the run page that enables you to examine the lanes and verify that there is one lane line centered in each lane on the gel.

The Verify Lanes window is shown below.



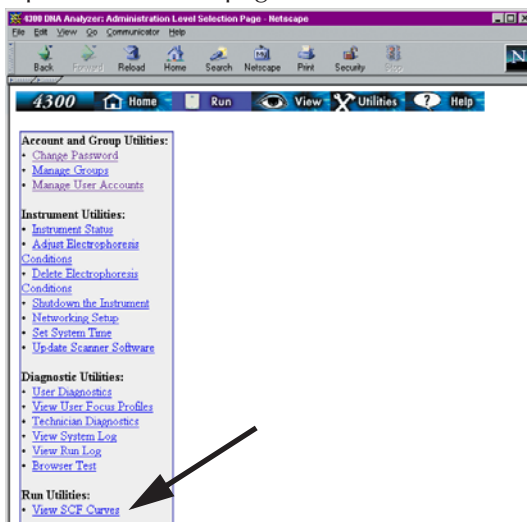
Occasionally, automatic lane finding may not find the lanes correctly due to electrophoresis anomalies that make the image data hard to interpret. When this happens, the Run page has a large, bold **Verify Lanes** link at the top that indicates a problem with lane finding. If you click this link, the Verify Lanes page opens so that you can change the lane positions. The *Troubleshooting Tips* section at the end of the chapter discusses how to manually adjust lanes.

IMPORTANT: Lane positions can only be corrected while the run is still in progress. Finished runs cannot be re-opened and sequenced. Make sure that lane finding is successful before leaving the instrument unattended. If lane finding fails, the images will not be sequenced unless the **Verify Lanes** link on the Run page is used to correct the lanes.

Viewing Sequence Data

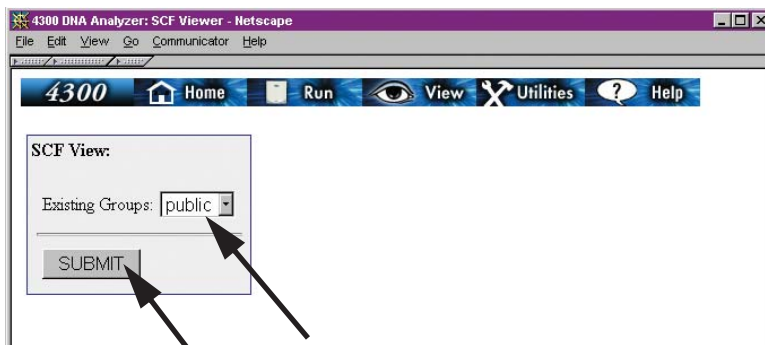
Sequence data can be viewed in real time, as the data are collected, or after the run is complete using the following procedure.

- 1) Open the Utilities page, click the **View SCF Curves** link.

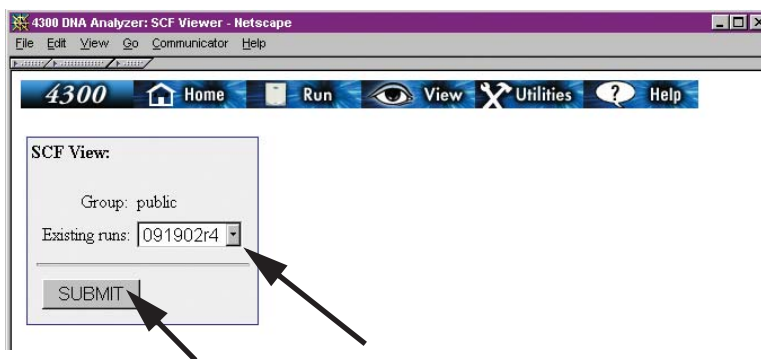


*While a run is in progress you can also view curves for a run by clicking the **SCF Curves** link on the Run page.*

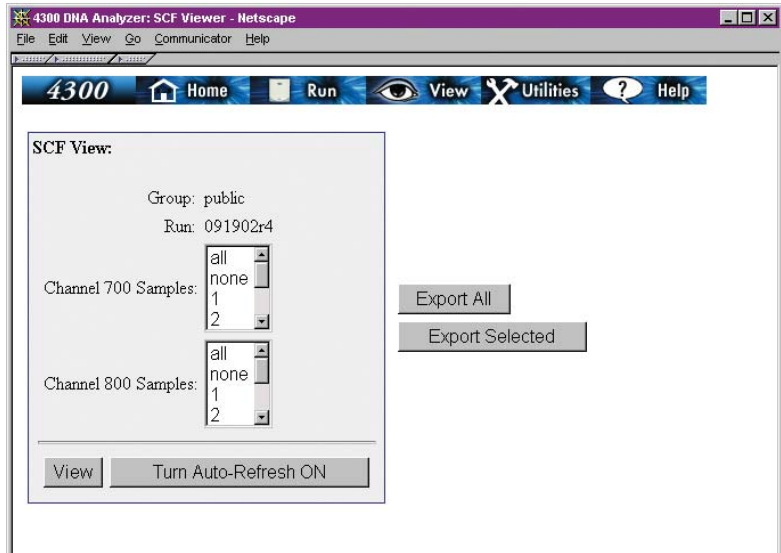
- 2) Select the Group containing the run you want to view and click **Submit**.



- 3) Select the run you want to view and click **Submit**.



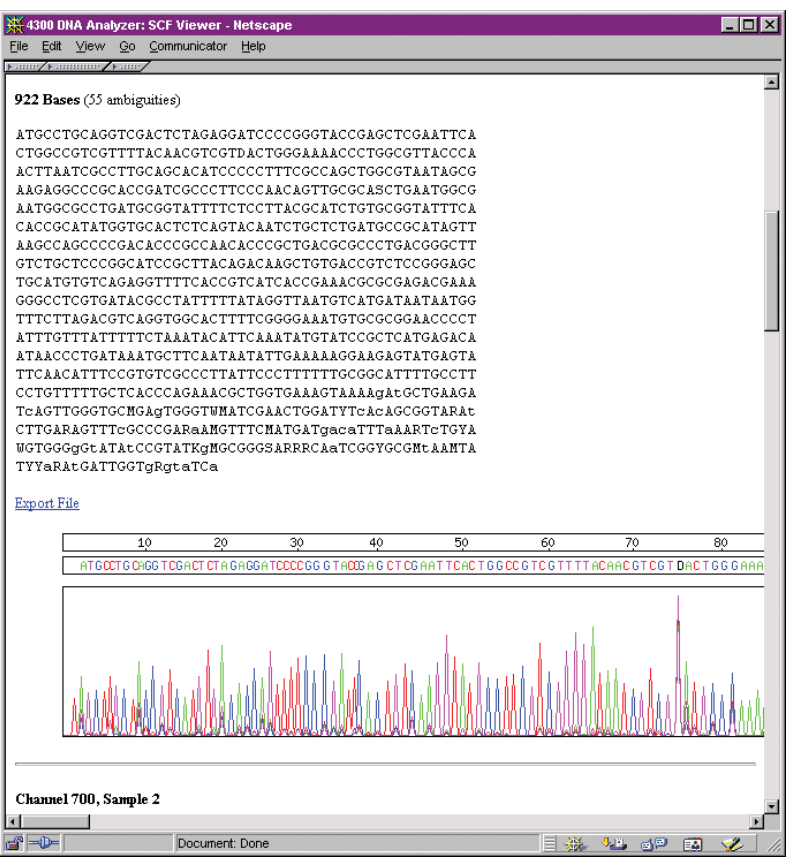
The SCF View page lets you view or export sequence data.



Viewing Samples

- 4) Begin by selecting the samples you want to view from the **Channel 700 Samples** and **Channel 800 Samples** fields. (Shift-click or Control-click to select multiple samples.)

- 5) Click the **View** button to display the selected samples and then scroll down to see sequence data and SCF curves for each sample.



If the run is still in progress, only a portion of the sequence data will be shown (it takes 1 to 1.5 hours for any data to be available). If you click the **Turn Auto-Refresh ON** button at the top of the page, the data will be updated periodically as sequencing progresses.

Exporting Data

To export sequence data, click and drag over the text in the browser window to select it. Copy the text and paste it into your analysis or database software.

An **Export File** link is shown above every SCF curve that can be used to export curve data in Standard Chromatogram File format (version 2) for that particular sample. SCF curve files are compatible with a wide variety of sequence assembly and analysis programs.

To export more than one SCF file, click the **Export All** or **Export Selected** buttons at the top of the page. **Export All** exports SCF files for all samples to a single “zip” file. **Export Selected** exports SCF files to a single “zip” file for the samples selected in the **Channel 700 Samples** and **Channel 800 Samples** fields. Utility programs to un-zip the files can be found on www.download.com and other shareware Internet sites.

Closing the Browser During the Run

After the run has started, the Model 4300 has complete control of electrophoresis and you can close the browser if you need the computer for other functions. To check on the run at a later time, open the browser, log in, and click the **Run** link on the home page. If the run is still in progress, the Run page will be displayed. If the run is complete, the Start Run page will be displayed. Completed runs can be viewed as described in Chapter 8.

Stopping Electrophoresis

Normally, a run is automatically stopped when the run time specified in the electrophoresis conditions has elapsed. If the browser is open when a run is automatically completed, the Run page is closed and the Start Run page is displayed again so that another run can be started. If you need to stop a run during electrophoresis, there are two

ways. Click **Stop Run** if you want to stop electrophoresis and save all image files before the full run time has elapsed. **Stop Run** is useful when there is still run time remaining, but no more bands are being detected and the image data being collected are meaningless.

If you need to completely abandon a run click **Cancel Run**. Canceling a run does not save any files, so it should not be used to finish a run early. A typical situation where **Cancel Run** might be used is when samples have failed and there is no need to collect more data or to keep the data that have already been collected.

Base Calling Description

When sequencing starts, the base caller analyzes the image to find the primer front, or the beginning of the bands. The software determines an initial point for lane finding that is generally a short distance above the first bands, near the bottom of the image. Lanes are found automatically at that initial point, but can be verified using the **Verify Lanes** link on the Run page.

The first base is called just above the initial point where lanes were found. Sequencing then proceeds "backwards" toward the bottom of the image. When sequencing backwards, a fixed amount of image data are processed, so depending on where the initial point was placed, the base caller may call bases all the way back to the first band on the image, or stop somewhere just above.

After finishing the initial step of calling bases backward toward the bottom of the image, sequencing begins again, starting at the first base called and proceeding toward the top of the image. The software compensates for lane migration and lanes where band migration may be faster or slower than other lanes. Sequencing terminates when the software determines that it is making too many calls with low confidence. This is similar to the **Auto Stop** sequencing setting in LI-COR's e-Seq software.

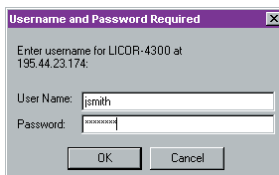
Important: Finished runs cannot be re-opened and sequenced. Images can only be automatically sequenced while a run is in progress. Make sure that lane finding is successful before leaving the instrument unattended. If lane finding fails, the images will not be sequenced unless the **Verify Lanes** link on the Run page is used to correct the lanes.

Starting a Run for Microsatellite or AFLP® Applications

This section describes how to use browser software to start runs for microsatellite or AFLP® applications.

Before you begin, make sure the electrophoresis gel apparatus is properly mounted and the instrument door is closed as described in the Model 4300 Applications Manual. Use the steps below to start a new run using an Internet browser:

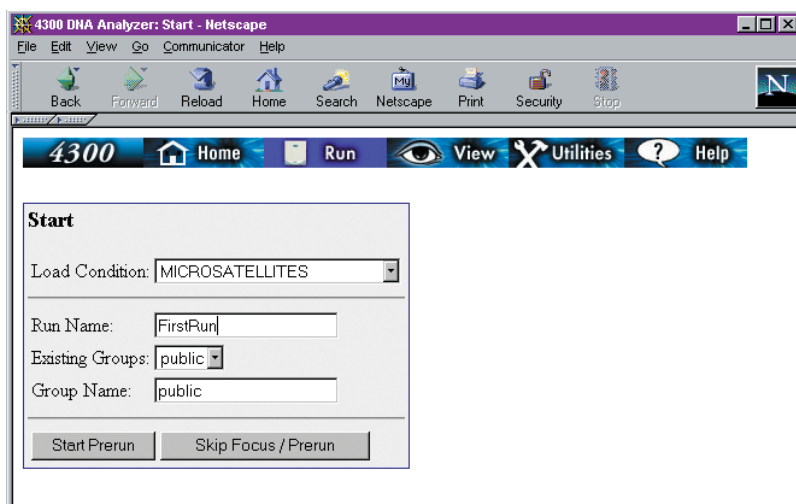
- 1) Log in to the Model 4300 using browser software (Chapter 5).
- 2) Click **Run** button on the home page.
- 3) In the password window, enter the **User Name** and **Password** assigned to you by your Model 4300 Administrator and click **OK**.



User Name and Password are case sensitive.

Entering Run Information

The Start page is used to choose a set of electrophoresis conditions, name the new run, and select a scan group in which to store the data.



- 4) Select a set of electrophoresis conditions from the **Load Conditions** drop-down list that matches your application (microsatellites or AFLP® analysis).

Note: If you accidentally choose a electrophoresis conditions file that has **Autosequence** selected, the Start page will include additional fields for lane order, number of samples, and the number of empty lanes between samples. These fields are described above in the section on 'Starting Runs That Use The Autosequence Software'.

- 5) Enter a name for the new run in the **Run Name** field.

Run names can be 1 to 32 characters long. Alphabetic or numeric characters, as well as dashes and underscores, are acceptable. Do not use any other characters.

- 6) Choose an existing group in which to store the run from the **Existing Groups** drop-down list, or create a new group by entering a group name in the **Group Name** field.

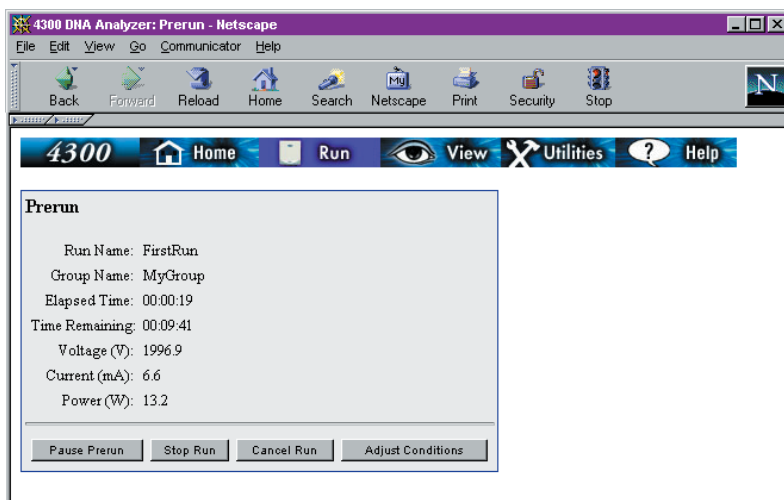
Starting the Pre-run

- 7) Click **Start Prerun** to begin the pre-electrophoresis procedure for the gel.

During the first 10 minutes of the pre-run, the laser/microscope is focused in the gel and the detection electronics are optimized for the run. For typical runs, the electrophoresis voltage and temperature used during the pre-run are the same as the voltage and temperature that will be used during the run.

Note: When reloading gels for microsatellites or AFLP[®] analysis, click **Skip Focus/Prerun** rather than **Start Prerun**. Clicking **Skip Focus/Prerun** bypasses the focusing of the laser microscope and pre-electrophoresis since it is assumed that these processes were done when the gel was run the first time. Do not skip the pre-run on the initial run or if the gel has been moved between runs. The microscope may not be focused if the gel has been moved. The Model 4300 Applications Manual has complete information on reloading gels for both microsatellites and AFLP[®].

When the pre-run starts the Prerun page is displayed. The actual electrophoresis conditions (voltage, current, and power) are displayed as well as the time remaining in the pre-run. The pre-run information is periodically updated with new values from the instrument.



If you need to pause the pre-run for some reason, such as opening the instrument door, click the **Pause Prerun** button. The **Pause Prerun** button will change to **Resume Prerun**. Click **Resume Prerun** to restart the pre-run from the beginning (you cannot resume a paused pre-run). The **Start (Y)** key on the instrument keypad can also be used to restart a paused pre-run.

If you need to abort the run rather than just pausing it, click **Cancel Run**. The **Stop Run** button also stops the pre-run, but it saves all files that have been created. **Stop Run** is useful for completing a run early once electrophoresis has started, but it is not generally used during the pre-run because no image data have been stored.

Occasionally you may get a focus error if the focus routine cannot accurately determine the center of the gel. The *Troubleshooting Tips* at the end of the chapter explain how to correct focus errors.

If you need to change the duration of the pre-run or the electrophoresis conditions, click **Adjust Conditions**.

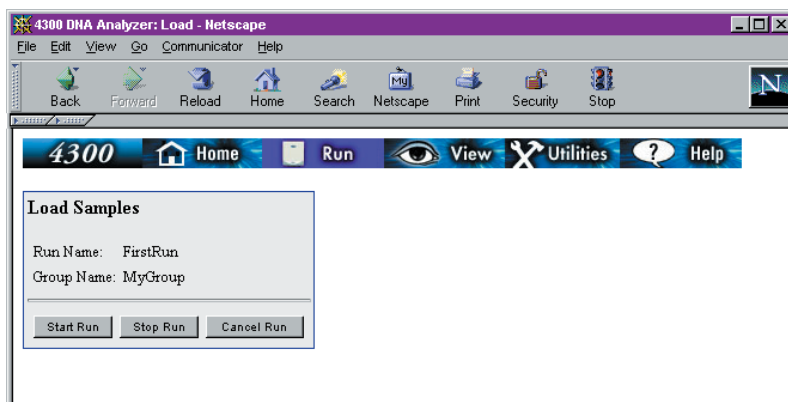
The screenshot shows a Netscape browser window titled "4300 DNA Analyzer: Electrophoresis Conditions - Netscape". The browser's address bar is empty, and the toolbar includes buttons for Back, Forward, Reload, Home, Search, Netscape, Print, Security, and Stop. Below the browser window, there is a navigation bar with buttons for Home, Run, View, Utilities, and Help. The main content area displays a form titled "Adjust Electrophoresis Prerun Conditions". The form contains the following fields and controls:

- Volts (0 - 5000) V: A text input field with the value "2000.0".
- Current (0 - 40) mA: A text input field with the value "25.0".
- Power (0 - 120) W: A text input field with the value "50.0".
- Enable Heater: A dropdown menu with "Yes" selected.
- Temperature (0 - 50) °C: A text input field with the value "45".
- Time (HH:MM): A time selection control with "00" for hours and "25" for minutes, separated by a colon. Up and down arrows are visible on either side of the colon.
- At the bottom of the form are two buttons: "Submit Adjustment" and "Cancel Adjustment".

Each parameter was described earlier in this chapter. To make changes, edit the fields and click **Submit Adjustment**. The electrophoresis power supply and heater plate temperature are adjusted immediately when the changes are submitted.

Loading Samples

When the pre-run time has elapsed, the “Load Samples” message is displayed indicating that it is time to load the samples on the gel. While this message is displayed, the electrophoresis power supply is turned off, but the heater plate remains on so that the gel temperature is maintained.



- 8) Load samples as described in the Model 4300 Applications Manual.

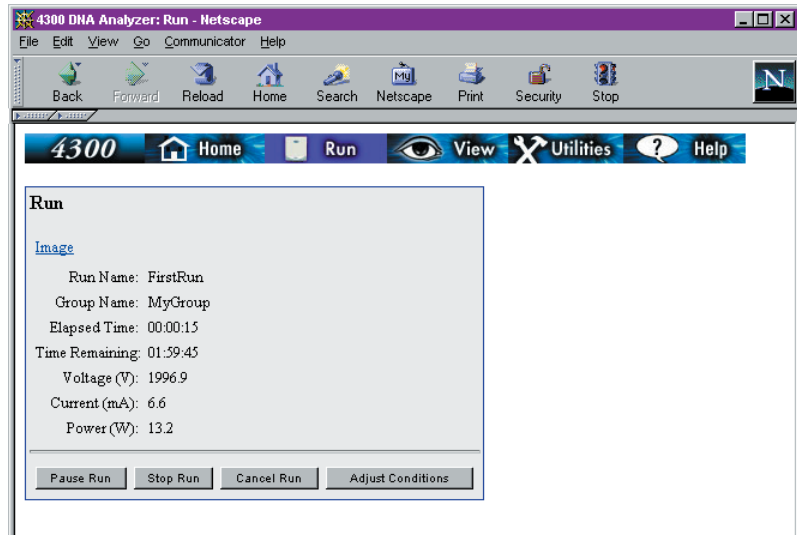
Starting the Run

- 9) After loading samples, click **Start Run** to begin electrophoresis.

As described above, the **Cancel Run** button aborts the run without saving files and the **Stop Run** button stops the run and saves any files that have been created.

When the run starts, the Run page is displayed. Like the Prerun page, the actual electrophoresis conditions (voltage, current, and power) are displayed as well as the time remaining in the run. The run information is periodically updated with new values from the instrument.

If you need to adjust the electrophoresis conditions, click the **Adjust Conditions** button and change the conditions as described above for the pre-run.



As electrophoresis progresses, you can view the image data that have accumulated by clicking the **Image** link on the Run page. Clicking **Image** opens the View page that lets you view and manipulate image data (described in Chapter 8).

Closing the Browser During the Run

After the run has started, the server software in the Model 4300 has complete control of electrophoresis and you can close the browser if you need the computer for other functions. To check on the run at a later time, open the browser, log in, and click the **Run** link on the home page. If the run is still in progress, the Run page will be displayed. If the run is complete, the Start Run page will be displayed. Completed runs can be viewed as described in Chapter 8.

Stopping Electrophoresis

If the browser is open when a run is automatically completed, the Run page is closed and the Start Run page is displayed again so that another run can be started. If you need to stop a run during electrophoresis, there are two ways. Click **Stop Run** if you want to stop electrophoresis and save all image files before the full run time has elapsed. **Stop Run** is useful when there is still run time remaining, but no more bands are being detected and the image data being collected are meaningless.

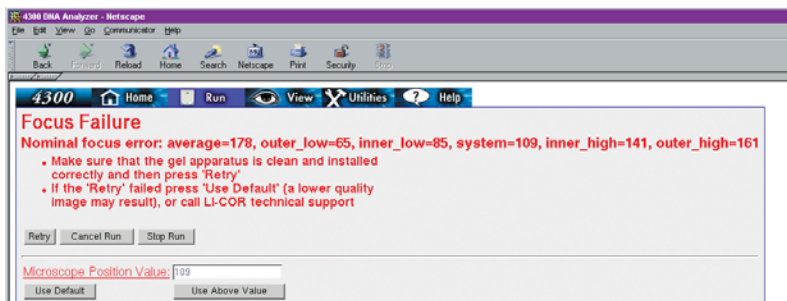
If you need to completely abandon a run click **Cancel Run**. Canceling a run does not save any files, so it should not be used to finish a run early. A typical situation where **Cancel Run** might be used is when samples have failed and there is no need to collect more data or to keep the data that have already been collected.

Troubleshooting Tips

Use this section to troubleshoot focusing or lane finding errors that may occur as you are trying to start the pre-run or run.

Correcting Focus Errors

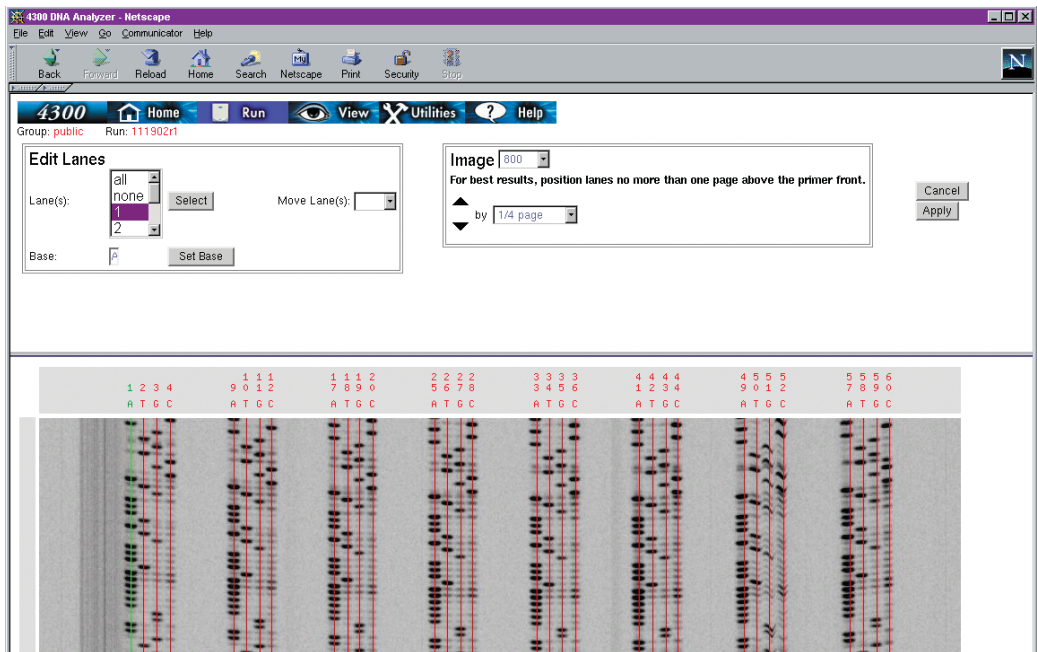
If you experience a focusing error, you will see a message something like that shown below.



Focus errors are most often caused by improper mounting of the gel apparatus, but can be caused by a variety of reasons. The first thing to do is to make sure the gel apparatus is hung correctly on the apparatus support brackets and that the rear gel plate is flush against the heater plate. After making any adjustments, click **Retry** to see if the problem has been corrected. If the problem is not corrected, you can either quit the run (**Cancel Run** button) or continue using a default focus position. The default focus position is pre-programmed into the Model 4300. You will generally get good image data if you click **Use Default** to set the microscope focus positions at the default values. You can also use the focus positions from the last run by clicking the **Use Above Value** button. This may not produce good images, however, if the last user used a different gel thickness than your gel or if the gel was slightly misaligned. The pre-run should proceed normally after selecting one of the pre-determined focus positions.

Correcting Lane Finding Errors for Sequencing Gels

Lane finding errors are indicated by the large “Verify Lanes” error link at the top of the Run page while a run is in progress. Click the link to open the Verify Lanes page shown below.



If the run is a two-dye run, the image being displayed is indicated in the **Image** field. The image can be changed by selecting a different image from the **Image** field.

Moving Lanes

Each lane should have a lane line approximately centered in the lane. If any lane lines are off-center, they should be moved. If a lane needs to be moved, start by determining its lane number, which is listed with vertically oriented numbers above the lane. Select the lane that needs to be moved by choosing it from the **Lanes** list and

clicking **Select**. The lane number you selected should turn green to indicate it is selected and ready to be moved. Lanes can be moved by using the **Move Lanes** drop-down list to select the number of pixels by which the lane should be moved. A positive number of pixels moves the lane to the right and a negative number moves the lane to the left. After making all lane changes, click **Apply**. Sequencing will proceed automatically.

Changing the Base Designation of a Lane

To change the base associated with a lane, select the lane as described above, type the new base letter (A, T, C, or G) in the **Base** field and click **Set Base**. You can also type an “X” character in the **Base** field to prevent a lane from being sequenced.

The “X” character is used to compensate for lane loading mistakes. Suppose you load two lanes of a sample and damage the third lane during loading. Bands will not form correctly in the damaged lane, so the lane should not be sequenced. The autosequencing software in the Model 4300 assumes samples are four contiguous lanes. In this example, your only choice would be to mark all four lanes of the sample with an “X” so that the lanes are not sequenced, and then reload the sample somewhere else on the gel, or on another gel.

Changing the Initial Point for Lane Finding

When lane finding starts, the base calling software designates an initial point on the image where lanes will be found and sequencing will be started. This initial point is above the first bands and is indicated by a green arrow in the left margin of the Verify Lanes page, near the bottom of the image. Since sequencing starts at the initial point, it is a good idea to examine the initial point and make sure there are no electrophoresis anomalies (fluorescent blobs, etc.) that may prevent the software from sequencing accurately. If the initial point is not clear of anomalous fluorescence, it can be moved by

using the “move” arrows below the **Image** field at the top of the window. First select how much you want to move the initial point (1/4 page, 1/2 page, etc.) in the drop-down list and then click the up or down arrow. **When possible, do not move the initial point more than one full page above the first bands at the bottom of the image or the primer front.** If the initial point is moved too far upward, some of the first bands at the bottom of the image may not be sequenced.

Gel Related Error Messages

The Model 4300 Applications Manual discusses gel-related errors in detail. Common errors are summarized below.

Gel Open Circuit Condition: This message is usually displayed because the level of buffer solution in the upper buffer tank has dropped below the electrode, or the buffer is not contacting the gel. The cause is generally improper cleaning or assembly of the gel apparatus, which causes buffer to “wick” up and over the top of the gel apparatus. This error can also be caused by improper apparatus assembly (missing buffer tank lid, high voltage cable, etc.).

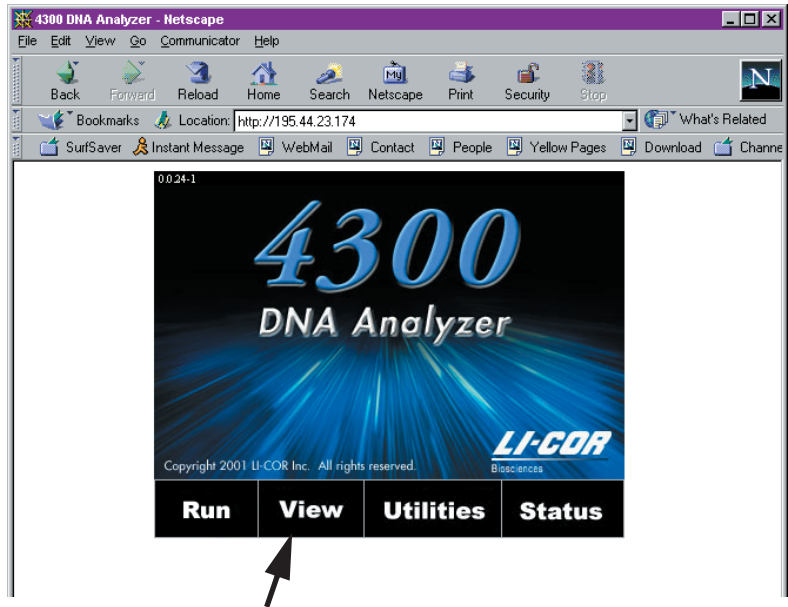
Voltage Arc Detect: If the level of buffer in one of the buffer tanks drops, it can cause an electrical arc. The high voltage power supply is immediately shut off if an arc is detected. Loss of buffer is generally caused by improper cleaning or apparatus assembly as described for the Gel Open Circuit Condition error or Probable Gel Leak error.

Probable Gel Leak: If the back gel plate is not clean and free of caked acrylamide or gel salts, wicking from the bottom buffer tank can occur. Once the wicking action causes moisture to contact the heater plate, the problem will be detected and the high voltage power supply will be turned off. Make sure the plates are clean and dry. Check the rear plate and make sure there is no acrylamide or gel salts along the edges of the rails.

Chapter 8: Importing and Editing Images

Opening the View Page

- 1) Open the home page using an Internet browser (Chapter 5).
- 2) Click the maximize button on the browser window. (Images are often large and it is best not to resize the browser window while viewing an image.)
- 3) On the home page, click the **View** link and enter your user name and password.



Opening a Run Stored on the Model 4300

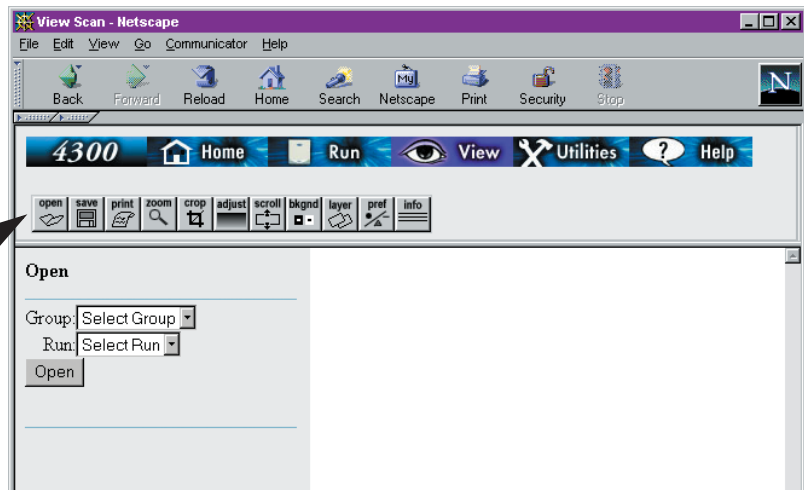
When the **View** link on the home page is clicked, the View page is displayed which can be used to open, edit, and save image files stored on the Model 4300. All editing and file management functions can be accessed using the toolbar in the top frame of the View page.

The **Open** button in the View toolbar can also be used to display the View page. After image files are open, they can be saved to a local storage drive using the **Save** button. Other buttons on the toolbar are used to change the appearance of the image while it is open.

Follow the steps below to open an image.

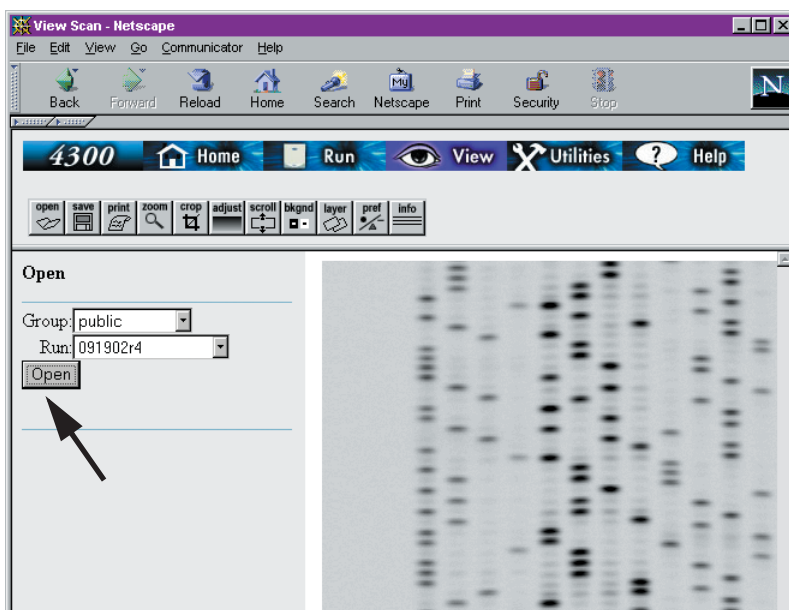
- 1) Select the scan group where the file is located from the **Group** drop-down list on the View page.

The View toolbar has buttons for opening and saving scans, as well as image editing.



Files are separated into scan groups. The **Group** drop-down list shows all scan groups that are available to the user who is logged in. See Chapter 6 for a discussion of scan groups.

- 2) Use the **Run** drop-down list to select one of the runs in the designated scan group.
- 3) Click **Open** to open the image file(s).

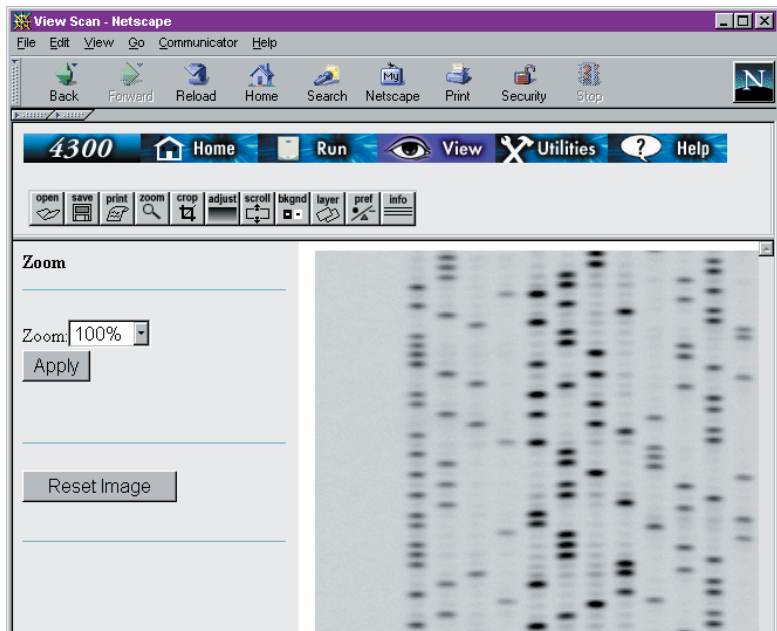


Images are initially displayed in the right side of the window at actual size (1:1). The images are displayed in color with both image channels overlaid. The 700 channel image is red and the 800 channel image is green. At pixel locations where fluorescence from both channels overlap, the image is some shade of yellow. Where the fluorescence on both images is equal and strong, the color will be bright yellow.

Changing the Appearance of an Image

Zooming In and Out

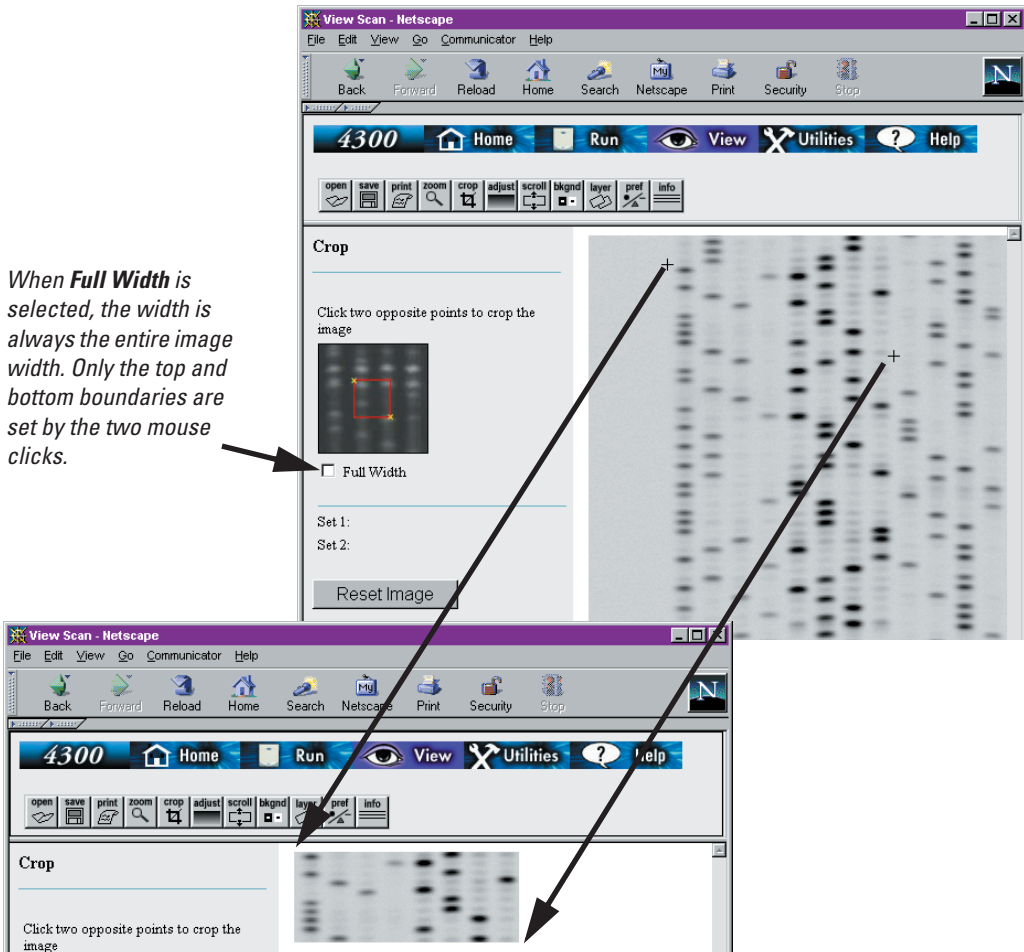
The Zoom page is opened by clicking the **Zoom** tool on the toolbar. In order to see more of the image, select a smaller percentage from the **Zoom** drop-down list. For example, selecting 50% reduces the image by 50% and displays twice as much image as the 100% zoom setting. Click the **Reset Image** button to return the image to the size it was when the window was opened.



Cropping an Image

To crop an image, start by clicking **Crop** in the toolbar. Next, define a rectangular crop area by clicking the mouse at the upper-left and lower-right corners of the area to be cropped, as shown below (crosshair marks are shown for illustration only).

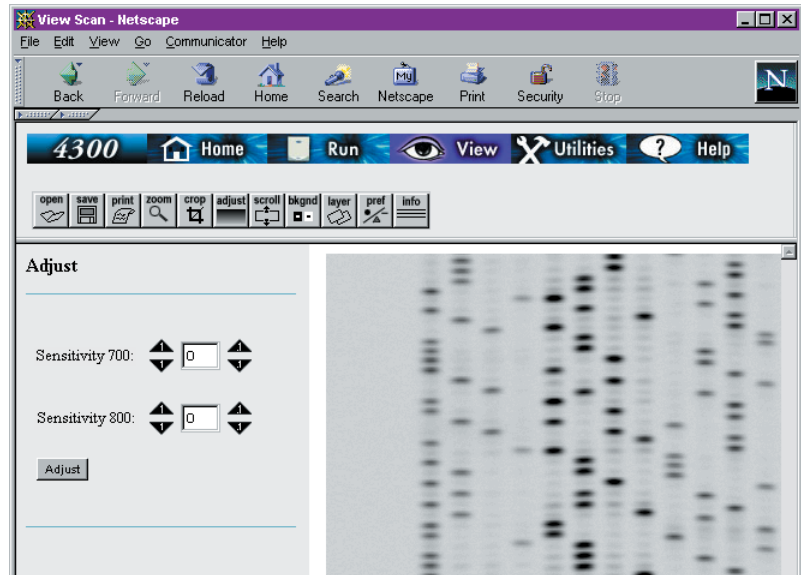
*When **Full Width** is selected, the width is always the entire image width. Only the top and bottom boundaries are set by the two mouse clicks.*



To display the entire image again, click the **Reset Image** button. Cropped images can be saved by clicking **Save** in the toolbar.

Changing Image Sensitivity

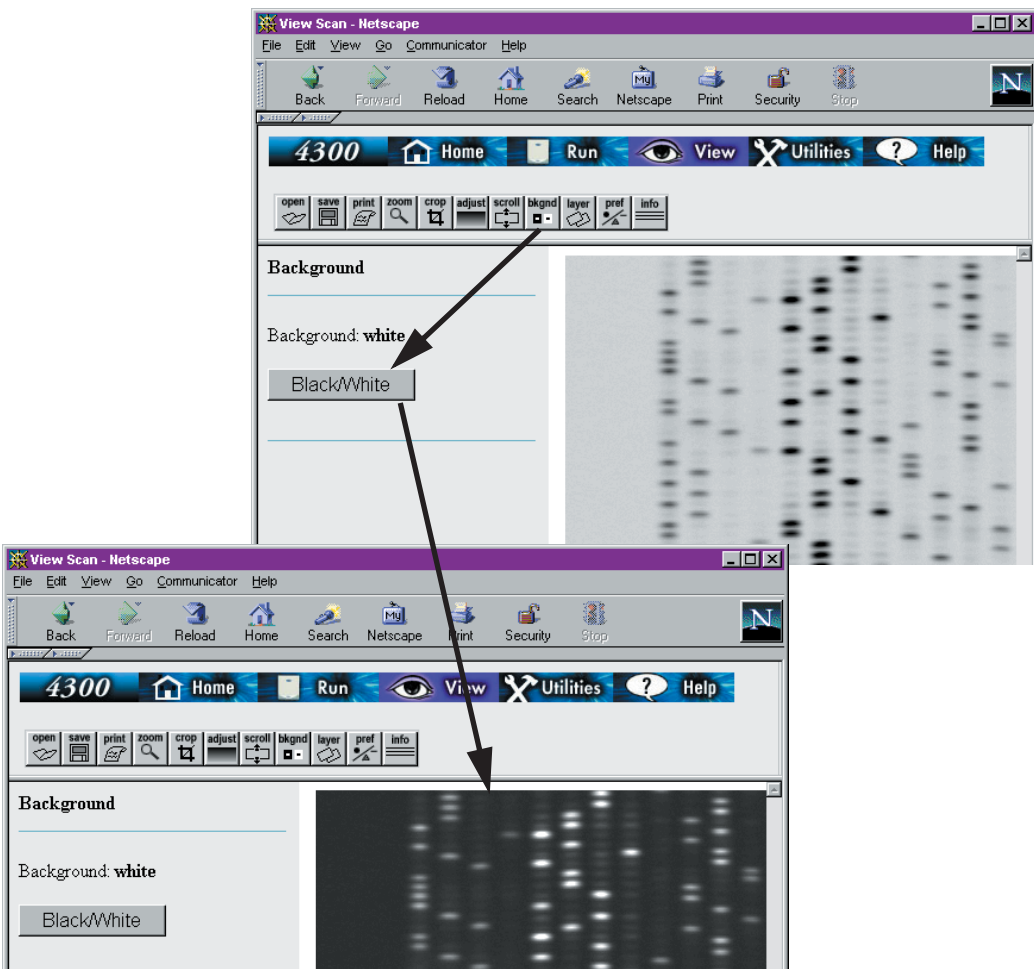
When an image is first opened the bands may appear too strong, too weak, or may not appear at all. The appearance of the image can be changed by clicking **Adjust** in the toolbar and changing the sensitivity.



The sensitivity settings change how the wide range of data values in the image are mapped to the comparatively small number of color or grayscale values on the display. The sensitivity of each channel can be adjusted independently by entering a value or by clicking the arrow buttons. The up and down arrows labeled "1" provide coarse sensitivity adjustment and the arrows labeled ".1" provide a finer adjustment. After changing the sensitivity, click **Adjust** to implement the change.

Inverting the Grayscale Map

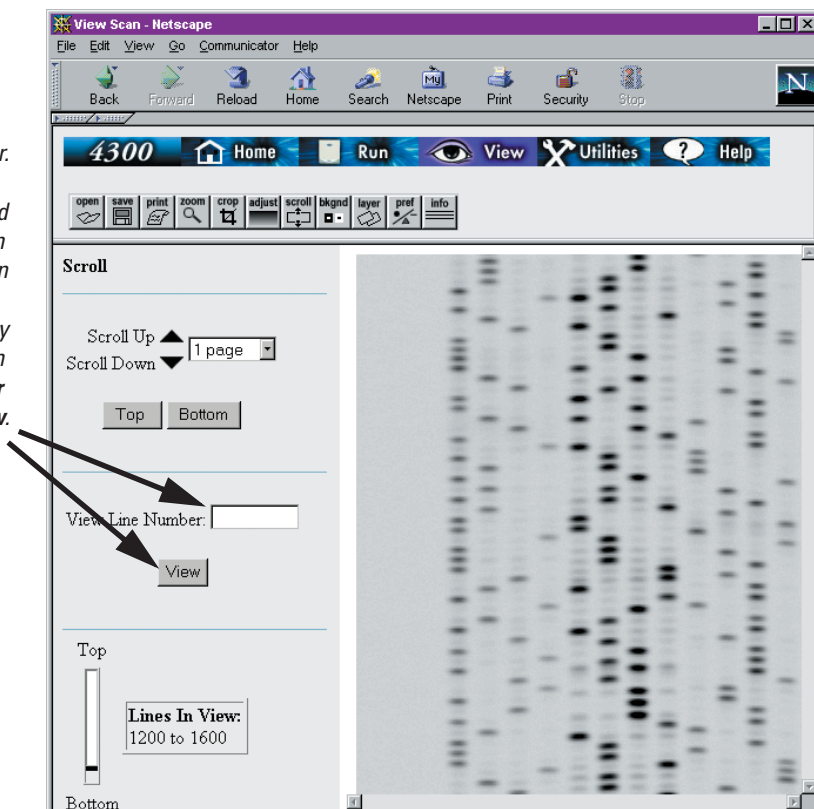
When only a single image channel is displayed (see Showing and Hiding Image Channels below), the grayscale image can be inverted by clicking **Bkgn**d in the toolbar and then clicking the **Black/White** button. Clicking **Black/White** toggles between a white image background with black bands and a black background with white bands.



Scrolling the Image

In addition to the standard scroll bars on the edge of the window, you can access additional scrolling functions by clicking **Scroll** on the toolbar. The **Top** and **Bottom** buttons scroll the image to the top or bottom. The **Scroll Up** and **Scroll Down** arrows scroll the image by the amount selected in the drop-down list. The portion of the image that is displayed is indicated by the black area in the **View** indicator. In the image below, a page starting at line 1200, near the bottom of the image, is displayed.

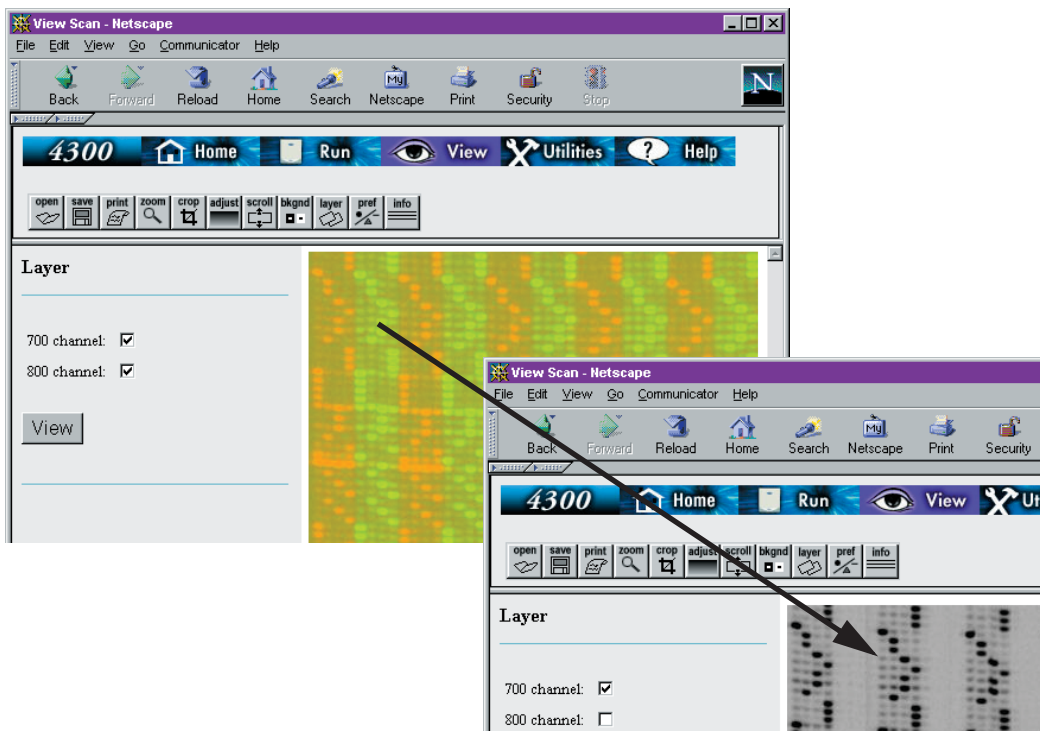
*Each row of pixels is assigned a line number. The current range of line numbers displayed is shown at the bottom of the window. You can scroll the image to a specific line number by entering the number in the **View Line Number** field and clicking **View**.*



Showing and Hiding Image Channels

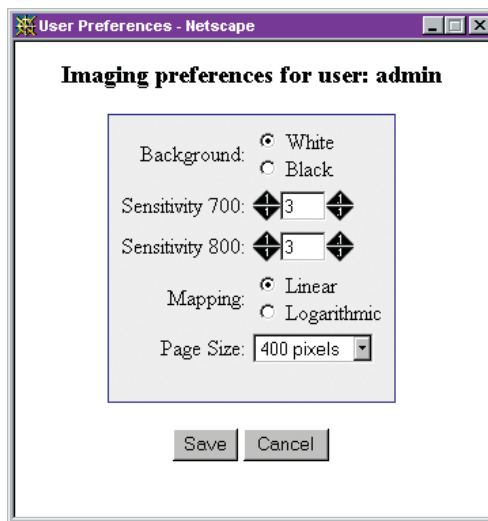
When a scan is opened, the images are initially displayed in color with both image channels overlaid. The 700 channel image is red and the 800 channel image is green. At pixel locations where fluorescence from both channels overlap, the image is some shade of yellow. Where the fluorescence on both images is equal and strong, the color will be bright yellow.

To view only one of the two images, click **Layer** in the toolbar. Next, deselect the image (**700 channel** or **800 channel**) that you don't want to see and click **View**. The image that remains selected is displayed in grayscale format.



Changing the Image Display Preferences

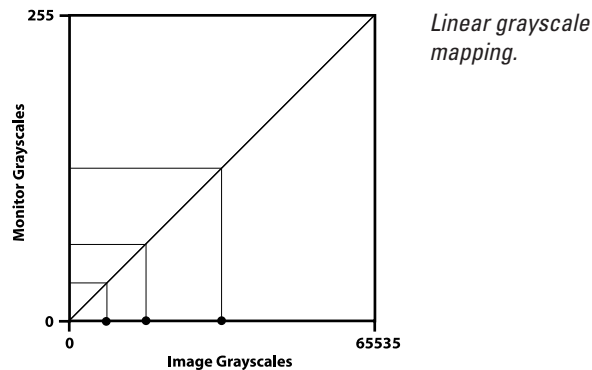
If you always change the appearance of images after opening them, you can save time by setting preferences that control how the images are displayed when opened. To open the preferences, click **Pref** on the toolbar. The image display style can be set to a white background with black bands or a black background with white bands by selecting the **White** or **Black** background setting, respectively. The default sensitivity can be adjusted for each image channel in increments of 1 or 0.1 using the up and down arrow **Sensitivity** buttons or by entering a number. The **Page Size** sets the number of rows of pixels by which the image scrolls up or down when you click in the image scrollbar.



Changing How Image Data Are Mapped to the Monitor

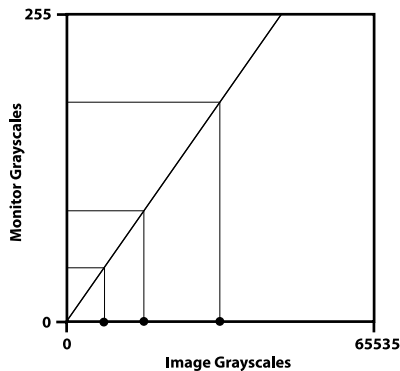
Image files collected by the Model 4300 contain over 65000 grayscale values, but the typical computer monitor can only display 256 grayscale values. This requires a scheme to “map” grayscale values in the image to the monitor. The **Mapping** buttons in the Imaging Preferences window let you select **Linear** or **Logarithmic** mapping.

The default setting is **Linear** mapping, which is preferred in most applications. Linear mapping can best be explained by considering a graph, which has image grayscale values on the X-axis and monitor grayscales on the Y-axis.



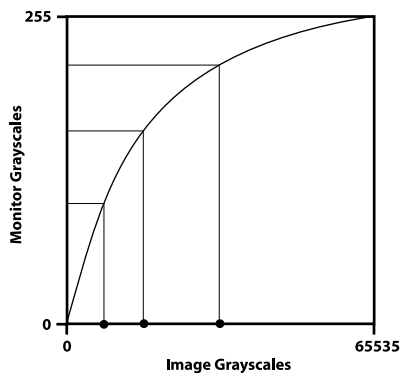
With **Linear** mapping, a given change in image intensity results in a proportional change in display intensity. The three circles on the X-axis above represent the grayscale values of three bands of relatively low intensity. When bands have low intensity on the monitor, the most common way to “intensify” the bands is to change

the sensitivity setting. This changes the slope of the linear response line as shown below. The result is that low intensity bands are displayed with higher grayscale values on the monitor.



Linear grayscale mapping after increasing sensitivity.

Increasing the sensitivity will produce satisfactory results as long as there are no bands on the image with high grayscale values. Bands with high grayscale value will saturate as the sensitivity is increased. When you have bands over the entire grayscale range but need to intensify weak bands on the monitor, **Logarithmic** mapping can help because it intensifies the display of weak bands while preventing strong bands from saturating.

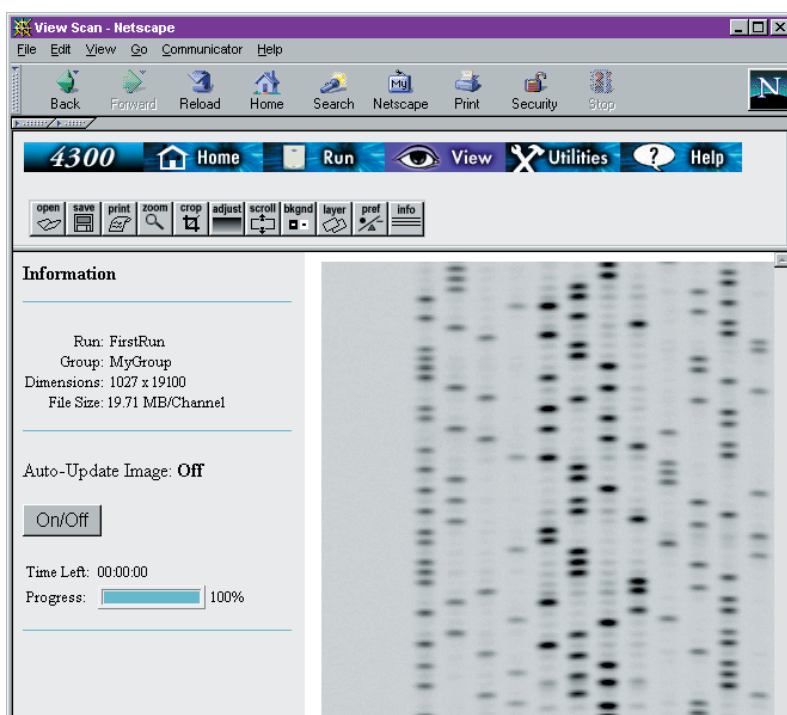


Logarithmic mapping intensifies weak bands while keeping strong bands from saturating.

Note: The **Mapping** preferences only influence image display and do not change how the image is analyzed during base calling.

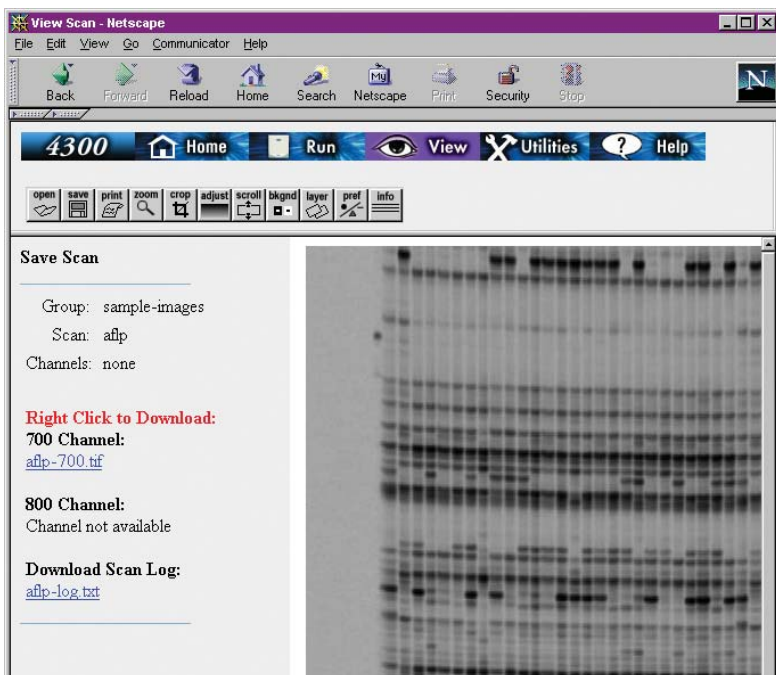
Displaying Run Information

To display information about the run, click **View** (if necessary) and click **Info** on the toolbar. The **Auto-Update Image** button lets you control whether the image updates automatically on the display during data collection. If a run is in progress and you want to observe data collection in real time, **Auto-Update** should be **ON**. If you are trying to perform other operations, you may want to turn **Auto-Update** off since updating the display takes computer resources and can slow down other operations. The time left in the run is displayed and the **Progress** bar shows the percentage of the run that has been completed.



Saving Images to the Computer

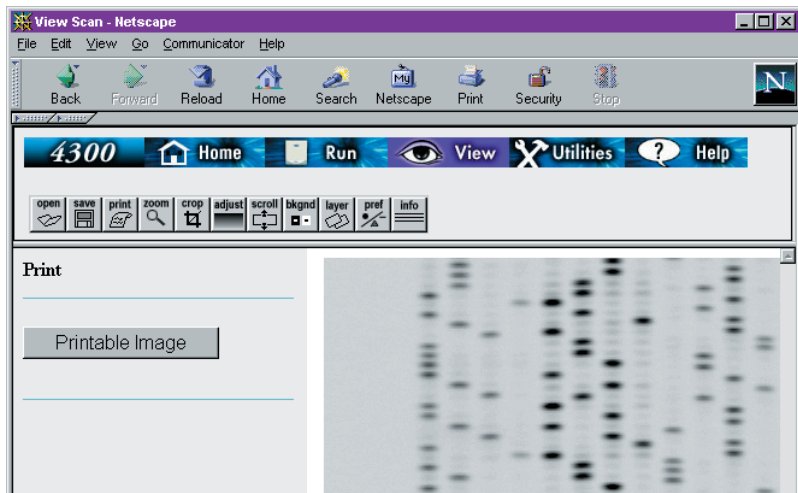
After images are open, they can be saved to a local storage drive by right-clicking the link for a given image channel. Choose “Save Link As” or “Save Link Target As” from the popup menu. In the Save File window, add a “.TIF” file name extension if necessary. If you also want to save the log file for the run, right-click the link for the scan log and select “Save Link As” or “Save Link Target As”. The log file lists the electrophoresis conditions and various instrument operations at various points during the scan. It can be useful for diagnosing problems.



Printing Image Files

After images are open, they can be printed by clicking **Print** in the View toolbar and clicking the **Printable Image** button. When **Printable Image** is clicked, the image is displayed in a new window that does not contain buttons and other user interface features. The image can then be printed by selecting **Print** from the browser **File** menu.

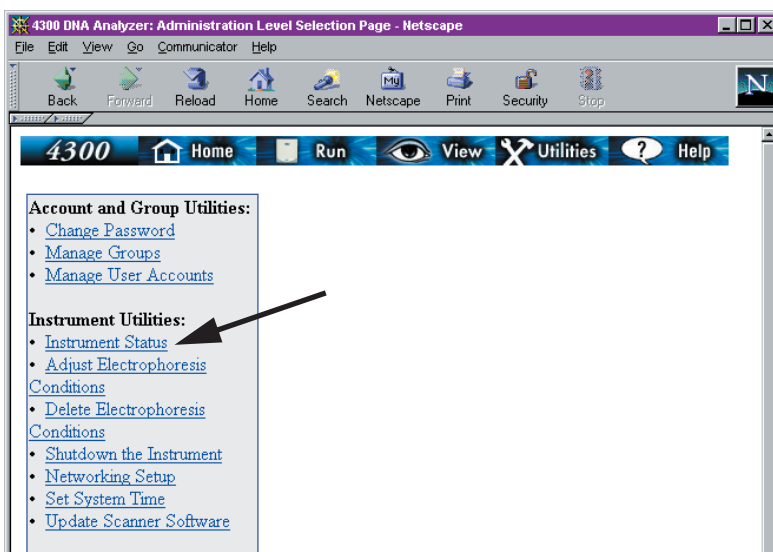
The image(s) are printed exactly as shown. If image channels are overlaid, a composite image of both channels is printed. If only a single channel is displayed, a grayscale image of one channel is printed.



Chapter 9: Instrument Utilities and Diagnostics

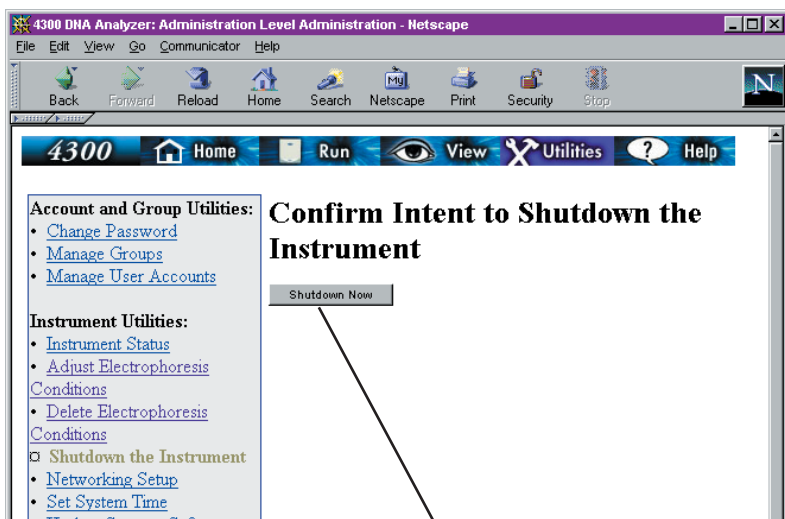
Instrument Status

Chapter 5 describes opening the Status page by clicking the **Status** link on the home page. That discussion describes how to use the Status page to determine whether the instrument is in operation, who the user is, and how much time remains in the current run. The Status page can also be accessed by clicking the **Instrument Status** link on the Utilities page.



Instrument Shutdown

The Model 4300 is normally shut down by pressing the **Power** key on the keypad, but it can also be shut down using the **Shutdown The Instrument** link on the Utilities page. Before shutting down the instrument, use the **Instrument Status** link to make sure there are no runs in progress.



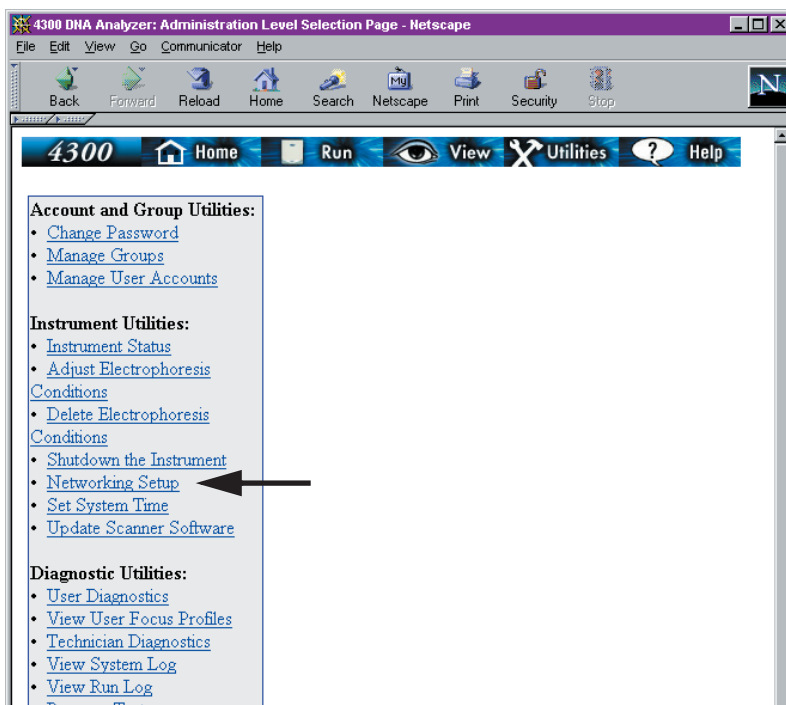
Click **Shutdown Now** to confirm that you want to shut down the instrument.

Note: You must be logged in using an account with administrator rights to use this function.

Editing Network Addresses

Network connections and cabling for both the Model 4300 and the system computer are discussed in the Installation Manual. Chapter 4 discusses how to set the network addresses using the front panel keypad for DHCP or static IP addresses. For some networks, you may also need to enter additional network parameters. All network parameters can be accessed through the **Networking Setup** link on the Utilities page.

- 1) Click the **Networking Setup** link on the Utilities page.



Note: You must be logged in using an account with administrator rights to use this function.

Before connecting to your local network, consult with your network administrator on how to fill in the Network Setup page. A brief description of each field is given below.

4300 DNA Analyzer: Network Configuration - Netscape

File Edit View Go Communicator Help

Back Forward Reload Home Search Netscape Print Security Stop

4300 Home Run View Utilities ? Help

Network Setup

Account and Group Utilities:

- [Change Password](#)
- [Manage Groups](#)
- [Manage User Accounts](#)

Instrument Utilities:

- [Instrument Status](#)
- [Adjust Electrophoresis Conditions](#)
- [Delete Electrophoresis Conditions](#)
- [Shutdown the Instrument](#)
- ☐ [Networking Setup](#)
- [Set System Time](#)
- [Update Scanner Software](#)

Diagnostic Utilities:

- [User Diagnostics](#)
- [View User Focus Profiles](#)
- [Technician Diagnostics](#)
- [View System Log](#)
- [View Run Log](#)
- [Browser Test](#)

Run Utilities:

- [View SCF Curves](#)

IP Address:

☐ Use DHCP

☒ Static IP

IP Address:

Net Mask:

MAC Address: 00:01:03:E8:31:C7

Host Name:

Name:

Gateway:

Address:

Domain:

Name:

Domain Name Servers:

Addresses:

DHCP vs. Static Addressing

Static IP addresses generally provide the most trouble-free operation with the Model 4300 since the address does not change. The issues related to static vs. dynamic addressing are discussed in the section titled “Changing Network Addresses” in Chapter 4.

Your network administrator will tell you whether to select **Use DHCP** or **Static IP**. If Dynamic Host Configuration Protocol (DHCP) is selected, a new IP address will be assigned by your network (can take as long as several minutes depending on the DHCP server) after you click the **Configure** button. To view the new address, repeatedly press **Next** on the Model 4300 keypad until the “IP” prompt and new address are displayed.

If **Static IP** is selected, you must enter the **IP Address** and **Net Mask** assigned by your network administrator.

The **MAC Address** listed below **Net Mask** is the address of the network card in the Model 4300. Your network administrator may request the MAC address during configuration.

Using Host Names

In order to log on to the Model 4300 using a browser, you must enter either the IP address or a host name assigned to the instrument. If your network has a domain name server, a host name may be a convenient alternative to entering the IP address. A host name is generally a common name that is easy to remember, rather than a series of numbers.

To use a host name, enter the host name assigned by your network administrator in the **Host Name** field. Enter the domain name in the **Domain** field and enter the IP address of the domain name server(s) that will be used to resolve the host name in the **Domain Name Servers** field. If there is more than one domain name server, enter only one address per line in the field.

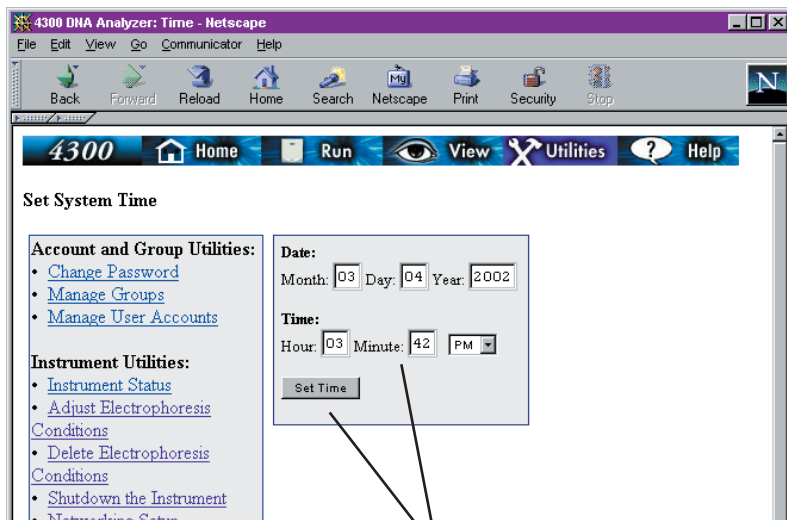
Entering a Gateway Address

In the **Gateway** field, enter the address of the router that connects your subnet to the rest of the network. A gateway address and Domain Name Server must be specified to enable direct e-mail of scan logs and system logs for troubleshooting.

Setting the Date and Time

All image files are assigned a date/time stamp that identifies when the files were created. Click the **Set System Time** link on the Utilities page to set the clock.

Important: Check the instrument status before setting date and time. If you attempt to set the clock during a run, the run will be aborted.



Enter **Date** and **Time** and
click **Set Time**.

Note: You must be logged in using an account with administrator rights to use this function.

A backup battery maintains the clock when power is disconnected, so it is generally only necessary to set the clock when the instrument is first installed.

Updating the Model 4300 Server Software

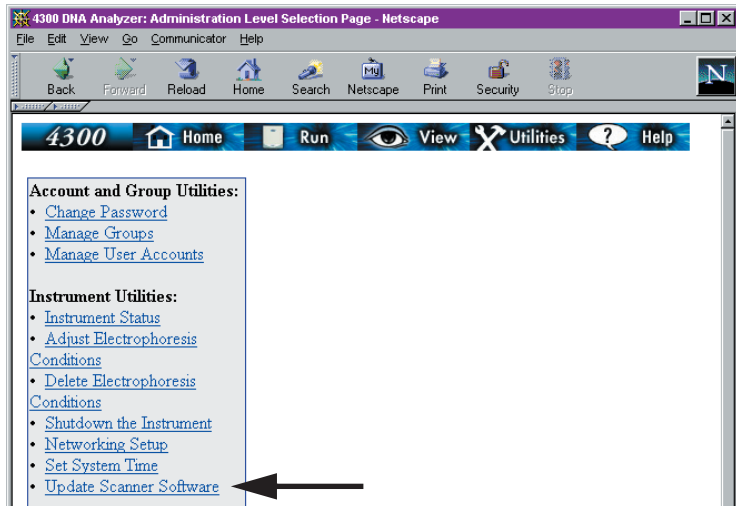
Updates to the Model 4300 server software will be posted on LI-COR's web site (Support section) and made available on CD-ROM. Users will be notified of updates and given instructions on how to get the update on CD-ROM if downloading the update file is not possible.

IMPORTANT: *The software update requires about 100 MB on the internal hard disk. If the hard disk is too full, the oldest runs will be deleted to make room for the update, so make sure you have downloaded all images and other run data before proceeding.*

Whether you have an update CD or have downloaded the update files, the update procedure is as follows:

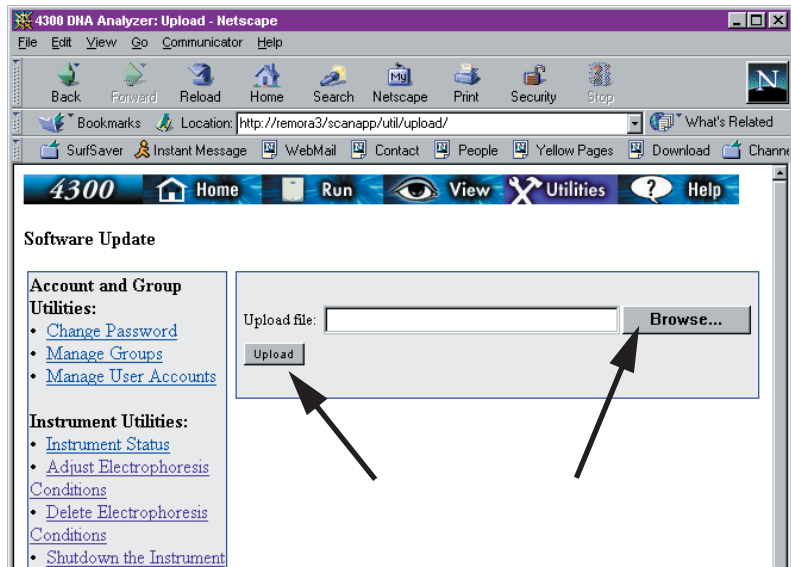
- 1) Use your browser to log on to the Model 4300. Click **Utilities** on the home page. Enter a **User Name** and **Password** with *administrator* rights.
- 2) Click the **Instrument Status** link and make sure the instrument is not being operated by another user.

3) Click the **Update Scanner Software** link.



4) If you have an update CD, insert the CD into the drive. If you downloaded the update file, make sure the file is accessible on your computer or network drive.

- 5) Click **Browse** and use the standard file selection window to select the update file from CD or disk drive. If the upload file name is not displayed, make sure that all file types are being displayed in the file selection window.



- 6) Click **Upload**. There will be a short period of inactivity while the update file is loaded.
- 7) Click **Install** to start the software installation.



The file (27897949 bytes) was placed on the scanner.

Click **Install** to install the software

When the update starts, a list of tasks is displayed with a yellow arrow next to the task being performed. As each task is successfully completed, a green check mark is placed next to the task.

Update list with two processes complete and a third process underway.

4300 Server Software Update (Version 1.2.3)

✔ Stopping Scanner Software

✔ Updating Components

➔ Updating Server

Updating User Database

Updating OS Database

Starting Scanner Software

Show Activity Log

If there was a problem during the update, a red “X” will be placed next to the task that failed and a failure message will be displayed. Contact LI-COR Technical Support if the update fails. A successfully completed update appears as shown below.

4300 Server Software Update (Version 1.2.3)

✔ Stopping Scanner Software

✔ Updating Components

✔ Updating Server

✔ Updating User Database

✔ Updating OS Database

✔ Starting Scanner Software

Success!

Go To Home Page

Show Activity Log

Details of the update can be examined by clicking **Show Activity Log**. If you have a failure during an update, LI-COR Technical Support may ask you to e-mail or print the activity log.

4300 Server Software Update (Version 1.2.3)

- ✔ Stopping Scanner Software
- ✔ Updating Components
- ✔ Updating Server
- ✔ Updating User Database
- ✔ Updating OS Database
- ✔ Starting Scanner Software

***Note:** Error and warning messages are a normal part of the activity log, even for successful updates. Only LI-COR Technical Support can interpret the activity log.*

Success!

[Go To Home Page](#)

Activity Log

```
Installing 4300 version 1.2.3...
Shutting down autosequencer: shutdown: Got exception: Socket: unable to connect to /
[FAILED]
Shutting down paneld: [ OK ]
Shutting down electrophoresisd: [ OK ]
Shutting down heaterd: [ OK ]
```

User Diagnostics

If you have a problem with your instrument, LI-COR Technical Support staff may ask you to perform the User Diagnostic function. If you suspect a problem with the instrument, the User Diagnostic can also be used to verify a problem before contacting technical support.

Before running the User Diagnostic, use the **Instrument Status** link to make sure the instrument is idle. To access the User Diagnostic, open the Utilities page and click the **User Diagnostics** link.

4300 DNA Analyzer: Diagnostics Viewer (User Level) - Netscape

File Edit View Go Communicator Help

Back Forward Reload Home Search Netscape Print Security Stop

4300 Home Run View Utilities Help

Account and Group Utilities:

- [Change Password](#)
- [Manage Groups](#)
- [Manage User Accounts](#)

Instrument Utilities:

- [Instrument Status](#)
- [Adjust Electrophoresis Conditions](#)
- [Delete Electrophoresis Conditions](#)
- [Shutdown the Instrument](#)
- [Networking Setup](#)
- [Set System Time](#)
- [Update Scanner Software](#)

Diagnostic Utilities:

- [User Diagnostics](#)
- [View User Focus](#)

To Run Diagnostics [Click Here](#) (Takes several minutes to run)

Transfer Options

Send To:

CC:

Reply Back To:(Your Return Email Address)

Comments:

Email Complete Session:

Download Complete Session:

To start the diagnostic, click the **Click Here** button at the top of the page.

At the end of the diagnostic, summary results are given at the bottom of the page for a variety of instrument parameters. All of these results should indicate **PASSED**. If any of these results indicate **FAILED**, contact LI-COR Technical Support (1-800-645-4260) or your local LI-COR Distributor.

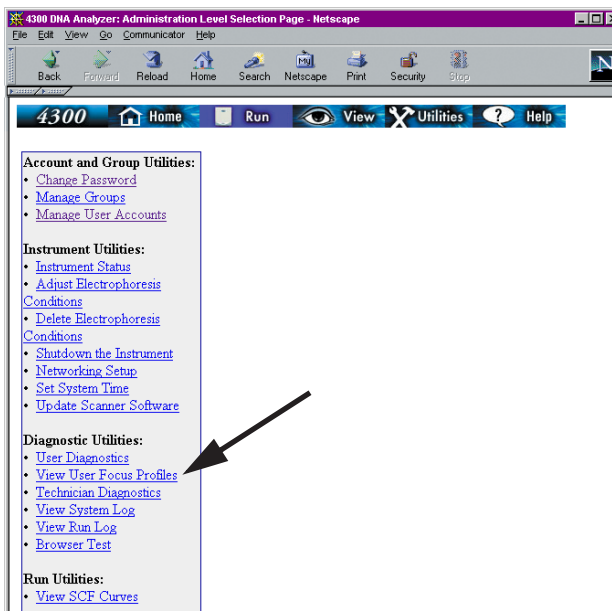
Instrument Information	PASSED
Motor Control Card	PASSED
Signals Board	PASSED
Motor Usage	PASSED
System Resources	PASSED
Focus Info	PASSED
Heater Diagnostics	PASSED
HWPS Diagnostics	PASSED
X-Motor	PASSED
Z-Motor	PASSED
DSP Card	PASSED
Active Signals Board	PASSED
Active Diagnostics	PASSED

A variety of tests are performed during the User Diagnostic. Most of these tests are meaningful only to LI-COR Technical Support staff, so only summary results are displayed. LI-COR or your Distributor may ask for a copy of the complete test results. If your network allows you to use the e-mail functions directly, you can enter your address in the **Reply Back To** field, add any descriptive comments, and then click **E-Mail**. (The default address in the **Send To** field should be 4300-tech@licor.com.) If you are using a dial-up account, make sure you are connected before clicking **E-mail**. Gateway and DNS addresses must be entered in the Network Setup page to use the e-mail features. If you cannot e-mail directly from the Model 4300, click the **Download** button to download all the diagnostic results into a "zip" file. Use the web page at <http://ftp.licor.com> to upload the "zip" file or other image files that exhibit a particular problem.

User Focus Profiles

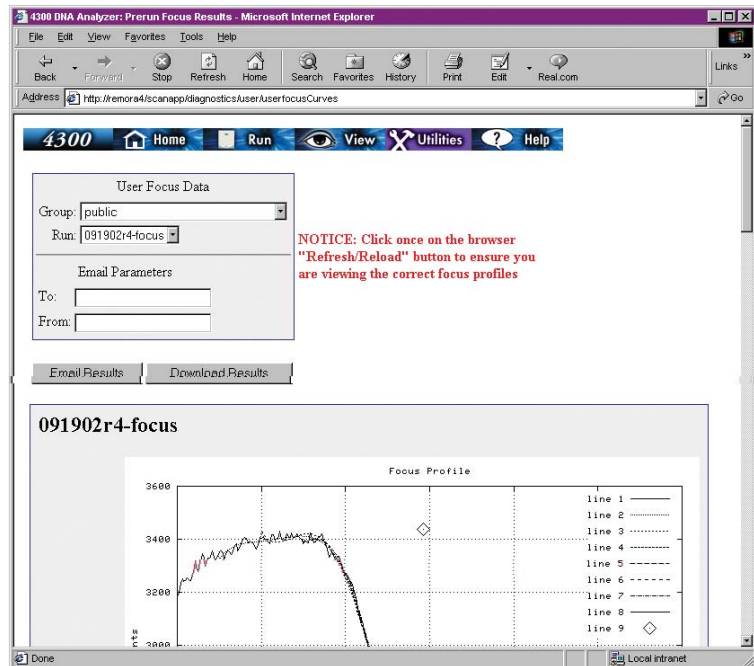
If you have frequent focus errors when starting runs, LI-COR Technical Support staff may ask you to use the View User Focus Profiles link on the Utilities page to e-mail focus profiles to LI-COR.

The focus profiles show the detector output as the focal point of the microscope is moved through one gel plate, into the gel, and then into the other gel plate. The lower fluorescence in the center of the gel usually allows the center of the gel to be determined accurately.



Note: You must be logged in using an account with at least control rights to use this function.

In the event that LI-COR asks you to send the focus profiles, begin by using the drop-down lists to select the group that contains the run. Then select the run. The run name, will have the word “focus” appended to indicate that it is the focus profile for the run.



If your network allows you to use the e-mail functions directly, you can enter your address in the **From** field, then click **E-Mail Results**. (The default address in the **To** field should be 4300-tech@licor.com.) Gateway and DNS addresses must be entered in the Network Setup page to use the e-mail features. If you cannot e-mail directly from the Model 4300, click the **Download Results** button to download all the related focus files into a “zip” file. Use the web page at <http://ftp.licor.com> to upload the “zip” file or other image files that exhibit a particular problem.

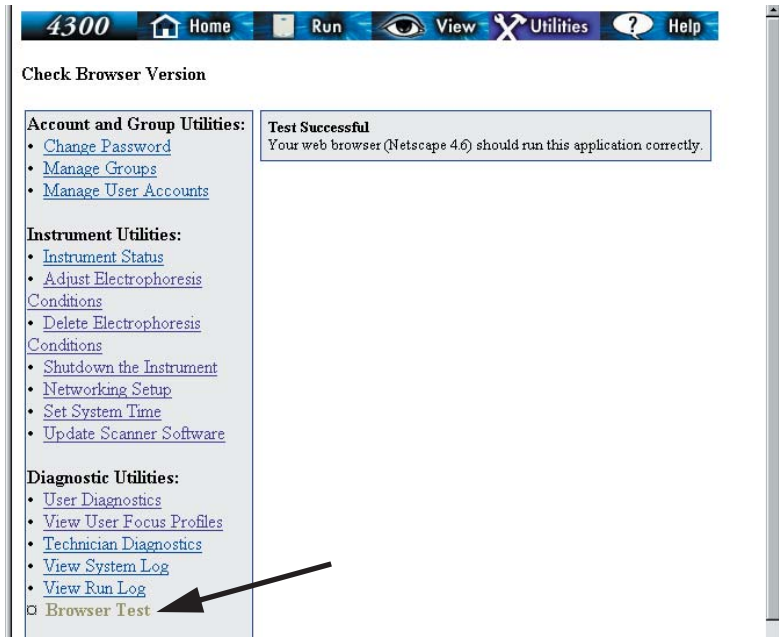
Technician Diagnostics

The Utilities page lists links for **Technician Diagnostics**, **View System Log**, and **View Run Log**. These functions are used only for troubleshooting and factory calibration. Do not use these functions unless directed to do so by LI-COR or LI-COR authorized Distributors.

Browser Test

Netscape Communicator (version 4.7 or above) or Internet Explorer (version 5.0 or above), or their equivalent, should be used with the Model 4300. If you don't know the version number of your browser or you are using some other browser, run the browser test on the Utilities page to verify that your browser has all the required capabilities. With some older browsers, certain pages may display correctly, but important functions like starting runs may not work correctly.

To run the browser diagnostic, open the Utilities page and click the **Browser Test** link.



If the test fails, download a current copy of Netscape Communicator (www.netscape.com) or Internet Explorer (www.microsoft.com) from the Internet.

Chapter 10: Appendix

Specifications

Laser/Microscope

Laser Lifetime: 40000-60000 hours typical.

700 Channel Laser Source: Solid-state diode laser at 685 nm.

800 Channel Laser Source: Solid-state diode laser at 785 nm.

Detectors: Two silicon avalanche photodiodes.

Scanning Speed: Five selectable speeds. Image quality is optimized by adjusting scan speeds. (Lower scan speeds produce higher quality images, but image size and scanning time are increased.)

Pixels per Scan Line: 1028 or 2055. User selectable.

Electrophoresis Power Supply

Maximum Output: 5000 V, 40 mA, 120 W.

Operating Specifications

Operating Conditions: For Indoor use only; operating temperature 15-35 °C and dew point not greater than 20 °C, non-condensing; altitude not to exceed 2000m.

Power Requirements: Automatic voltage selection at 90 - 250 VAC and 47-63 Hz. 3 Amps at 120 V; 380 W maximum.

- 4300S Dimensions:** 59H x 51W x 47D cm (23" x 20" x 18"). 102 cm (40") wide with the door fully open.
- 4300S Weight:** 29 kg (64 lb).
- 4300L Dimensions:** 84H x 51W x 47D cm (33" x 20" x 18"). 102 cm (40") wide with the door fully open.
- 4300L Weight:** 36.3 kg (80 lb).

Gel Electrophoresis Apparatus

<u>Glass Height</u>	<u>Separation Dist.</u>	<u>Plate Width</u>	<u>Spacer Thickness</u>
66 cm	56 cm	25 cm	0.2, 0.25 mm
41 cm	31 cm	25 cm	0.2, 0.25 mm
25 cm	15 cm	25 cm	0.2, 0.25 mm
18 cm	9cm	25 cm	0.2, 0.25 mm

Internal Server

- Storage:** Internal hard disk.
- Network Protocol:** TCP/IP.
- Network Connection:** RJ-45. 10Base-T.
- Security:** Password protected access.