FMBIO®

Fluorescent Image Scanning Unit and Analytical Software

Read Image for Windows v1.5

Image Analysis for Windows v3.0

Operation Manual

Please read this manual carefully before operating the scanning unit.

Keep this manual near the scanning unit or in a location that is both easily accessible and under your control.

This product had been tested and complies with the limits for a Class A digital device in accordance with the specifications in Subpart B, Part 15, of FCC rules.
This product has been tested and complies with the limits for a Class A digital device in accordance with the specifications in VDE rules.
This product has been certified and found to comply with the specifications for a Class I laser device of Japanese Industrial Standards JIS6802 and Title 21, USA code of Federal Regulations, Subchapter J, of FDA rules.
This product has been certified and qualified as a strategic object/technology as set out in the Foreign Exchange and Foreign Trade Control Law. In accordance with the above mentioned law, permission from the Japanese government is required for purposes of exportation or delivery outside Japan.
This product contains devices and technology regulated under USA Export Control Rules and requires additional permission from the American government for exportation to certain countries.

Exemption From Any Obligation

Hitachi Software Engineering Co., Ltd. shall not be liable for any damage that occurs as a result of mistakes or omissions in this manual.
The new duty of guarantee shall not be appended by advise provided by the staff or the representative of Hitachi Software Engineering Co., Ltd.
Hitachi Software Engineering Co., Ltd. and the manufacturer shall not in any case be liable for the nonperformance of agreement, disobedience of guarantee, fault, responsibility for strict liability, special, indirect, consequential or other similar damages in law. These damages include the loss of a profit, the loss or revenue, the exchange expenses of the instruments, the equipment and the services, the period of failure, the period of the customer, the claim by the third party with the customer and the sufferer included, the infringement of the property.

Guarantee

For the period of ONE YEAR, Hitachi Software Engineering Co., Ltd. guarantees that there is no defect in the quality of the material and the production of the equipment.
While the guarantee is valid, if the defect or the failure occurs in the equipment, Hitachi Software Engineering Co., Ltd. may repair the equipment. If this happens, please contact Hitachi Software Engineering service representative while the guarantee is valid.
The customers shall not make any representative actions and/or any guarantees through the manufacturer, Hitachi Software Engineering Co., Ltd. and its licenser.
The guarantee may apply only in proper use, may not apply to the following:
Any damage caused by an improper use of the equipment or use not described in this manual.
Any damage caused by disasters of fire, flood, storm, earthquake, thunder and other natural causes.

Any damage caused by moving the equipment after it has been installed at the customer site.
Any damaged caused by addition, modification, reverse engineering, and/or are not permitted, or any other articles.
Any damage caused by setting up the Scanning Unit and FMBIO System improperly.
Any damage caused by improper control or mismanagement of the equipment.
Any damage caused by improper uses, faulty operation, abnormal uses or mistakes of application of the equipment.
Expendable supplies.
Any damage caused by the peripheral devices.
Any damage caused by products purchased from other than Hitachi Software Engineering Co., Ltd. or its authorized dealers.

Exclusions

Hitachi Software Engineering Co., Ltd.'s FMBIO instruments are to be used **FOR RESEARCH PURPOSES ONLY** and is NOT to be used for diagnostic purposes. Hitachi Software Engineering shall not in any case be held liable for special, incidental, consequential, indirect or other similar damages ensuing from the use of this product. This product is designed to operate safely and reliably. Hitachi Software Engineering Co., Ltd. will not be held responsible for any complications resulting from the use of this product. For more information about the software license and limited warranty of this system, please refer to the software license and warranty agreement.

Trademarks and Patents

Contents

For Safe Operations: Precautions

Preventing Mechanical Failure, Electric Shock, Fire and Exposure to Hazardous Laser Radiation

For your safety, operate this equipment only as prescribed by the instructions in this manual. Operation outside these parameters may subject the purchaser to waive of any liabilities by Hitachi Software Engineering Co., Ltd.

In addition, any claims deemed to arise due to failure to thoroughly read this manual may result in the waiver of any liabilities by Hitachi Software Engineering Co., Ltd.

The following symbols are related to safety concerns. Please ensure that you understand the meaning and implications of each symbol before continuing to read this manual.

WARNING!

This symbol indicates the possibility of loss of life or serious injury if you accidentally operate the equipment in ignorance of the information highlighted by this symbol.

CAUTION!

This symbol indicates the potential for personal injury or damage to equipment if the equipment is operated in ignorance of the information highlighted by this symbol.

Examples of Schematic Symbols

Each symbol indicates the type of warning illustrated.

Cautions the user of a particular hazard (in this case, of potential electrical shock).

This symbol represents prohibited actions.

WARNING! Proper Handling

Do Not Disassemble or Repair

Please do not disassemble or attempt repairs. Tampering with the internal components risks exposure to such hazards as electrical shock and laser radiation.

Shield the Scanning Unit from Water

Shield the Scanning Unit from water. Never place objects containing water or other fluids on the Scanning Unit. A spill can lead to short circuits or electric shock. If there is a spill, immediately turn the power switch off, disconnect the power cord and contact your nearest authorized Hitachi Software Engineering Service representative.

No Foreign Objects

Keep foreign objects out of the Scanning Unit. Do not allow paper clips, hairpins, metallic objects, paper or other combustible material to fall in or otherwise enter the Scanning Unit. Foreign objects in the Scanning Unit can lead to short circuit, fire or electric shock. If this should happen, turn the power switch OFF, disconnect the power cord and contact your nearest authorized Hitachi Software Engineering service representative.

WARNING! Handling the Electric Power Unit

Use Only the Specified Power Cable

Ground the Scanning Unit with the proper power cord with the three-prong plug provided with the Scanning Unit. Before turning on the Scanning Unit, check that the power cord is NOT damaged. Using the damaged power cord can lead to short circuits, fire or electric shock. If the power cord is damaged, turn the power switch OFF, disconnect the power cord and contact your nearest authorized Hitachi Software Engineering Service representative for a replacement. Use only a properly grounded three-prong power cord as a temporary replacement.

Do Not Use a Non-standard Power Supply

Use the specified power supply (AC100 V and 50/60 Hz). Use only with the proper voltage and frequency indicated on the specification plate on the rear of the equipment. Use of other than the proper power specifications may lead to accidents and equipment malfunction.

1 Overview

Advantages of the FMBIO System

Thank you for purchasing Hitachi Software Engineering Company, Limited, FMBIO scanning unit, a device for reading the electrophoresis patterns of fluorescent dye-marked samples. FMBIO represents a maturing of fluorescent technology that has been applied to life sciences labs since the 1960's. Some of the major benefits associated with the system are improved safety, speed, accuracy, and lower cost.

Read this manual carefully before attempting to operate the system and carefully heed all safety warnings.

Improved Safety

The FMBIO system eliminates the risks to lab personnel, the need for radioactive shielding and the storage and procurement problems associated with handling I^{125}, C^{14}, S^{35}, and P^{32}.

Faster Results

With the FMBIO, speed of experimental throughput is substantially greater because scanning and analysis are independent of electrophoresis. Many more gels can be run and scanned with the FMBIO than with a dedicated electrophoretic sequencer.

The FMBIO reads gels, membranes, and plates without the need for x-ray film or costly phosphor screens. Over- and under-exposure problems are eliminated. Scan times for the FMBIO are typically five to ninety minutes, considerably less than the eight hours development time required for conventional radiography. FMBIO images are processed without removing glass plates, drying gels, or developing film, saving more time. Using multi-wavelength scanning, samples labeled by two or more different fluorophores can be read in one scan.

Greater Accuracy

Accuracy, sensitivity, and flexibility of experimental operations are improved with the FMBIO system. Sixteen-bit imaging allows detection of signal intensity over a much greater linear dynamic range than isotope-based systems. This enables both dark and light bands to be read in the same experiment. Many of the errors that stem from sample handling are virtually eliminated because glass plates are not removed, gels are not dried, and readability of results does not depend on exposure time. Because scanning does not require removing the gel from the glass plate, gels can be run, then scanned, then run and scanned again, increasing the number of readable bands per gel.

Lower Costs

When the FMBIO system is in place, the expense associated with X-ray film, storage phosphor screens, shields, badges, Geiger counters, low temperature freezers, and disposal services is eliminated.

Simplicity

The FMBIO IIe consists of a scanning unit and two software applications that run on Microsoft Windows 95 and above. The Read Image scanning software controls the operation of the scanner, and the Image Analysis software carries out the analysis of the scans. The two software applications may be run on separate computers if desired.

FMBIO IIe System

Scanning Unit Features

The FMBIO IIe scanning unit detects laser-induced fluorescent signals on gels, blots and thin layer chromatograms. It accommodates plate sizes up to 200 x 475 mm, with a maximum reading size of 200 x 430 mm. The excitation source is a YAG 532-nm solid state 50mW laser.

The laser's optical unit moves across the sample (Y direction) as its laser beam is directed onto the sample (X direction) via a polygon mirror rotating at high speed. The resulting fluorescent light signals emitted from the excited fluorophores are then collected by two optical fiber arrays.

The instrument also features two photomultiplier tubes, a large scan area of 20x43 cm, and a linear dynamic range of four orders of magnitude. The FMBIO produces extremely high resolution images capable of resolving even single-base microvariants.

Fluorescent probes that are responsive to the frequency of the laser beam emit fluorescent signals upon being excited. The emitted light is collected by a lens and directed into a fiber optic array which passes it to an interference filter. The targeted fluorescence wavelength passes through the interference filter and then to a photomultiplier where the light signal is converted to a digital signal. Conversion of the digital signal to experimental data takes place in the data acquisition circuits, where fluorescence detected at the photomultiplier is synchronized with the angle of rotation of the scanner mirror and with the horizontal motion of the optical unit to produce positional values in the sample. See Figure 1-1.

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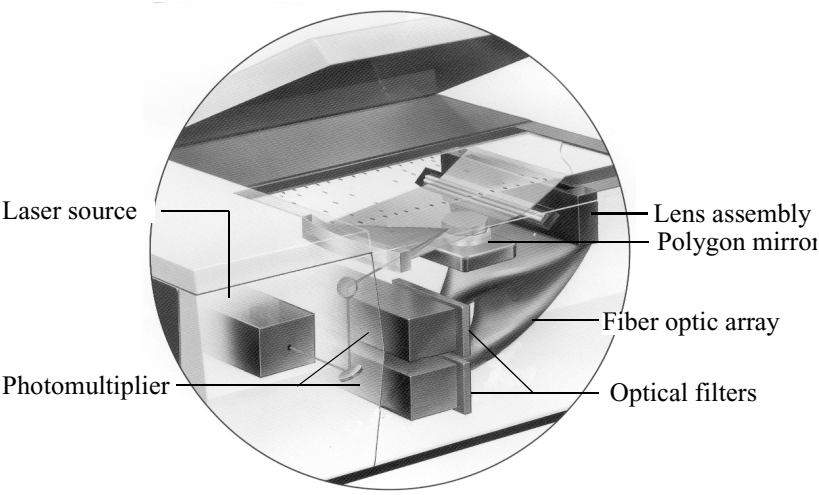


Figure 1-1. Interior view of FMBIO scanning unit

Multicolor imaging is achieved using band-pass detection filters to discriminate light from fluorescent dyes emitting between 500 and 700 nm. Up to four filters can be stored in the instrument and accessed through software. Two filters can be used simultaneously to detect emissions from two different dyes.

In three-color analyses, the gel is scanned after electrophoresis using a 505 nm band-pass filter to detect fluoresceinated amplification products, a 585 nm band-pass filter to detect products labeled with TMR, and a 650 nm band-pass filter to detect the CXR-labeled internal size standards. These images can be overlaid into a three-color image or viewed separately.

FMBIO Specifications

Weight	55 kg
Outside dimensions	87 cm (W) x 65 cm (D) x 35 cm (H)
Operating temperature	10 - 30 °C
Operating humidity	20 - 80% relative humidity
Laser excitation wavelength	532 nm
Emission wavelength	Selectable: 500 - 700 nm
Detection sensitivity (with polyacrylamide gel)	Rhodamine: 0.1 fmol/band Fluoroscein: 0.2 fmol/band
Plate size (max)	295 mm (W) x 470 mm (D) x 5 mm (H)
Scanning area (max)	200 x 430 mm
Dynamic range (signal readability)	Up to five orders of magnitude
Resolution	83 µm maximum spatial resolution; 16 bit (65536 grey-scale levels)
Laser power	~ 50mW, solid state YAG laser
Power frequency	50/60 Hz
Power consumption	~ 100 W
Power supply (AC)	100/110/115/120/220/240 V

Ensure that the voltage and frequency of the power source correspond to FMBIO power settings. Check the power settings on the specification panel on the rear of the machine. Verify that the building power supply has a functional ground.

Software Features

The FMBIO software consists of two modules: FMBIO Read Image and Image Analysis. These applications function independently and need not be installed on the same computer.

FMBIO Read Image. This application controls the FMBIO scanning

hardware, synchronizes the fluorescent input signal with the scanning mirror and optical unit, and converts this data into a bitstream. Read Image is used to easily set the scan area, scan resolution, image orientation, and photomultiplier sensitivity. The user can also choose filters, add comments to be saved with the scanned image, and perform hardware checks. The experimental data collected are converted by Read Image into a 16-bit digital TIFF file for analysis with the Image Analysis software. The scanning software provides a sixteen-bit gray scale image of fluorescence intensity, with a broader linear dynamic range than possible with conventional autoradiography.

Image Analysis. This application offers a user-friendly interface for viewing and analyzing images. The software features analysis functions that include automatic band detection to facilitate data processing, quantitation of peak height or peak area, and band sizing through comparison to size standards.

With fluorescent dyes for sample labeling, and the FMBIO software the system can analyze:

- Gels (1D and 2D)
- Membranes (Southern, Northern, Western, dot, slot, TLC)
- low- to medium-density arrays

Multi-wavelength analysis makes it possible to create and analyze multi-color signal images. You can run fluorophores of multiple spectra in the same lanes, and simultaneously scan their emission signals. This function is useful for comparative allele analysis or, when a marker of known size is added to the lane, for highly accurate size determination.

FMBIO software contains spatial analysis functions that accurately estimate fragment migration distances. It also contains signal intensity analysis functions, so that it can perform densitometry to estimate band or spot intensity.

Fluorescent Labels

With the FMBIO, you can select from a wide range of fluorophores, from rhodamine red to fluorescein green and including tetramethylrhodamine, X-rhodamine, beta-phycoerythrin, ethidium bromide, propidium iodide. Because of the powerful intensity of the FMBIO laser, you can use fluorophores with emission wavelengths closer to the excitation wavelength. Table 1-1 shows a list of dyes and stains that can be used with the FMBIO, along with recommended filters.

Almost all molecular species absorb and then re-emit light under certain conditions, giving a broad emission spectrum, up to 100 nm wide in condensed media. When the emission process (the excited state) takes place at the nanosecond or a slower time scale, the phenomenon is called fluorescence.

Table 1-1: Dyes and Stains Detected with the FMBIO

Dye or Stain	Excitation (nm)	Emission (nm)	*Filter (nm)	Vendor
Protein Stains				
Sypro Orange	472	570	585	Molecular Probes
Sypro Red	530	625	625	Molecular Probes
DNA Post Stains				
Ethidium Bromide	300	511	605	A reliable source
Sybr Green I	490	520	505	Molecular Probes
High Sensitivity	532	605	605	Molecular Probes
Dye Labels				
FITC Fluorescein	490	520	505	A reliable source
FAM	495	535	505	Perkin-Elmer
JOE	526	548	585	Perkin-Elmer
HEX	529	560	585	Perkin-Elmer
TAMRA	559	578	585	Perkin-Elmer
Tetramethyl rhodamine	546	572	585/605	Molecular Probes
Rhodamine X	574	595	605	Molecular Probes
Texas red	578	602	605	Molecular Probes
ROX	580	605	605	Perkin-Elmer
Bodipy	563	602	605	Molecular Probes
POPRO3	270, 593	567	585	Molecular Probes
BOPRO3	271, 575	600	605	Molecular Probes
BOBO3	245, 572	602	605	Molecular Probes
Cy2	489	505	505	Amersham
Cy3	552	565	585	Amersham
Cy3.5	581	596	605	Amersham
FluorX	494	520	505	Amersham
Alk. Phos. Substrates				

*Use these filters for maximum sensitivity.

Dye or Stain	Excitation (nm)	Emission (nm)	*Filter (nm)	Vendor
Attophos	430-440	560	505	JBL
Vector red			605	Vector Labs

*Use these filters for maximum sensitivity.

2 Installation

Preparing for Installation

WARNING!  **Failure to follow proper installation procedures could result in physical harm to personnel and damage to the equipment. Review the warnings and cautions detailed after the Table of Contents at the beginning of this manual.**

Contents of FMBIO Shipment

Before beginning installation, check that you received the following items with the FMBIO shipment.

1	Scanning Unit
1	Power cord, 2 m long
1	SCSI terminator
2	Dust caps, mounted to SCSI connector
1	Optical filter, 605 nm

Installing the Scanning Unit

Instrument and software installations are performed by qualified Hitachi Service personnel. Any unauthorized attempt to install the scanning unit could void the instrument warranty.

Prepare a level, sturdy, non-vibrating surface in a well-ventilated, temperature-stable area for the FMBIO scanning unit. The unit should not be placed in a dusty or damp location, or in an area where it will be subject to vibration or impact. Allow at least 10 cm of clearance next to the rear and left panels. Do not place heavy objects on top of the scanning unit. Keep the scanning unit away from flammable or corrosive gases.

Important: The laser on the FMBIO requires a constant power source. If the power specifications on page 1-5 are not met, a power shortage could result and cause instrument failure.

Installing the FMBIO Software

FMBIO system requirements:

- IBM® or 100% IBM®-compatible personal computer
- Windows 95 or later
- Pentium II 300 MHz or greater is recommended
- 64 MB RAM minimum (128 MB or more recommended)
- 10 MB free (minimum) of hard disk storage space is required for program loading. Image and spreadsheet files could require substantially more storage space
- CD-ROM disc drive or a 3.5 inch floppy disk drive is required for installation
- Resolution: 1024 x 768 or higher, 16-bit color display or higher
- Mouse or other pointing device
- Keyboard

The FMBIO software can be obtained on a CD or 3.5 inch floppy disks.

To install the FMBIO software contained on a CD:

1. Turn off the screensaver.
    - In the Windows Taskbar, click on the Start button. Choose Settings > Control Panel.
    - Double-click the Display icon.
    - Click the Screensaver tab, then select None.
2. Disable all virus protection programs.
3. On the CD, open the ReadMe file to find out about changes and updates.
4. Click the Start button, then choose Run....
5. Enter the pathname for the setup.exe file on the CD (for example, D:\setup.exe), then click OK.
6. Follow the instructions on the screen.

File Compression

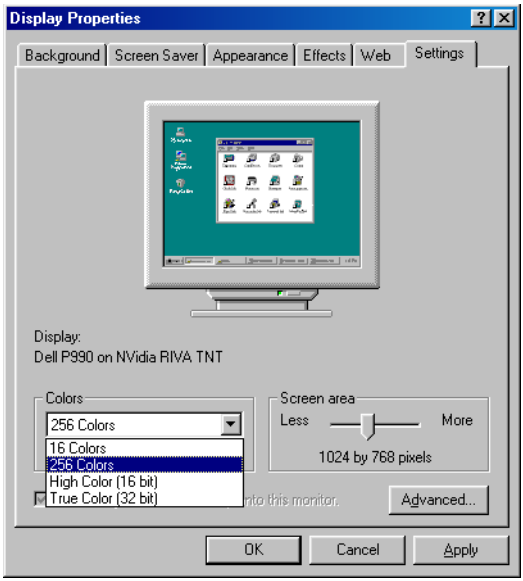
A single image file may consume up to 10 MB or more of disk storage space. FMBIO software save functions include optional file compression to allow disc space to be conserved. Depending on the file, compression reduces file size to 25-50 percent of its original size. Hard disk capacity is scanned prior to image reads to ensure that adequate storage space exists.

Understanding Personal Computers

Users of the FMBIO system should be familiar with basic IBM-compatible computer and Windows system functions. New users should familiarize themselves with Windows system operation before attempting to operate the FMBIO system. Refer to your manuals and the online help installed with Windows system.

Setting Color Display

1. In the Windows Taskbar, click on the Start button. Choose Settings > Control Panel.
2. In the Control Panel window, double-click the Display icon.
3. Click the Settings tab, then select the 256 Colors setting or higher.



Note When 256 colors are selected, only the active window is displayed with sharpness and clarity, while background windows may have a rough appearance. When High Color (16-bit) or True Color (32-bit) is selected, all windows are displayed with sharpness and clarity, but at the cost of slower screen drawing. The number of colors you choose depends on the complexity of your images and the speed of your system.

Printer

Images generated by the FMBIO system can be printed on conventional postscript laser printers with 600-dpi resolution. While the images rendered by such machines are adequate for many reference and record-keeping purposes, much information is necessarily left out of images printed on such machines. Always remember that images printed on such machines are schematics that do not faithfully represent all the information available in the digital image in the FMBIO file.

High quality images that more faithfully represent in hard copy the information present in the FMBIO image file can be created using a high-quality image printer. Image printer capability should include 256 grayscale, 3-color dye sublimation, and 300 or higher dpi resolution.

Peripheral Storage Devices

A single digital image file may be 10 MB or larger. To conserve hard drive space, you may store files on a peripheral storage device, such as a magneto optical drive or another removable storage drive. Connect the optional storage device to the FMBIO using the appropriate 50-pin SCSI cable.

3 Operating the Scanning Unit

This chapter describes the steps you should follow to operate the scanning unit. These steps are summarized below:

1. Start ReadImage software. See page 3-2.
2. Turn on power to the FMBIO scanning unit and wait for the self-diagnosis routine to finish. See page 3-2.
3. Check that the 605-nm filter is in Channel 1 for autofocusing. See page 3-5.
4. Clean the sample plates and scanner sample stage. See page 3-8.
5. Load the sample on the FMBIO sample stage. See page 3-10.
6. Choose the appropriate parameter set in the ReadImage dialog box and, if necessary, adjust the scanning parameters. See Chapter 4, *Read Image Software*.
7. Use ReadImage software to begin scanning the sample. A scan may take from five to ninety minutes.

See page 3-14 for Guidelines to Scanning with the FMBIO ReadImage software.

Starting the FMBIO Scanner

Before turning on the power to the scanning unit, turn on the computer and start the FMBIO ReadImage software.

- To start the ReadImage program, double-click the ReadImage icon.

The FMBIO scanning unit has two electrical switches:

- The Main switch, located on the rear panel next to the SCSI cable port
- A Power switch, located inside the front panel

For both electrical switches, the Off position is marked with a circle (**O**) and the On position is marked with a vertical line (|). The locations of the power switches are shown in the following two figures.

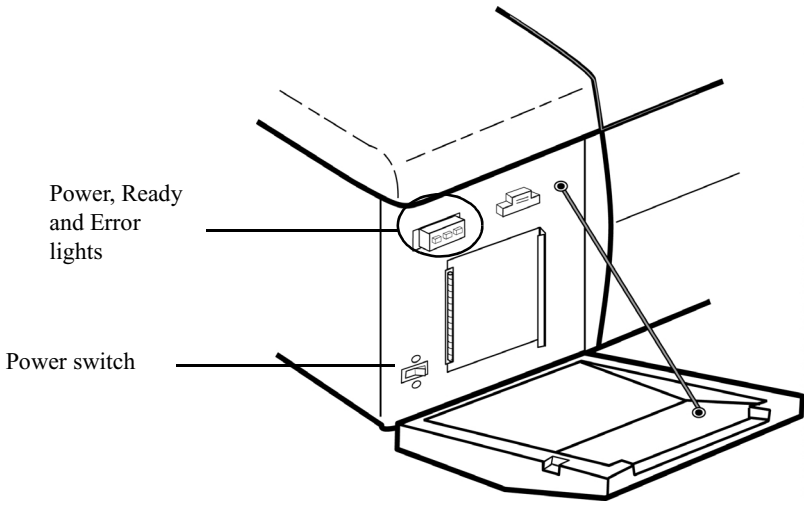


Figure 3-1.Inside front panel with Power switch

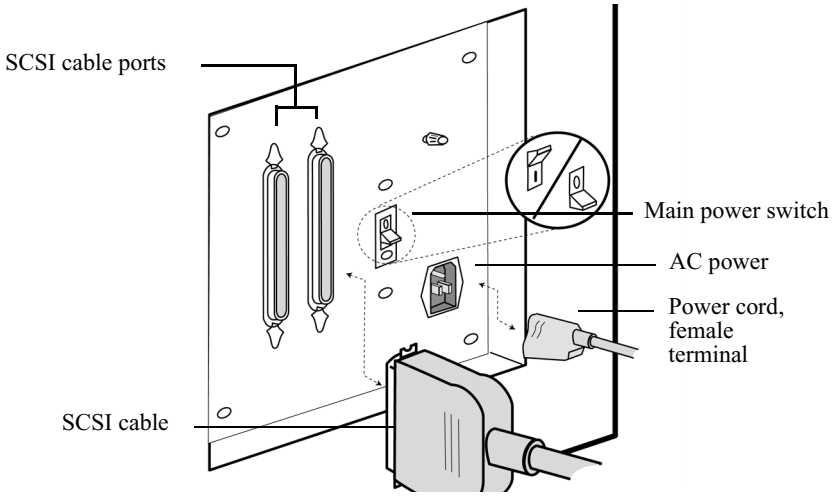


Figure 3-2. Rear view of FMBIO scanning unit

1. Turn on the computer that is connected to the scanner by a SCSI cable.
2. Set the Main switch to the On (|) position.
3. Open the front panel of the scanning unit and turn on the Power switch.

   When the scanning unit is first turned on, it automatically performs a self-diagnostic routine. During the routine, the Ready light on the front panel blinks.

Self-diagnosis Normal

When the Ready light shines steadily, without blinking, the self-diagnostic routine has been completed without detecting a problem. You may now use the scanning unit.

Error Detected

When the self-diagnostic routine detects an error, the Ready light shuts off and the Error light begins to glow. Turn off the Power switch for at least five seconds, then turn on the Power switch. The FMBIO repeats the self-diagnostic routine. If the routine detects an error again, contact your nearest authorized Hitachi Software Engineering service representative.

CAUTION!

Spills on the scanning unit may damage the electronics or laser inside the FMBIO**.** Heavy objects on top of the unit may throw the scanning surfaces out of alignment. Avoid placing objects on top of the scanning unit.

WARNING!

Normal FMBIO IIe operation uses a 50-mW micro-laser beam. Exposure to laser radiation can seriously damage eyes and vision. A set of built-in safety switches places a shutter in front of the laser beam for your protection.

- **Do not override the laser safety interlock system.**
- **Do not look directly into the open optical filter door.**
- **Do not remove the external panels and covers of the FMBIO at any time.**

WARNING!

Unauthorized repair attempts may expose you and your coworkers to extremely high voltage and hazardous laser light, and will also void your warranty or service contract. Always call Hitachi Software Engineering for repairs and service.

Autofocus Calibration

After a power-up, the FMBIO automatically performs an autofocus before beginning the first scan. Channel 1 does not have to be active during an autofocus, but it must contain the 605-nm filter.

After the initial autofocus, you may remove the 605-nm filter and replace it with a filter that accommodates a detection range compatible with the stain or dye you are using on your sample.

Inserting Optical Filters

CAUTION!

Scratched or dirty filters interfere with precise laser detection. Avoid directly touching the surface of the optical filter. Use a lens blower to remove dust on the filter. For more stubborn dirt, use lens cleaning paper.

Prepare for a scan by placing the appropriate filters in each channel. Only active channels collect image data during the scan. See *Selecting Active Channels* on page 4-14 for information about active channels.

The FMBIO scanning unit can accommodate two filter holders. Each filter holder can hold two filters. You must place the appropriate filters in the scanning unit before beginning a scan. Once the scan begins, the optical unit moves out of home position and the filters are no longer accessible.

Note If this is the first scan after turning on power to the FMBIO unit, insert the 605-nm filter in the Channel 1 position.

If the automatic initial autofocus has been completed, turn off Auto Focus in the FMBIO menu, and replace the 605-nm filter with a filter appropriate to your scanning needs.

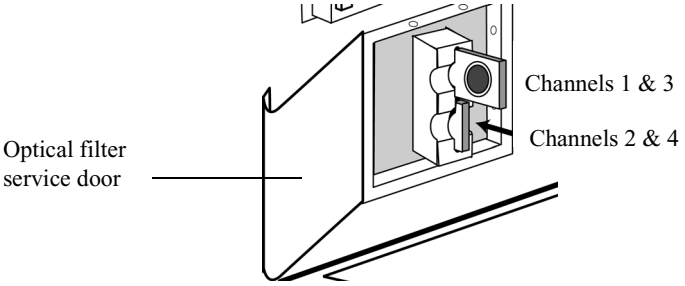
Important: If the FMBIO scanning unit has begun a scan, or if the unit is in cleaning mode, the optical unit does not return to home position when you click the Pause button. You cannot change filters when the scanning unit is not in home position. See Table 1-1 on page 1-7 for filter information.

1. To stop the scan, click the Pause button.
2. A dialog box opens to display three options: Save, Don't Save, and Cancel:
   - To stop scanning, return the optical unit to home position and save the scan data, click Save. Continue to step 3.
   - To stop scanning and discard the scan data, click Don't Save and continue to step 3.
   - To continue scanning, click Cancel to close the dialog box, then click Resume.
3. In the FMBIO menu, turn off Autofocus. See the directions for setting an autofocus on page 4-13.
4. Replace the 605-nm filter and restart the scan. See the directions for changing the optical filters below.

To insert or change filters:

1. Choose Eject Filter in the FMBIO menu.

2. Open the front panel, then open the optical filter service door.



3. Pull out the appropriate filter holder.

   The filter holder in the top position holds filters for Channel 1 and Channel 3.

   The filter holder in the bottom position holds filters for Channel 2 and Channel 4.

4. Hold the filter holder in your left hand, so that the arrow on the edge faces you and points to the right and the ball bearing on surface of the holder faces up. See Figure 3-3 on page 3-7.
5. In this position, find the two set screws in the groove on the right edge of the filter holder. There is one set screw adjacent to each optical filter position.
6. Use a 1.5-mm Allen wrench to loosen the set screw that holds in place the filter you want to change.

Note One full turn loosens the screw sufficiently; do not remove the set screw.

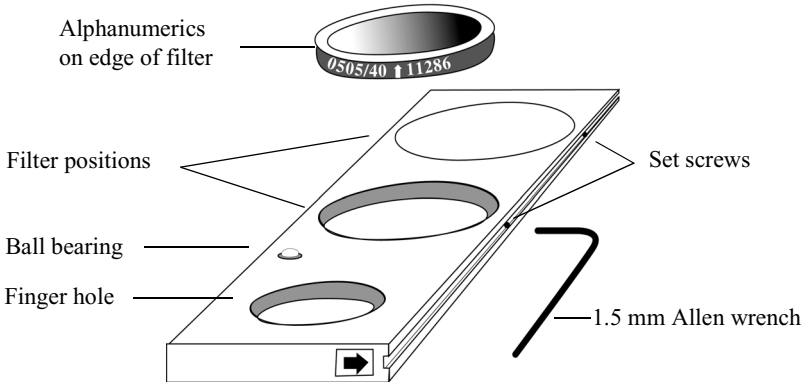


Figure 3-3. Changing optical filters

7. After one full turn of the set screw, hold the filter holder over a clean, lint-free tissue and gently tilt the holder upright until the filter falls out. Set the filter aside.
8. Return the filter holder to a horizontal position and hold the replacement filter over the appropriate hole in the filter holder. The alphanumerics on the edge of each filter should be upright. If there is an arrow on the edge of the filter, it should point up. Drop the filter in place.
9. Turn the set screw one full turn to tighten the filter.

Important: To avoid breaking the filter, do not overtighten the set screw.

10. Return the filter holder to its place in the scanning unit. Be sure the finger hole in the filter holder is nearest to the service door. Close the optical filter service door.

Preparing for Scanning

You can use the FMBIO system to scan and generate an image of:

- Gels —1D and 2D
- Membranes —Southern, Northern, Western, dot, slot, and TLC
- Low- and medium-density arrays

Opening the Cover

Below is a figure that shows the location of the button which, when pressed, causes the top cover to open.

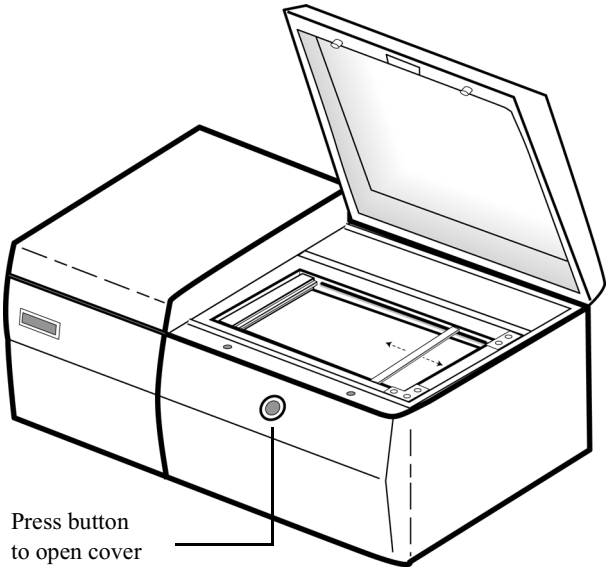


Figure 3-4. Front view of FMBIO scanning unit

When the cover is open, you can access the glass plates and the scanner sample stage.

Important: When the cover is open, the scanning unit cannot scan and the computer cannot start a scanning operation. When closing the cover, be sure to firmly press it down so that it is securely closed and the scanning unit can operate.

Glass Plates

The scanning unit accommodates a glass plate that is 200 mm wide x 475 mm long and 5.0 mm thick ± 0.1 mm. For best results, only use low-fluorescence borosilicate glass of "Tempax" or similar grade. The use of other grades or sizes of glass may result in poor scanning quality.

The sample stage supports the sample plates on two sides only to allow the maximum area to be scanned, and when the glass plate is properly seated on the scanner bed, the scannable area is 200 mm (width) x 430 mm (length).

Be careful when loading the sample stage; use both hands, lower the sample plate gently onto the stage supports, and close the scanning cover gently. Sample plates break easily, and broken glass inside the instrument exposes the operator to cuts and the equipment to mechanical damage.

Fingerprints, reagents and other foreign objects on the glass plate surface can cause noise in the image and may even show up as signal.

- Keep the surfaces of the plates clean. Use soap and water, plain water, or 70% methanol (MeOH) to clean plates. Avoid using ammonia-based glass cleaners. Wipe plates until dry to avoid streaks.

Scanner Sample Stage

Dust and particles on the sample stage reflect stray light which can increase background signal or cause loss of signal.

- Keep the sample stage clean, particularly the autofocus strip and adjacent areas.
- Regularly use the cleaning mode to clean the mirror and lens that passes below the sample.
- Wipe the stage and interior areas with a damp, low-lint cloth.
- Immediately wipe off any gel or samples that fall onto the sample supports or into the unit itself.

Loading the Sample

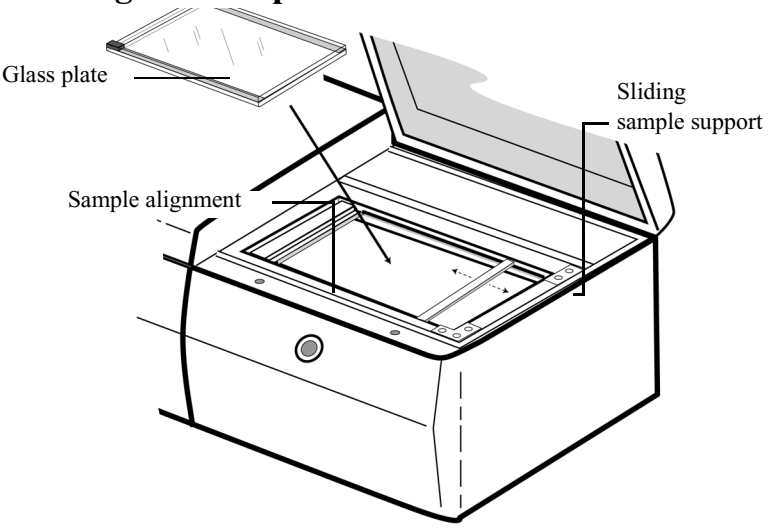


Figure 3-5. Loading the glass plates on to the scanner bed

To maximize the scannable area on the glass plates, the scanner supports the sample plate only on two ends. Take great care when placing the glass plates on the scanner bed to avoid breaking the glass plate and damaging both the sample and the scanning unit.

Orientation of glass plates. Scanning begins in the upper left corner of the scanner bed, near the sample alignment surface. (See Figure 3-5.) The longer of the two glass plates should rest on the sample supports.

Removing spacers. It is not necessary to remove the spacers on the glass plates.

Important: For optimal results and to minimize image file size, do not include the spacers in the defined scan area.

Note Begin the scan as soon as possible after electrophoresis to ensure a clear scan image.

1. Set the Power switch to the On position and open the cover of the scanning unit.
2. Clean and thoroughly dry the surfaces of the glass plates. You may want

to mark the front of the plate so that it can be distinguished on the scan.

3. Before putting the glass plate on the scanner bed, adjust the sliding sample support so that it is ready to accommodate the length of the plate.
4. Carefully and firmly place the end of the longer glass plate up against the sample alignment surface. See Figure 3-5 on page 3-10.
5. As you carefully begin to lower the rear end of the glass plate to lay flat on the scanner bed, finely adjust the position of the sliding sample plate so that it offers full support.
6. When both ends of the glass plate are firmly supported on the scanner bed, gently close the scanning unit cover to avoid vibrations that might unsettle the glass plate. You can hear a clicking sound when the cover shuts.

WARNING!

Laser radiation can leak from the scanning unit when the cover is not closed tightly. Stray radiation can damage vision and interfere with accurate image scanning. Carefully check that the cover is closed tightly before beginning a scan.

Scanning the Image

The sensitivity of the scanning unit is dependent on a number of factors including: the dyes or stains used; the quality of the reagents; gel density and the diffusion rate of the sample in the gel; protocols and technique. You can fine-tune the image scan by adjusting the scanning parameters in the ReadImage dialog box.

Before beginning a scan, read Chapter 4, *Read Image Software*, to become familiar with the features of this software.

Beginning the Scan

Once you are satisfied with the ReadImage scanning parameters, click the Read button on the ReadImage dialog box to begin the scan.

Auto Focus. After the Power switch is first turned on, the FMBIO ReadImage software automatically performs an autofocus before it begins the first scan. During autofocusing, the rotating mirror and other signal tracking and processing elements are adjusted to optimize optical alignment. The entire process requires about thirty seconds to complete. The 605-nm optical filter should be in Channel 1 during autofocusing.

After the initial autofocus, you can turn autofocusing off and on with ReadImage software. See page 4-13 for more information.

After the initial autofocus routine, you may stop the scan and replace the 605-nm filter with a filter of a different wavelength. See *Autofocus Calibration* on page 3-4 for more information.

Stopping a Scan

When you stop a scan in progress, you either save or delete the partially scanned image.

1. To stop a scan in progress, click Pause.
2. A dialog box offers three actions after you click Pause: Save, Don't Save, and Cancel:
    - Click Save to save scan data and end the scanning process.
    - Click Don't Save to discard scan data and end the scanning process.
    - Click Cancel to close the dialog box, then click Resume to continue

the scanning process.

Scanning Guidelines

Following is a list of guidelines for use when scanning with FMBIO ReadImage.

Auto Focus. After the FMBIO is turned on, it performs an autofocus before it begins the first scan. The 605-nm filter must be in Channel 1 position although Channel 1 does not have to be active during the autofocus.

PreRead. Use PreRead to identify the area of interest on the sample and to make sure that spacers and other reflective items are not included in the scan area.

Scan area. The rectangle on the Scan Control window represents the total possible scan area. You may specify a smaller rectangle within the total scan area to represent a pre-defined scan area. By default, ReadImage displays the last scan area used with each Parameter Set.

Time. The amount of time it takes to perform a scan is a product of the area of the scan, the resolution (dpi), and the number of repeats per scan line.

Repeats. As you increase the number of repeats per scan line, the signal to noise (background) ratio increases.

Signal. At lower resolutions, the amount of visible signal may increase, but resolution of bands and spots decreases.

Resolution. The resolution of bands and spots improves as you increase both resolution (dpi) and repeats per line.

Saving parameters. You can save a set of customized scanning parameters as a ReadImage file. You can open this file and reapply the saved settings to new scans.

Multiple scans of the same gel. If necessary, you can remove a gel from the FMBIO scanning unit, remount it in the gel box for additional electrophoresis, and then rescan the gel.

4 Read Image Software

The Read Image software controls the FMBIO scanning unit as it generates digital images of gels, membranes, blots, and microtiter plates. ReadImage converts the experimental data into a digital file which you can store on the computer hard disk or on a peripheral storage device. You can then use Image Analysis software to analyze the image.

Note This manual does not discuss sample preparation. Refer to standard texts such as Ausubel or Maniatis, and to the documentation accompanying your fluorophores.

With Read Image software you can:

- Define the type of material being scanned and the scan area
- Set scan resolution (dpi) and number of repeats
- Adjust PMT sensitivity
- Adjust cutoff thresholds for background and signal
- Adjust the focusing point
- Set and save an autofocus routine
- Add comments to be recorded and saved with the scanned image
- Create and save customized scanning preferences

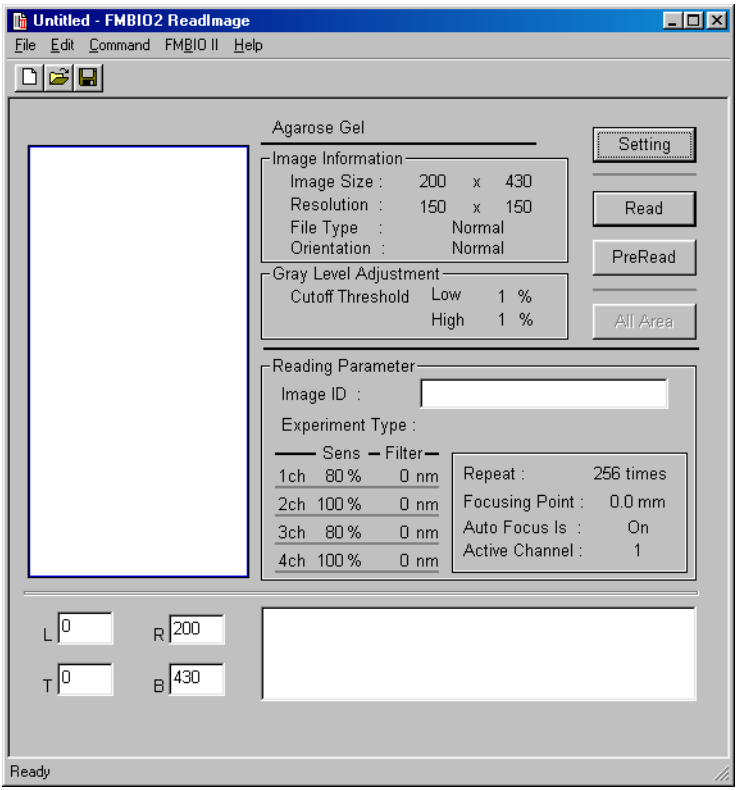
The procedures discussed in this chapter can be used with all types of scannable materials.

An example which illustrates some of the procedures begins on page 4-27. It is followed by a troubleshooting guide on page 4-31 and a list of error codes on page 4-32.

Opening Read Image

Read Image provides default scan settings for four types of scannable material: agarose gels, acrylamide gels, membranes, and TLC (thin layer chromatography). You can modify the default settings and save the changes as a custom file.

- To start the Read Image program, double-click the Read Image icon. When the program opens, a untitled Scan Control window is displayed.



Use this window to do the following:

- Pre-read the scan object
- Define the scan area

- Read the scan object
- Provide a unique name for the image
- Enter comments

For more information about this window, see page 4-15.

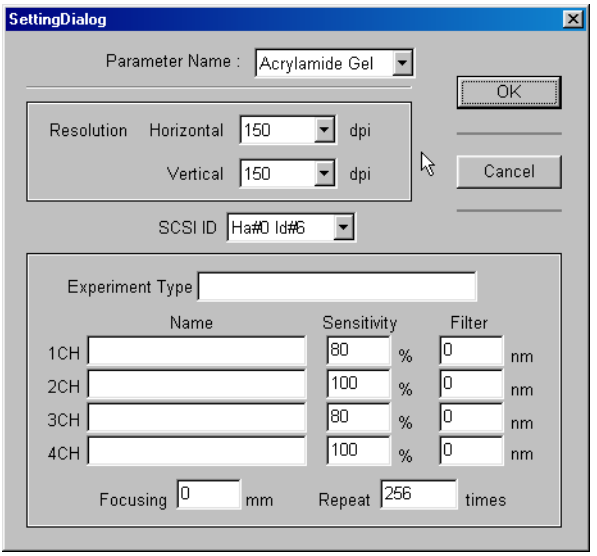
Read Image Files

You can save all the Read Image parameters in a file. When you open a saved Read Image file, the Scan Control window is displayed. Read Image automatically displays the scanning parameters. You may modify these parameters or apply them unchanged to a new scan.

- Use the commands in the File menu to create, open, and save Read Image files. See page 4-20 for a description of this menu.

Setting the Parameters

To set the FMBIO II parameters, choose Set Parameters... in the Command menu.



Use the Setting dialog box to specify:

- Parameter name
- Scanning resolution
- SCSI ID
- Experiment type
- Channel name, sensitivity, and filter
- Focusing height
- Scan line repeats

For more information about this window, see page 4-6.

Additional Scanning Parameters

Use items in the Command menu to specify:

- Gray level adjustment settings, including high and low cutoff thresholds
- Orientation of the scanned image

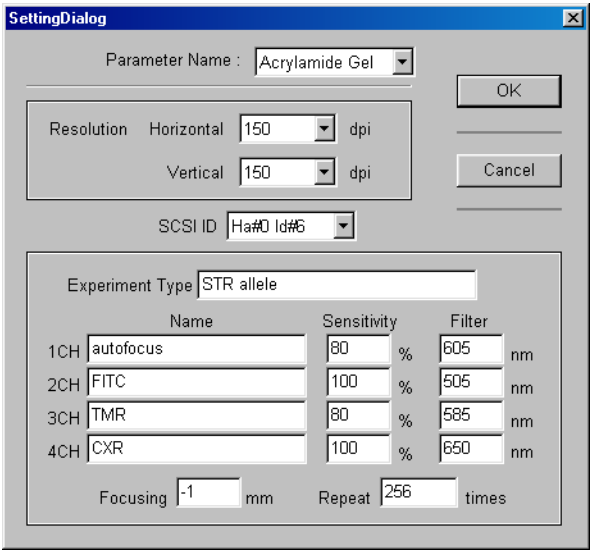
See page 4-10 for more information about this menu.

Use items in the FMBIO II menu to choose:

- Autofocus
- Active channels

See page 4-13 for more information about this menu.

Features of the Parameters Window



Name	Description
Parameter Name	Parameters for the scan.
Reading Resolution	Horizontal and vertical scan resolution.
SCSI ID	SCSI ID assigned to the FMBIO scanning unit.
Experiment Type	User defined label for experiment.
Channel Name	User defined label for channel.
Reading Sensitivity	Percentage of the collected laser signal is amplified by the PMT at each channel.
Filter	Filter used for the channel.
Focusing	Focal length (range: -2 to +3 mm).
Repeat	Number of laser passes per scan line.

The most appropriate parameter values for your experiment depend on image quality (signal to noise ratio), sample volume (heavy bands or faint bands), and fluorophore chemistry. Improper scan parameters may lead to images saturated with signal or images so faint that missing important elements, such as weak bands, are missing. If in doubt, it may be helpful to scan a portion of the image a number of times using different parameters, then choosing one set of parameters for the entire image.

1. In the Parameter Name pull-down list, choose the material type you want to scan.

   The each parameter name is associated with default settings for the scanning parameters.

2. Examine the parameters in the window and modify them as needed to accommodate your particular experimental conditions.

3. Click OK to apply the scanning parameters to your scan. See page 4-15 for information about defining scan area.

Defining the Parameter Name

The Parameter Name list contains various types of scannable material. Five parameter names are available: agarose gel, acrylamide gel, membrane, and TLC (thin layer chromatography). Each parameter name is linked to a pre-defined set of default scanning parameters.

	Agarose	**Acrylamide**	**Membrane**	**TLC**
Horiz. dpi	150	150	150	150
Vert. dpi	150	150	150	150
Repeat	256	256	256	256
1 Ch	80	80	45	45
2 Ch	100	100	55	55
3 Ch	80	80	45	45
4 Ch	100	100	55	55

You can modify any of these parameters as needed to accommodate your particular gel conditions. You may also specify and save a customized set of parameters for repeated use. See page 4-20.

Selecting SCSI ID

The SCSI ID of the FMBIO scanning unit is set to 6 at the factory. If you have any other peripherals attached to the SCSI port of the computer, make sure they do not share the same SCSI ID number.

- Verify that the SCSI ID is set to Auto or 6 in the ReadImage software.

Setting the Reading Resolution

The higher the resolution, or dots per inch, of the scanned image, the more information is collected during the scan. The resulting file for a scan collected at a higher resolution requires more storage space. You can specify resolution for both vertical and horizontal scan directions. Each parameter name has a limited number of resolution choices for each direction.

- Select horizontal and vertical scan resolutions.

Reading Sensitivity

The intensity of the charging voltage generated by the photomultiplier tube (PMT) influences how much of the collected light signal is considered as viable data. The higher the voltage, the greater the sensitivity and the more background signal is included in the data. Material with inherent high background signal, such as membranes, require lower reading sensitivity.

When you use more than one filter to produce an image, one or more fluorescent signals may be noticeably stronger than the others. You can adjust the sensitivity of each channel to equalize the intensity of the signals.

Important: You must use the FMBIO menu to activate all the channels used in the scan. See page 4-13.

- Enter a value between 0-100% for each channel. Read Image automatically assigns a value of 0% to sensitivity fields that are left blank.

Setting Repeats

During a scan, the laser scans across each line repeatedly before moving on to the next. ReadImage software retains median signal values for each line and throws out high and low values. This process of throwing out extreme values reduces anomalies due to temperature shifts in equipment and sample during processing.

The ideal number of repeats for any sample is difficult to predict and is dependent on a number of factors, including gel density and sample concentration. Increasing the number of scan repeats per line increases the ratio of signal to noise and also increases the scan time. You may begin with 150 to 256 repeats per line, but do not hesitate to use values outside this range.

- Enter a number in the Repeat field.

Focusing Point

When you modify the focusing point value, ReadImage software adjusts the height of the focusing point at the scanning bed to read thick samples, based on 5.0-mm glass plates. For most acrylamide gels (0.35 - 0.4 mm thick) and membranes, the focusing point value should be set to zero (0). For a 5-mm agarose gel, you may raise the focusing point to + 1.2 mm.

WARNING!

The FMBIO scanning unit can accommodate a focusing point range from +3.0 to -2.0 mm. Do not enter a focusing point value outside of this range or severe damage may result.

Settings in the Command Menu

The Command menu contains two kinds of commands:

- Commands that correspond to buttons on the Scan Control window.
- Settings that affect the format and appearance of the scanned image. These are displayed in the Scan Control window.

Preparing to Scan

Before beginning a scan, check the following settings displayed in the Scan Control window:

- Gray Level Adjustment, including the Adjustment Type and the High and Low Cutoff Thresholds
- Image File Type
- Orientation

Setting Gray Level Adjustment

Proper setting of the Gray Level Adjustment parameters enhances signal and suppresses noise, resulting in clean, readable images. Improper setting of these parameters might result in either saturated or faint images. The Gray Level Adjustment parameters modify the signal intensity of each “dot,” or pixel, in the scanned image. A pixel, is the smallest unit of a scanned image. FMBIO ReadImage files contain 16 bits per pixel, or 65,536 grayscale levels (2^{16} colors [black and white] = 65,536 shades of gray). Most computer monitors are 8-bit and can therefore only display 256 (2^8) shades of gray.

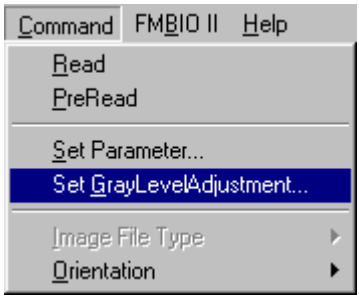
The Gray Level Adjustment tool tells your computer monitor how best to display your image. It allows you to optimize your image by assigning these 256 shades of gray to the region of sample signal only. All pixels below the sample signal range will be shown as white and all pixels above as black.

Cutoff Thresholds. Cutoff thresholds are applied to the range of 2^{16}— or 65,536—shades of gray that are generated during a scan. Background, or noise, lies at the low end of this range; signal lies near the high end of this range.

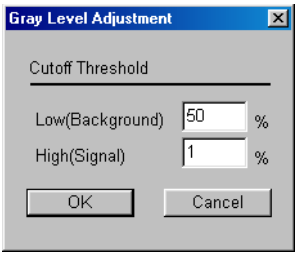
By adjusting the cutoff thresholds, you are adjusting the range between the highest and the lowest acceptable gray shades. When the high cutoff is too low, dark band images may be blurry, with indistinct edges. When the low cutoff is too high, faint images are lost in the background.

When you change the percentages for high and low cutoff thresholds, you change how the image appears on your monitor. You do not change how much imaging data is collected. Once the image file has been saved, you can use Image Analysis software to further experiment with the high and low cutoff thresholds.

1. Choose Gray Level Adjustment... in the Command menu.



2. Enter cutoff threshold values in the fields labeled Low (Background) and High (Signal).

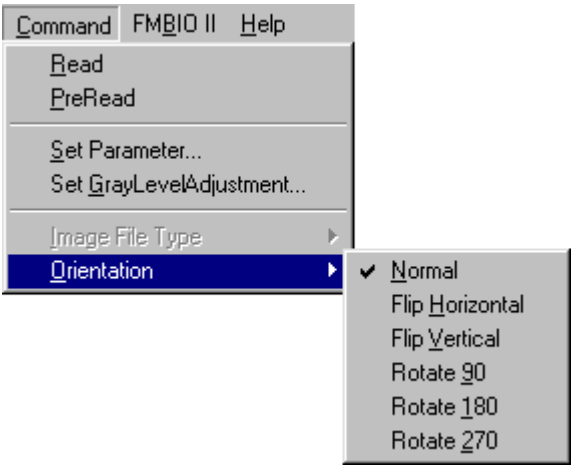


3. Click OK to save the Gray Level Adjustment settings.

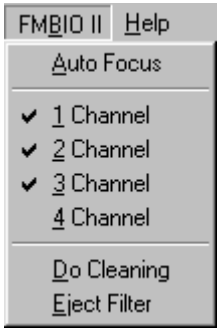
Defining Image Orientation

You change the viewing orientation of the image in the saved file. You can change this orientation again in the Image Analysis software.

- Choose Command > Orientation, then choose an orientation.



Settings in the FMBIO II Menu



The FMBIO II menu contains these commands:

- Requesting an Autofocus before each scan
- Determining which channels are active during a scan
- Preparing the FMBIO scanning unit for cleaning
- Ejecting the filter

Performing an Autofocus

During an autofocus, ReadImage adjusts the focusing mirror and other signal tracking and processing elements to find the best optical alignment. This procedure takes approximately thirty seconds. After the initial autofocus, the procedure does not need to be repeated unless the scanning unit is moved or bumped. The FMBIO always performs an autofocus at the beginning of the first scan after the unit is turned on.

If you do not check the Auto Focus command, an autofocus occurs only once, at the beginning of the first scan after you turn on the power switch, which is on the front of the FMBIO scanning unit.

When a checkmark appears before the Auto Focus command, an autofocus occurs before each scan.

The 605-nm filter should be in Channel 1 during the autofocus.

Performing an autofocus as part of a routine scan. Choose the parameter settings and load a sample for a routine scan. When FMBIO completes the

autofocus, it begins the scan. If Channel 1 is active, ReadImage creates an image file for the emission wavelength that passes through the 605-nm filter.

1. Place the 605-nm filter in Channel 1. See page 3-6 for directions.
2. Select Auto Focus in the FMBIO menu.
3. Select the appropriate ReadImage settings and load the sample.
4. Click the Read button on the Scan Control window.

Before the FMBIO scanning unit begins the scan, it performs the autofocus.

Note You can stop the scan after the autofocus. See page 3-12.

Selecting Active Channels

The FMBIO scanning unit has two optical filter holders and each filter holder can hold two filters. As a result, the FMBIO scanning unit can simultaneously read four different emission wavelengths in the same lane or on an entire gel.

Read Image collects emission data from all active channels during a scan. Active channels are preceded by a checkmark in the FMBIO menu and are listed in the Scan Control window. Channel 1 is the default active channel.

Note Channel 1 does not have to be active if it is only in place for an autofocus.

1. Before beginning a scan, place the appropriate filters in the filter holders.
2. Choose the corresponding channels in the FMBIO menu to make them active. Read Image collects emission data from each active channel during the scan.
3. Check the reading sensitivity setting for each active channel in the FMBIO Parameter window. See page 4-8.

Important: Each active channel must have an associated filter in the filter holder.

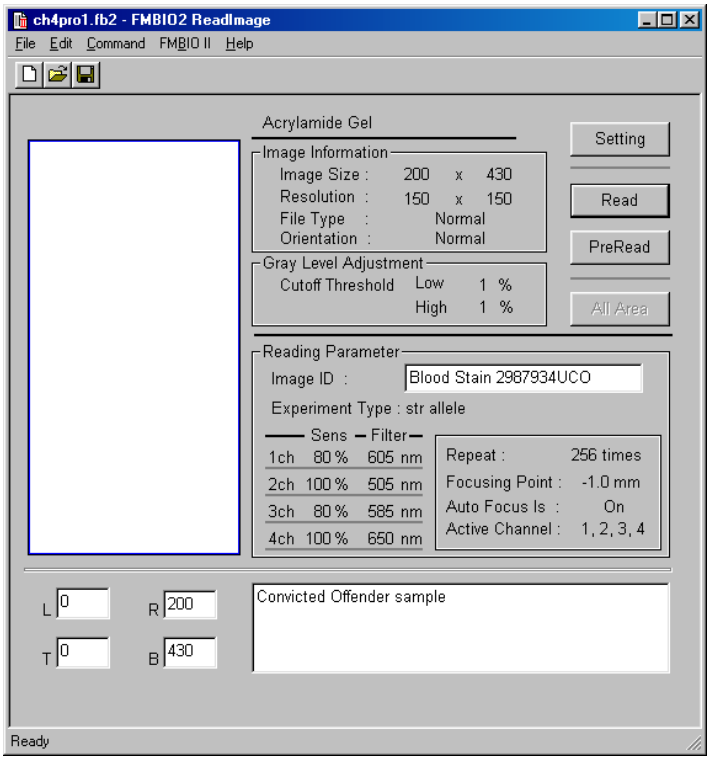
If you make a channel active in Read Image, the corresponding channel on the FMBIO scanning unit must contain a filter. During a scan, if an active channel does not contain a filter, or if the filter holder is not in place, the resulting scanned image is white. If an active channel contains an opaque, plastic barrier, the resulting scanned image is black.

See *Inserting Optical Filters* on page 3-5 for directions on changing optical filters.

See page 4-22 for information about the other commands in the FMBIO menu.

Scan Control Window

Feature Name	Description
Total scan area	Displays available scan area and a pre-defined scan area.
Scan area coordinates	Four values that define the scan area.
Setting button	Opens the FMBIO Parameters window.
Read button	Begins an image scan. Changes to Pause during a scan. Changes to Resume during a Pause.
PreRead button	Begins a prescan. Changes to Cancel during a scan.
All Area button	Sets the scan area to the total scan area.
Comment field	Stores your comments.



Using the Scan Control Window

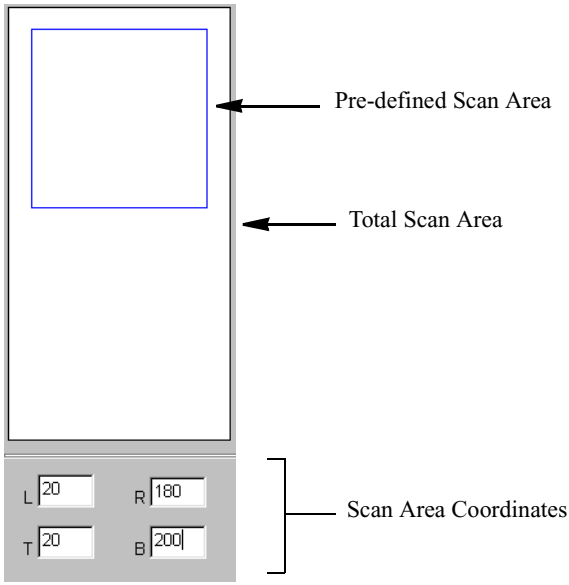
In addition to providing the tools for defining the scan area and performing scans, the Scan Control window displays:

- Scan settings specified in the Parameters window. See page 4-6.
- Image file type, orientation, gray level adjustment—settings defined by items in the Command menu. See page 4-10.
- Autofocus and the active channels—settings defined by commands in the FMBIO menu. See page 4-13.

Important: Before defining the scan area and beginning the scan, review the settings displayed in the text of the Scan Control window. Verify the gray level adjustment and active channels are appropriate for your scan.

Defining Scan Area

The PreRead Display in the Scan Control window represents the total scan area of the FMBIO scanner bed. The material you are scanning is positioned within the total scan area, but may be smaller than the total area. When there is a rectangle inside the total scan area, it represents a pre-defined scan area that is smaller than the total scan area.



When you choose PreRead, ReadImage produces a low-resolution, rasterized image of the scan area in the PreRead Display. You can then modify the scan area to include only essential information.

The total scan area is 200 mm x 430 mm. The minimum scan area is 20 mm x 20 mm.

Note To reduce the file size of a scanned image, avoid including the glass plate spacers and areas outside the sample in the scan area.

During a PreRead scan, the optical unit moves under the scanning bed and a rasterized image begins to appear on the monitor.

To preview the *entire* scan area:

- If the PreRead display is empty, the All Area button is not active. Click the PreRead button to preview the entire scan area.
- If the PreRead display contains a smaller, pre-defined scan area, the All Area button is active. Click All Area to preview the entire scan area.

To preview a *pre-defined* scan area:

1. Click the PreRead button to preview the pre-defined scan area.
2. To redefine the scan area, place the cursor in the scan area and drag it to the area of interest. You can change the size of the scan area by dragging the sides of the rectangle.

Adding Comments

You may enter memo information about the scan, such as date, researcher's name, or particular experiment features, in the Comment field in the Scan Control window. The comment field holds up to 255 characters. Comments are saved in a small text file associated with the image file. You can also use Image Analysis to add comments to the image file.

Read

When you have defined the scan area and all the scan settings, you can begin scanning the sample.

- To begin a scan, click the Read button.

  Before the scan begins, a Save As dialog appears. Use this window to name the image and select its folder location.

  When you click the Read button, ReadImage estimates the size of the image file that will be created and compares that size to available disk space. If the amount of available free disk space is inadequate, an alert box appears to warn you of insufficient space and ReadImage cancels the scan. If there is adequate disk space, the scan continues.

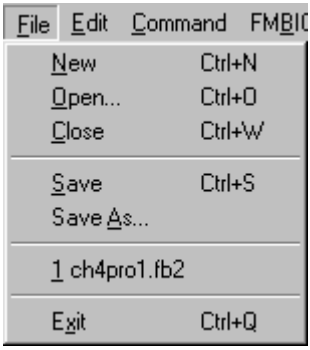
Depending on the size of the image and the stringency of the scan parameters, scanning may take between five and ninety minutes. As the scan progresses, a low-resolution raster image appears in the PreRead Display window.

When scanning is complete the data is assembled into the digital image file. This last step takes a short time.

Additional Features

The menu bar in Read Image software contains many standard commands found in IBM-compatible programs, as well as specific commands for operating the FMBIO scanning unit, modifying scan settings, and opening the Image Analysis software. These additional features are discussed in this section of the manual.

File Menu



Most of the commands in the File menu are standard Windows commands.

New. Opens a new Read Image file.

Open. Opens a saved Read Image file.

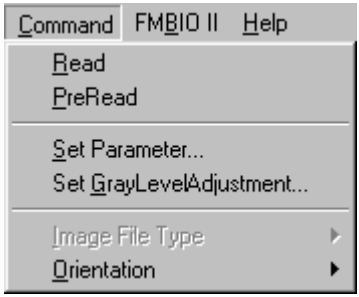
Close. Closes the active Read Image file.

Saving Read Image Files. Two commands in the File menu provide a way to save Read Image files: Save and Save As. You can use these commands to assign a unique name to a unique collection of settings that you plan to use repeatedly.

Recent Files. Files that have been opened recently are listed. Choose a file to open it.

Exit. Closes only the ReadImage software.

Command Menu



The Command menu contains commands to do the following:

- correspond to buttons on the Scan Control window.
- modify settings that appear in text on the Scan Control window.

See page 4-15 for information about the Scan Control window.

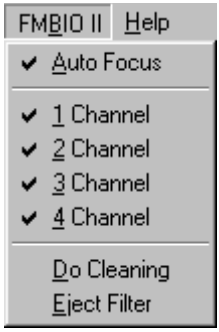
Read. Corresponds to the Read button on the Scan Control window.

PreRead. Corresponds to the PreRead button on the Scan Control window.

Set Parameter... Corresponds to the Setting button on the Scan Control window. Choose this command to open the FMBIO Parameters window.

Set Gray Level Adjustment..., Orientation. For a discussion of these commands, see page 4-10.

FMBIO Menu



The commands in the FMBIO menu directly manipulate the FMBIO scanning unit. These commands only work if your system has been configured by qualified Hitachi Service personnel.

Auto Focus. See page 4-13 for a description of this command.

Channels. Checkmarks precede the active channels. See page 4-13 for a discussion of active channels.

Do Cleaning. Moves the optical unit to allow access to the inside of the scanning unit. You cannot change filters when the scanning unit is in the cleaning position. See page 9-1 for more information about cleaning the scanning unit.

Eject Filter. Moves the filter holders to make filters accessible for removal.

Note Use the Eject Filter command only when the optical filter service door is closed.

Imaging Basics

In the scanning process, light signals are converted into a bitstream. The FMBIO scanning unit generates 16-bit images. Each pixel in the image contains 2^{16} or 65,536 possible signal intensity levels. Sixteen-bit imaging can produce very fine-grained images, in which very small or subtle differences in the elements of the image may be discerned.

The resolution, or density of pixels (dots) in the digital image may be 75, 150, or 300 dots per inch (dpi) in each direction. Dot density of the image is determined in ReadImage before scanning.

Due to two limitations inherent in video display monitors, the image as it appears on the monitor is a rough representation of the actual image data present in the digital file. First, video monitors use 8-bit processing which means only 2^8, or 256, signal intensity (grayscale) levels are displayed per pixel. Thus file signals that are close but not identical to one another in grayscale value appear to have the same intensity on the monitor. Signals that are slightly above the low cutoff threshold may also be submerged in background on the monitor.

Second, most video monitors have a resolution of 72 dpi. Thus an image scanned at 150 x 300 dpi would contain 45,000 pixels/in^2 in the digital file, while the monitor is capable of displaying only about 72 x 72, or 5184, pixels/in^2. File pixels are averaged together for display purposes with a consequent loss of resolution, in this case at a resolution loss ratio of nearly 9:1. The combined effect of these two limitations is that display images are apt to appear coarser, grainier, or blurrier than they actually are.

You can compensate for these limitations in a number of ways:

- Adjust grayscale and gamma controls to fit the particular conditions of an image. With Image Analysis, you can modify the image to reveal a very broad range of signal intensities. Just remember that considerably more information is latent in the image than can be displayed with any particular set of display parameters.
- Many factors contribute to the success of DNA detection. Variables include: gel type and concentration, sample preparation, band size, dye concentration, staining time, destaining time, gray level settings, and laser focusing point. Further optimization of protocols may result in higher detection sensitivity.

- For best scanning results, avoid dust specks by using only powderless gloves, rinsing gloves with distilled water, thoroughly cleaning all containers used for staining, and filtering all buffers and solutions with a 0.45µ filter.
- Loading buffers containing xylene cyanol and bromophenol blue fluoresce strongly when excited by the FMBIO laser, and can interfere with the gel image. For best results, use loading buffers containing no xylene cyanol, and bromophenol blue concentrations decreased 1:10.
- Gels can be cast with ethidium bromide to save time after electrophoresis. However, electrophoretic mobility of linear double-stranded DNA is reduced by up to 15% in the presence of ethidium bromide and background staining is not uniform when casting agarose gels with ethidium bromide. Therefore, post-staining is highly recommended.
- Detection sensitivity is higher in acrylamide gels than in agarose gels, due to lower background.
- Ethidium bromide can be used to detect both single-and double-stranded DNA or RNA. The affinity for double-stranded DNA is higher than for single-stranded DNA or RNA.
- Longer staining times do not necessarily increase sensitivity.
- Shortening the ethidium bromide staining time or lengthening the water destaining time may decrease detection sensitivity.
- Keep all solutions containing ethidium bromide covered by aluminum foil to prevent bleaching of the dye by ambient light.
- Ethidium bromide is a powerful mutagen. Follow usage precautions as described on the MSDS.

Multi-Color Images

Multi-wavelength analysis makes it possible to create and analyze signals from multi-color gel images. Two or more fluorophores of different emission spectra can be run in the same lanes, or on the same gel. This function is useful for comparative allele analysis, or for highly accurate size determination by running a marker in the same lane with a sample.

To scan material with more than one emission wavelength requires that you scan with multiple channels, with the appropriate filters in place.

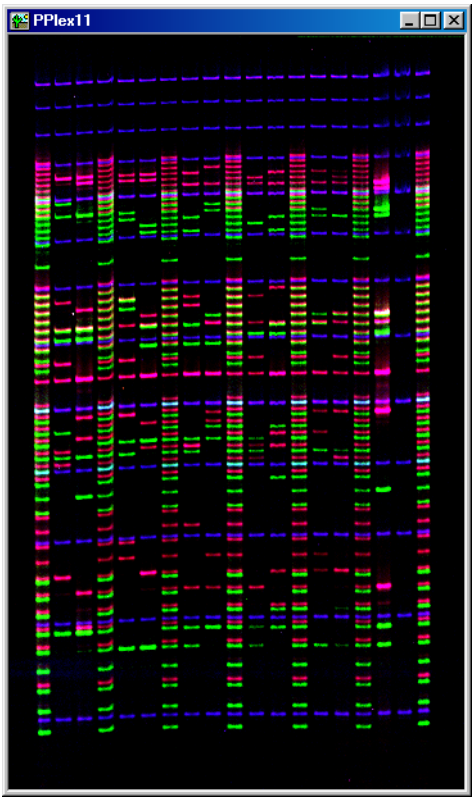
Multi-color analysis depends on the use of appropriate filters. Each filter captures one emission wavelength by screening out the others. To load filters, see page 3-5.

Long Pass Filters

Long pass filters reject shorter wavelength light while allowing longer wavelengths to pass through. Long pass filters are named for the wavelength at the midpoint of the transition range, for example, OG 610. The midpoint of the sigmoid curve that describes the transition from 0 to maximum transmission. The narrower this transition range is, the better, especially when the two fluorophore emission frequencies are close.

The name of the long pass filter may also include letters such as OG (orange glass), RG (red glass), E (emission), LP (long pass), EFLP (edge filter long pass). OG and RG are colored glass absorption filters, whereas E, LP, and EFLP filters are coated interference filters. Colored glass filters are cheaper and have broader transition slopes than coated interference filters.

Match filters to fluorophores in such a way as to maximize the amount of signal separation. This multi-color image contains red, green and blue signals, which were created with FITC, TMR and CXR dyes matched with 505, 585 and 650 filters, respectively.


PPlex11

Example Scanning Process

This section describes the process of scanning, using as an example the detection of fluorophore-labeled str alleles.

WARNING!  **This is only an example. These procedures are not meant to describe the only or optimal method to carry out the scanning of any material. It is included only to illustrate the application of various FMBIO II functions.**

1. Turn on power to the computer.
2. Turn on power to the FMBIO II. (The switch is behind the pull-down panel, on the front left).
3. After electrophoresis has been performed, keep the polyacrylamide gel-plate assembly together. Carefully clean and dry the outside of the plates. The gel/plate assembly does not need to be taken apart, unless post-staining is necessary for your application.

Note Maintaining the integrity of the gel-plate assembly can allow for better separation of larger STR loci, because the gel can be returned to the electrophoretic apparatus after scanning for additional electrophoresis.

If you have an agarose gel, polyacrylamide mini-gel or membrane, place them on a 5mm thick, low-fluorescent glass plate. Avoid dripping by removing excess buffer on stain. Smooth out bubbles under gels, as they can interfere with the scan. Membranes should be mounted wet, face down between two pieces of glass. Polystyrene and other clear plastics may also be acceptable for holding sample(s), but may result in high levels of background.

4. Place the gel-plate assembly in the scanner.
   a. Press the button on the top right of the front of the instrument, and open the sample door.
   b. Slide the right side stage bar to the proper distance to support the plate, without obscuring the area to be scanned.
   c. Place the plate assembly (long plate face down) on the stage, resting the plate assembly on the black bars at each end and between both of the stabilizing screws on the bars. Make sure the plate is level

and secure. For polyacrylamide gels, place long plate face-down.

5. Start the FMBIO II Readlmage program.
6. Click the Setting button. The Read Image Parameter dialog box is displayed.
7. Set the Scanning parameters:

Note The example show described below is for illustration only, and must not be interpreted as a suitable procedure for any particular case.

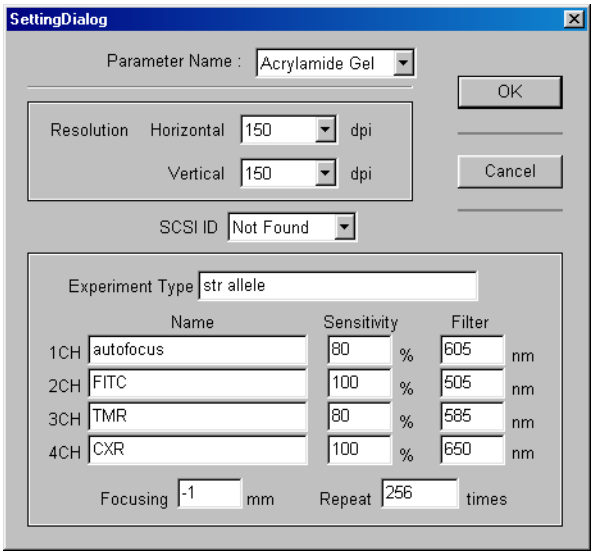
a. Choose the Parameter Name. In this example, Acrylamide is entered.

b. Choose the resolution. 150 dpi is an example for the initial setting.

c. Enter the sensitivity. In this example, 80% is enter for Channels 1 and 3, and 100% for Channels 2 and 4. One of the factors influencing this choice is the types of filter to be used.

d. Enter the focusing. 0 is shown in this example. It may need to be lower for the thinner plates used in pre-cast gels. Note that the range of focusing is ONLY - 2 mm to + 3 mm. Use of values outside of this range can seriously damage the instrument.

e. Choose the number of repeats. A example initial setting is 256.



a. Click OK to return to the Scan Control window.

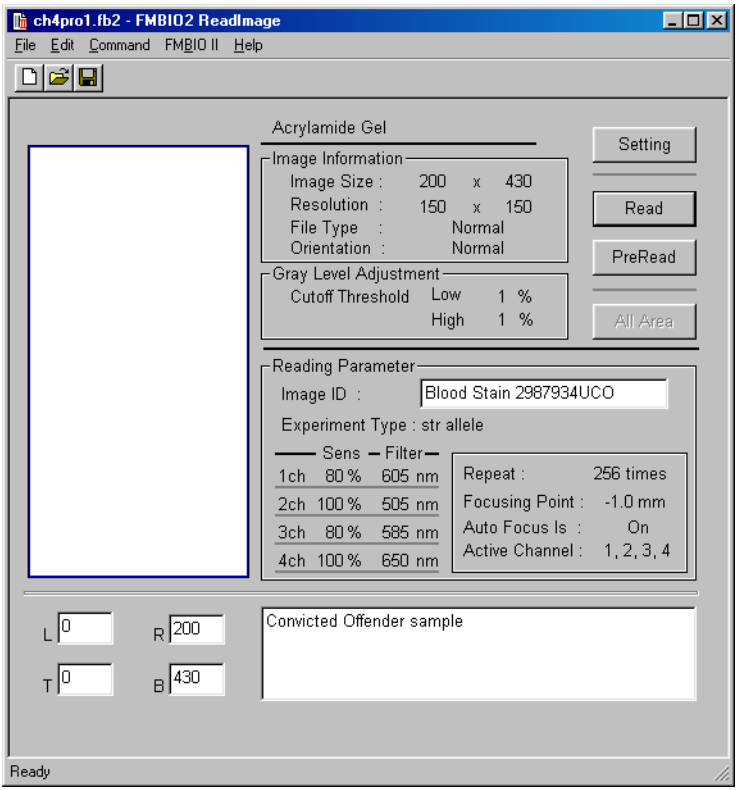
8. In the FMBIO II menu, set the active channels. A separate image is collected for each channel.

9. Since the instrument will always autofocus before the first read after power-up, turn Auto Focus off in the FMBIO II menu to save time during subsequent scans. The 605 nm filter must be installed in Channel 1 for autofocusing.

10. In the Command menu, set the gray level adjustment for polyacrylamide gels. Examples might be 50% for Low and 1% for High.

11. Make any changes needed to Orientation in the Command menu.

Note To save the Read Image parameters for future scans, choose Save from the File menu, assign a filename and location, and then click Save. Double-click on the saved parameter file to launch the Read Image program and return the user to the saved scanning parameters.



12. Add any comments to an image file by typing those comments in the text box in the lower section of the Scan Control window.
13. Click the PreRead button to quickly identify the gel area to scan.

Note If a small scanning box is already set from a previous scan, click on the All Area button before prereading. If you need to terminate an in-progress prereading, click Cancel.

14. Designate the scan area by dragging with the mouse from top left to lower right in the viewing window.

Note DO NOT include SPACERS or other solid objects in this box, because they can distort the gray scale balance of the image.

15. Click Read. Specify a file name, and a location for the saved file.

Note You can terminate a scan before completion by clicking Cancel, then save or discard the image data.

16. Wait for the instrument to complete all of its scans. The FMBIO II will autofocus and might scan twice depending on the channels chosen. After the scan begins, a calculated time for completion is noted in the lower right of the Read Image Window. This time is per scan, and calculation accuracy improves as the scan progresses
17. To view the results, use the Image Analysis software.
18. Adjust the grayscale to optimize the image.

Note See *Gray Level Adjustment* on page 5-14 for information about grayscale adjustment.

19. If the image is satisfactory, proceed to Chapter 5.
20. If the grayscale adjustment does not produce an adequate image, make the adjustments suggested in the Image Troubleshooting Guide at the end of this chapter, then rescan the image.

Table 4-1: Image Troubleshooting Guide

Problem	Suggested Adjustments
Poor band definition	• increase repeats and/or resolution • adjust focussing point
Too much signal	• lower the sensitivity
Low signal	• increase sensitivity • adjust focusing point
Grainy appearance	• increase sensitivity • increase repeats • adjust focusing point
High Background	• use low fluorescent glass • de-stain gel or membrane • adjust focusing point

ReadImage Error Codes

In the process of using ReadImage software, you may occasionally see an error code number appear on the screen.

Error code = 161, 162, 163, 164, 165, 167, or 169. Perform the following steps:

1. Press the Power switch to the Off position and wait at least 5 seconds.
2. Press the Power switch to the On position.

   The FMBIO performs a self-diagnostic routine.

   - If the error code appears again, repeat steps 1 and 2 one more time.
3. If the error code persists, call Hitachi Software Engineering service personnel.

Error code =166. Insert a filter holder in either Channel 1 or Channel 2, and then restart the scan.

Error code = 168. Firmly close the scanning unit cover.

Error code = 192. There is an internal memory shortage.

Error code = 193. There is an external memory shortage.

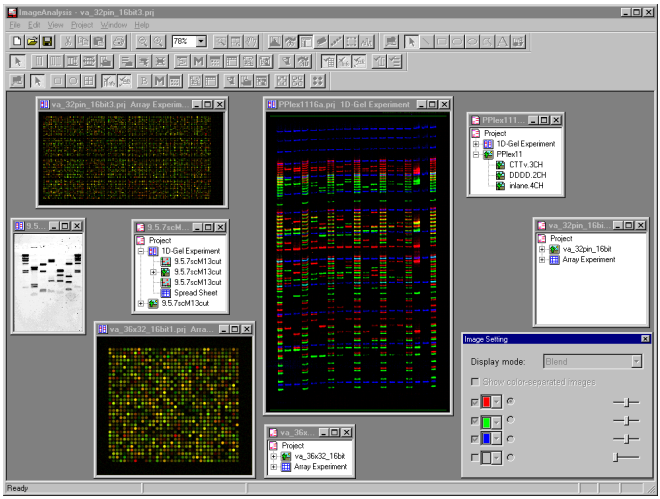
Error code = 198. The optical unit did not return to the home position.

5 Image Analysis Tools

With the FMBIO scanning unit and Read Image software, a single scan can create a multi-color image of a gel that contains one, two, or more fluorophores. In Image Analysis, you can view and analyze the image.

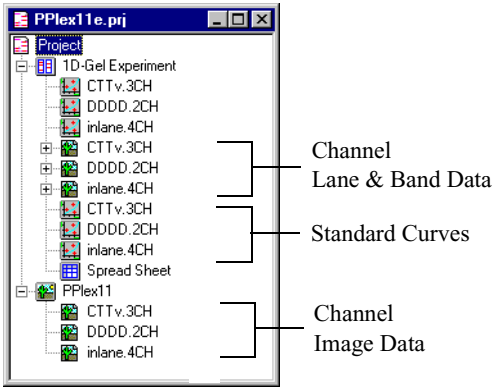
You can run fluorophores of two or more different emission spectra in the same lane or gel for comparative allele analysis. Highly accurate size determinations can be achieved when a internal lane standard is run in the same lane with a sample. If a sample contains two or more fluorophores and you designate two or more channels as active during the scan, FMBIO Read Image creates a single multi-color image.

In Image Analysis, you create a project to analyze your 1D or array experiments. You can create a project for a single- or multi-color image. You can also display images and data from more than one project simultaneously.



You can view all the colors in the image simultaneously, or select the layers you want to view. You can assign a color to each layer in the project and modify the intensity of each color, or display all layers in grayscale.

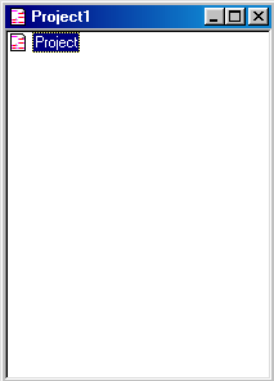
This chapter describes the tools you can use to modify and analyze the scanned image.



Note While the following chapter describes each of the analysis tools in the order of use it is not meant to serve as an procedural guide. Please refer to Chapter 6, *1D-Gel Analysis*, for step-by-step procedures.

Image Analysis Projects

Double-click the Image Analysis shortcut icon to start the Image Analysis program. When Image Analysis window opens, a new project is automatically created for you and a new Project window is displayed.



You can use this new project, create another new project, or open an existing project.

Creating a New Project

- To create a new project, click the New Project button or choose New Project from the File menu.

Opening a Project

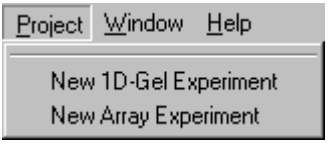
- To open an existing project, click the Open Project button or choose Open Project from the File menu.

Saving a Project

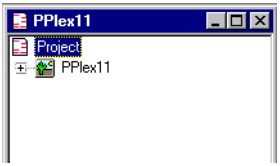
- To save a project, click the Save Project button or choose Save Project from the File menu. Give the project a name and click Save.

Image Menu and Tools

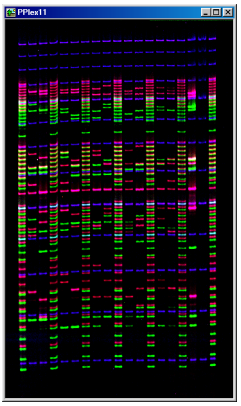
1. In the Project menu, choose New 1D-Gel Experiment or New Array Experiment.



2. Select an image file from the directory window. The image icon is added to the project tree.

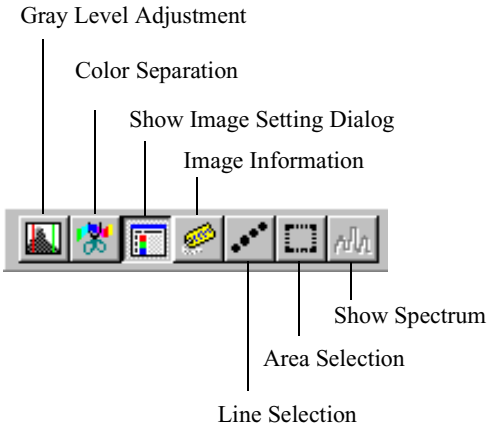


3. Double-click the image icon to display the selected image.



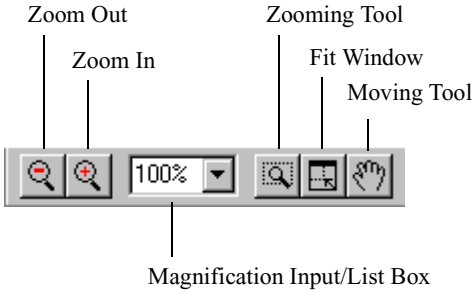
Once an image is displayed, the Image menu appears in the menu bar and the Image Tool buttons become active.


Image
1D-Gel
Window
Help
Gray Level Adjustment...
Color Separation...
Show Image Setting Dialog
Get Information...
Save Image as ...
Transparent Background
Line Selection
Area Selection
Show Spectrum



Command	Description
Gray Level Adjustment	See *Gray Level Adjustment* on page 5-14.
Color Separation	See *Color Separation* on page 5-30
Show Image Setting Dialog	See *Image Setting Dialog Box* on page 5-11.
Image Information	View sample, image and scanning information from the FMBIO II Read Image file.
Save Image As	Save an image or selected area as a TIFF or BMP file. See *Saving an Image* on page 5-7.
Transparent Background	See *Transparent Background* on page 5-36
Line Selection	Draw a line to select a location on the image
Area Selection	Draw a rectangle to select an area on the image.
Show Spectrum	See *Spectrum* on page 5-26

In addition, you can use the Zoom tools to magnify or reduce the image.



Tool	Description
Zoom Out	Each click reduces the image.
Zoom In	Each click to enlarges the image.
Magnification Input/List Box	Displays current magnification. Enter the desired magnification or select a magnification from the drop-down list.
Zooming Tool	1. Click the button. The cursor transforms into a magnifying glass with a "+" sign. 2. Drag the magnifying glass cursor to create a rectangle surrounding a region of the image. The image is magnified to show the region.
Fit Window	Once a magnification is selected, the image fills the entire window.
Moving Tool	Click the button, and then drag the image with the hand cursor.

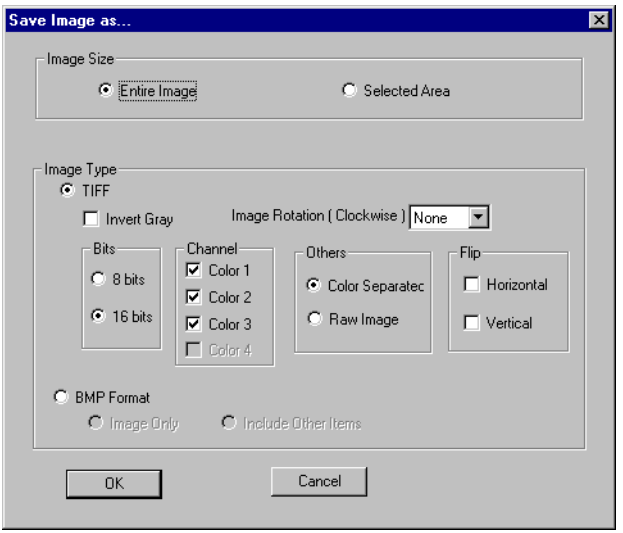
Saving an Image

You can save the entire image or a selected area of an image as a TIFF or BMP file.

1. If you want to crop the image, click the Select area to draw but-

ton  or choose Area Selection from the Image menu. Place the crosshair pointer on a corner of the rectangular area you want to save, and click-and-drag the mouse to the opposite corner of the area. Release the mouse.

2. Choose Save Image as... from the Image menu. The Save Image as dialog box is displayed.



Parameter	Description
Image Size	Save the entire image or a selected area. Select the area using the Area Selection tool.
Image Type	TIFF or BMP
Invert Gray	Inverts the image grayscale values.
Image Rotation	None, 180, 270, or 360.

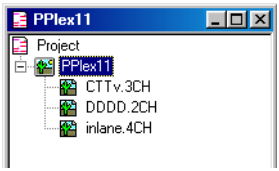
Parameter	Description
Bits	8-bit, or 16-bit.
Channel	Save any of the channels represented by colors 1, 2, 3, and 4.
Color Separation	If color separation has been performed in Image Analysis, save the color separated image or the raw (pre-color separated) image.
Flip	Flips image horizontally or vertically.
BMP Format	Save the image only or include other items, such as band and lane markers.

3. Select the appropriate parameter settings, and click OK. The Save As dialog box is displayed.
4. Specify a location for the file in the Directory window, and enter a filename
5. Click the Save button.

Viewing a Multi-Color Image

A multi-color image is a combination of two or more scanned image channels that share the same scan area and orientation. FMBIO Read Image automatically creates a multi-color image file whenever two or more channels are active during a scan. When Image Analysis opens the image file, it displays the combined image channels as one multi-color image.

1. Click the folder expansion  symbol next to the image icon to display the names of each layer in the image.

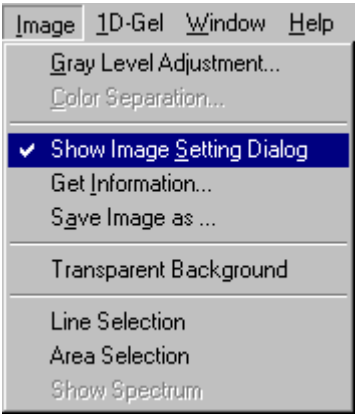


2. In this example, during one multi-color scan CTTv, DDDD, and inlane-CXR were the active channels.

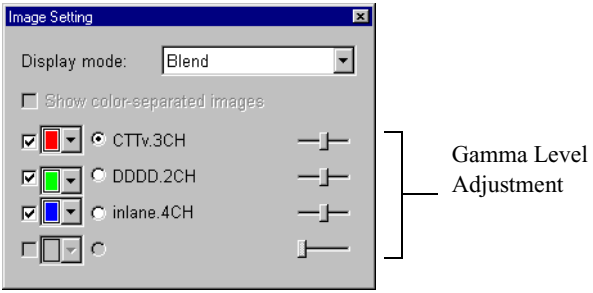
Image Setting Dialog Box

You use the Image Setting dialog box to change how the image is displayed on the screen.

- By default the Image Setting dialog box is displayed. If it is not displayed, click the Show Image Setting Dialog button  or choose Show Image Setting Dialog from the Image menu. A checkmark indicates the dialog box is displayed.



The Image Setting dialog box looks like this:



Note The Show color-separated images option is active only after you have performed a color separation on the image. See *Color Separation* on page 5-30.

Display Mode

The Image Setting dialog box assigns a unique color to each channel in the multi-color image. The colors available depend on the display mode. The Display Mode list box provides three options: Blend, Over, and Mono. Use the Display Mode drop-down list box to choose how you want a multi-color image displayed. The colors available in the Image Setting dialog box change as the display mode option changes.

Mode	Colors Available
Blend	Red, Green, Blue. Colors in each channel blend together when they overlap to make another color. Red blends with green to make yellow; blue blends with green to make cyan; red blends with blue to make magenta.
Over	Red, Green, Blue, Yellow. Colors in each channel remain distinct when they overlap. Use this mode when the multi-color project contains a yellow channel.
Mono	All channels display 65,535 shades of gray. All channels in the multi-color image appear in shades of gray.

Displaying a Channel

You can choose to display or hide the image data from one or more channels.

- Select the channel to show the channel image data. To hide a channel's image data, remove the checkmark adjacent to the channel.

Note You cannot turn off the channel display in Mono display mode.

Changing Channel Color

You can change the color associated with a channel in the multi-color image. The Color Chooser is the colored square next to the channel name. No two channels may have the same color assignment.

- Click the Color Chooser for the channel you want to change. A pop-up menu of available colors is displayed. Click the desired color.

Changing Intensity Level

You can change the intensity of each color associated with a channel.

- Drag the gamma level bar to the right to increase intensity. Slide the bar to the left to decrease intensity.

Displaying Detected Bands

After you use Image Analysis to define bands on all channels, you can choose which channel displays the band definition lines.

- Click the radio button adjacent to the channel name. The black dot in the radio button indicates the active channel. In the image, Image Analysis displays the band definition lines for the active channel.

For more information on detecting bands, see page 5-63.

Gray Level Adjustment

The proper setting of the Gray Level Adjustment parameters enhances signal and suppresses noise, resulting in clean, readable images. Conversely, improper settings might result in either saturated or faint images.

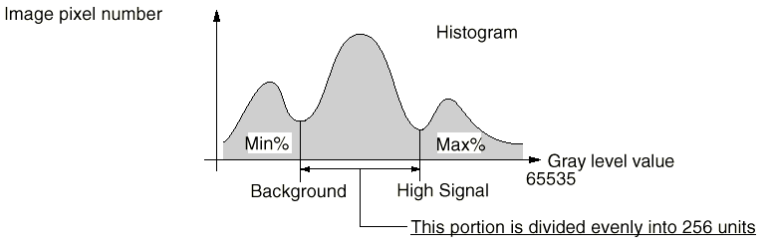
The Gray Level Adjustment parameters modify the signal intensity of each "dot," or pixel, in the scanned image. A pixel, is the smallest unit of a scanned image and each channel in an FMBIO file contains 16 bits per pixel, or 2^{16} = 65,536 grayscale levels (starting at 0). Most computer monitors are 8-bit and can therefore only display 256 shades of gray.

Image Analysis transforms the 16-bit image for display on an 8-bit monitor, averaging the additional 16-bit values within each of the 256 8-bit grayscale levels to give an 8-bit value. The Gray Level Adjustment tool tells your computer monitor how best to display your image. It allows you to optimize your image by assigning these 256 shades of gray to only the region containing sample signal.

Note The raw image data is not affected by the gray level settings.

Cutoff Thresholds. Image Analysis assigns a background cutoff threshold and a high signal cutoff threshold for each image. Cutoff thresholds are applied to the range of 65,536 shades of gray generated during a scan. Background, or noise, lies at the low end of this range; signal lies near the high end of this range.

Signals below the background cutoff threshold appear white in the image; signals above the high signal cutoff threshold appear as black. The range between the background and high signal cutoff thresholds signals is transformed into 256 gray levels.



To obtain greater clarity of an entire image or certain selected bands within it, you can modify the range of gray levels in each channel. In the Gray Level Adjustment window, you can use a histogram to adjust the cutoff threshold values. Alternatively, you can designate reference background and high signal areas on the scanned image. Using these reference areas, Image Analysis automatically adjusts all gray levels on the image.

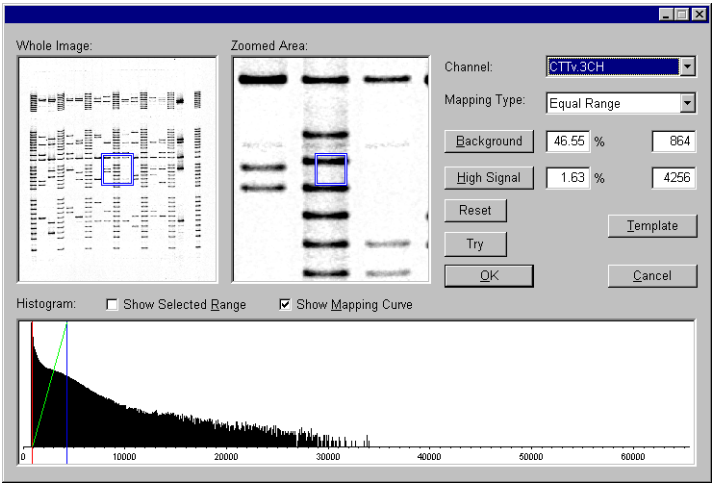
By adjusting the cutoff thresholds, you are adjusting the range between the highest and the lowest acceptable gray shades. When the high signal cutoff is too low, dark band images may be blurry, with indistinct edges. When the background cutoff is too high, faint images are lost.

Because signal intensities can vary greatly from one scan to the next, it is difficult to assign default values for high signal and background cutoff thresholds. Images that contain only faint bands against a clean background can be enhanced with high signal and background cutoff thresholds set to relatively high values. Images that contain heavily stained or optically dense regions may suffer substantial loss of content even with low threshold values.

The diagnostic guide on page 5-25 provides guidelines for threshold adjustment. See also *Imaging Basics* on page 4-23.

Gray Level Adjustment Window

- Click the Gray Level Adjustment button  or choose Gray Level Adjustment from the Image menu. The Gray Level Adjustment window is displayed.



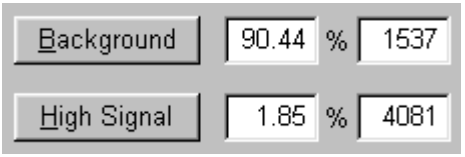
Channel Selector.

The gray level must be adjusted for each individual channel.

- Select the channel from the Channel drop-down list box. The Gray Level Adjustment window displays the image data for the selected channel.

Background and High Signal cutoff thresholds.

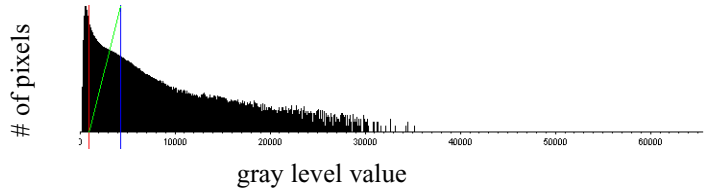
The values in the High Signal Percent and High Signal Number fields change to reflect the selected region. In the following example, 1.85% of the pixels have a value between 4,081 and 65,535. These pixels will appear on the monitor as black.



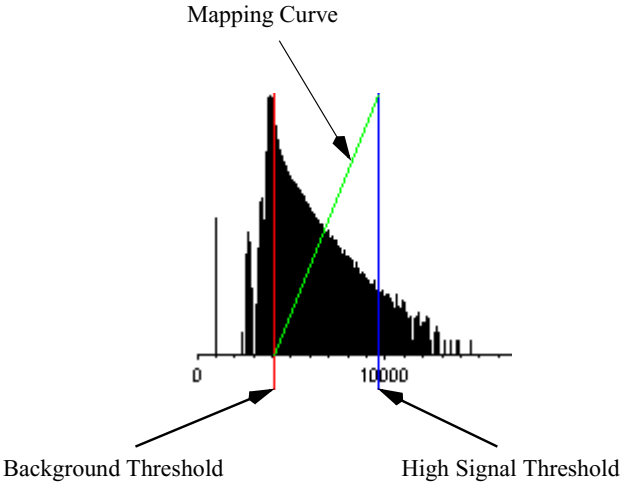
All regions of the image with gray level values below the background cutoff threshold value will appear white. As shown, 90.44% of the pixels have a value between 0 and 1,537. These pixels will appear on the monitor as white. All other elements of the image that fall within the range between the high signal and background cutoff thresholds appear as shades of gray.

Histogram.

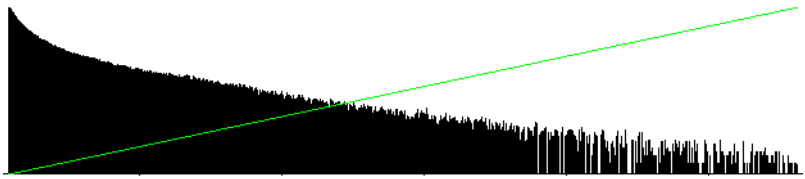
The Gray Level Histogram shows the 16-bit gray level gradations (0 to 65,535) on the X axis and the number of pixels in the image on the Y axis.



Boundary lines indicate the high signal cutoff threshold and background cutoff threshold. The red line slider indicates the background cutoff threshold value. The blue line slider shows the high signal cutoff threshold value. The green line on the histogram displays the current mapping curve. By default, the mapping curve is displayed.

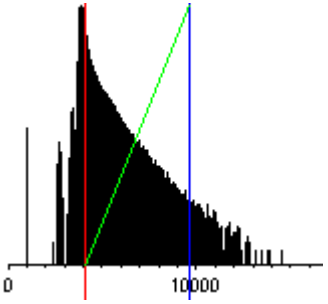


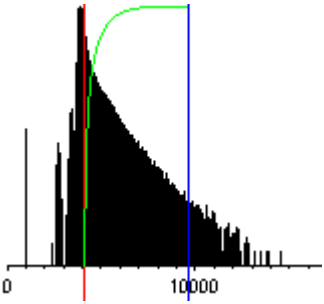
Show Selected Range. This option magnifies the histogram to show the range between the high signal and background cutoff thresholds.

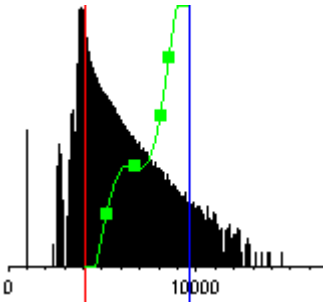


Mapping Type Selector.

The mapping type defines the method used to transform 16-bit gradation (0 to 65535) into 8-bit gradation (0 to 255). The gray level gradations between the high signal cutoff threshold and the background cutoff threshold are divided into 255 equal parts. To view the mapping curve, select the Show Mapping Curve option.

Mode	Description
Equal Range	• Range of the histogram between the high signal cutoff threshold and background cutoff threshold divided into 256 equal parts.  Note: Use the Equal Range mode to display of the relative concentration of the bands.

Mode	Description
Equal Area	• Area of the histogram between the high signal cutoff threshold and background cutoff threshold divided into 256 equal parts. In this mode, more pixels are contained in the very dark gray parts than in the Equal Range mode. Therefore, faint bands are displayed darker than their actual pixel values.  Note: Use the Equal Area mode to enhance detection of faint bands; this mode is NOT recommended for determining the relative concentration of bands.

Mode	Description
User Defined	• Drag any of the four rectangular points to define the mapping curve.  Note: Use the User Defined mode to customize your mapping curve.

Procedure

The gray level for each channel is adjusted by setting the background and high signal cutoff thresholds. You can set these thresholds in three different ways:

- Drag threshold boundary lines in the histogram.
- Visually defining signal and background pixels.
- Entering numbers for the high signal and background values.

Using the histogram.

1. Select a channel and mapping type.
2. To adjust the high signal cutoff threshold value, place the pointer over the blue line. The pointer transforms into a horizontal double-headed arrow.
3. Drag the line along the gray level axis until the desired value is displayed. The new high signal cutoff threshold value is applied to the Whole Image and Zoomed Area windows and a new High Signal Percent value is calculated.
4. To adjust the background cutoff threshold value, place the pointer over

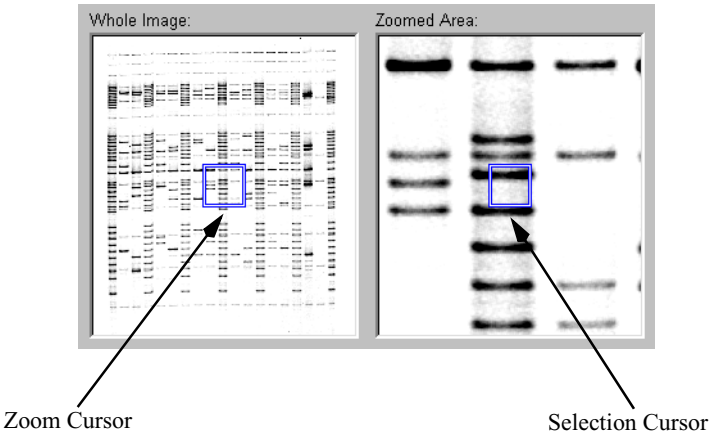
the red line. The pointer transforms into a horizontal double-headed arrow and the value appears in the upper left corner of the window.

5. Drag the line along the gray level axis until the desired value appears. The new background cutoff threshold value is applied to the Whole Image and Zoomed Area windows and a new High Signal Percent value is calculated.
6. Click the Try button to apply the modified gray levels to the actual image. To return to the original settings, click Reset.
7. Continue to try different gray level settings until your are satisfied with the signal and background levels.
8. Repeat the procedure for each channel.
9. When you are finished, click OK.

Image Analysis recalculates the gray levels for the channels using these values as high signal and background limits.

Note The raw image data is not affected by the gray level settings.

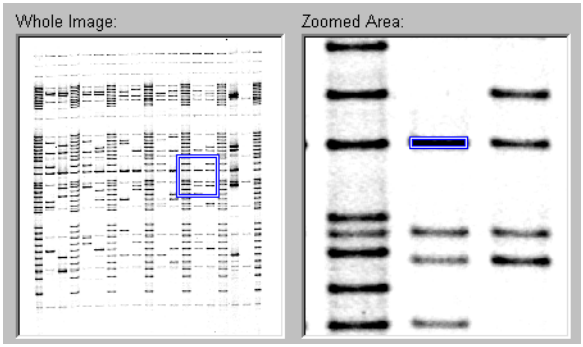
Visually defining signal and background pixels. You can define the signal and background intensities using the Zoom and Selection cursors.



1. Select a channel and mapping type.
2. In the Whole Image window, drag the Zoom Cursor to a region con-

taining average intensity bands/background. If necessary, adjust the size of the Zoom Cursor to include the desired area. The area appears in the Zoomed Area.

3. In the Zoom window, surround a band with the Selection Cursor.



4. Click the High Signal button. The image displays appear with the new high signal cutoff threshold and the values are displayed in the High Signal Percent and High Signal Number fields.
5. Move the Selection Cursor to a background area away from the band.
6. Click the Background button. The selected background cutoff threshold is applied to the Whole Image and Zoomed Area windows and the values appear in the Background Percent and Background Number fields.
7. Click the Try button to apply the modified gray levels to the actual image. To return to the original settings, click Reset.
8. Continue to try different gray level settings until your are satisfied with the signal and background levels.
9. Repeat the procedure for each channel.
10. When you are finished, click OK.

Image Analysis recalculates the gray levels for the entire image using these values as high signal and background limits.

Using the percent and number fields.

You can also adjust the high signal and background cutoff thresholds by entering values in the percent and number fields.

Select a channel and mapping type.

1. Enter numbers in either the percent or absolute value fields, and then click OK.
2. Click the Try button to apply the modified gray levels to the actual image. To return to the original settings, click Reset.
3. Continue to try different gray level settings until your are satisfied with the signal and background levels.
4. Repeat the procedure for each channel.
5. When you are finished, click OK.

Image Analysis recalculates the gray levels for the entire image using these values as high signal and background limits.

Creating a template.

If you have a series of gels that have been run under the same conditions and produce similar images, you might want to save your gray level adjustment values as a template for future use:

1. Click the Template button.
2. Click the Create New button. The Template dialog box is displayed.
3. Type a unique name for the template, then click the Close button.

Note When a Gray level Adjustment Template is created, only the mapping type, high signal cutoff threshold value, and background cutoff threshold value are saved. The percent values are not saved. Therefore, it is imperative that a Gray Level Adjustment template is created for each channel.

To apply a gray level adjustment template:

1. Select a channel.
2. Click the Template button.
3. Select the template name, then click the Apply button.

Image Diagnostic Guide

Image Quality	Cutoff threshold
Entire image is too dark.	Increase background and high signal thresholds.
Entire image is too faint.	Decrease background and high signal thresholds.
Image signal blurred with solid color.	Increase high signal threshold.
Image background is blank.	Decrease background threshold.
Image is chaotic and unclear, due to noise.	Decrease background threshold and increase high signal threshold.
Image and signals unclear.	Increase background threshold and decrease high signal threshold.
Image has too much contrast.	If the value settings are too close together then you may be spreading your 256 shades of gray across too small an area. Try using the cursor to draw a box that encloses a dark band.
Scan shows spacers only.	If highly fluorescent objects (such as spacers, combs, or gel thermometers) are included in the image, the High Signal value will be set too high. Enter a lower number in the value column. To avoid this problem in future scans, eliminate these objects from your scan area.

If, after these steps, you cannot achieve a satisfactory image, you may have too much signal. Try rescanning the sample at a lower photomultiplier tube (PMT) sensitivity, scan using a different filter or, if stains were used, destain the gel.

Spectrum

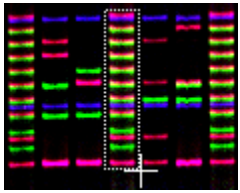
With the FMBIO system you can accurately quantify signal strength at particular locations on the image. This quantification is more accurate than conventional radiography followed by scanning densitometry because the intermediate film and scanning steps are eliminated. The FMBIO spectrum function analyzes the pixel and grayscale data in the digital image and calculates the signal strength at a given location.

You can use two methods to select the location on the image for spectrum analysis.

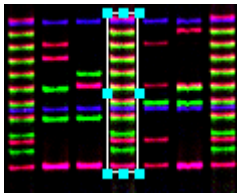
- Draw a rectangle on the image. The spectrum displays the signal strength of the bands within the rectangle.
- Draw a straight line on the image. The spectrum displays the signal strength at each point on the line.

Area Selection Method

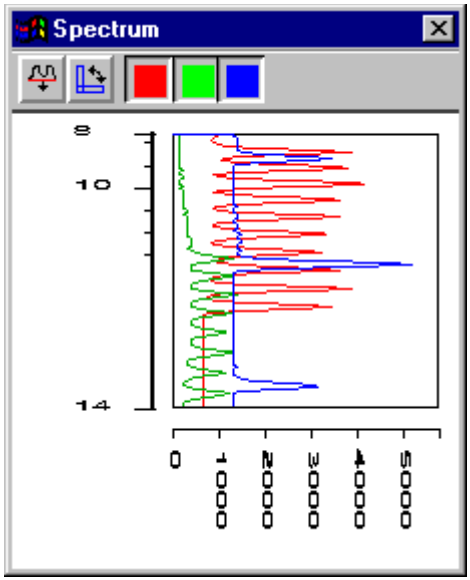
1. Click the Area Selection button  or choose Area Selection from the Image menu.
2. Position the tool at a corner of the group of bands you want to select. Drag the cursor down to the opposite corner until the bands are contained in the rectangle.



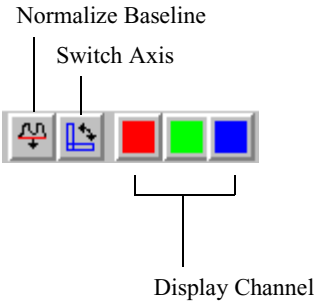
3. Release the mouse button. A selection rectangle is displayed. If necessary, adjust the rectangle by dragging the selection handles.



4. Click the Spectrum View button  or choose Show Spectrum from the Image menu. The Spectrum window displays an overlay trace of the signals, with the areas of signal intensity represented by peaks.



5. To change the dimensions of the graph, resize the window.



Tool	Description
Normalize Baseline	Normalizes vertical range.
Switch Axis	Switches between the horizontal and vertical spectrum views.
Display Channel	Toggles display of spectrum for individual channels.

Normalize Baseline tool. Click the Normalize Baseline tool to normalize the vertical range by converting the low end of the range to 0 (zero) and the upper end of the range to 255. Use this feature to compare lane traces from different image files or from files created by different filters.

Before the vertical range is normalized, it represents the range between the high and low Cutoff Thresholds on the Gray Level correction. The low and high ends of the vertical range before normalization lie somewhere within the range of available pixels in a 16-bit file, from 0 to 65535. On this range, spots on the image that appear black or “saturated” may actually have a signal above the high cutoff threshold.

However, when the vertical range is normalized, it is compressed to fit to the range of available pixels in an 8-bit file, from 0 to 255. The low cutoff threshold becomes 0; the high cutoff threshold becomes 255. When Image Analysis encounters signal above the high cutoff threshold, it extrapolates a

value for these spots that may actually be greater than 255. The upper end of the vertical range reflects the value assigned to these high signals.

Straight Line Method

1. Click the Area Selection button  or choose Line Selection from the Image menu.
2. Drag the cursor in a straight line over the location you want to analyze.
3. Release the mouse button. A selection line is displayed. If necessary, adjust the line by dragging the selection handles.
4. Click the Spectrum View button  or choose Show Spectrum from the Image menu.

Color Separation

In multi-color fluorescence imaging, up to four fluorescent dyes (fluorophores) can be detected in a single image. Although each fluorophore exhibits its own characteristic emission spectrum, with a distinct maximum at specific wavelength, there may be some overlap (bleed-through) between the spectra of the different fluorophores.

The purpose of color separation is to identify these areas of emission spectra overlap by quantifying the proportion of each fluorophore that is known to overlap with the other fluorophores.

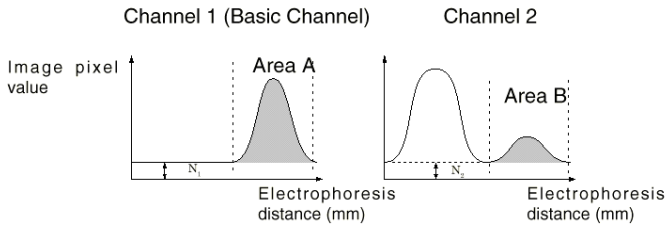
Image Analysis allows two methods for color separation:

- Color separation by individual band selection
- Multi-band color separation

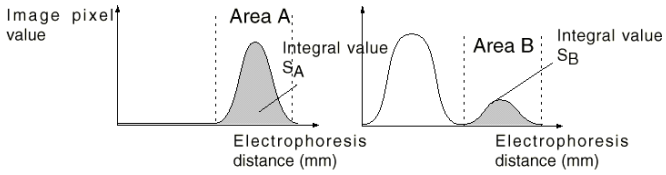
Multi-band color separation is a feature of the 1D-Gel analysis tools. See page 5-43.

Color Separation Theory

The following is a theoretical treatment of color separation for an image with two channels. Below are shown the spectra for an image from a lane with two channels. In this example, the pixels in region B of Channel 2, bleed through into area A of Channel 1 so the following type of color separation is performed.



Remove the background portion (N_1, N_2) from each channel.



1. Calculate the bleed-through rate:

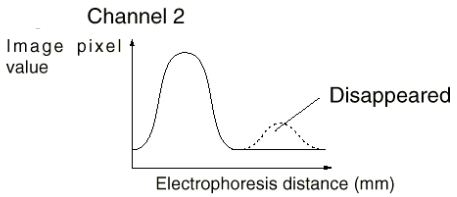
   Bleed-through rate Rab = Sa / Sb

2. The bleed through rate (Rab) is calculated for each pixel value in Channel 2 and the corresponding pixel value in Channel 1. This rate is multiplied by the image pixel values in Channel 2, and the result is subtracted. Then the spectra in Channel 2 is as follows:

   For any point on the image P (x,y), the image pixel value in Channel 1 is I_1 and the image pixel value in Channel 2 is I_2. After color separation, the pixel value in Channel 1 is:

   $I_2' = I_2-(I_1-N_1)$ x Rab, provided $(I_1-N_1) > 0$

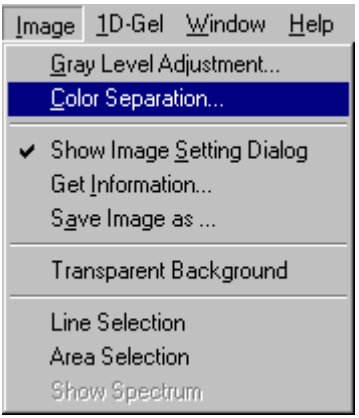
3. The operations explained above for Channel 1 are then applied to Channel 2.



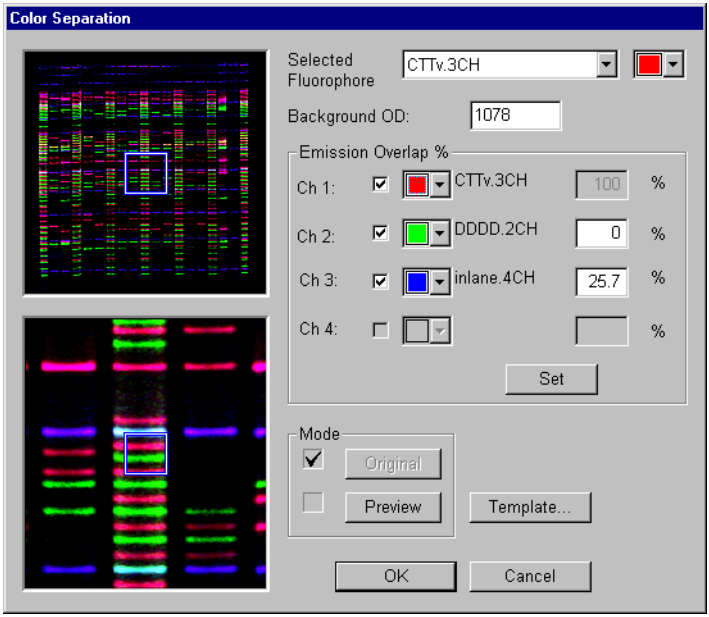
Individual Band Color Separation

To separate the colors in an image using individual bands, you must select at least one non-overlapping, medium intensity, representative band of each color.

1. Click the Color Separation button  or choose Color Separation from the Image menu.



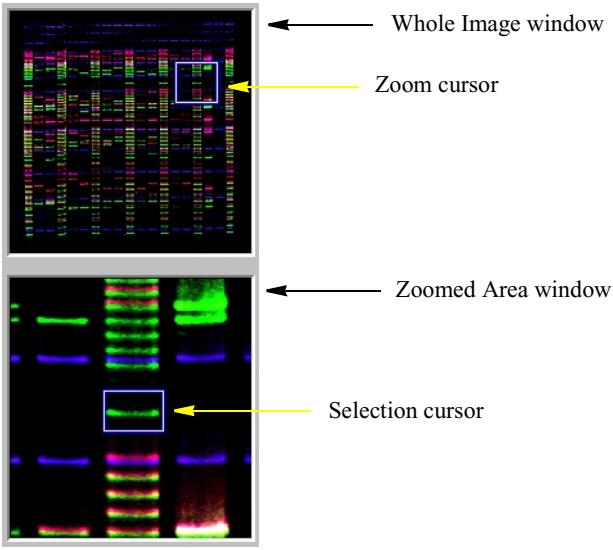
The Color Separation window is displayed.



2. Choose a channel from the Selected Fluorophore drop-down list box, and select a color.

Note In the Blend mode, the color associated with each channel appears in the box adjacent to it.

3. In the Whole Image window, move the Zoom Cursor to an area with medium intensity bands of that color.
4. In the Zoomed Area window, pick a band of medium intensity that matches the color, but does not overlap any other band. Surround the band with the Selection Cursor. Make the Selection Cursor larger than the band to include a portion of the image background.



5. Click the Set button to make the color of the selected band represent the fluorophore. Image Analysis calculates the percent overlap between the selected fluorophore and each of the other channels. The percentages are displayed in the Emission Overlap fields.
   - You can click the Preview button at any time to test the color separation process.
   - Click the Original button to revert to the original color settings.
6. Use one of the other channels in the Selected Fluorophore list.
7. Repeat this process for each of the remaining channels.
8. If the Color Separation parameters are to be used again in the future, click the Template... button. Click Create New... and assign the template a unique name.
9. When you are satisfied with the color settings, click OK.
10. If you want to remove the color separation, uncheck the Show Color-Separated Images checkbox in the Image Setting dialog box. You can now repeat the color separation process.

Using a Color Separation Template

To perform this function a previously created Color Separation template must have been saved.

1. Click the Color Separation button  or choose Color Separation... from the Image menu.
2. Once the Color Separation window has opened click the Template... button. The Template dialog box is displayed.
3. Select a template, then click the Apply button.
4. Click Close.
5. Click OK. The selected Color Separation template is applied to the new image.

Modifying Color Separation Results

On some gel images, the color separation process can result in over-subtraction of a dye that has minimal bleed-through signal.

For example, a gel image might contain three dyes: tetramethyl rhodamine, carboxy-x-rhodamine, and fluorescein. The two rhodamine derivatives have overlapping emission curves, but fluorescein has minimal overlap with the other two dyes. The following procedure explains how to exclude the non-overlapping dye, fluorescein, from the color separation process.

1. In the Image Setting dialog box, turn off the Show color-separated images option.

   When the option is off, the checkbox is empty.

2. Click the Color Separation button  or choose Color Separation... from the Image menu.
3. Choose one of the overlapping dyes (carboxy-x-rhodamine) from the Selected Fluorophore drop-down list.
4. In the Target Channel section, enter a zero (0) in both the Background and Percent entry fields for the fluorescein channel.
5. In the Mode section, click Preview to examine the new values in the Whole Image window and Zoom window. You may see a slight color

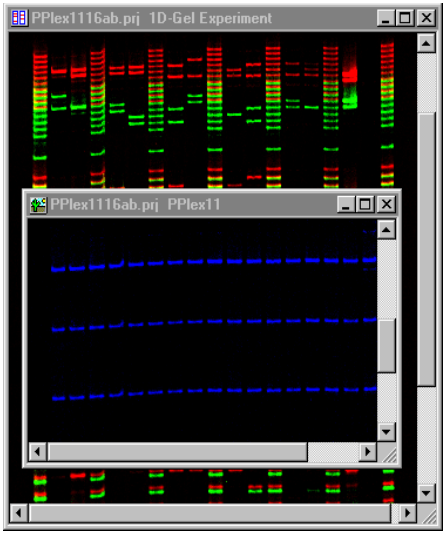
change.

6. In the Selected Fluorophore list, use the drop-down list to choose the second overlapping dye (tetramethyl rhodamine).
7. Enter a zero (0) in both the Background and fluorescein Percent entry fields.
8. Preview the color separation.

   If you are satisfied with the results, click OK on the Color Separation box. When the message “Create the Separated Images this time?” appears, click OK again.
9. If you want to remove the color separation, uncheck the Show Color-Separated Images checkbox in the Image Setting dialog box. You can now repeat the color separation process.

Transparent Background

You can choose to make the background of an image transparent and position it over another image to compare bands.



To make the background of an image transparent, select Transparent Background from the Image menu.

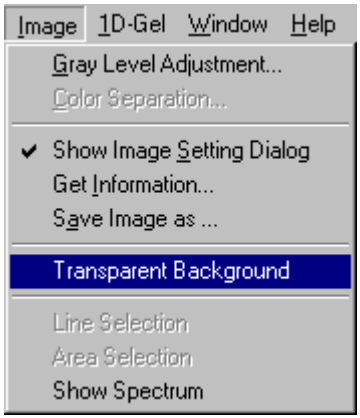
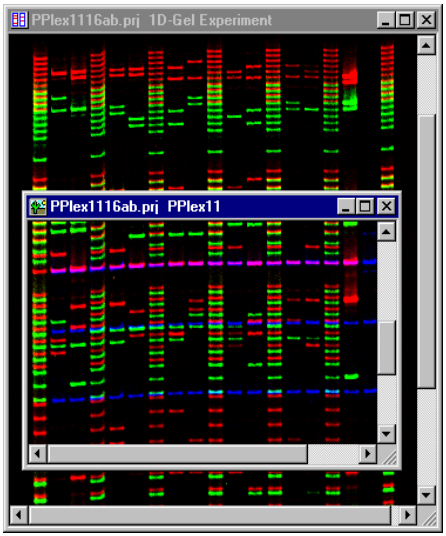


Image
1D-Gel
Window
Help
Gray Level Adjustment...
Color Separation...
Show Image Setting Dialog
Get Information...
Save Image as ...
Transparent Background
Line Selection
Area Selection
Show Spectrum

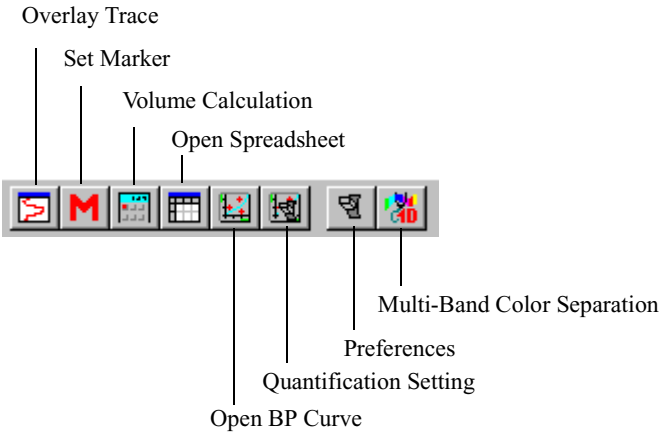

PPlex1116ab.prj 1D-Gel Experiment
PPlex1116ab.prj PPlex11

1D-Gel menu and Tools

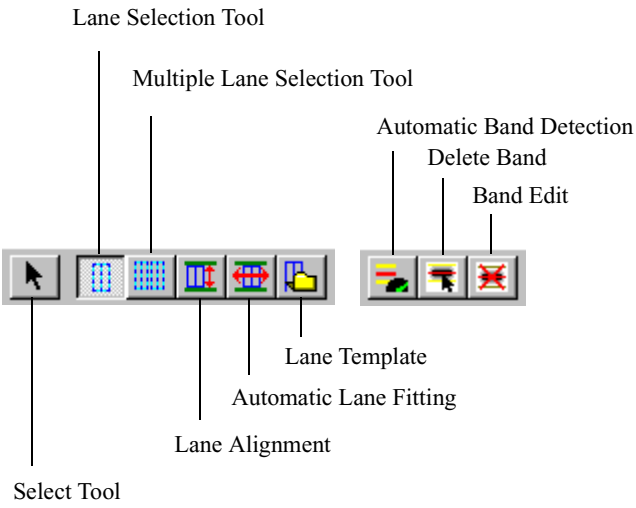
1. Click the New Project button  or choose New Project from the File menu.
2. Choose New 1D-Gel Experiment from the Project menu.

The 1D-Gel Experiment window, Image Setting dialog box, 1D-Gel menu and its corresponding tool bar appear.

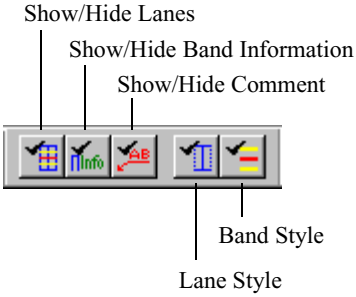

1D-Gel
Window
Help
Tool
Function
View
Lane Style
Band Style
Set Marker...
Volume Calculation
New Overlay Trace
Open Spreadsheet
Preferences...
Lane Template...
Quantification Setting...
Open Standard Curve
Open BP Curve
Multi-Band Color Separation
Batch Control
Export Lane Trace ...



Tool	Description
Set Marker	Displays Marker Setting window.
Volume Calculation	Performs a volume calculation.
Overlay Trace	Displays new overlay trace of selected lanes.
Open Spreadsheet	Displays spreadsheet.
Preferences	Displays Preferences window.
Lane Template	Displays Lane Template dialog box
Open Standard Curve	Displays standard curve.
Quantification Setting	Displays Quantification Setting window.
Multi-Band Color Separation	Displays Multi-Band Color Separation window.



Tool	Description
Select	Selects objects.
Lane Selection	Defines a single lane.
Multiple Lane Selection	Defines multiple adjacent lanes.
Lane Alignment	Aligns lanes with migration lines.
Automatic Lane Fitting	Automatically adjusts lane boundaries.
Lane Template	Creates a template for lane definition.
Automatic Band Detection	Detects selected bands automatically.
Delete Band	Delete all bands in the selected lanes.
Band Edit	Add, edit, or delete bands.



Tool	Description
Show/Hide Lanes	Toggles display of lane boundary lines.
Show/Hide Band Information	Toggles display of band information, e.g., OD,MM, Bp value.
Show/Hide Comments	Toggles display of label information entered in spreadsheet.
Lane Style	Toggles among different display options: center line with lane borders, center lines only, lane boundaries only, and lane display off.
Band Style	Toggles among the different band styles: band display off, peak and range bands displayed, and only peak bands displayed.

Multi-Band Color Separation

In a 1D-Gel Experiment, Image Analysis lets you perform separation of all colors simultaneously using multiple bands within a lane. The colors assigned to the bands collectively constitute a Multi-Band Separation Parameter Set, which is used to calculate the spectral overlap between the individual fluorophores.

Band Selection Parameters

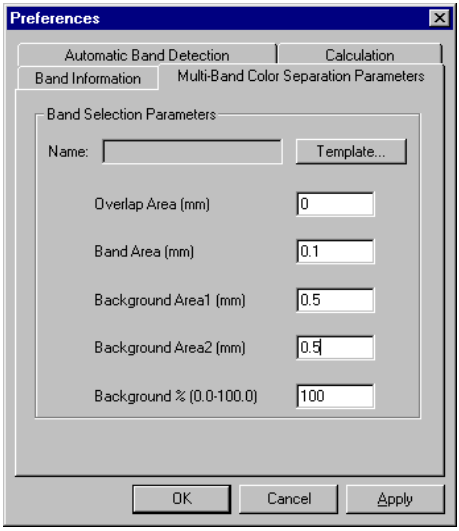
Before performing multi-band color separation can be performed, you assign values for the Band Selection Parameters, which define the criteria for calculating the background, band area, and overlap between bands in a lane.

1. Choose New 1D-Gel Experiment from the Project menu.

   The 1D-Gel Experiment window, Image Setting dialog box, 1D-Gel menu and its corresponding tool bar appear.

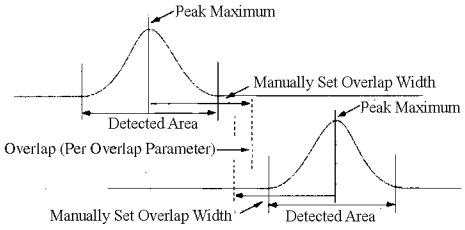
2. Click the Preferences button  or choose Preferences... from the 1D-Gel menu.
3. Click the Multi-Band Color Separation tab. This filecard displays the default Band Selection Parameter set.
4. Try these settings and, if necessary, adjust them for different gels:
   - Overlap = 0.0 mm
   - Band Area = 0.1 mm
   - Background Area 1 = 0.5 mm
   - Background Area 2 = 0.5 mm
   - Background % = 100%

Note If you plan to use these parameter settings in the future, it is necessary to save a template with a unique name.



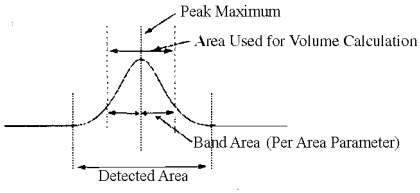
Overlap. Specifies the width of the band used in the area calculation when checking for band overlap.

- If this Overlap value is 0.0 mm, the overlap will be the width detected by the Auto Band tool.
- If there is no overlap, the band area is the total that is calculated by the Auto Band tool.
- When the Overlap value (X) is greater than 0 mm the width used to calculate the area will -X to +X from the peak maximum.
- Overlap values may range from 0-99.9 mm with a minimum increment of 0.1 mm.



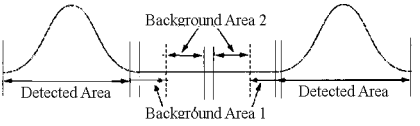
Band Area. Specifies the area that is used to calculate the volume for color separation.

- If this Band Area value is 0 mm, the band area will be the area detected by the Auto Band tool.
- If the Band Area value (X) is greater than 0 mm the band area will be -X to +X from the peak maximum.
- Band Area values may range from 0 to 99.9 mm with a minimum increment of 0.1 mm.



Background Area 1. The area directly adjacent to either side of the band of interest that is used in the background calculation.

Background Area 2. The area between bands of interest that is used in the background calculation.



Background %. Specifies the correction value for the calculated background value. This Background % value should range from 1 to 100%.

Multi-Band Color Separation Window

To use the multi-band color separation window, you must select a lane containing a minimum of one medium intensity band of each color.

1. Find a portion of a lane containing a minimum of one non-overlapping band of each color.

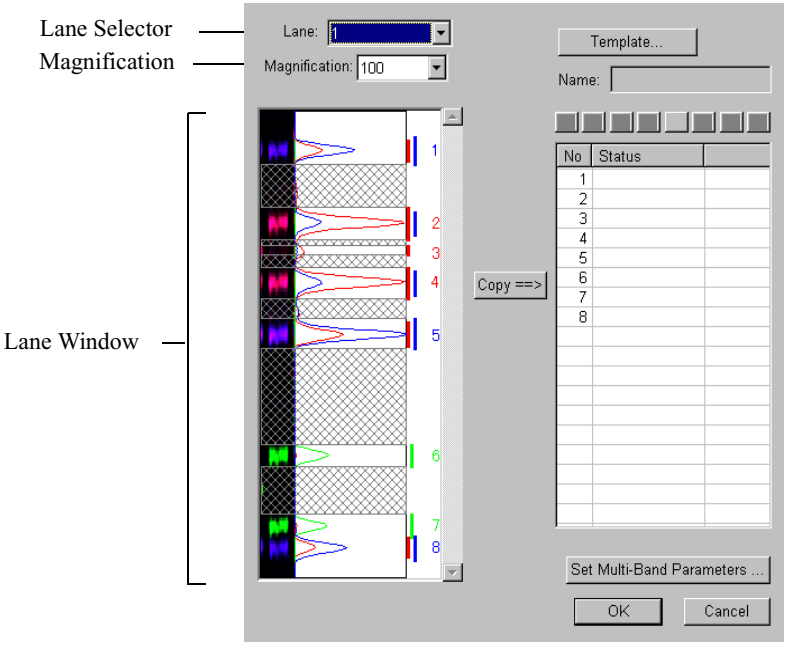
Note Two or more bands of each color is recommended.

2. Click the Lane Selection Tool button  or choose 1D-Gel > Tool > Lane Selection. Drag down and then across that portion of the lane.

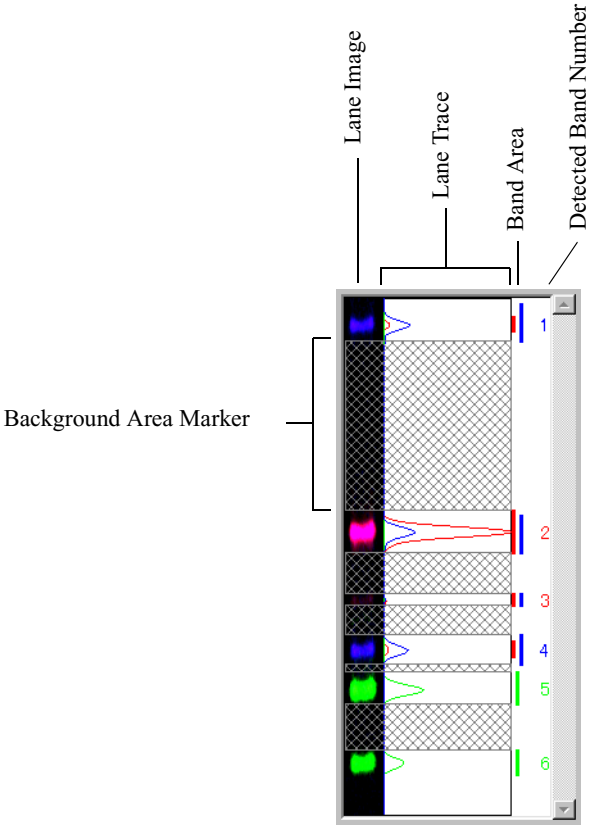
3. Click the Automatic Band Detection button  or choose 1D-Gel > Function > Auto Band.

Note Auto Band detects bands in all channels. To view the detected bands in a channel, select the channel in the Image Setting dialog box. See *Displaying Detected Bands* on page 5-13.

4. After Image Analysis has automatically detected the lane, click the Multi-Band Color Separation button  or choose Multi-Band Color Separation... from the 1D-Gel menu. Using the Band Selection parameters, Image Analysis assigns a color to each band, and the Multi-Band Color Separation window is displayed.



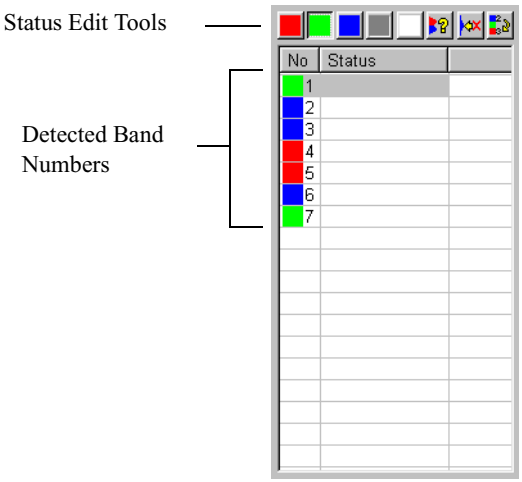
Lane Window. The Lane window displays all detected bands and a spectrum trace of the selected lane. The color of the Detected Band number indicates the color assigned by Image Analysis.



Magnification Selector. To view the Lane window at a higher magnification, select a desired value from the drop-down list box.

5. If you are not satisfied with the separation of the colors, you can adjust the Multi-Band Color Separation preferences. Click the Set Multi-Band Parameters... button, and change the parameter values. When you click OK, the colors are automatically separated according to the new values.

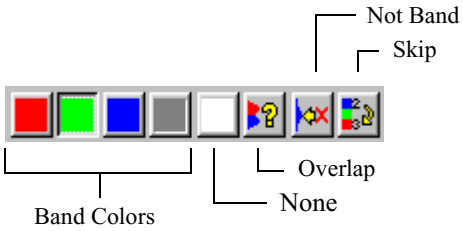
6. On the right side of the window the Status Edit Sheet displays the detected band numbers.
   - If you want to use the colors assigned by the Multi-Band Color Separator, click the Copy ==> button.
   - If you want to load a previously saved Multi-Band Separation template, click Template.... Select a template, and then click Apply. The detected bands appear in the Status Edit Sheet.



7. Review each band in the Status Edit Sheet, verifying that the assigned colors are representative of the colors viewed in the gel image.

   Typically, the Multi-Band Color Separator accurately selects bands and assigns colors to them. In a rare instance, you might want to edit the bands displayed.

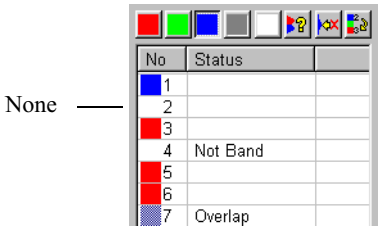
8. If you want to change the color or status of a band, click its row in the Status Edit Sheet. The Status Edit Sheet toolbar is activated.



Tool	Description
Band Colors	Designates band color for the selected band.
None	No color is assigned. Excludes band from multi-band color separation.
Overlap	Overlapped band. Excludes band from multi-band color separation.
Not Band	Band is not listed in template. Color list is shifted down.
Skip	The Detected Band numbering skips the band.

- If the band was not assigned the appropriate color, click the appropriate color. The new color is displayed.
- If the band is overlapped by another band, click Overlap. The band is grayed out.
- If you do not want to use the band, click None. The band is de-selected and no color is displayed.
- If you have applied a template to the Status Edit Sheet and the band is not listed in the template, click Not Band. The band is deselected and the color list is shifted down.
- If you do not want to use the band, click Skip. The Detected Band numbering skips the band.

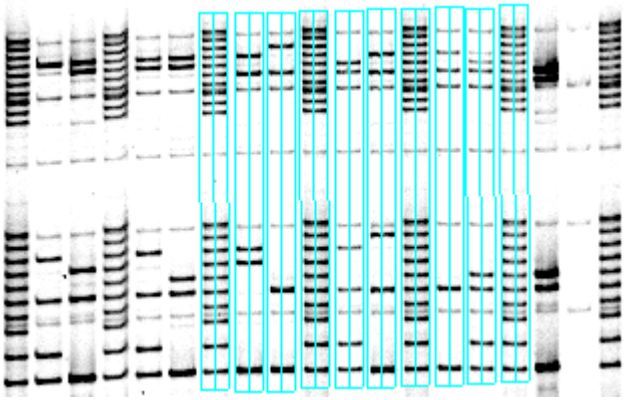
Any band that has an Overlap, None, Not Band, or Skip status is not used for the multi-band color separation.



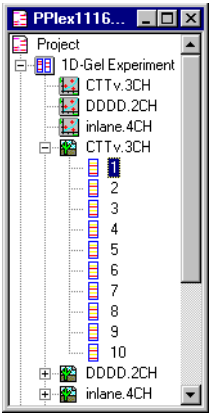
9. If this Multi-Band Separation Marker is to be used in the future (i.e. Controls such as K562, 9947A, etc.), click the Template... button. Click Create New... and assign it a unique name.
10. Once the bands have been verified, then click OK. The Color Separation window is displayed with the bleedthrough values that were calculated during the multi-band color separation.
11. Click Preview. Image Analysis performs the multi-band separation on the images in the Color Separation windows.
12. If the Color Separation parameters are to be used again in the future, click the Template... button and give the parameter set a unique name.
13. Click OK. Image Analysis creates a set of color separated images, each channel displaying only the signal from a single dye.
14. If you want to remove the color separation, uncheck the Show Color-Separated Images checkbox in the Image Setting dialog box. You can now repeat the color separation process.

Indicating Lanes

To analyze the data in a lane, the lane must first be defined with lane boundaries. Image Analysis automatically numbers each defined lane. Any data outside the lane boundaries are not included in the analysis. You can define single lanes or multiple lanes in sequence. Adjustment to lane position, width and shape can be made after the lanes are set. You can also create a lane template for use on other gels.



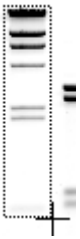
The Project window lists the lanes defined for each channel.



Defining a Single Lane

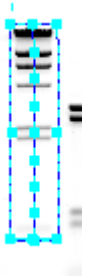
To define a single lane:

1. Click the Lane Selection Tool button  or choose 1D-Gel > Tool > Lane Selection.
2. Position the tool at a corner of the lane. Drag the cursor down to the opposite corner below the bottom migration line until the bands are contained in the boundary.



By default, Image Analysis displays a line down the center of the lane with a line on either side of the center defining the line width. When the

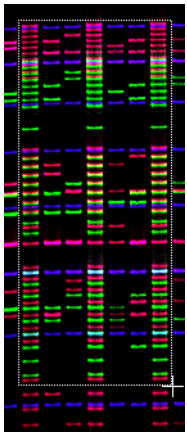
lane is selected, square edit handles are displayed on the lane lines.



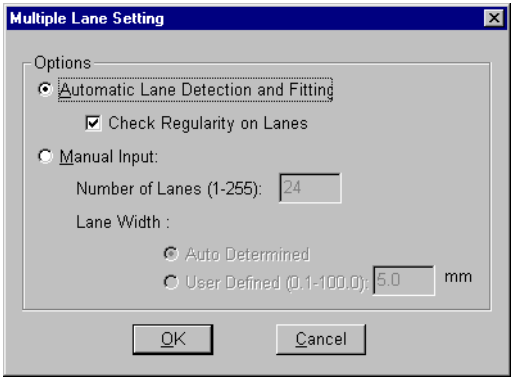
Defining Multiple Lanes

You can define up to 255 adjacent lanes simultaneously. To define multiple sequential lanes:

1. Click the Multiple Lane Selection Tool button  or choose 1D-Gel > Tool > Multiple Lane Selection.
2. Position the tool above the top migration line at the corner of the first lane in a sequence of lanes.
3. Drag the tool below the bottom migration line to the opposite corner of the last lane in the sequence.



4. Release the mouse. The Multiple Lane Setting dialog box is displayed.



5. Choose either Automatic Lane Detection and Fitting or Manual Input.

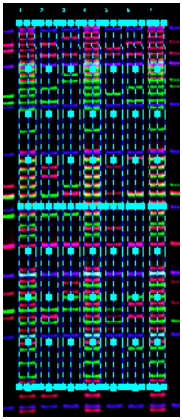
   If you choose Automatic Lane Detection, you can also choose to check the uniformity of the lanes.

Note We do not advise using automatic lane detection on lanes that are not clearly defined, such as lanes produced by sharks tooth combs.

If you choose Manual Input, enter the number of lanes in the sequence

then choose to either automatically determine the lane width or specify a width. Lane width may range from 1.0 to 100.0 mm, in 0.1-mm increments.

6. Click OK. By default, Image Analysis displays a central line down each defined lane, with additional lines marking the lane width. When the lane is selected, square edit handles are displayed on the lane lines.



Displaying Lane Lines

The Lane Style button  toggles among different display options:

center line with lane borders, center lines only, lane boundaries only, and lane display off. You can also choose lane styles from the 1D-Gel menu.

Editing a Lane Boundary

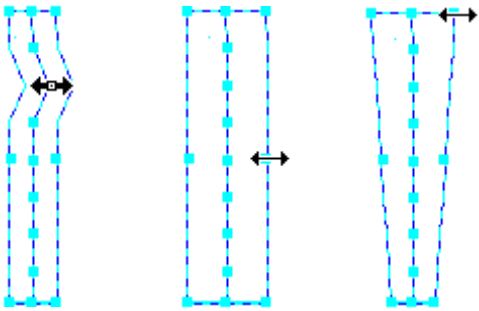
You can change the length, width, location, or angle of each defined lane.

- Click the Select Tool button  or choose 1D-Gel > Tool > Select,

  and then click on the lane. Six handles appear in the lane boundary. Handles also appear on the center line to adjust the lane boundaries for curved lanes.

There are several methods for editing the lane boundaries:

- Place the cursor inside the lane, then drag the entire lane to the new location.
- The top center handle moves the position of the top boundary.
- The bottom center handle moves the position of the bottom boundary.
- An interior center handle shifts a segment of both side boundaries in the same direction.
- The side handles change the width of the lane. Drag a handle away from the center to make the lane wider; drag the handle toward the center to make the lane narrower.
- The corner handles change the lane width at the top or bottom of the boundary.



Copying and Deleting Lanes

- To make a copy of a selected lane, choose Duplicate from the Edit menu or press Ctrl + C on the keyboard.
- To delete a lane boundary, select the lane and press the Delete key.

Selecting Multiple Lanes

- Hold the Shift key as you click each lane.
- To select all lanes, choose Select All from the Edit menu.

Lane Templates

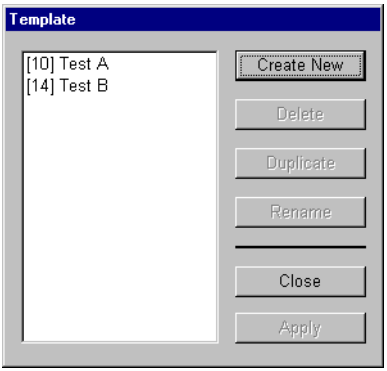
Image Analysis allows you to save lane templates for future use. When preparing a lane template, it is recommended that the total length of each

lane extends beyond the lowest band in the gel to allow for increased migration distance in future gels. A lane template may be applied to any 1D-Gel experiment.

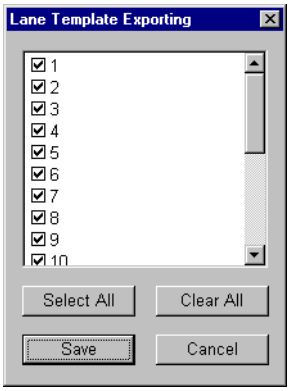
Note When defining lanes for a template, it is suggested that the total length of each lane extend beyond the lowest band in the gel to allow for increased migration distance in future gels.

To create a lane template:

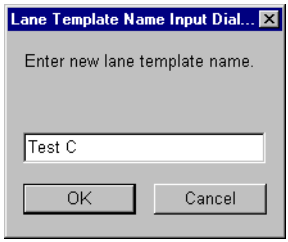
1. Click the Lane Template button  or choose Lane Template... from the 1D-Gel menu. The Template dialog box is displayed.



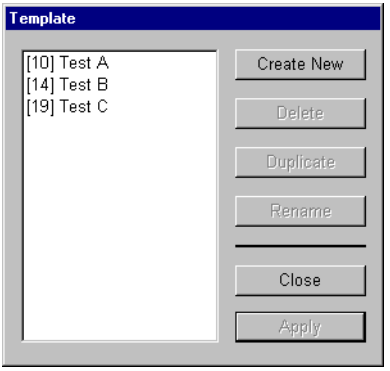
2. Click the Add button. In the Lane Template Saving dialog box, deselect any lanes you do not want to save.



3. Click Save.
4. Assign a unique name when prompted, then click OK.

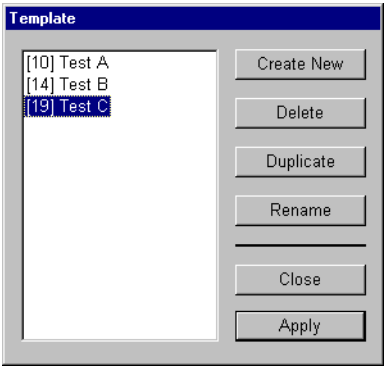


5. The template name is listed with the number of saved lanes in brackets.



To apply a lane template:

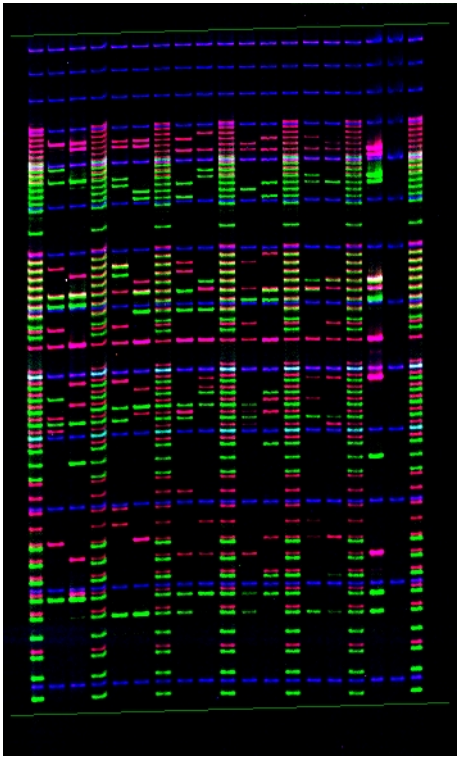
1. Click the Lane Template button  or choose Lane Template... from the 1D-Gel menu, then select the template from the list.



2. Click Apply.

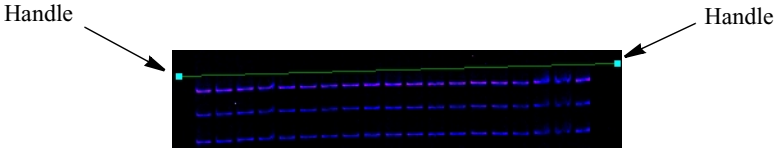
Defining the Migration Area

You can adjust the top and bottom migration lines according to your laboratory's protocol. Some labs adjust the lines to encompass only the sample bands. Other labs set the markers above the top band in the internal ladder.



To adjusting the migration lines:

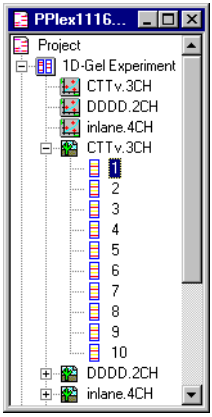
1. Click on a migration line. Each migration line has a handle at each end.



2. Click the left or right handle and drag it to change the angle of the migration line with respect to the migration direction axis of the gel. This is useful for gels that are misaligned, as in pulse field gels, where migration paths may deviate from the gel axis.
3. Click on any other point along the migration line and drag it up and down to move the migration line perpendicular to the gel.

Defining Bands

Once lane boundaries are defined, you can define bands in each lane. Use the Automatic Band Detection (Auto Band) tool to let Image Analysis automatically assign bands. You can also use the Band Edit tool to manually define bands. The Project window lists all defined bands in each channel.



Automatic Band Detection

Band recognition depends on four parameters: Gradient Start, Gradient End, Duration, and Noise Level. Image Analysis can automatically detect bands by calculating the linear differential coefficient of the spectrum curve. It recognizes a signal peak as a band if the linear differentiation of the spectrum curve is greater than the value set for the gradient start, and continues to exceed that value across a specified duration. The gradient and duration values must also exceed a baseline, or noise level.

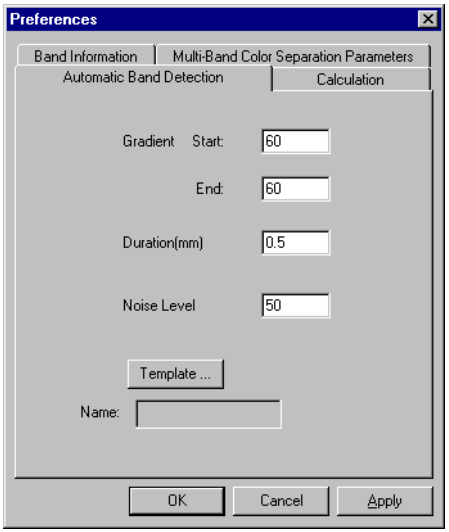
You can adjust these four parameters in the Preferences dialog box. Values you enter in the Automatic Band Detection filecard depend on the quality of your gel. Heavily loaded and smeared lanes require more gradual gradients, longer gradient durations, and higher background settings. Gels with faint or partially hidden bands require more stringent settings for these parameters.

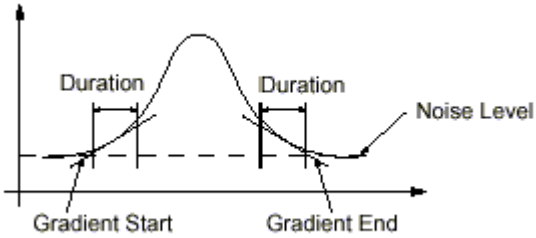
Note For optimum band identification, adjust the gray level to improve band appearance in the 1D-Gel Experiment window. See *Procedure* on page 5-21.

Automatic Band Detection Preferences.

To set the Automatic Band Detection preferences:

- Click the Preferences button  or choose Preferences... from the 1D-Gel menu, and then click the Automatic Band Detection tab.





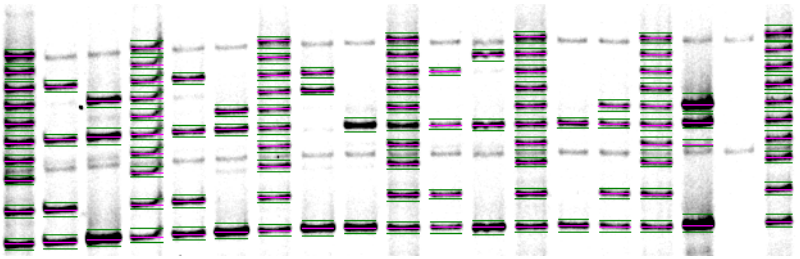
Name	Description
Gradient Start	Defines the minimum change in signal intensity necessary to signal the leading edge of the band. This parameter, combined with the duration parameter, defines the slope of the function.
Gradient End	Defines the minimum change in signal intensity necessary to signal the trailing edge of the band. This parameter, combined with the duration parameter, defines the slope of the function.
Duration	Defines the gradient length in the leading and trailing edges of the band.
Noise Level	Defines the baseline signal intensity. Gradient and duration must exceed noise level if the signal is to be recognized as a band. Choose a value between 0 and 255. Choose higher values for gels with dirty or “noisy” background. Choose lower values for clean or “quiet” gels. Evaluate the noise level of your gel by trying different Gray Scale Adjustment values (see *Procedure* on page 5-21).

Using the Auto Band Tool.

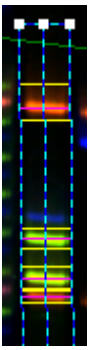
To use the Automatic Band Detection tool:

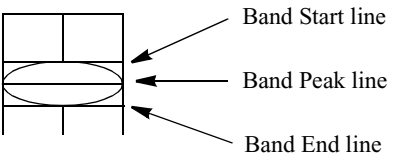
1. Choose Select All from the Edit menu to select all lanes for automatic detection, or hold down the Shift key and click the lanes.

Click the Automatic Band Detection button  or choose 1D-Gel > Function > Auto Band.

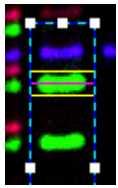


2. For each detected band, Auto Band creates a Band Start line, Band Peak line, and Band End line. The migration distance is also calculated.





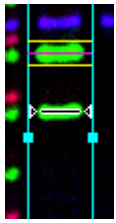
3. Check the accuracy of the automatic detection. Switch from one to the other channel by selecting the desired channel on the Image Window Setting dialog box. See *Image Setting Dialog Box* on page 5-11.
4. Magnify the image to view individual bands more closely. If you still cannot view a detected band, increase the gamma level for the channel.
5. If necessary, you can manually add, move, and delete band lines. See *Manual Band Input* on page 5-67.
6. To customize you auto band detection, adjust the Automatic Band Detection preferences, page 5-64.



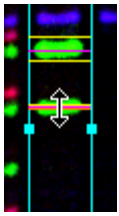
Manual Band Input

Bands that are faint, partially obscured, or highly smeared may not be recognized by the Automatic Band Detection tool. You can manually add bands that have not been detected.

1. Double-click on the lane you want to edit. The lane is selected and the Band Edit button  is activated.
2. Magnify the image with the Zoom tools to view the band.
3. Align the Band Edit cursor over the center of the band image.



4. Click on the band. Image Analysis places the band lines onto the band, and the cursor transforms into a two-headed arrow. Initially, the active (violet) band line is the Band End line.



5. Drag the Band End line down to the desired position, then release the mouse button.
6. Go back and click on the group of band lines. The Band Start line becomes active. Drag the Band Start line up to the desired position, then release the mouse button.

Moving bands.

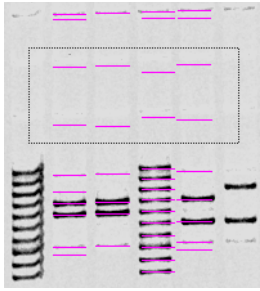
1. Double-click on the lane you want to edit. The lane is selected and the Band Edit button  is automatically selected.
2. Click on the band line to select it. The band line changes from yellow to violet.
3. Drag the band line to the new position.

Deleting bands.

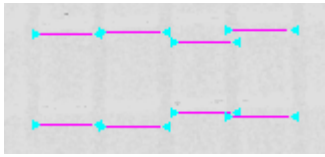
There are two methods to delete bands:

Area Selection method

1. Draw a rectangle around the bands you want to delete.



The edit handles for the bands are displayed.



2. Press the Delete key.

Note If you want to delete bands in multiple areas, hold down the Shift key while selecting the additional areas.

Band Edit Method

1. Double-click on the lane you want to edit. The lane is selected and the Band Edit button  is automatically selected.
2. Click on any band line to select it. The band line changes from yellow to violet.
3. Press the Delete key.

Hiding bands.

The Show/Hide Band Information button and the 1D-Gel > View > Band Information menu item control the display of the band migration values and lane numbers.

Displaying band lines.

The Band Style button  toggles among the different band styles: band display off, peak and range bands displayed, and only peak bands displayed. You can also choose a band style from the 1D-Gel menu.

Standard Markers

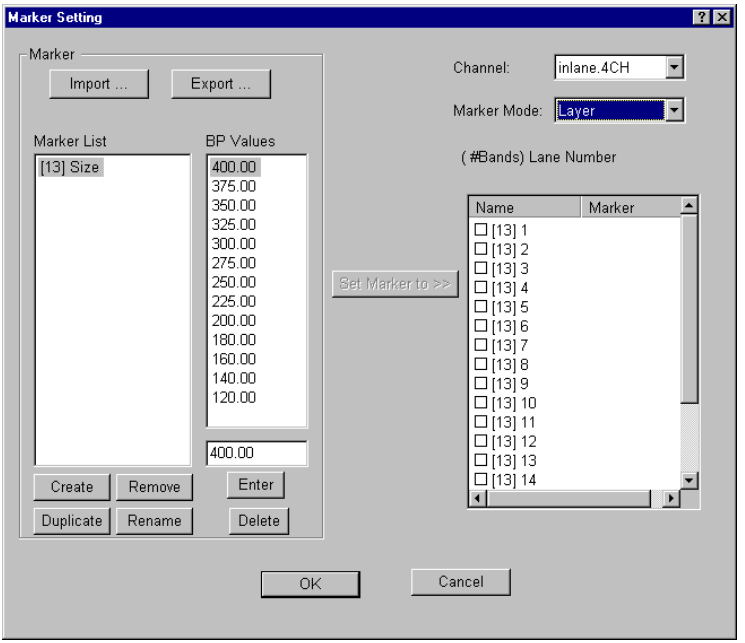
Standard markers serve as reference points for base pair length calculations. You may load more than one marker in the same lane. You can load markers in sample lanes, and intersperse marker lanes with sample lanes. If you are using an internal standard (such as Promega's CXR Fluorescent Ladder), choose Layer and select the corresponding channel. After the bands have been detected and edited, the length of the fragments in each band can be calculated.

Registering Markers

To register markers in your gel:

1. Click the Marker Setting button  or choose 1D-Gel > Marker....

   A Marker Setting dialog box is displayed. The Marker Setting dialog box lists the defined lanes. In brackets adjacent to each lane is the number of defined bands.



Note This is a good time to again check the number of expected bands in each marker lane. Compare the number to the actual number of defined bands in the lane.

2. Select a channel and a marker mode from the drop-down list boxes. There are three marker modes:

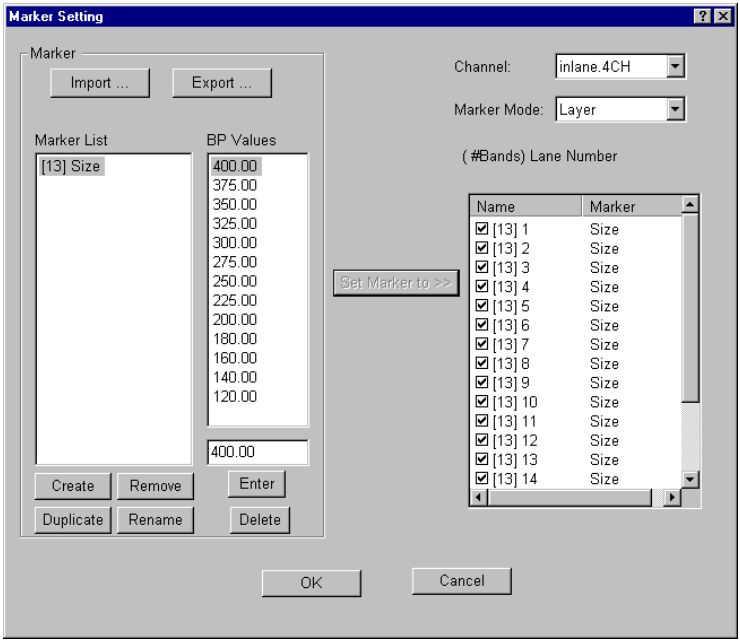
   Separate —markers are selected for each channel. The results based on markers for other channels are ignored.

   Mix —selected markers are used to calculate the results for all channels.

   Layer —markers are selected for each channel. The calculated results for the channels are layered.

3. Click on the lane numbers in the list that contain the same marker. To select more than one lane, hold down the Ctrl key, then click each lane.
4. Select the marker from the Marker list.
5. Click the Set Marker to >> button.
6. Continue this process for each marker or marker group.

Note To deselect a marker lane, remove the checkmark adjacent to its lane number.



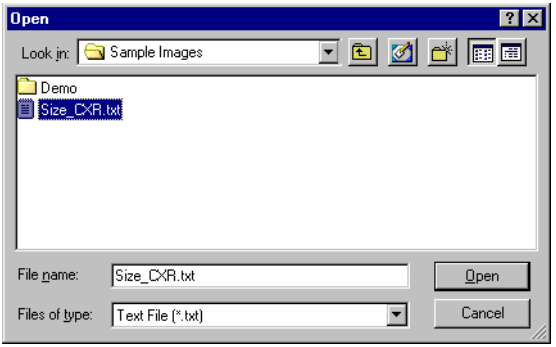
7. Click OK to complete the marker registration.

Importing and Exporting Markers

You can import a set of markers, including markers exported from the Macintosh FMBIO Analysis software, or export a set of markers.

To import a marker set:

1. Click the Import... button. The Open File dialog box is displayed.



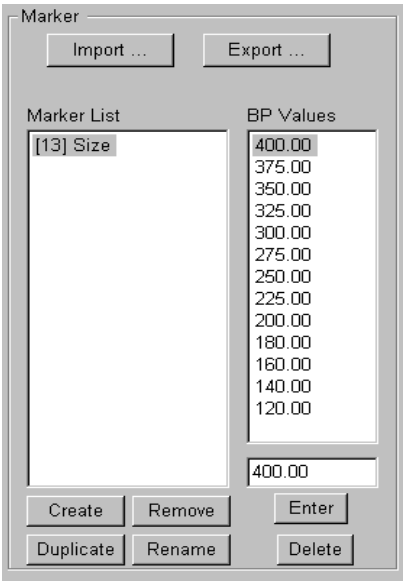
2. Select a marker set file, and click the Open button. The size markers are added to the BP Values list.

To export a set of markers:

1. Click the Export... button. The Save As dialog box is displayed.
2. Enter a name for the marker set, and click Save.

Working with Marker Templates

You can edit an existing marker, add new markers, duplicate, rename, or delete markers.



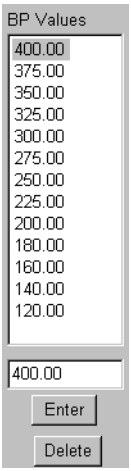
Adding Marker Templates

To add new marker templates:

1. Click the Add button. The New Marker dialog box is displayed.

2. Enter the name of the marker template, then click OK.

3. Select the new marker, then enter the length of the marker band in the BP field.
4. Click Add. The new band length appears in the BP list.

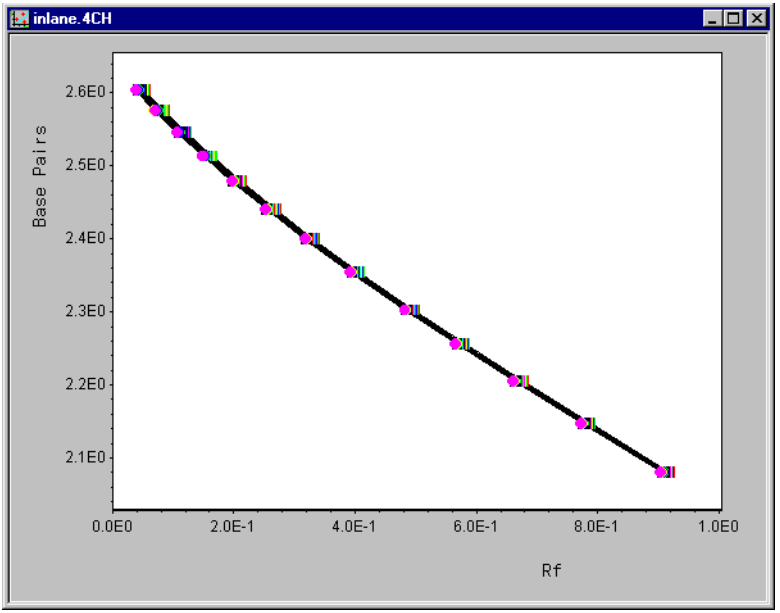


5. Repeat this process for each band in the new marker.

Base Pair Standard Curve

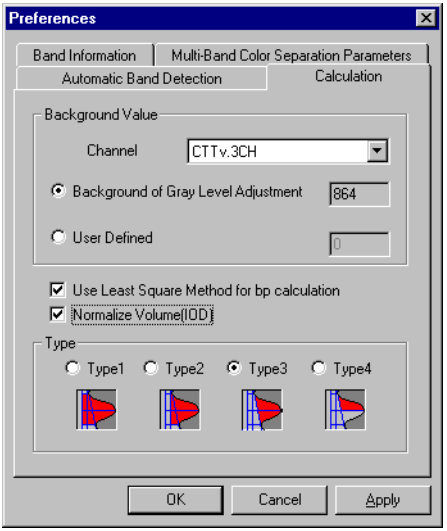
Image Analysis automatically creates a standard curve using the base pair markers as a reference.

1. Click the Open BP Curve button  or choose Open BP Curve from the 1D-Gel menu. The curve is fitted to the base pair values (Y-axis) versus Rf (X-axis).



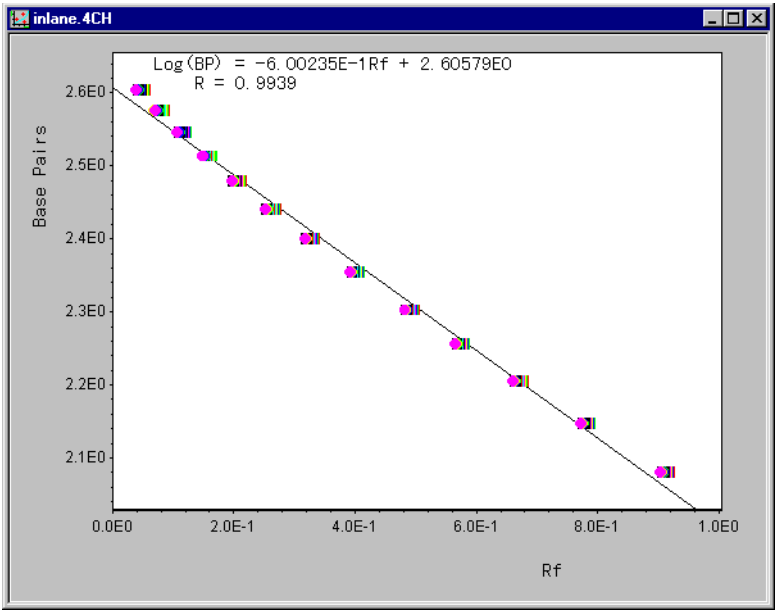
You can also choose to use the Least Squares curve fitting:

1. Click the Preferences button or choose Preferences... from the 1D-Gel menu, then click the Calculation tab.
2. Click on the Use Least Squares Method for bp calculation option to place a checkmark in the box.



3. Click OK. The calculation is performed automatically.

4. Click the Open BP Curve button  or choose Open BP Curve from the 1D-Gel menu. In addition to the base pair curve, the correlation coefficient is displayed.


inlane.4CH
Log(BP) = -6.00235E-1Rf + 2.60579E0
R = 0.9939
Base Pairs
2.6E0
2.5E0
2.4E0
2.3E0
2.2E0
2.1E0
0.0E0
2.0E-1
4.0E-1
6.0E-1
8.0E-1
1.0E0
Rf

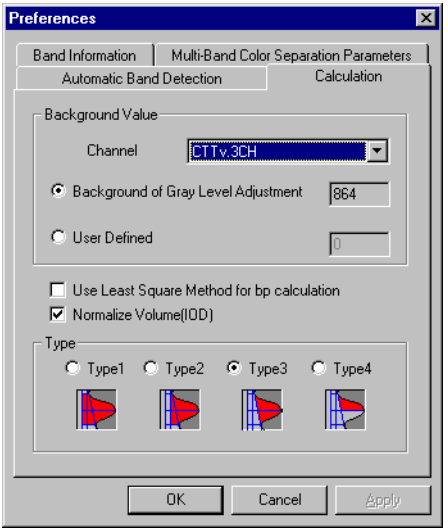
Calculating Volumes and Peak Heights

Image Analysis automatically calculates the volume (IOD) and/or peak height (OD) of each designated band. A standard curve is created based on the marker IOD values. These reference values are used to determine the concentration of each sample band. Once the peak area is defined, Image-Analysis multiplies the area by the number of grayscale bits in that area to determine the volume.

Note You cannot compare volume calculations until you have used the commands in the 1D-Gel menu to define bands. See *Defining Bands* on page 5-63.

To select the Calculation parameters:

- Click the Preferences button or choose Preferences... from the 1D-Gel menu, then click the Calculation tab.



Background Value

The background value defines a cutoff value, in grayscale bits per pixel, below which signal will not be calculated. Specify the background value for each channel in your image.

Background of Gray Level Adjustment. This option allows you to use the background cutoff threshold value for volume calculations. It is automatically entered.

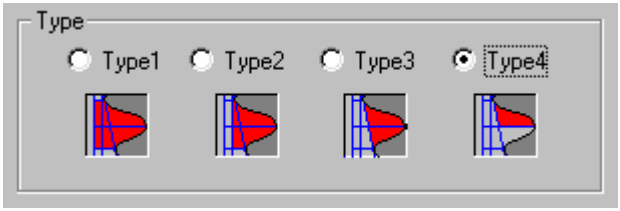
User Defined. This option allows you to enter a background value.

Calculating Normalized Volume (IOD)

Image Analysis can subtract the background signal for more accurate volume (IOD) calculation. Use this feature with any of the four background types.

Volume Calculation Types

Image Analysis offers four types of volume calculation. Each type offers a different method of defining the area of the selected peak relative to the background.



Type 1. The area incorporates any signal that is greater than the background on either side of the selected peak. This gives the largest value of the four calculation types. Use Type 1 for bands or spots that are distinctly separated from others, or for quantifying isolated faint bands with signal intensities that are close to background value.

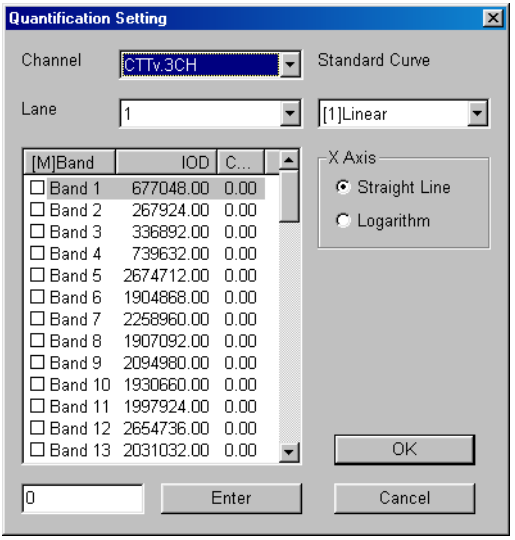
Type 2. The area incorporates any signal that is greater than the spectrum valleys on either side of the designated peak. The value of the deeper of the two valleys on either side of the peak is used as the local background value. Use Type 2 to quantify individual bands or spots that are packed in with others in a group.

Type 3. The peak is split and the lower of the two valleys is defined as the local background value. The area is defined as half of the peak, between the highest and the lowest signal. Use Type 3 volume to quantify border regions of spots or bands, where the signal fades into background.

Type 4. Local background is defined by a line that connects the spectrum valleys at their lowest point on either side of the peak. The area incorporates signal above this line and below the peak. Use Type 4 calculation to determine relative volumes of adjacent bands or spots that are packed in with others in a group.

Setting Quantification Markers

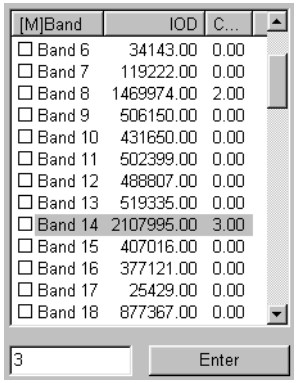
1. Click the Quantification Setting button  or choose Quantification Setting... from the 1D-Gel menu.



2. Select a channel from the drop-down list box.
3. Select a lane number containing standard markers from the drop-down list box. The defined bands in the lane appear in the list box.
4. Click on a marker band to highlight it, then enter a concentration value

in the field below the list box.

5. Click Enter. The value appears in the Concentration column.
6. Continue to enter concentration values for all marker bands.

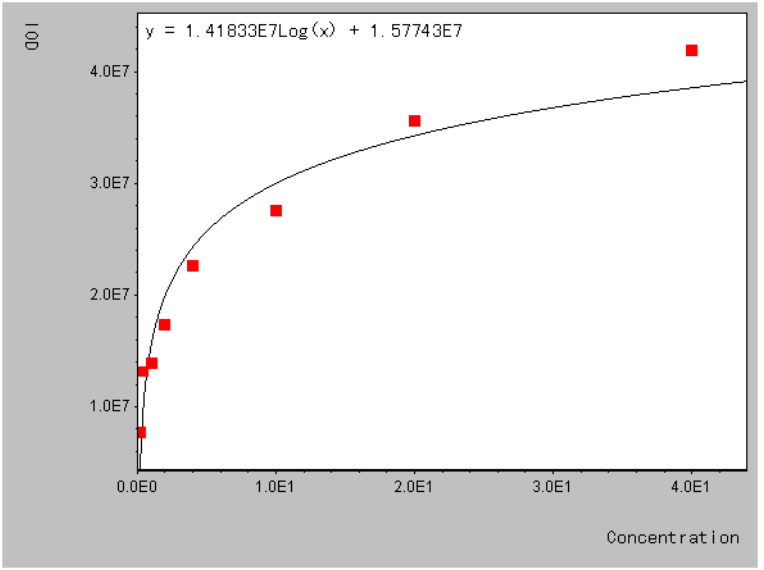


7. Place a checkmark in the box next to each marker band you want to use.
8. Select a standard curve fit in the drop-down list.

   Linear —straight line least squares regression

   Second Order —second order curve least squares regression

   Logarithmic —natural logarithms least squares regression
9. Select a straight line or logarithmic X-axis scale.
10. Click OK.
11. Click the Open Standard Curve button or choose Open Standard Curve from the 1D-Gel menu. The curve is fitted to the marker IOD values (Y-axis) versus concentration (X-axis).



Volume Calculation

After the lanes, bands, and markers have been established, Image Analysis can compare the known markers to unknown sample bands. Using the markers as reference points, the volume (IOD), concentration, and fragment length of the sample bands is calculated.

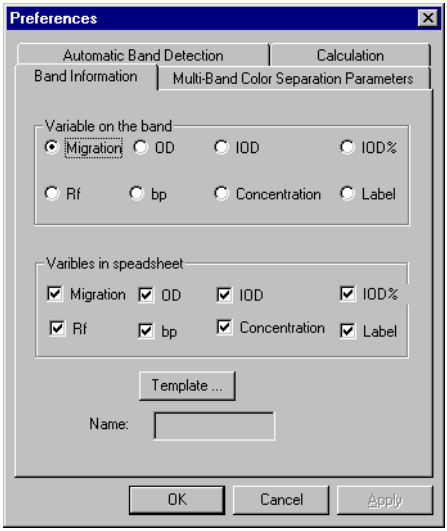
- To initiate the analysis, click the Volume Calculation button or choose Volume Calculation from the 1D-Gel menu.

Displaying Results

You can display the results of the calculation in a spreadsheet and on the image.

To choose the variables to display:

1. Click the Preferences button or choose Preferences... from the 1D-Gel menu, then click the Band Information tab.


Preferences
Automatic Band Detection
Calculation
Band Information
Multi-Band Color Separation Parameters
Variable on the band
Migration
OD
IOD
IOD%
Rf
bp
Concentration
Label
Varibles in speadsheet
Migration
OD
IOD
IOD%
Rf
bp
Concentration
Label
Template ...
Name:
OK
Cancel
Apply

Variable	Contents
mm	Migration distance between the top migration line and band peak line.
OD	Optical density of the peak fluorescent signal on the band peak line.
IOD	Amount of fluorescent signal in a band. Band volume.
IOD%	Band volume as a percentage of the total volume of all bands in the lane.
Rf	Relative electrophoresis mobility. When the top migration line is 0% and the bottom migration line is 100%, it is the ratio of the band peak migration relative to the total length.
bp	Fragment length in base pairs determined with standard markers. Enter numeric values.
Concentration	Concentration determined with standard markers. Enter numeric values.
Label	Enter any alphanumeric label or comment for a band.

2. Select the variables to display.

To save your selections as a template:

1. Click the Template... button. The Template dialog box is displayed.
2. Click the Add... button, and type in a name for the template. The default name for the first template is info1.tmp, and each additional template is numbered sequentially.
3. Click OK, and then click Close.

Spreadsheet Display. The results of the analysis are shown in a spreadsheet that can be exported. Image Analysis can display the OD, IOD, IOD%, Rf, base pair, concentration value or a user-defined label.

1. To display a spreadsheet of results, click the Open Spreadsheet button  or choose Open Spreadsheet from the 1D-Gel menu. The Spreadsheet window is displayed, and the SpreadSheet menu appears in

the menu bar.

2. Select a channel by clicking its tab at the bottom of the spreadsheet.

Each band is listed in order of migration distance. Image Analysis displays a set of band information columns for each lane.

Entering Spreadsheet Data. You can enter and edit data in the base pair, concentration, and label fields.

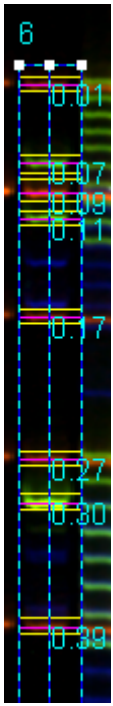
1. Click on the field.
2. Enter the data.

Note Enter numeric values in the base pair and concentration fields. Enter alphanumeric values in the label field.

Band Information display. In the 1-D Gel Experiment window, Image Analysis can display the OD, IOD, IOD%, Rf, base pair, concentration value or a user-defined label next to the band.

To display values in the 1D-Gel Experiment window:

1. Click the Preferences button  or choose Preferences... from the 1D-Gel menu, then click the Band Information tab. Select the item you want to display. See page 6-6 for an explanation of these parameters.
2. Select the lanes. The information is displayed next to the band.



The Show/Hide Band Information button  or 1D-Gel > View > Band Information menu item toggles the display on and off.

Updating Spreadsheet Values

Changes you make to your 1D-Gel experiment may affect the values displayed in the spreadsheet. These changes include:

- Adding or deleting values
- Changing units of measurement
- Moving, adding, or deleting bands or lanes

Whenever you make these changes, you must be sure the corresponding values in the spreadsheet are updated.

To update spreadsheet values after changes in the 1D-Gel Experiment window:

- Click the Volume Calculation button  or choose 1D-Gel > Volume Calculation.

Exporting the Spreadsheet

You can save the spreadsheet as a tab-delimited text file for export to other programs, such as Microsoft Excel and Microsoft Word.

To export a spreadsheet:

1. Click the Open Spreadsheet button  or choose Open Spreadsheet from the 1D-Gel menu.
2. Choose Export... from the SpreadSheet menu.

3. In the Save As dialog box, select a location for the file, and then enter a unique name.
4. Click Save.

Overlay Trace

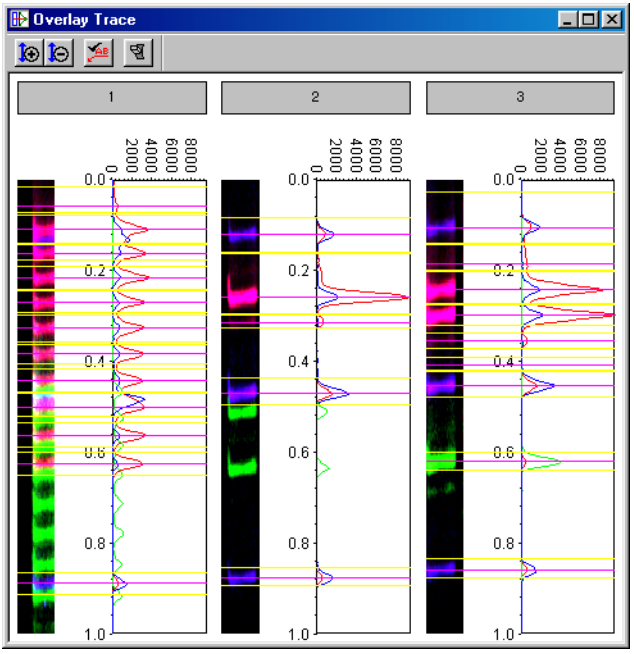
In an Overlay Trace window, you can compare the spectrums of different lanes. This window also allows you to edit a band while viewing the graphic trace of its signal. To use Trace Overlay, you must first use the1D-Gel analysis tools to define one or more lanes in an 1D-Gel Experiment window. See *Analyzing 1D-Gels* on page 6-6.

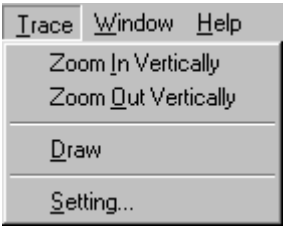
Note Your images must have defined lanes, but creating an overlay trace does not require previously defined bands.

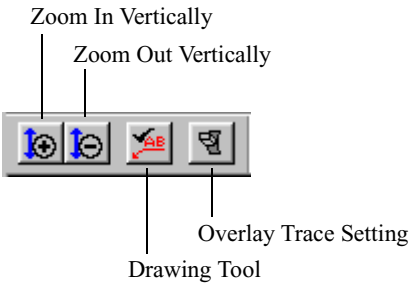
Overlay Trace Window

1. Open one or more 1D-Gel Experiment windows that have defined lanes.
1. Select the lane or lanes of interest by clicking the Arrow cursor on the desired lane. Shift-click to select more than one lane.
2. A maximum of 16 lanes may be converted into Lane Trace format at one time.

3. Click the New Overlay Trace button  or choose New Overlay Trace from the 1D-Gel menu. The Overlay Trace window is displayed and the Trace menu appears in the menu bar.


Overlay Trace
1
2
3
0
2000
4000
6000
8000
0.0
0.2
0.4
0.6
0.8
1.0


Trace
Window
Help
Zoom In Vertically
Zoom Out Vertically
Draw
Setting...



Tool	Description
Zoom In Vertically	Magnify in vertical direction.
Zoom Out Vertically	Reduce in vertical direction.
Drawing	Use drawing tools for Overlay Trace window.
Overlay Trace Setting	Displays Overlay Trace Setting window.

Vertical Zoom tools. You can proportionally increase or decrease the vertical range of all traces in the window simultaneously.

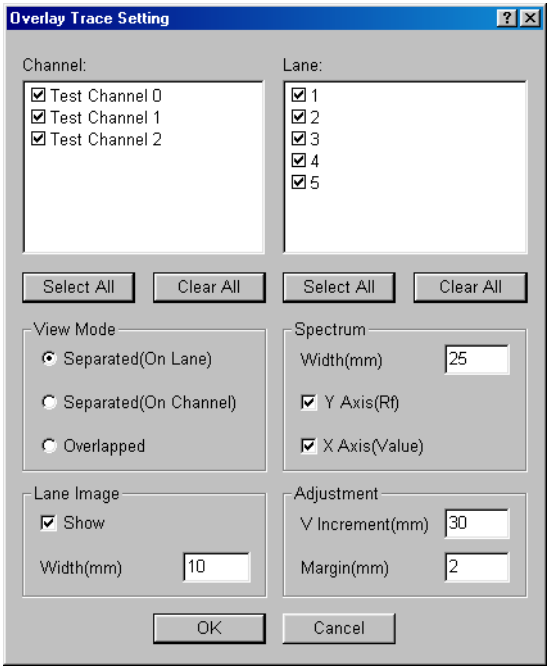
Note Use the Image Zoom tools to control the vertical magnification. See page 5-7.

Drawing tools. You can draw and type text on your overlay trace.

1. Click the Drawing Tool button  or choose Draw from the Trace menu. The Drawing Tool bars become active and the Draw menu appears in the menu bar.
2. Use the Drawing tools to draw and type text on the overlay trace. See Chapter 8, *Drawing Tools*.
3. When you have finished, choose Save Project... from the File menu. An Overlay Trace icon is added to the 1D-Gel Experiment tree.

Overlay Trace Setting Window

To display the Overlay Trace Setting dialog box, click the Overlay Trace Setting button  or choose Setting... from the Trace menu.



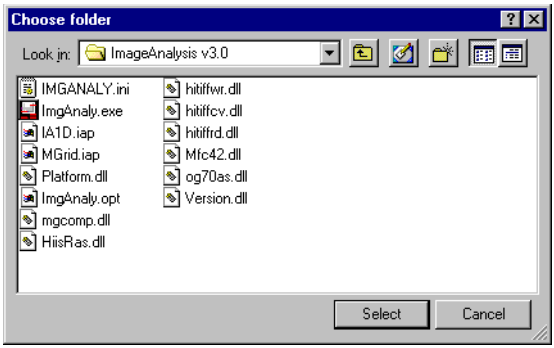
Item	Description
Channel	Place a checkmark in the box next to each channel you want to display. You can also select channels in the Image Setting dialog box.
Lane	Place a checkmark in the box next to the lane you want to display.

Item	Description
View Mode	Separated (On Lane) —The spectra for each lane is shown. One spectral graph is shown for each lane, and spectra from all channels are shown. Separated (On Channel) —One spectral graph shows one channel, and all lanes are shown overlapped. Overlapped —The spectra of all channels are shown overlapped.
Lane Image	Show —Select the show lane image/hide image check box. Width —Enter the width of the lane image.
Spectrum	Enter the width of the spectral graph. Show/Hide Y (Rf) axis. Show/Hide X (Value) axis.
Adjustment	Margin —Enter the spacing for lanes and spectra. V Increment —Enter the units for magnification/ reduction for the overlay trace. To increase magnification, enter a higher value.

Exporting Lane Trace Data

In some cases it may be desirable to export the Lane Trace data from Image Analysis into an Excel spreadsheet. The data will be displayed in the spreadsheet as migration distance (mm) and intensity (OD).

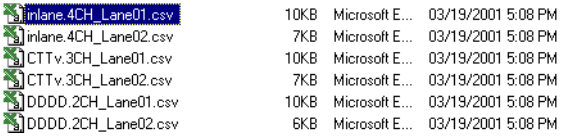
1. Define the desired lanes in the images.
2. View the lane traces if desired.
3. The lane trace data can be exported to a folder as a set of Microsoft Excel spreadsheets by choosing Export Lane Trace... from the 1D-Gel menu. The Select Folder dialog box is displayed.



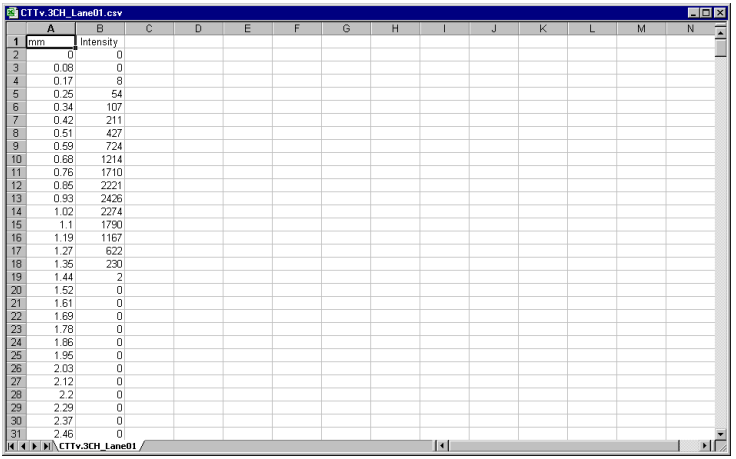
4. Specify the folder to store the files, and click Select.

Note The window will automatically choose the Image Analysis program folder as the default folder and create filenames using previously entered information. (i.e. “Lane #. Project Name.#CH.csv”).

5. Open the desired file in Microsoft Excel.



6. The spreadsheet displays the migration distance (mm) and the intensity (OD) of the trace.



CTTv.3CH_Lane01.csv

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	mm	Intensity												
2	0	0												
3	0.08	0												
4	0.17	8												
5	0.25	54												
6	0.34	107												
7	0.42	211												
8	0.51	427												
9	0.59	724												
10	0.68	1214												
11	0.76	1710												
12	0.85	2221												
13	0.93	2426												
14	1.02	2274												
15	1.1	1790												
16	1.19	1167												
17	1.27	622												
18	1.35	230												
19	1.44	2												
20	1.52	0												
21	1.61	0												
22	1.69	0												
23	1.78	0												
24	1.86	0												
25	1.95	0												
26	2.03	0												
27	2.12	0												
28	2.2	0												
29	2.29	0												
30	2.37	0												
31	2.46	0												

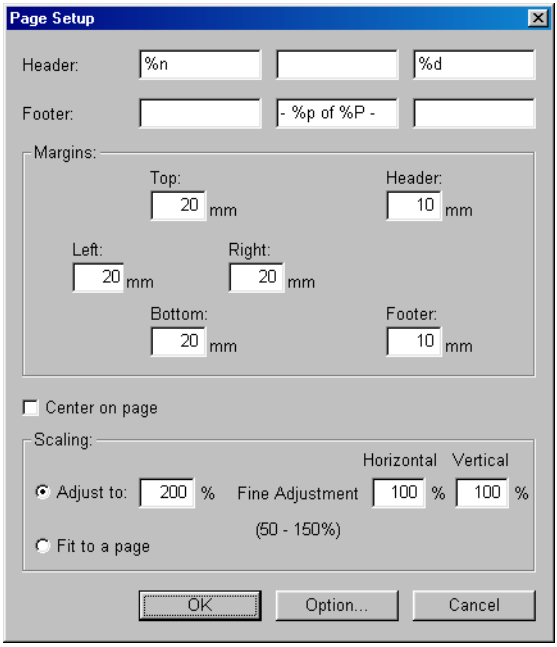
CTTv.3CH_Lane01

Printing

Image Analysis allows you to print an image, experiment, standard curve, spreadsheet, or overlay trace. You can also customize the print job in several ways.

To customize a print job:

- Choose Print Setup from the File menu. The Page Setup window is displayed.



Feature	Description
Header, Footer, Align Left, Align Center, Align Right	%n =project name %d =current date %t =current time %p =page number %P =total number of pages
Margins	Top, Left, Right, Bottom, Header, Footer
Scaling	Adjust to —Enter scaling (whole numbers) Fit to a page —Scaling determined by paper size, e.g. if the figure is reduced if it is larger than the page size
Fine Adjustment	Horizontal and vertical (50 - 150%, real numbers).

Print Setup. To set the print options, click the Options... button.

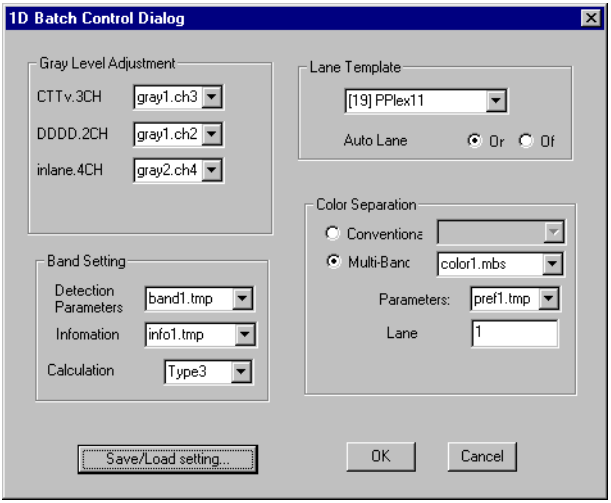
Print Preview. To preview your print job, click Print Preview.

Printing. To begin printing, click the Print button  or choose Print from the File menu.

Batch Analysis

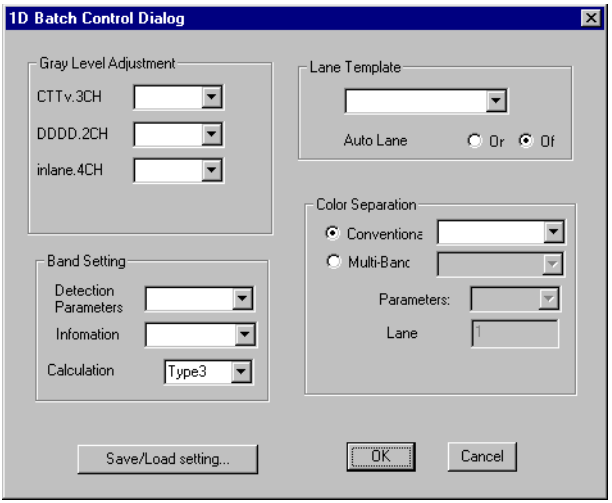
A set of templates can be saved and loaded for use in a different analysis. There are four sections:

- Gray Level Adjustment
- Band Settings
- Lane Template
- Color Separation



Procedure

1. Choose Batch Control from the 1D-Gel menu. The 1D Batch Control dialog box is displayed.



Item	Description
Gray Level Adjustment	Select a template from the drop-down menu. *Note* Special care should be given to match the template with the correct channel.
Band Setting	Select the templates for the Band Selection Parameters and Band Information from the drop-down menus. Select the calculation type from the drop-down menu.
Lane Template	Select the Lane template from the drop-down menu. If you want to use auto lane detection, click the On radio button to select it.
Color Separation	Choose the Conventional or Multi-Band separation method. If you choose the Conventional method, select a template from the drop-down menu. If you choose the Color Separation method, select a Color Separation template and a Parameters template from the drop-down menus.

2. Choose the templates and enter the appropriate parameters in the fields.
3. If you want to use this set of Batch Control settings again, click on the Save/Load Setting... button. The Template dialog box appears.
4. Click the Add... button, and enter a name for the Batch Control template.
5. Alternatively, if you want to Load a previous Batch Control template, select the template by clicking on it's filename in the list box. Then click the Apply button.
6. When you have finished entering the parameters, click OK in the Batch Control dialog box. Image Analysis processes the 1D-Gel experiment.

6 1D-Gel Analysis

One-dimensional (1D) analysis entails studying the migration patterns of molecules that have been separated and spread out in one linear dimension by the passage of a voltage through a gel. Image Analysis provides a versatile assortment of display options and tools for 1D gel analysis.

Chapter 5, *Image Analysis Tools*, describes how to use Image Analysis tools to create single and multi-color image project files. This chapter describes how to use the 1D-Gel tools to analyze these single- and multi-color images. The following summaries are provided:

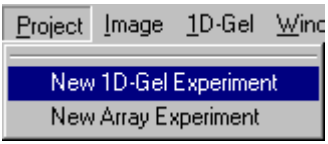
- Single-color analysis example. See page 6-8.
- Multi-color analysis example. See page 6-16.

You can also use the Drawing tools to enhance the results of 1D analysis. See Chapter 8, *Drawing Tools*.

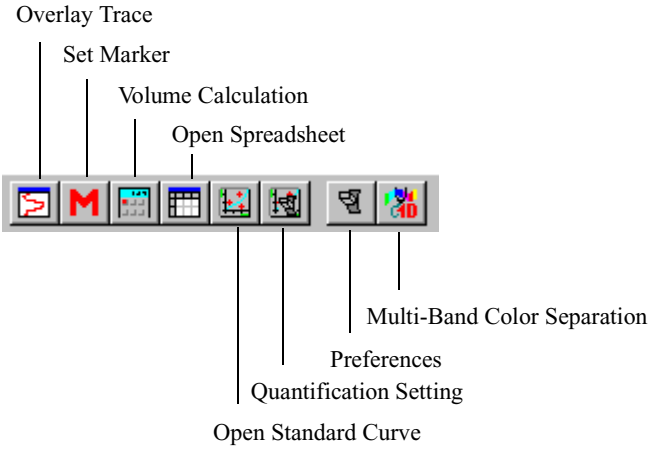
Note The procedures and parameters settings contained in this chapter are recommendations that can be modified to achieve the best results for your images.

1D-Gel Menu and Tools

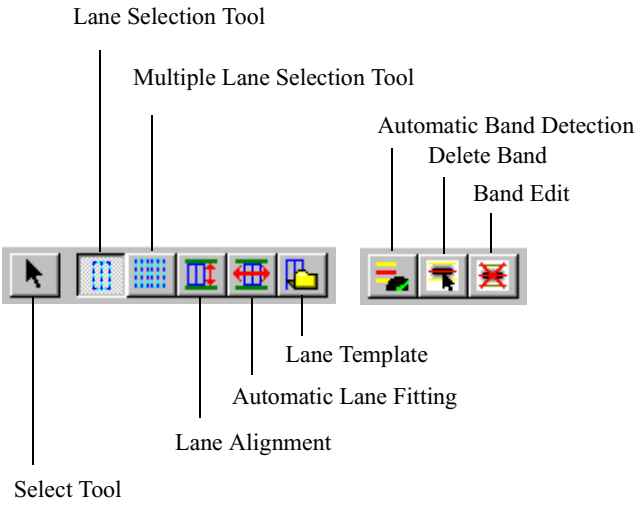
1. Choose New 1D-Gel Experiment from the Project menu.



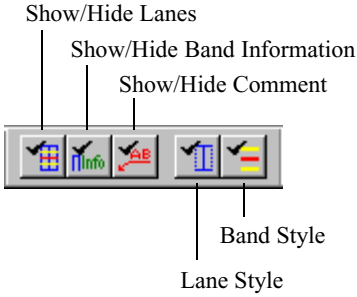
The 1D-Gel Document window, Image Setting dialog box, 1D-Gel menu and its corresponding tool bar appear.



Tool	Description
Set Marker	Displays Marker Setting window.
Volume Calculation	Performs a volume calculation.
Overlay Trace	Displays new overlay trace of selected lanes.
Open Spreadsheet	Displays spreadsheet.
Preferences	Displays Preferences window.
Lane Template	Displays Lane Template dialog box
Open Standard Curve	Displays standard curve.
Quantification Setting	Displays Quantification Setting window.
Multi-Band Color Separation	Displays Multi-Band Color Separation window.



Tool	**Description**
Select	Select objects.
Lane Selection	Define a single lane.
Multiple Lane Selection	Define multiple adjacent lanes.
Lane Alignment	Aligns lanes with migration lines.
Automatic Lane Fitting	Automatically adjusts lane boundaries.
Lane Template	Saves all lanes on image.
Automatic Band Detection	Autoband selected lanes.
Delete Band	Delete all bands in the selected lanes.
Band Edit	Add, edit, or delete bands.



Tool	Description
Show/Hide Lanes	Toggle display of lane boundary lines.
Show/Hide Band Information	Toggle display of band information.
Show/Hide Comments	Toggle display of label information entered in spreadsheet.
Lane Style	Toggle among different display options: center line with lane borders, center lines only, lane boundaries only, and lane display off.
Band Style	Toggle among the different band styles: band display off, peak and range bands displayed, and only peak bands displayed.

Analyzing 1D-Gels

With the tools and commands in the 1D-Gel menu, you can identify lanes and bands of interest. Image Analysis then calculates the following band parameters:

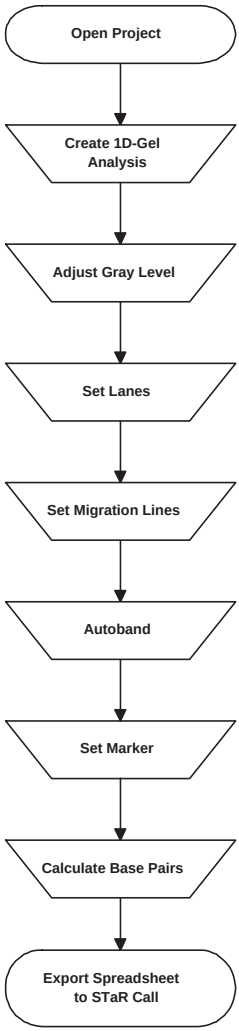
- mm—migration distance of the band from the top limit line, in millimeters
- OD— optical density of the peak fluorescent signal, relative to a standard marker
- IOD—average band volume, based on the fluorescent signal intensity
- IOD%—band volume as a percentage of the total volume of all bands in the lane
- Rf—relative electrophoretic mobility
- bp—estimated size of the fragment in basepairs (DNA only)
- concentration

Summary of Analysis Steps

Image analysis of a 1D gel consists of these steps:

1. Open project.
2. Create 1D-Gel analysis.
3. Adjust gray level.
4. Set lanes.
5. Set migration lines.
6. Perform color separation (if required).
7. Review image.
8. Autoband.
9. Set markers.
10. Calculate base pairs.
11. View results.

Single-Color Experiment Flowchart

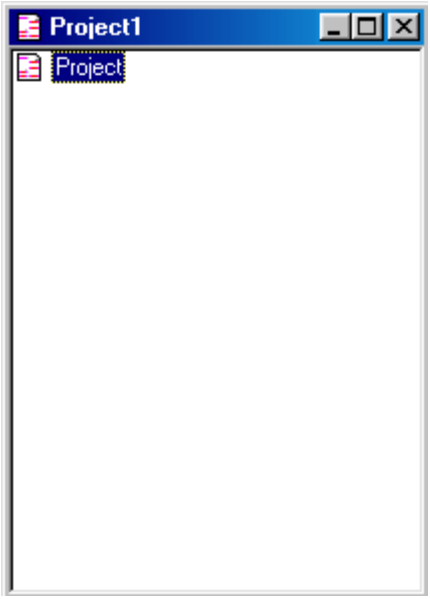


Single-Color Experiment Procedure

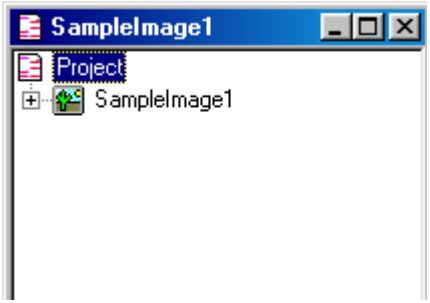
The following section is a summary of steps used in the analysis of a single color image. While this summary utilizes diagrams from a single color multiplex, the process is also applicable to other types of single color images including gels stained with ethidium bromide.

Create a New Project (page 5-3).

1. In the File menu, click the New Project button or choose New Project. A new Project window is displayed.



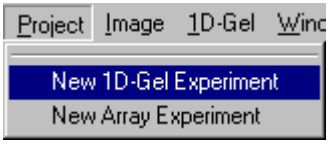
2. In the Project menu, choose Add Image..., then select a file that contains a digital image of a 1D gel. Image Analysis adds an icon representing the image file to the project tree.



The Image menu appears in the menu bar and its corresponding tool buttons become active.

Create 1D-Gel analysis (page 5-14).

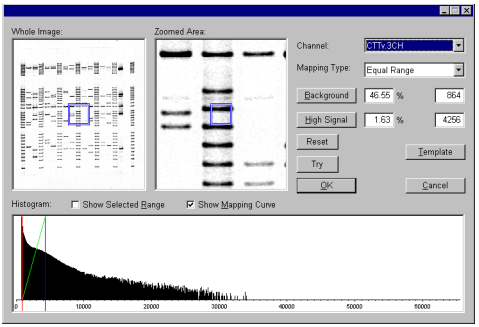
- Choose New 1D-Gel Experiment from the Project menu.



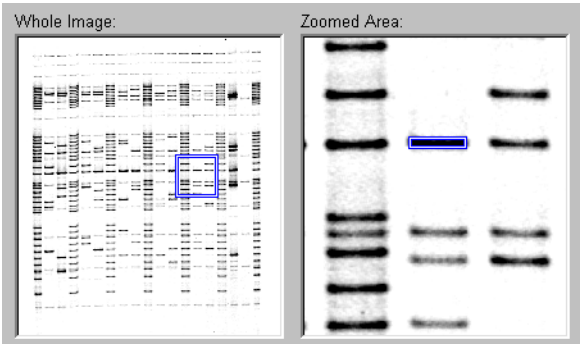
The 1D-Gel Document window, Image Setting dialog box, 1D-Gel menu and its corresponding tool bar appear.

Adjust Gray Level. (page 5-14).

1. Click the Gray Level Adjustment button  or choose Gray Level Adjustment from the Image menu. The Gray Level Adjustment window is displayed.



1. In the Full Image Display, drag the Zoom Cursor to a region containing average intensity bands/background. If necessary, adjust the size of the Zoom Cursor to include the desired area.
2. The area appears in the Zoom Display.
3. In the Zoom Display, surround a band with the Selection Cursor.

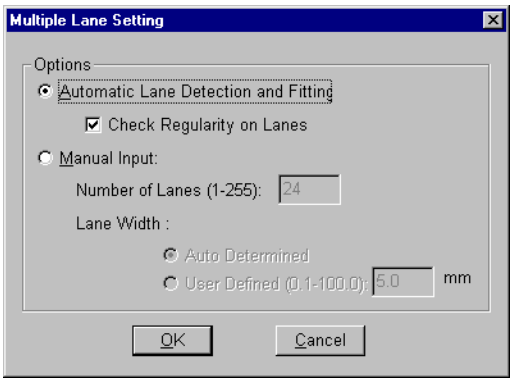


4. Move the Selection Cursor to a background area away from the band, then click the Background button. The calculated Background cutoff threshold is applied to the Full Image and Zoom windows and the values appear in the Background Percent and Background Value boxes.
5. Use the High Signal and Background threshold marker lines in the histogram to adjust the gray scale range.

6. When you have finished, click OK.

Set Lanes (page 5-52).

1. Click the Multiple Lane Selection Tool button  or choose 1D-Gel > Tools > Lane Selection.
2. Create a rectangle surrounding the group. Position the tool above the top migration line at the corner of the first lane in a sequence of lanes.
3. Drag the tool below the bottom migration line to the opposite corner of the last lane in the sequence.
4. Release the mouse. The Multiple Lane Setting dialog box is displayed.



5. Choose either Automatic Lane Detection and Fitting or Manual Input.

   If you choose Automatic Lane Detection, you can also choose to check the uniformity of the lanes.

   If you choose Manual Input, enter the number of lanes in the sequence then choose to either automatically determine the lane width or specify a width. Lane width may range from 1.0 to 100.0 mm, in 0.1-mm increments.
6. Click OK. By default, Image Analysis displays a central line down each defined lane, with additional lines marking the lane width. When the lane is selected, square edit handles are displayed on the lane lines.
7. If any of the lanes need adjustment, click the lane to highlight it and

move it to the correct position. The Lane Style button  toggles among different display options: center line with lane borders, center lines only, lane boundaries only, and lane display off. You can also choose lane styles from the 1D-Gel menu.

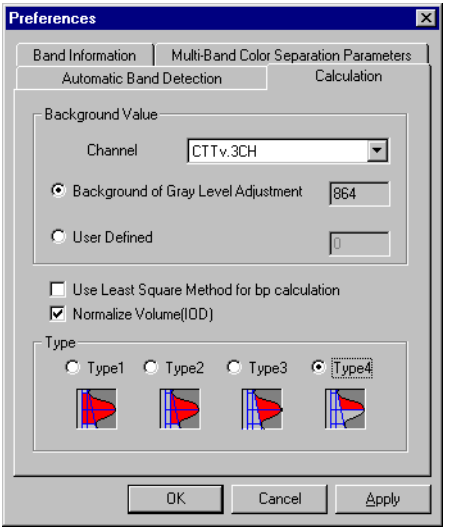
Set Migration Lines (page 5-52).

- Adjust the top and bottom migration lines according to the user's laboratory protocol.

Autobanding (page 5-63).

With the lanes selected and the migration lines adjusted, autoband determination can be performed.

1. Select Normalized Volume (IOD) and choose Type 4 as the calculation type in the 1D-Gel Preferences dialog box.



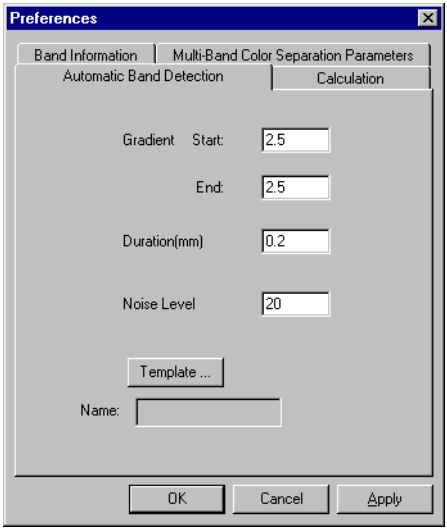
2. Click the Automatic Band Detection tab to edit the autobanding parameters.
3. Try these settings and adjust the settings as needed for different gels:

   Gradient Start = 2.5

End = 2.5

Duration = 0.2

Noise Level = 20



4. Choose Edit > Select All to select all the lanes.

5. Click the Automatic Band Detection button  or choose 1D-Gel > Function > Auto Band. You might need to adjust the Image Analysis automatic band calls. The Band Style button  toggles among the different band styles: band display off, peak and range bands displayed, and only peak bands displayed. You can also choose a band style from the 1D-Gel menu.

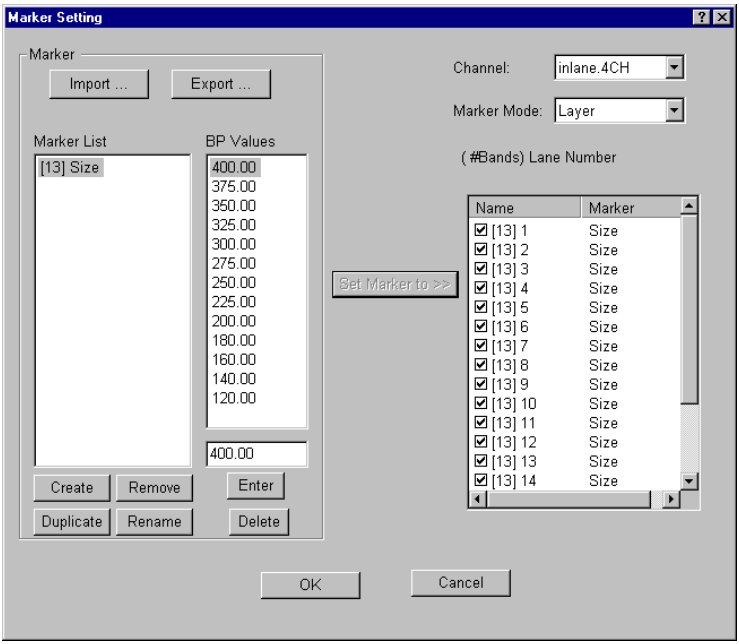
6. Click the Band Edit button  or choose 1D-Gel > Tool > Band Edit, then use the cursor to place bands.

Set Marker (page 5-71).

1. Click the Quantification Setting button  or choose Quantification Setting... from the 1D-Gel Menu.

   Select the standard curve fit from the drop-down list box. Logarithmic is the correct standard curve fit for most applications.

2. Click the Marker Setting button  or choose Set Marker... from the 1D-Gel menu.
3. Highlight the lanes containing the marker by holding down the Shift key while clicking on them with the mouse. Select the correct marker from the Template list, then click Adopt. The selected marker appears next to each lane in the list.



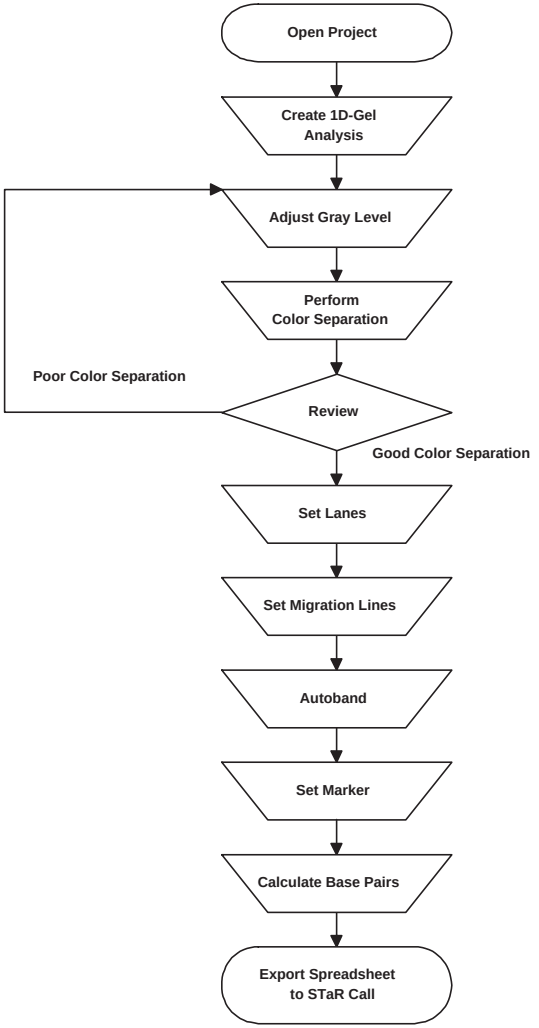
Calculate Base Pairs (page 5-80).

1. Click the Volume Calculation button  or choose Volume Calculation from the 1D-Gel menu to calculate base pair sizes in reference to the assigned marker.

2. Click the Open Spreadsheet button  or choose Open Spreadsheet from the 1D-Gel menu to view the table of base pair values for each layer. Click the tabs at the bottom of the window to switch among channels.

3. Choose Export from the SpreadSheet menu to save the spreadsheets as comma-delimited text files that can be exported to STaR Call ™.

Multi-Color Experiment Flowchart

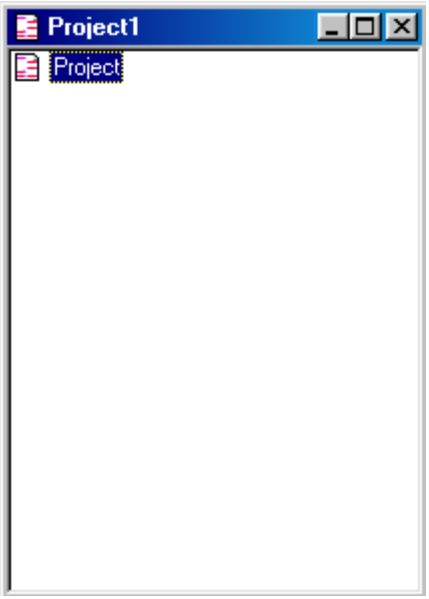


Multi-Color Experiment Procedure

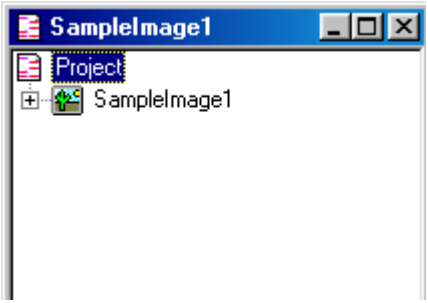
The following section is a summary of steps used in the analysis of a multi-color image. While this summary uses diagrams from the Promega 1.1 Powerplex, the process is also applicable to other types of multi-color images.

Create a New Project (page 5-3).

1. In the File menu, choose New Project. A new Project window is displayed.

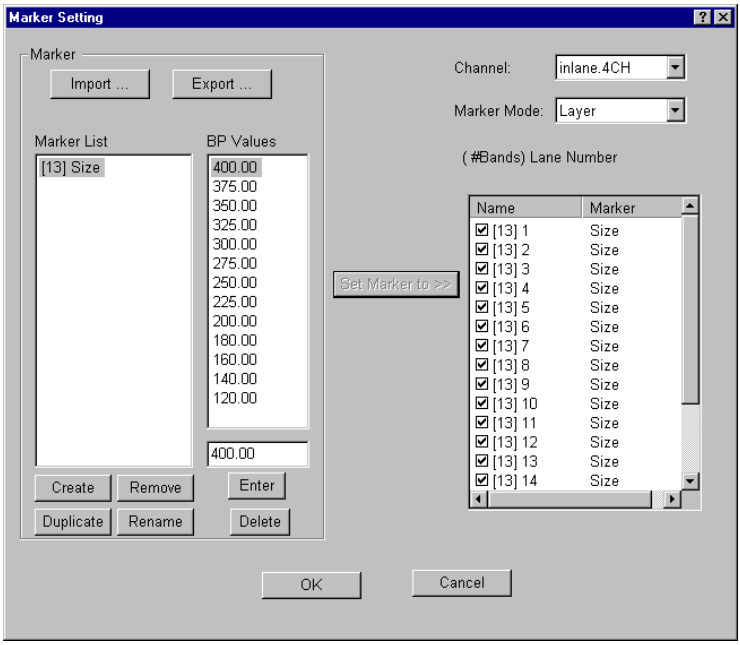


2. In the Project menu, choose Add Image..., then select a file that contains a digital image of a one-dimensional gel. Image Analysis adds an icon representing the image file to the project tree.



3. The Image menu appears in the menu bar and its corresponding tool buttons become active.

4. Click the Marker Setting button  or choose Set Marker... from the 1D-Gel menu.



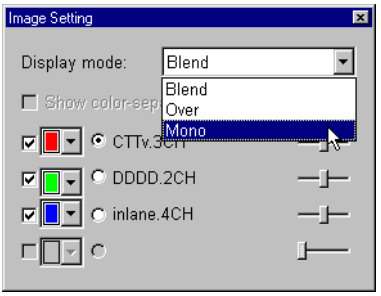
5. If you are using an internal lane standard (such as Promega's CXR), select the channel used to scan the internal lane standard, and then select Layer from the Marker Mode drop-down list box.
6. If an internal lane standard was not used, select the channel used to scan the any standard markers on the gel, and then select Separate from the Marker Mode drop-down list box.

Create 1D-Gel analysis (page 5-14).

- Choose New 1D-Gel Experiment from the Project menu. The 1D-Gel Document window, 1D-Gel menu, and its 1D-Gel tool bar appear.

Adjust Gray Level. (page 5-14).

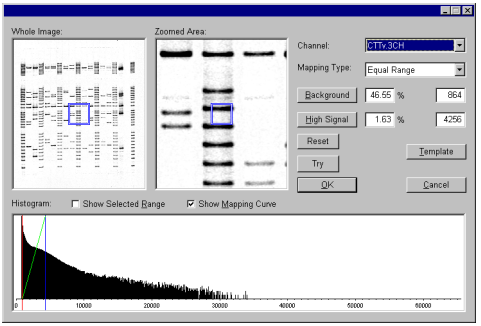
1. In the Image Setting dialog box, select Mono as the display mode.



When a black and white image is displayed, the user is ready to adjust the grayscale.

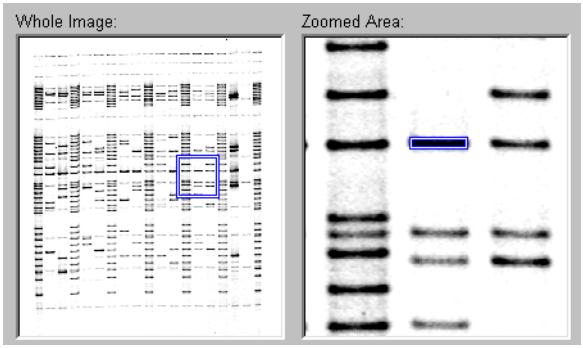
2. Click the Gray Level Adjustment button  or choose Gray Level

Adjustment from the Image menu. The Gray Level Adjustment window is displayed.



3. In the Full Image window, drag the Zoom Cursor to a region containing average intensity bands/background. If necessary, adjust the size of the Zoom Cursor to include the desired area. The area appears in the Zoom window.
4. In the Zoom window, surround a band with the Selection Cursor, then

click the High Signal button. The new High Signal cutoff threshold is applied to the Full Image and Zoom windows and the values appear in the High Signal Percent and Background Number boxes.



5. Move the Selection Cursor to a background area away form the band, and then click the Background button. The new Background cutoff threshold is applied to the Full Image and Zoom windows and the values appear in the Background Percent and Background Number boxes.
   - All regions of the image with gray level values below these Background values will appear white.
   - All regions of the image with gray level values above the High Signal values will appear black.
   - All other elements of the image that fall within the range between High Signal and Background values appear as shades of gray.
6. If these gray level adjustment parameters is to be used again in the future click the Template button and assign a unique name.
7. Repeat the gray scale adjustment for each channel. Switch to a different channel by placing a checkmark next to it in the Image Setting dialog box.
8. Click OK to apply these values to the image.

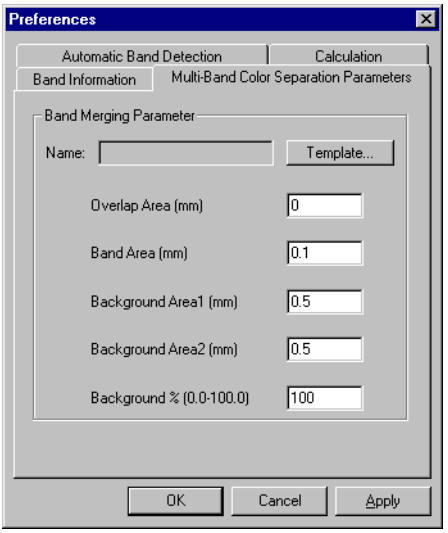
Perform Color Separation (page 5-30).

In this example, color separation is performed using the Multi-Band Color Separation window. You can also use the Color Separation window (see *Indi-*

vidual Band Color Separation on page 5-32), or a previously saved template (see *Multi-Band Color Separation* on page 5-43).

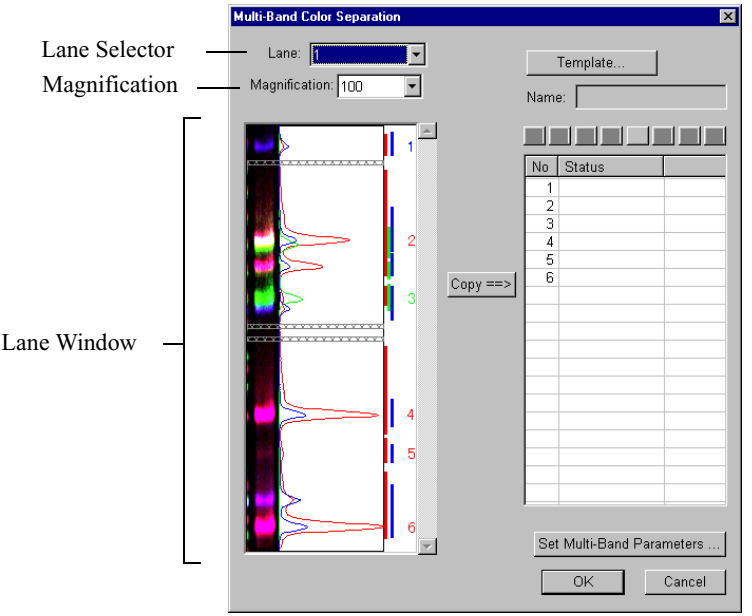
You must select a lane containing at least one non-overlapping, medium intensity, representative band of each color.

1. Select Blend as the display mode.
2. Review the Multi-Band Separation parameters. Click the Preferences button  or choose Preferences... from the 1D-Gel menu, then click the Multi-Band Color Separation tab.
3. Try these settings and, if necessary, adjust them for different gels:
   - Overlap = 0.0 mm
   - Band Area = 0.1 mm
   - Background Area 1 = 0.5 mm
   - Background Area 2 = 0.5 mm
   - Background % = 100%



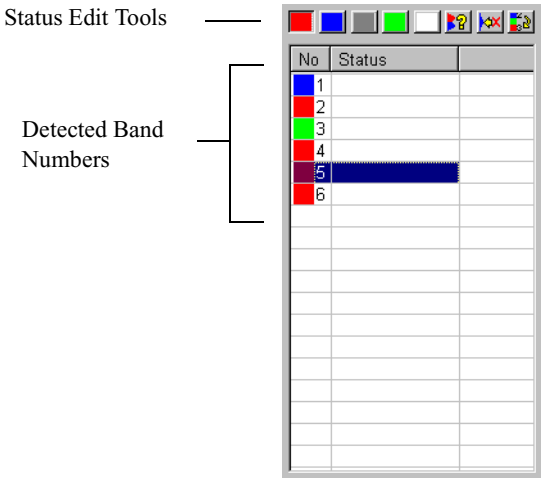
4. Click the Lane Selection Tool button  or choose 1D-Gel > Tool > Lane Selection.
5. Find a lane that contains a minimum of one band of each color.
6. Position the tool at a corner of the lane above the top migration line. Drag the cursor down to the opposite corner below the bottom migration line until the bands are contained in the boundary.
7. Click the Automatic Band Detection button  or choose 1D-Gel > Function > Auto Band.
8. After Image Analysis has automatically detected bands in the lane, click the Multi-Band Color Separation button  or choose Multi-Band Color Separation... from the 1D-Gel menu.

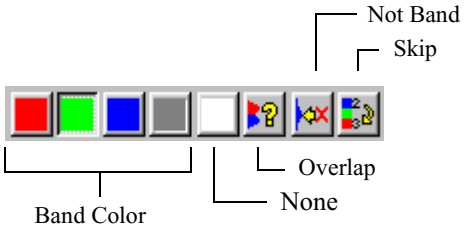
   Using the Band Merging parameters, Image Analysis assigns a color to each band, and the Multi-Band Color Separation window is displayed. The color of the Detected Band number indicates the color assigned by Image Analysis. The numbers of the detected bands appear in the Status Edit Sheet.



9. If you are not satisfied with the separation of the colors, adjust the Multi-Band Color Separation preferences. Click the Set Multi-Band Parameters... button, and change the parameter values (see *Band Selection Parameters* on page 5-43). When you click OK, the colors are automatically separated according to the new values.
10. After the Multi-Band Color Separation window is displayed, click the Copy>> button or load a previously generated Multi-Band Separation Marker template. The band colors appear in the Status Edit Sheet.

    Typically, the Multi-Band Color Separator accurately selects bands and assigns colors to them. In a rare instance, you might want to edit the bands displayed.
11. Click the row of the band you want to edit. The Status Edit buttons are activated.



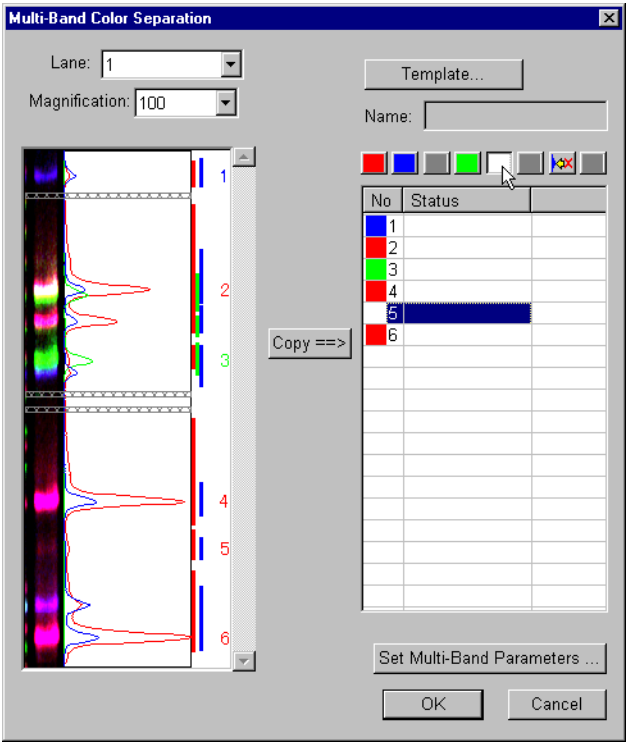


- If the band was not assigned the appropriate color, click the appropriate color. The new color is displayed.
- If the band is overlapped by another band, click Overlap. The band is grayed out.
- If you do not want to use the band, click None. The band is deselected and no color is displayed.
- If you have applied a template to the Status Edit Sheet and the band is not listed in the template, click Not Band. The band is deselected and the color list is shifted down.

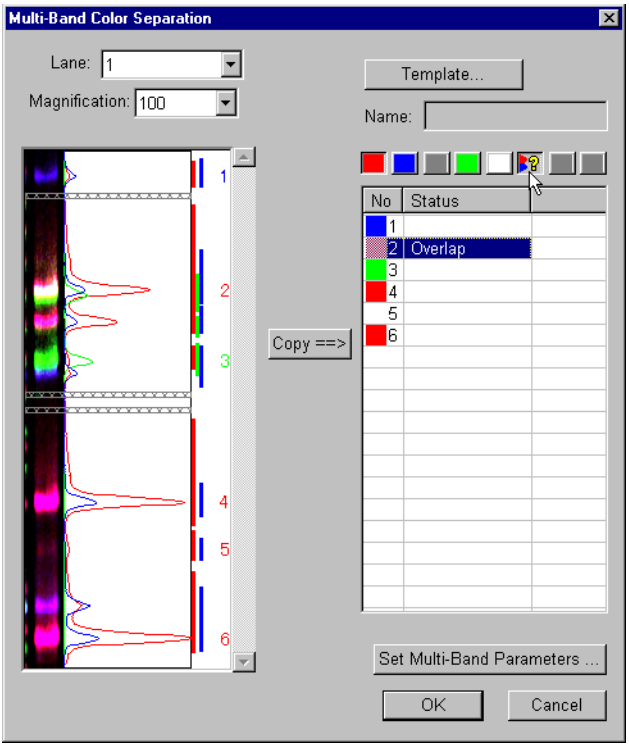
- If you do not want to use the band, click Skip. The Detected Band numbering skips the band.

Any band that has an Overlap, None, Not Band, or Skip status is not used for multi-band color separation.

In this example, Band 5 is an overlapping signal, so click the band row to select it and then click the None button from the tool bar.



In addition, Band 2 is actually two overlapping bands, so click Band 2 to select it and then click the Overlap button.



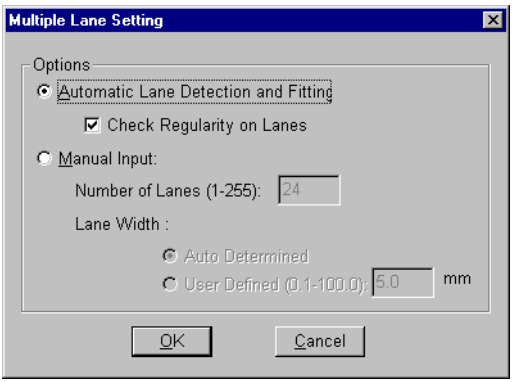
12. If this Multi-Band Separation Marker is to be used in the future (i.e. Controls such as K562, 9947A, etc.) click the Template button and assign the template a unique name.
13. Once the band colors have been verified, click OK. The Color Separation window is displayed.
14. Click OK to perform the color separation. Image Analysis creates an image for each channel that displays only the signal from a single dye.

Set Lanes (page 5-52).

1. Click the Multiple Lane Selection Tool button  or choose 1D-Gel > Tool > Multiple Lane Selection.
2. Create a rectangle surrounding the group. Position the tool above the top

migration line at the corner of the first lane in a sequence of lanes.

3. Drag the tool below the bottom migration line to the opposite corner of the last lane in the sequence.
4. Release the mouse. The Multiple Lane Setting dialog box is displayed.



5. Choose either Automatic Lane Detection and Fitting or Manual Input.

   If you choose Automatic Lane Detection, you can also choose to check the uniformity of the lanes.

   If you choose Manual Input, enter the number of lanes in the sequence then choose to either automatically determine the lane width or specify a width. Lane width may range from 1.0 to 100.0 mm, in 0.1-mm increments.
6. Click OK. By default, Image Analysis displays a central line down each defined lane, with additional lines marking the lane width. When the lane is selected, square edit handles are displayed on the lane lines.
7. If any of the lanes need adjustment, click on the lane to highlight it and move it to the correct position. The Lane Style button  toggles among different display options: center line with lane boundary, center line only, lane boundary only, and lane display off. You can also choose a lane style from the 1D-Gel menu.

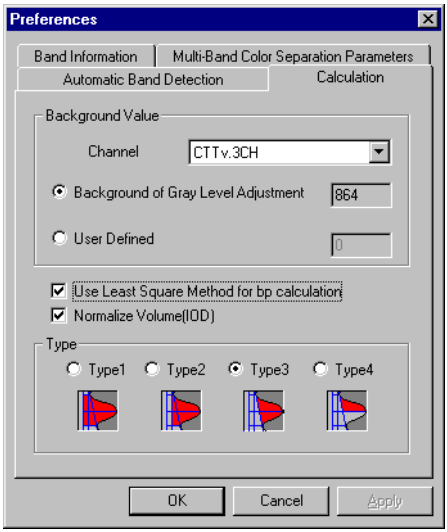
Set Migration Lines (page 5-52).

- Adjust the top and bottom migration lines according to the user's laboratory protocol.

Autoband (page 5-63).

With the lanes selected and the migration lines adjusted, autoband determination can be performed.

1. Select Normalized Volume (IOD) and choose Type 4 as the calculation type in the 1D-Gel Preferences dialog box.



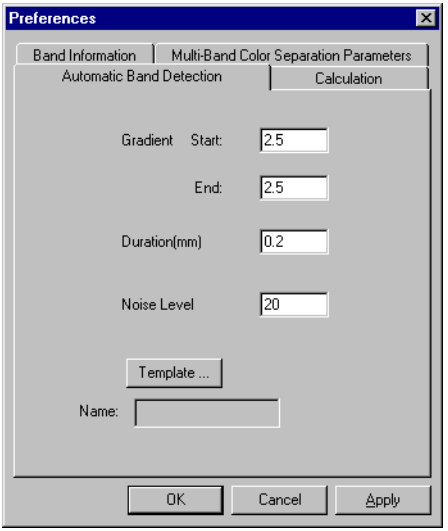
2. Click the Automatic Band Detection tab to edit the autobanding parameters.
3. Try these settings and adjust the settings as needed for different gels:

   Gradient Start = 2.5

   End = 2.5

   Duration = 0.2

   Noise Level = 20



4. Choose Edit > Select All to select all the lanes.

5. Click the Automatic Band Detection button  or choose 1D-Gel > Function > Auto Band. You might need to adjust the Image Analysis automatic band calls. The Band Style button  toggles among the different band styles: band display off, peak and range bands displayed, and only peak bands displayed. You can also choose a band style from the 1D-Gel menu.

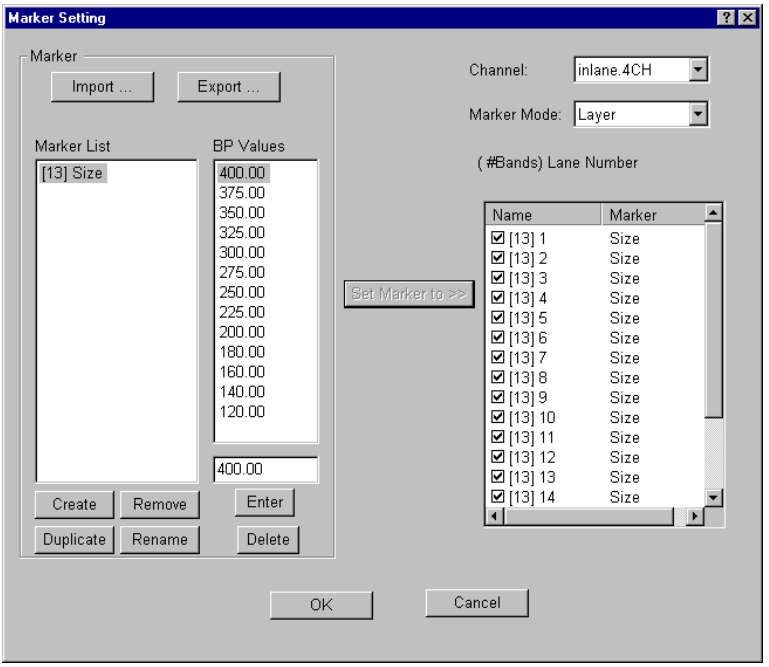
6. Click the Band Edit button  or choose 1D-Gel > Tool > Band Edit, then use the cursor to place bands.

Set Marker (page 5-71).

1. Click the Quantification Setting button  or choose Quantification Setting... from the 1D-Gel Menu.
   Select the standard curve fit from the drop-down list box. Logarithmic is

the correct standard curve fit for most applications.

2. Click the Marker Setting button  or choose Set Marker... from the 1D-Gel menu.
3. Highlight the lanes containing the marker by holding down the Shift key while clicking on them with the mouse. Select the correct marker from the Template list, then click <<Adopt Marker. The selected marker appears next to each lane in the list.



Calculate Base Pairs (page 5-80).

1. Click the Volume Calculation button  or choose Volume Calculation from the 1D-Gel menu to calculate base pair sizes in reference to the assigned marker.

2. Click the Open Spreadsheet button  or choose Open Spreadsheet from the 1D-Gel menu to view the table of base pair values for each layer. Click the tabs at the bottom of the window to switch among channels.
3. Choose Export from the SpreadSheet menu to save the spreadsheets as comma-delimited text files that can be exported to STaR Call™.

7 Array Analysis

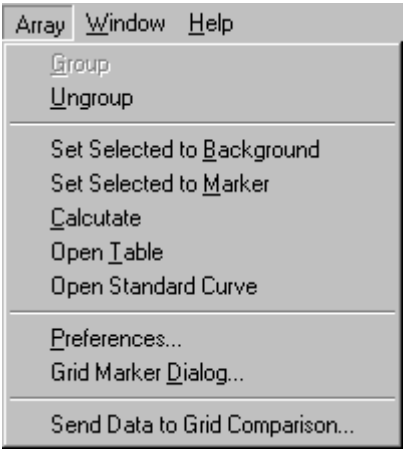
Image Analysis can extract information from two-dimensional (2D) objects as small as one pixel by one pixel. This capability is especially useful when you need to analyze arrays and other tightly-spaced objects.

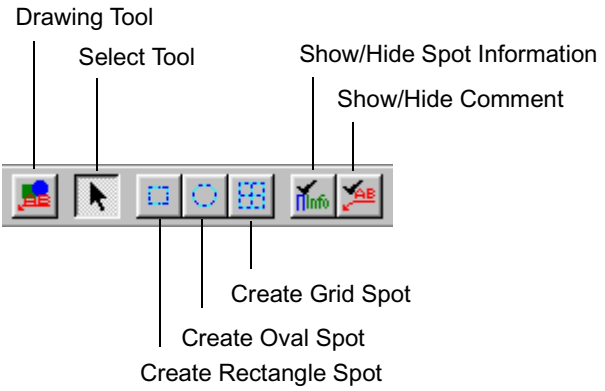
Using the analysis tools in the Array menu, you can analyze dot blots, slot blots, gels or arrays. These tools allow you to calculate the following parameters, and display them in a table:

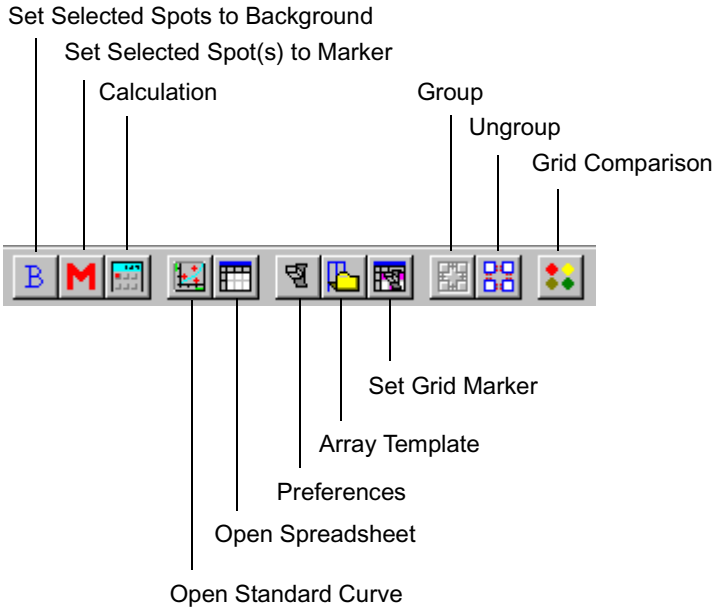
- Spot coordinates (Position)
- Spot area (Area)
- Average signal intensity of the fluorescence of the pixels in a designated spot area (Mean)
- Volume of relevant pixels in a spot area. (Volume)
- Quantity in spot, e.g., micrograms DNA (Mol)
- Standard deviation of signal intensity range in each designated spot area.

Note For quantification of 1D-gels, see *Setting Quantification Markers* on page 5-82.

Array Menu and Tools






Set Selected Spots to Background
Set Selected Spot(s) to Marker
Calculation
Group
Ungroup
Grid Comparison
Set Grid Marker
Array Template
Preferences
Open Spreadsheet
Open Standard Curve

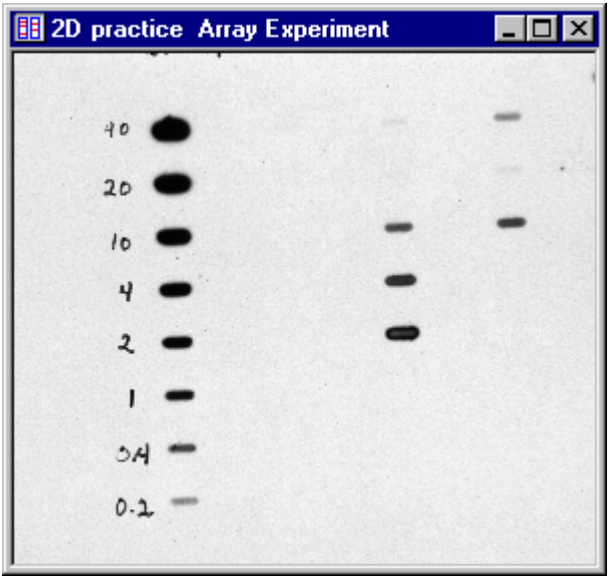
Creating an Array Analysis

To create an Array analysis experiment:

1. Create a new project.
2. Choose New Array Analysis in the Project menu.

3. Select a file that contains a digital image. A new Array experiment is added to the project tree and the Array menu appears in the menu bar.

4. Double-click the Array Experiment icon. An Array Experiment window containing the image is displayed.



5. To deselect an item, remove the checkmark adjacent to it.
6. Click OK.

Analyzing Spots in an Image

To analyze spots in an image, you must first create a boundary surrounding each spot of interest. You can either create a single boundary or create an evenly spaced grid over a region with many spots. A single spot can be a rectangle or an oval. In addition, you can specify a region to analyze within the spot area.

Creating a rectangle or oval spot boundary.

1. Click the Create Rectangle Spot or Create Oval Spot  .

2. Drag the cursor to draw a rectangle or oval around each spot of interest.

Modifying the boundary of a spot.

To move a boundary:

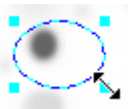
1. Click the Select Tool button  or choose Array > Tool > Select.

2. Place the cursor inside the boundary until it transforms into a crosshair, then drag the crosshair to the desired location.

To enlarge or compress a boundary:

1. Click the Select Tool button  or choose Array > Tool > Select.

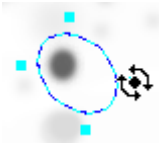
2. Place the cursor over a transformation handle (initially the upper left or lower right handle), and then drag the cursor until you have the desired shape.



To rotate a boundary:

1. Click the Select Tool button  or choose Array > Tool > Select.

2. Place the cursor over a rotation handle (initially an upper right or lower left handle), and then drag the cursor about the midpoint.



Duplicating a spot boundary.

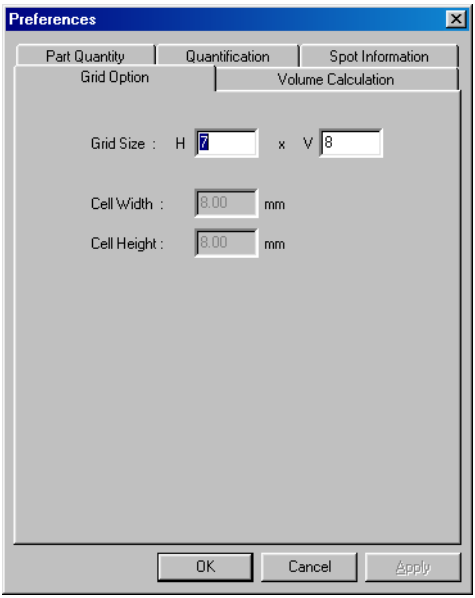
You can duplicate a spot boundary by using the Copy and Paste function in the Edit menu.

Creating a Grid

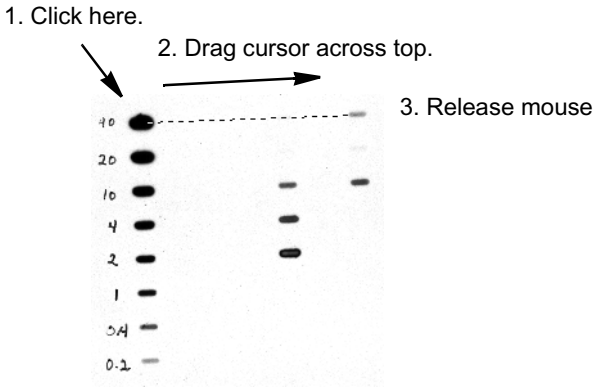
To create a grid over a group of spots:

1. Click the Preferences button  or choose Preferences... from the Array menu.

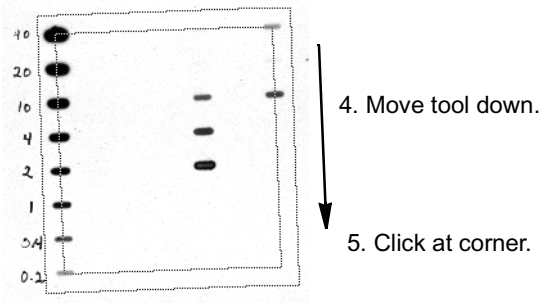
2. In the Grid Option filecard, enter the number of cells in the H (horizontal) and V (vertical) direction. By default, the grid dimensions are 12 (horizontal) by 8 (vertical) boxes.



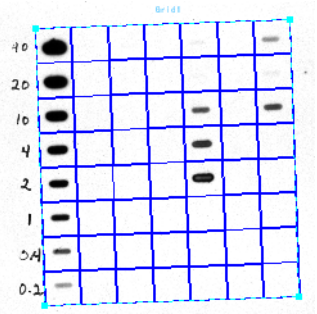
3. If you entered 1 as the number of cells horizontally, enter a value in the cell width field.
4. If you entered 1 as the number of cells vertically, enter a value in the cell height field.
5. Click OK.
6. Click the Create Grid Spot button  .
7. Place the crosshair cursor in the center of the spot at the top corner, and then drag the cursor through the spots in the top row.



8. Release the mouse button, then move the cursor down until the inner rectangle passes through the spots on the four sides.



9. Then click the mouse button. The grid is created according to the grid options set in the Preferences dialog box.



Modifying a Grid

You can use the Select Tool  to modify the grid size, position, and rotational angle.

To move the grid:

- Use the up, down, left, and right arrow keys on the keyboard.
- Place the Select Tool cursor inside the grid, and then drag the crosshair to move the shape.

To enlarge or compress a grid:

- Place the Select Tool cursor over a transformation handle (initially the upper left or lower right handle), and then drag the cursor until you have the desired shape.

To rotate a grid:

- Place the Select Tool cursor over a rotation handle (initially an upper right or lower left handle), and then drag the cursor about the midpoint.

Grouping Spots and Grids

You can group spots and grids to change their parameters simultaneously.

To Group spots/grids:

1. Hold down the Shift key, and then click the single spots or grids you want to group. Any type of spot or grid may be selected.

2. Choose Group from the Array menu.

3. Change the spot parameters. Your changes will affect all spots in the group.

To Ungroup spots/grids:

- Click the group to select it, and then choose Ungroup from the Array menu.

Note In the Array menu, if Group is grayed out and Ungroup is active, then the spots/grids are already grouped. If Group is active and Ungroup is grayed out, then the spots/grids are not grouped.

Creating a Template

Image Analysis allows you to save spots or grids as templates for future use. You can also duplicate and rename saved templates.

To create an Array template:

1. Click on the Array Template button  . Array Template dialog box is displayed.
2. Click Add. The Select Spots and Grids dialog box lists the spot and grid elements in the image. Select the spots and grids you want in the tem-

plate by clicking the checkboxes or using the Select All and Clear buttons.

3. When you are finished, click the Save button. The template appears in the list preceded by the number of elements contained in it.

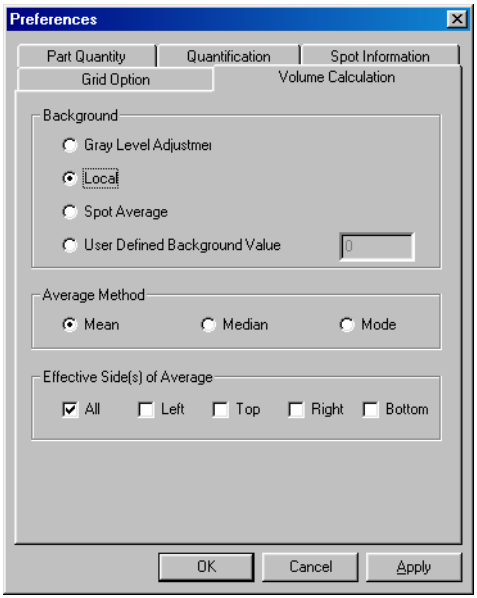
To apply a template:

4. Click on the Array Template button  . The Array Template dialog box is displayed.
5. Click on the desired template in the list, and click Apply.

Background Calculation

You can specify the background value, the method for averaging a background marker, and the side of a rectangular spot to use as the background.

1. Click the Preferences button  or choose Preferences... from the Array menu.
2. In the Preferences dialog box, click the Volume Calculation tab.


Preferences
Part Quantity
Quantification
Spot Information
Grid Option
Volume Calculation
Background
Gray Level Adjustmer
Local
Spot Average
User Defined Background Value
0
Average Method
Mean
Median
Mode
Effective Side(s) of Average
All
Left
Top
Right
Bottom
OK
Cancel
Apply

Background Option	Description
Gray Level Adjustment	Uses gray level background value for the image.
Local	Uses average pixel value of spot/grid cell boundary. Does not use the background marker.
Spot Average	Uses average pixel value of background spot marker.
User Defined Background	Uses value entered in the field.
Average Method (Local or Spot Average)	Mean = (total volume)/(number of pixels) Median = middle pixel value, e.g., 1, 2, 5, 15, 16*, 20, 300, 2000, 5000 Mode = most frequent pixel value
Effective side(s) of Average (Local Background)	Side of cell used to calculate local background: All, Left, Top, Right, Bottom. For example, if there is significant horizontal overlap of signal between spots, you might want to select the top of each cell.

Registering the Background Value

1. Create a rectangle or oval spot surrounding the area that represents the background.
2. Click the Set Selected Spots to Background button  or choose Set Selected to Background from the Array menu.
3. The boundary color changes to yellow.

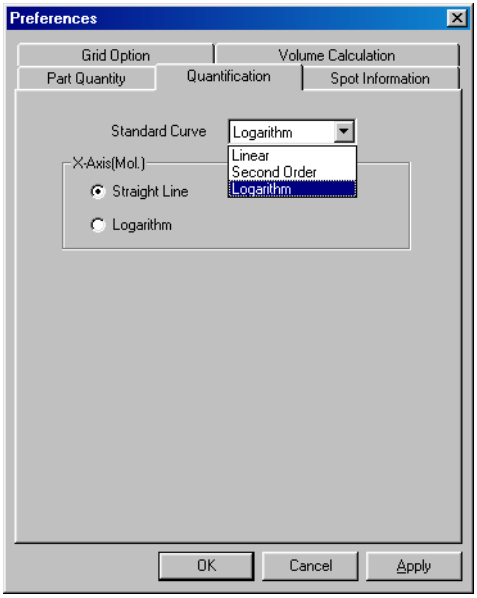
Registering Markers

You can assign known amounts to spots and use these spots as markers to quantify unknown samples.

Standard Curve Type.

To set the type of standard curve:

1. Click the Preferences button  or choose Preferences... from the Array menu.
2. In the Preferences dialog box, click the Quantification tab.



3. Select the desired standard curve in the list box, then select a straight or Logarithmic scale for the X-axis.
4. Click OK.

Spot Markers.

To set any combination of spots as markers:

1. Select any spot or group of spots, then click the Set Selected Spot(s) to Marker button  or choose Set Selected to Marker from the Array menu. The boundary color of each spot changes to violet.

2. Click the Open Spreadsheet button  or choose Open Spreadsheet from the Array menu.

3. Enter a quantity value for each marker spot in the column labeled "Mol.". If necessary, use the bottom scroll bar to view the right side of the Channel table.

Channel 1

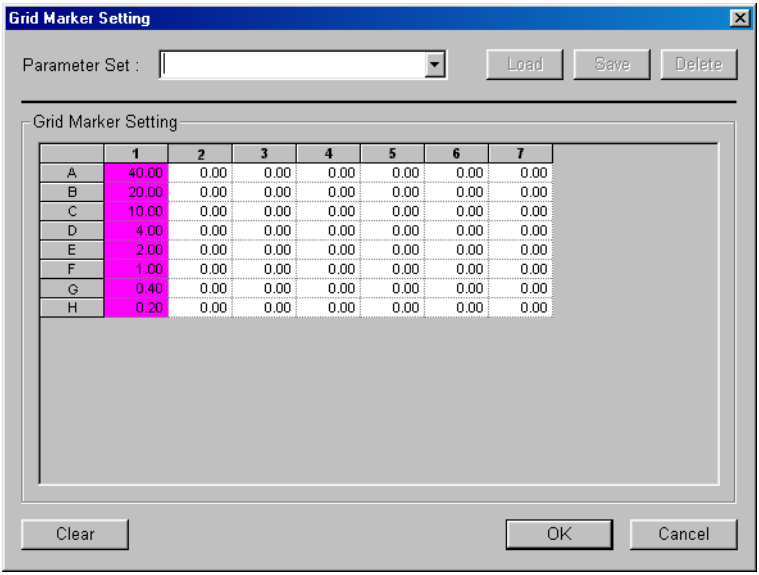
	x	y	Width	Height	Area	Mean	Std.	Vol.	Mol.	Label
Spot1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	
Spot2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	10.00	
Spot3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	
Spot4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	1.00	
Spot5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	
Spot6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	

Grid Cell Markers.

To set cells in a grid as markers:

1. Double-click on the grid containing the marker cells. The Grid Marker dialog box is displayed.

2. In the Grid Marker table, enter the amount of each marker corresponding table cell. Any cell containing a value greater than zero is set as a marker.



3. If you plan to use these grid markers again, save the parameters as a template.
   - Enter a name in the Parameter Set list box.
   - Click Save.
4. Click OK. The boundary color of the cells changes to violet.

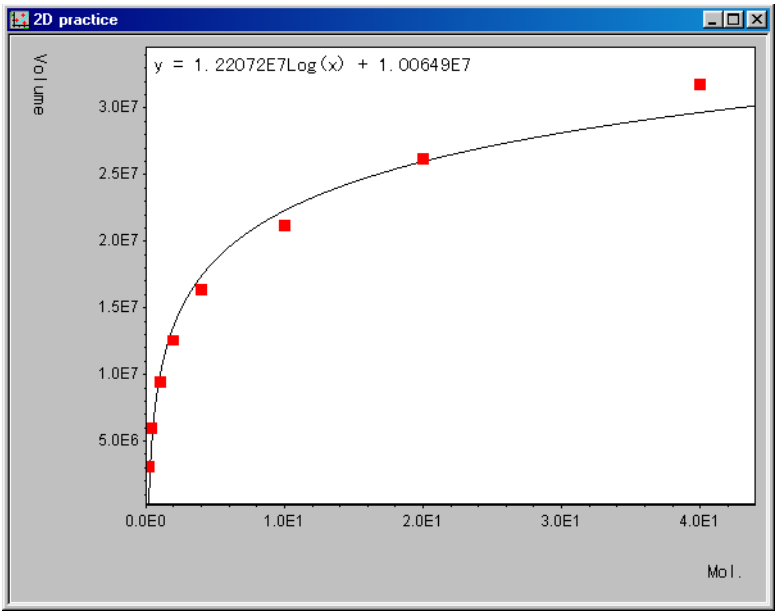
Grid Templates.

To use a Grid Marker Template:

1. If you want to use an existing grid marker template, select the template from the Parameter Set list box.
2. Click Load.

Displaying the Standard Curve.

- Click the Open Standard Curve button  or choose Open Standard Curve from the Array menu. The standard curve window is displayed.

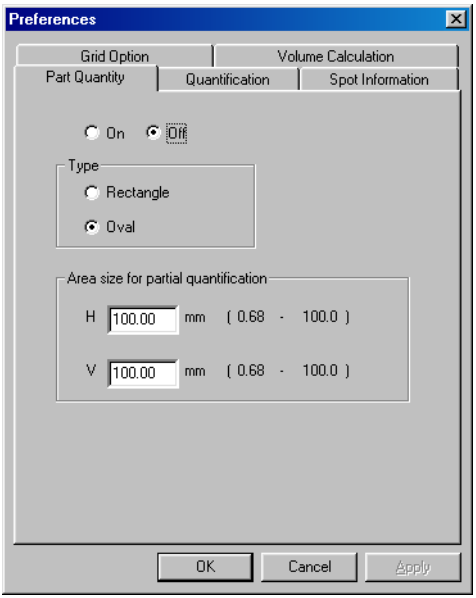


Setting a Partial Quantity

You can restrict the area of a grid cell that is used for volume calculations. For example, this feature is useful when the spots in a grid are far apart relative to their size.

To specify a partial quantity:

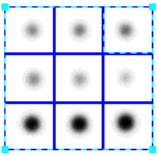
1. Click the Preferences button or choose Preferences... from the Array menu.
2. In the Preferences dialog box, click the Part Quantity tab.

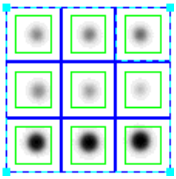
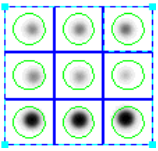


3. Click On.

Note When Part Quantity is turned off, the entire area of the spot/grid cell is used.

4. Choose the type of partial boundary, Rectangle or Oval.
5. Enter the horizontal (0.68 - 100) and vertical (0.68 - 100) dimensions. The following examples illustrate these settings:

Type/Area Size	Grid Area
Part Quantity = Off	

Type/Area Size	Grid Area
Part Quantity = On Rectangle H = 8 mm V = 8 mm	
Part Quantity = On Oval H = 8 mm V = 8 mm	

Mol Calculation

Image Analysis calculates the mean signal intensity, standard deviation, and volume of each spot. If you have specified quantification markers, Image Analysis also calculates the quantity of sample in each spot.

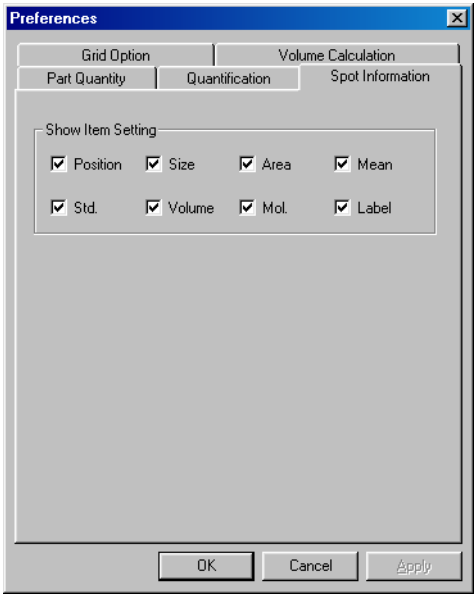
- Click the Calculation button  or choose Calculate from the Array menu.

Note Values are only quantified if they fall within the highest and lowest standard marker values.

Displaying a Spreadsheet

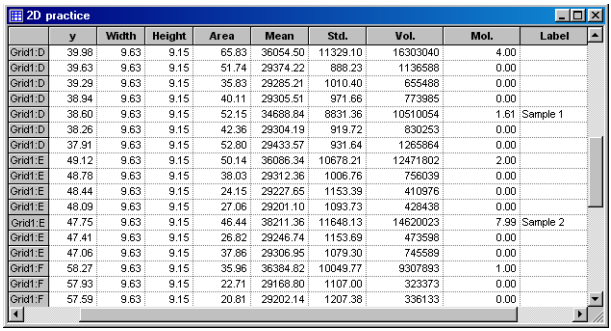
You can select the spot information you want displayed in the Channel spreadsheet.

1. Click the Preferences button  or choose Preferences... from the Array menu.
2. In the Preferences dialog box, click the Spot Information tab.



The calculated values appear in the spreadsheet and the volume of each spot/ grid cell is displayed on the image.

3. Click the Open Spreadsheet button  or choose Open Spreadsheet from the Array menu.



2D practice

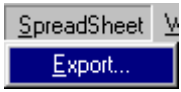
	y	Width	Height	Area	Mean	Std.	Vol.	Mol.	Label
Grid1:D	39.98	9.63	9.15	65.83	36054.50	11329.10	16303040	4.00	
Grid1:D	39.63	9.63	9.15	51.74	29374.22	888.23	1136588	0.00	
Grid1:D	39.29	9.63	9.15	35.83	29285.21	1010.40	655488	0.00	
Grid1:D	38.94	9.63	9.15	40.11	29305.51	971.66	773985	0.00	
Grid1:D	38.60	9.63	9.15	52.15	34688.84	8831.36	10510054	1.61	Sample 1
Grid1:D	38.26	9.63	9.15	42.36	29304.19	919.72	830253	0.00	
Grid1:D	37.91	9.63	9.15	52.80	29433.57	931.64	1265864	0.00	
Grid1:E	49.12	9.63	9.15	50.14	36086.34	10678.21	12471802	2.00	
Grid1:E	48.78	9.63	9.15	38.03	29312.36	1006.76	756039	0.00	
Grid1:E	48.44	9.63	9.15	24.15	29227.65	1153.39	410976	0.00	
Grid1:E	48.09	9.63	9.15	27.06	29201.10	1093.73	428438	0.00	
Grid1:E	47.75	9.63	9.15	46.44	36211.36	11648.13	14620023	7.99	Sample 2
Grid1:E	47.41	9.63	9.15	26.82	29246.74	1153.69	473598	0.00	
Grid1:E	47.06	9.63	9.15	37.86	29306.95	1079.30	745589	0.00	
Grid1:F	58.27	9.63	9.15	35.96	36384.82	10049.77	9307893	1.00	
Grid1:F	57.93	9.63	9.15	22.71	29168.80	1107.00	323373	0.00	
Grid1:F	57.59	9.63	9.15	20.81	29202.14	1207.38	336133	0.00	

Exporting a Spreadsheet

You can save the spreadsheet as a tab delimited text file for export to other programs, such as Microsoft Excel and Microsoft Word.

To export a spreadsheet:

1. Choose Export... from the SpreadSheet menu.



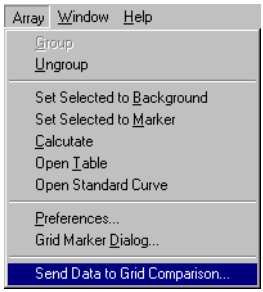
2. In the Save As dialog box, select a location for the file, and then enter a unique name.
3. Click Save.

Comparing Grids

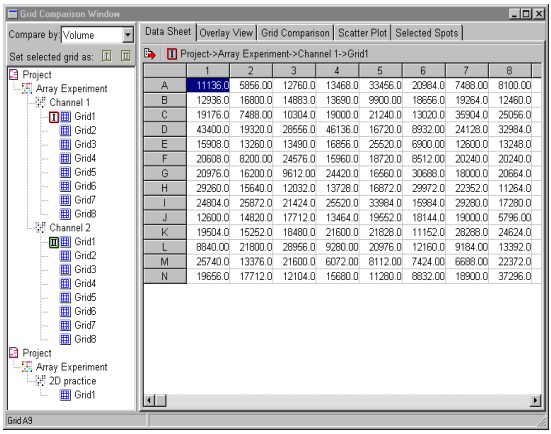
Image Analysis allows you to compare grid data. You can compare data from two channels in the same grid or two different grids, including grids in different projects. For example, you can conduct data mining for gene expression by concentration or volume. The resulting images and spreadsheets can be pasted into other applications.

To compare grid data:

1. Choose Send Data To Grid Comparison... from the Array menu.

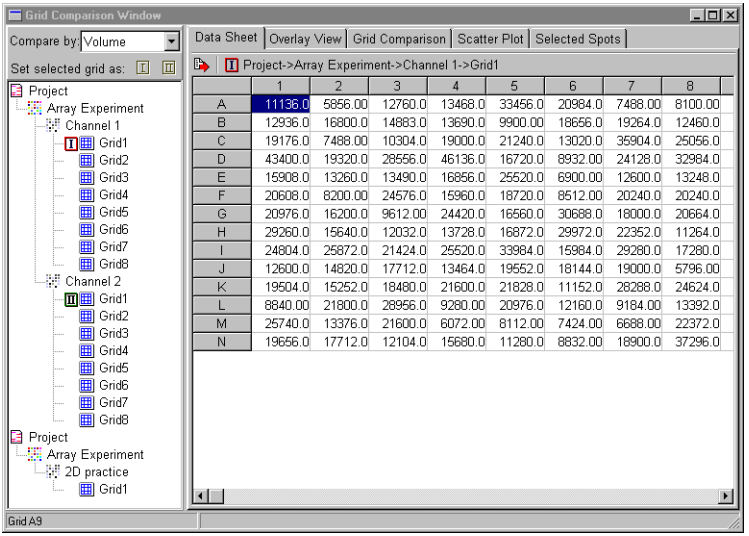


The Grid Comparison window is displayed. The list box contains all open projects with grid data.



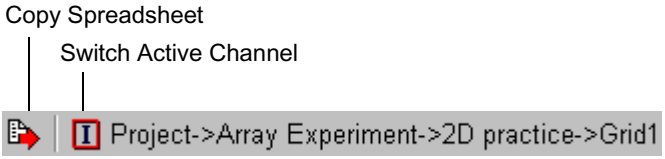
Data Sheet Filecard

You can view the numerical data for each grid in the Data Sheet. You can also copy the spreadsheet and use it in another application, such as Microsoft Excel.



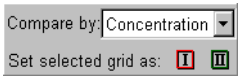
	1	2	3	4	5	6	7	8
A	11136.0	5856.00	12760.0	13468.0	33456.0	20984.0	7488.00	8100.00
B	12936.0	16800.0	14883.0	13690.0	9900.00	18656.0	19264.0	12460.0
C	19176.0	7488.00	10304.0	19000.0	21240.0	13020.0	35904.0	25056.0
D	43400.0	19320.0	28556.0	46136.0	16720.0	8932.00	24128.0	32984.0
E	15908.0	13260.0	13490.0	16856.0	25520.0	6900.00	12600.0	13248.0
F	20608.0	8200.00	24576.0	15960.0	18720.0	8512.00	20240.0	20240.0
G	20976.0	16200.0	9612.00	24420.0	16560.0	30688.0	18000.0	20664.0
H	29260.0	15640.0	12032.0	13728.0	16872.0	29972.0	22352.0	11264.0
I	24804.0	25872.0	21424.0	25520.0	33984.0	15984.0	29280.0	17280.0
J	12600.0	14820.0	17712.0	13464.0	19552.0	18144.0	19000.0	5796.00
K	19504.0	15252.0	18480.0	21600.0	21828.0	11152.0	28288.0	24624.0
L	8840.00	21800.0	28956.0	9280.00	20976.0	12160.0	9184.00	13392.0
M	25740.0	13376.0	21600.0	6072.00	8112.00	7424.00	6688.00	22372.0
N	19656.0	17712.0	12104.0	15680.0	11280.0	8832.00	18900.0	37296.0

Data Sheet Buttons.



Item	Description
Switch Active Channel	Switch the active data channel.
Copy Spreadsheet	Copies spreadsheet to clipboard

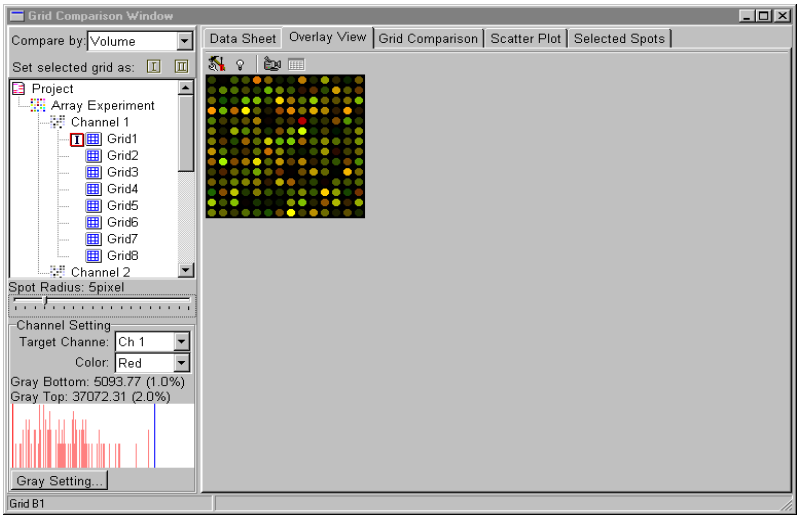
2. Select the grids to compare by first clicking the first grid to highlight it.
3. Click the Channel 1 button  .



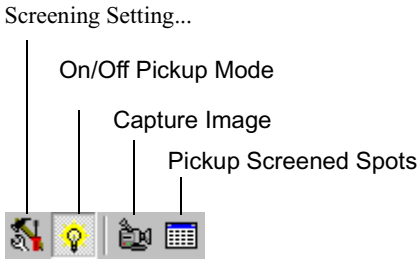
4. Highlight the second grid, and click the Channel 2 button  .
5. In the Compare by drop-down box, select a Concentration, Volume, Standard, Mean, or Area.
6. Click the Switch Active Channel button  to display the grid data for each channel.
7. If you want to copy the spreadsheet to the clipboard, click the Copy Spreadsheet button  .

Overlay View Filecard

You can overlay the two grids and to visually screen each spot for differences in the data values. You can adjust the gray scale for each channel and enhance the detection of spots with small values. The Screening feature allows you display only spots that exceed a minimum Channel 1/Channel 2 ratio and view the numerical data for those spots. The Overlay View is particularly useful for quickly filtering spots for quantitative changes in expression.

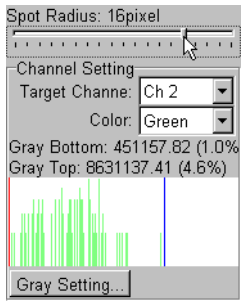


Item	Description
Spot Radius	Adjusts the spot radius.
Channel Setting	Select the target channel and color.
Histogram	Slider adjusts the top and bottom gray scale signal threshold.
Gray Setting...	Displays the Gray Setting dialog box.

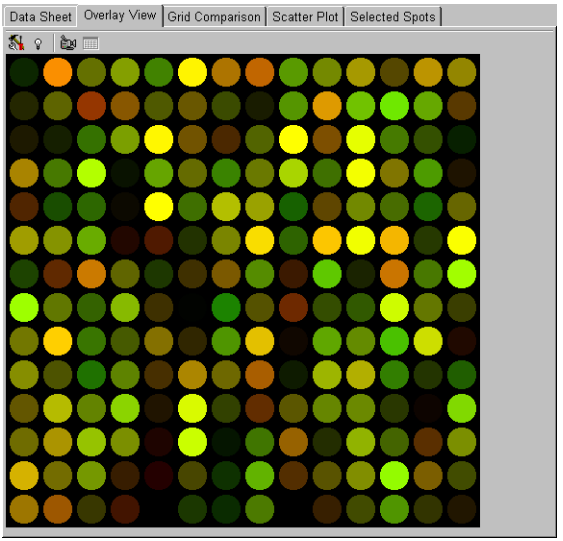


Item	Description
Screening Setting...	Displays the Screening Setting window.
On/Off Pickup Mode	Applies the screening settings to the overlay to detect spots.
Capture Image	Copies overlay image to the clipboard.
Pickup Screened Spots	Displays the comparison data in the Selected Spots spreadsheet.

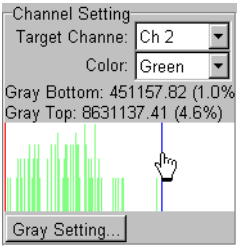
1. Adjust the spot radius by using the sliding control bar.



The spot size changes dynamically in the overlay image.

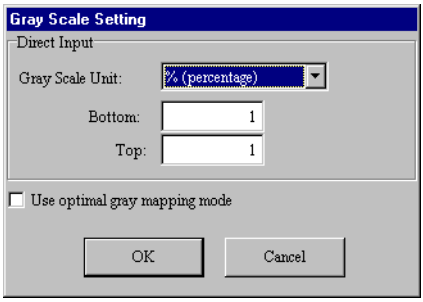


2. Choose the target channel and its color from the drop-down list boxes.
3. The histogram displays the # pixels vs. raw gray scale value. Using the blue slider on the histogram to adjust the top gray scale threshold and red line slider to adjust the bottom gray scale threshold for the target channel. The threshold values and percentages change dynamically as you move the sliders. For an explanation of the histogram and thresholds, see *Gray Level Adjustment* on page 5-14.



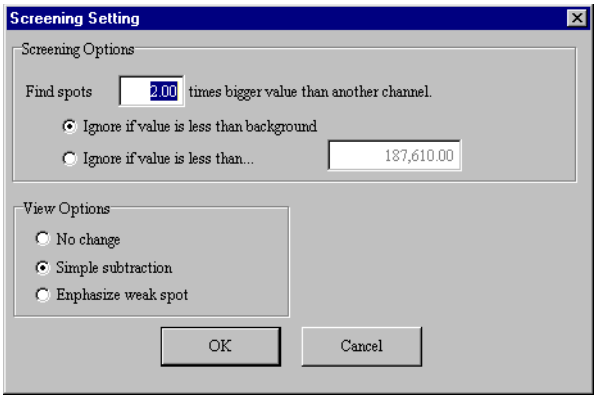
Note You can also use the Gray Settings dialog box to adjust these threshold values.

4. Click the Gray Scale... button. The Gray Scale Setting dialog box is displayed.

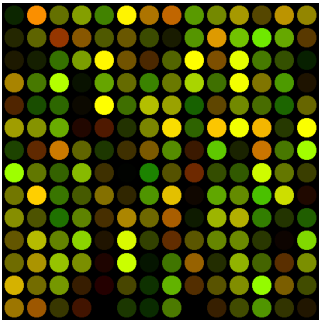


5. Choose to use % (percentage) of total pixels or raw value as the gray scale unit for the thresholds.
6. Type values for the top and bottom gray scale thresholds in the fields.
7. If desired, you can choose to use the optimal gray mapping mode. This mode is equivalent to the Equal Area mode. See *Mapping Type Selector* on page 5-18
8. Click the Screen Setting button  . The Screen Setting dialog box is displayed.
9. Specify the Screening and Viewing options.

Note You can choose to view a simple subtraction of the Channel 2 value from the Channel 1 value for each spot, or increase the gray scale value of weak spots. The latter can be used for detection, but is not recommended for quantitation by visual inspection.

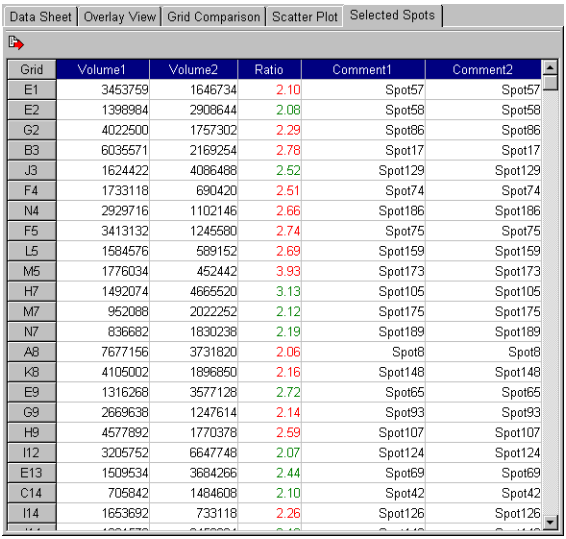


10. Apply the screening settings to the grid overlay by clicking the On/Off Pickup Mode button  .



11. If desired, you can copy the image to the clipboard by clicking the Capture Image button  .

12. Click the Pickup Screened Spots button  . The Selected Spots spreadsheet is displayed containing the comparison data for the screened spots.



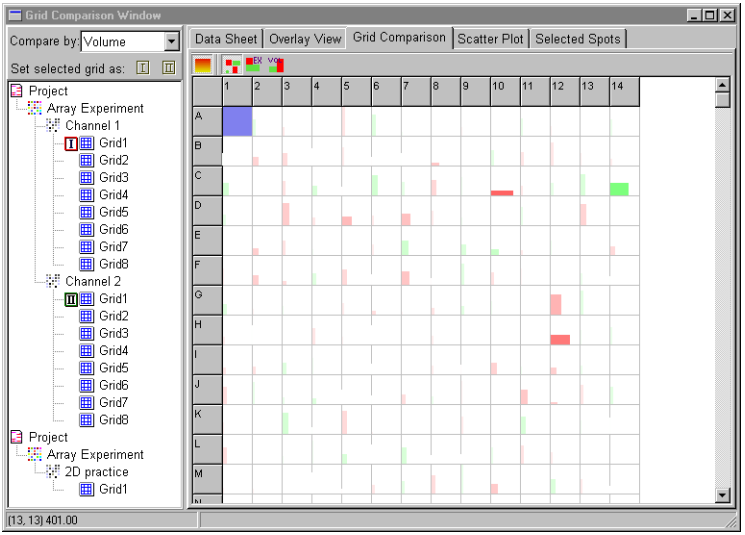
Data Sheet | Overlay View | Grid Comparison | Scatter Plot | Selected Spots

Grid	Volume1	Volume2	Ratio	Comment1	Comment2
E1	3453759	1646734	2.10	Spot57	Spot57
E2	1398984	2908644	2.08	Spot58	Spot58
G2	4022500	1757302	2.29	Spot86	Spot86
B3	6035571	2169254	2.78	Spot17	Spot17
J3	1624422	4086488	2.52	Spot129	Spot129
F4	1733118	690420	2.51	Spot74	Spot74
N4	2929716	1102146	2.66	Spot186	Spot186
F5	3413132	1245580	2.74	Spot75	Spot75
L5	1584576	589152	2.69	Spot159	Spot159
M5	1776034	452442	3.93	Spot173	Spot173
H7	1492074	4665520	3.13	Spot105	Spot105
M7	952088	2022252	2.12	Spot175	Spot175
N7	836682	1830238	2.19	Spot189	Spot189
A8	7677156	3731820	2.06	Spot8	Spot8
K8	4105002	1896850	2.16	Spot148	Spot148
E9	1316268	3577128	2.72	Spot65	Spot65
G9	2669638	1247614	2.14	Spot93	Spot93
H9	4577892	1770378	2.59	Spot107	Spot107
I12	3205752	6647748	2.07	Spot124	Spot124
E13	1509534	3684266	2.44	Spot69	Spot69
C14	705842	1484608	2.10	Spot42	Spot42
I14	1653692	733118	2.26	Spot126	Spot126

Grid Comparison Filecard

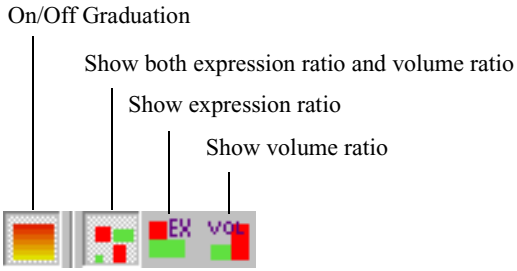
In the Grid Comparison filecard you can compare histograms of the expression and volume ratios in the grids.

1. Click on the Grid Comparison tab. The expression and volume ratios for the channels is displayed in histograms.



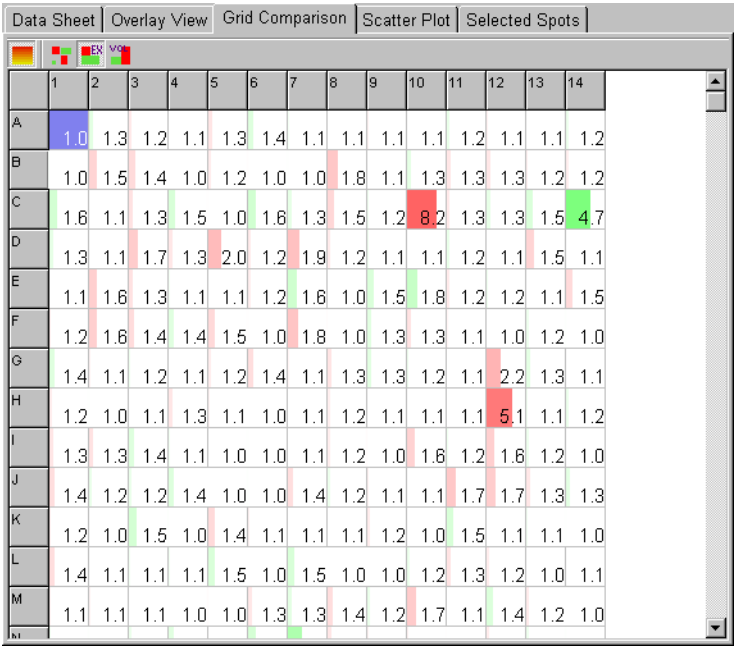
The color of the channel with the greater expression and volume is displayed. The width represents its relative expression, and the height represents its relative volume.

Grid Comparison Buttons. The Grid Comparison buttons allow you to view the expression and volume ratios separately.



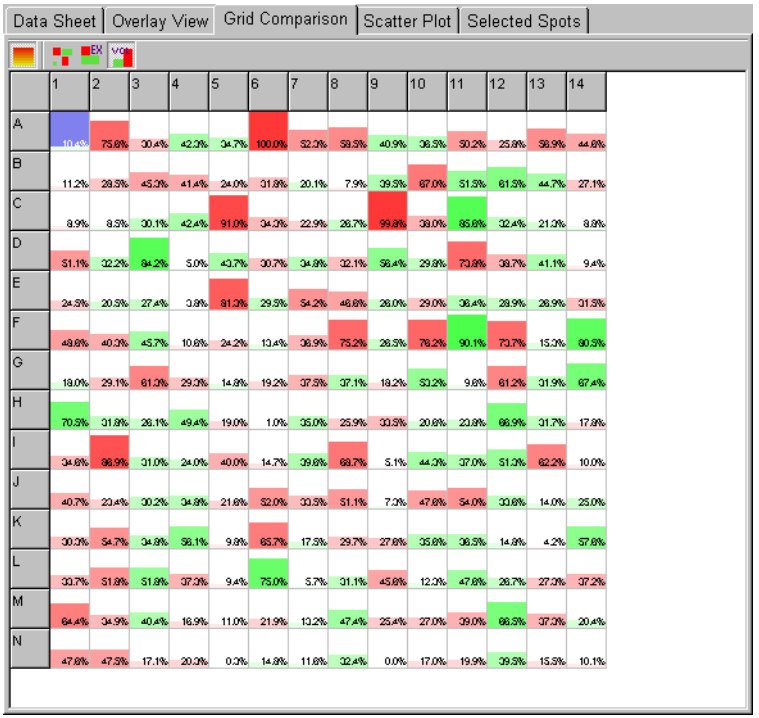
1. Click on the Show expression ratio button 

. The expression ratio histograms and numerical values for the two channels are displayed.



The color of the channel with the greater expression is displayed. The width represents its relative expression.

2. Click on the Show volume ratio button  . The volume ratio histograms and numerical values for the two channels are displayed.

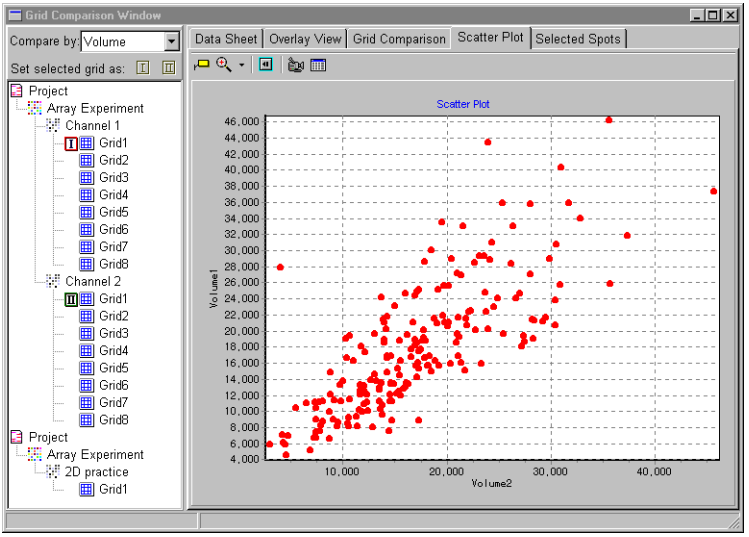


The color of the channel with the greater volume is displayed. The height represents its relative volume.

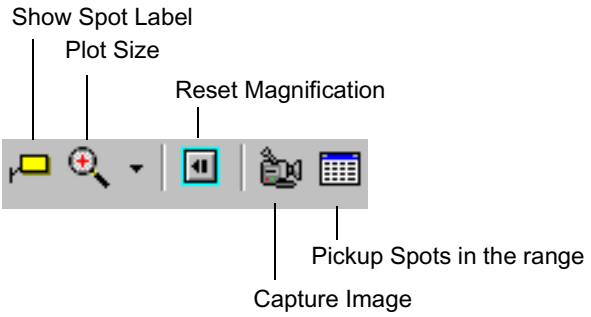
Scatter Plot Filecard

You can view a scatter plot of the data from the two grids, and automatically track spots of interest to the grid in the Overlay View. An area of the plot can be magnified to view selected spots, and numerical spot data viewed in a spreadsheet.

1. Click on the Scatter Plot tab.

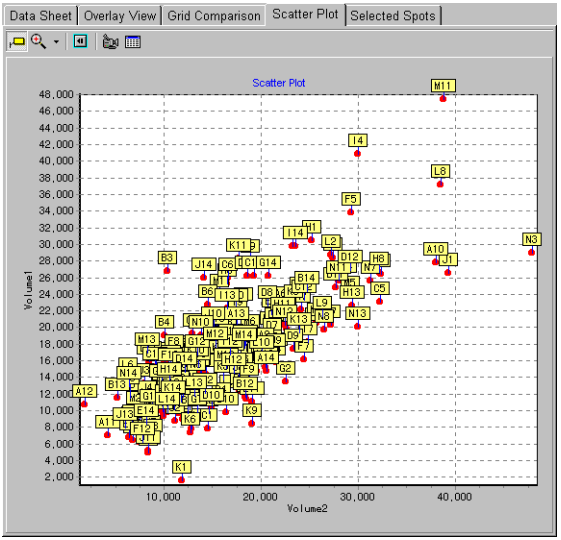


Scatter Plot Buttons.

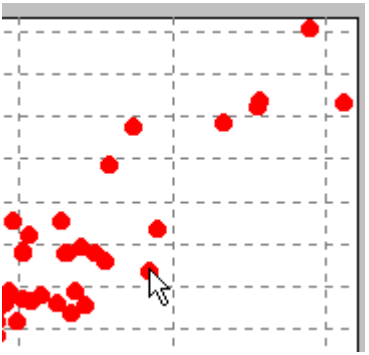


Item	Description
Show Spot Label	Display grid coordinates on each spot.
Plot Size	Adjust the spot radius.
Reset Magnification	Reset to the original magnification
Capture Image	Copy the graph to the clipboard.
Pickup Spots in the range	Display the spot data in the Selected Spots spreadsheet.

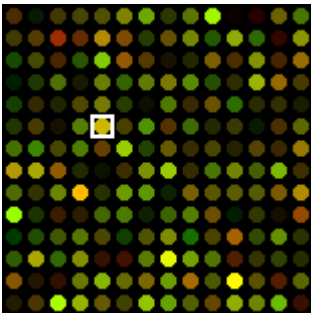
2. Click the Show Spot Label button  . A label with the grid coordinate appears on each spot.



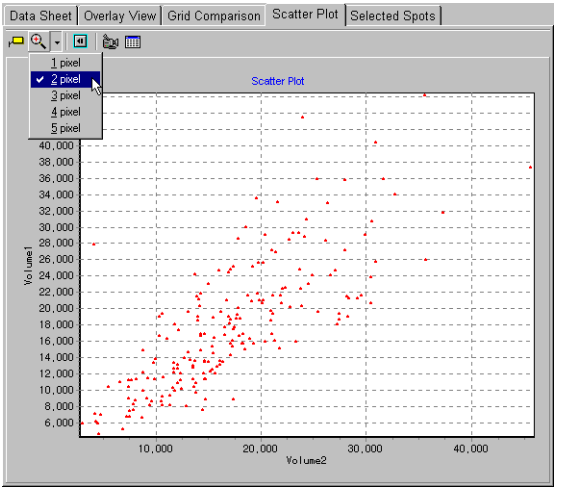
3. Double-click on a spot.



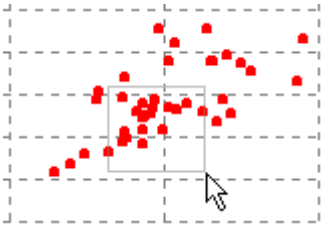
The Overlay View filecard is displayed and the spot is selected on the grid



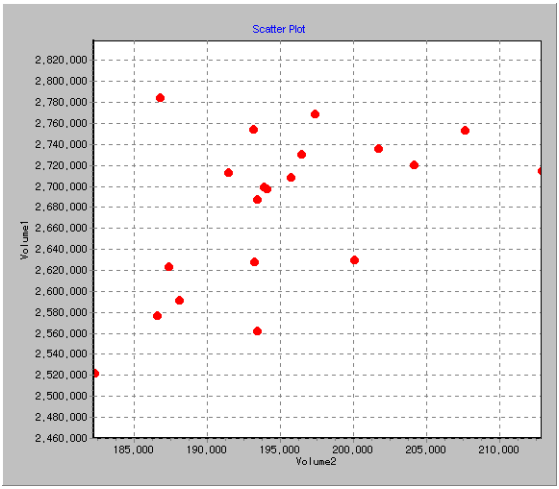
4. To change the size of the spots, click the down arrow next to the Spot Size button  , and choose the spot radius from the drop-down menu.



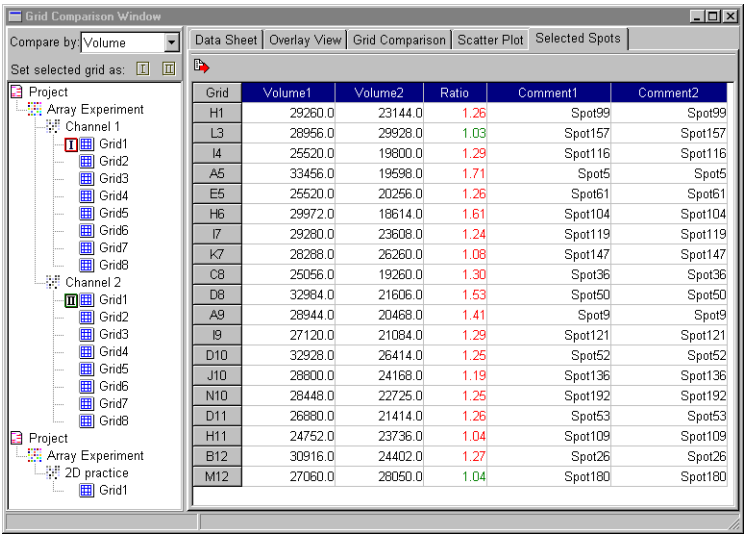
5. To magnify an area of the plot, drag the cursor to form a rectangle enclosing the area of interest.



6. Release the mouse button. The plot is magnified so that the area occupies the entire graph.



7. Click the Pickup Spots in the Range button  . The comparison data for the spots is displayed in the Selected Spots spreadsheet.



Grid	Volume1	Volume2	Ratio	Comment1	Comment2
H1	29260.0	23144.0	1.26	Spot99	Spot99
L3	28956.0	29928.0	1.03	Spot157	Spot157
I4	25520.0	19800.0	1.29	Spot116	Spot116
A5	33456.0	19598.0	1.71	Spot5	Spot5
E5	25520.0	20256.0	1.26	Spot61	Spot61
H6	29972.0	18614.0	1.61	Spot104	Spot104
I7	29280.0	23608.0	1.24	Spot119	Spot119
K7	28288.0	26260.0	1.08	Spot147	Spot147
C8	25056.0	19260.0	1.30	Spot36	Spot36
D8	32984.0	21606.0	1.53	Spot50	Spot50
A9	28944.0	20468.0	1.41	Spot9	Spot9
I9	27120.0	21084.0	1.29	Spot121	Spot121
D10	32928.0	26414.0	1.25	Spot52	Spot52
J10	28800.0	24168.0	1.19	Spot136	Spot136
N10	28448.0	22725.0	1.25	Spot192	Spot192
D11	26880.0	21414.0	1.26	Spot53	Spot53
H11	24752.0	23736.0	1.04	Spot109	Spot109
B12	30916.0	24402.0	1.27	Spot26	Spot26
M12	27060.0	28050.0	1.04	Spot180	Spot180

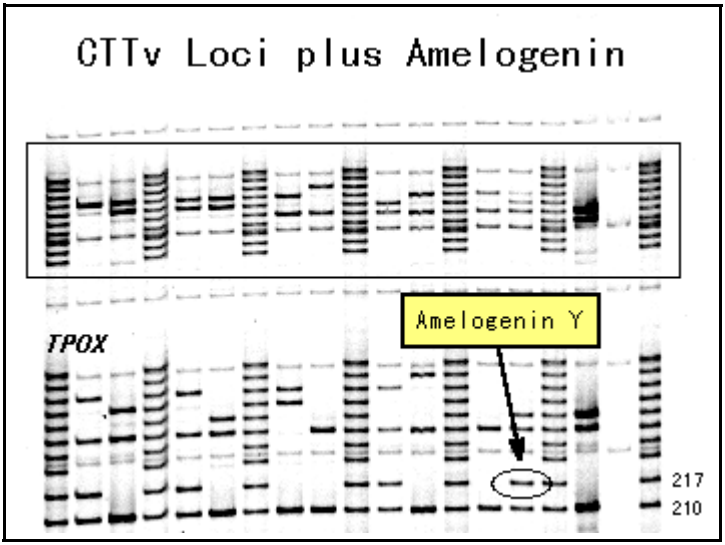
8. To return to the original magnification, click the Reset Magnification button  .

9. To copy the image to the clipboard, click the Capture Image button  .

8 Drawing Tools

You may use the Drawing tools before or after analysis functions to create text and graphics elements for inclusion any document or overlay trace. With the these tools, you can do the following:

- Call attention to faint or targeted bands.
- Include comments with the scanned image.
- Package experimental results with a professional appearance.



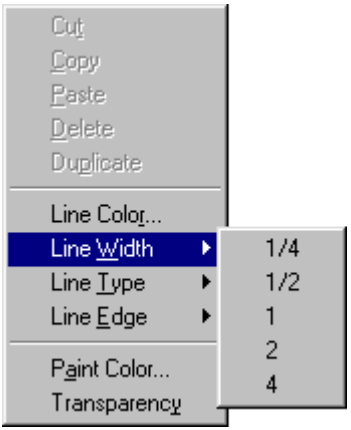
Draw Menu and Tools

1. To use the drawing tools, click the Drawing Tool button  or choose Drawing from the Tools menu. The Draw menu appears in the menu bar and the Drawing tool buttons are activated.

2. In the View menu, choose to display the Draw 1, Draw 2, Draw 3, and Text Attribute toolbars.

Right-click Menu

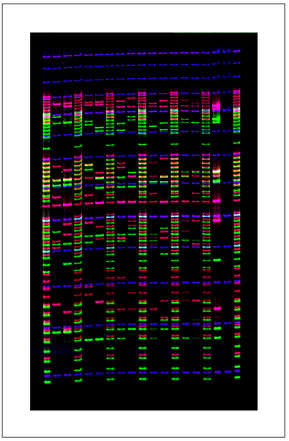
You can also choose the line and paint attributes by placing the cursor over the window and right-clicking the mouse.



Setting the Margins

To create a margin for annotations around the image:

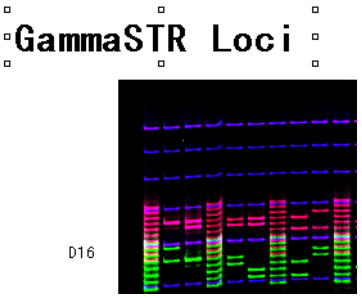
1. Click the Paper Position  . The Comments Area Settings dialog box is displayed.
2. Type in the desired top, bottom, left, and right margins in pixels, and Click OK. The margins are added to the image.



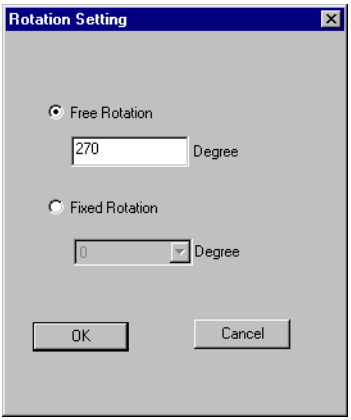
Rotating Text

To rotate text boxes:

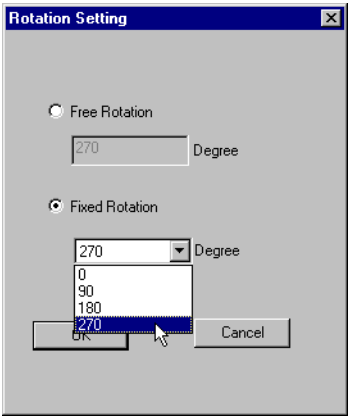
1. Select the text box you want to rotate.



2. Click the Rotate text... button  . The Rotation Setting dialog box is displayed.

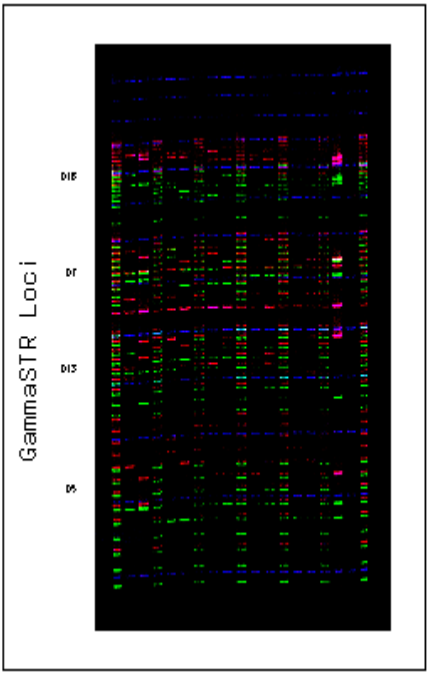


3. Choose to enter a free rotation angle or select a fixed rotation from the drop-down list box, and specify the angle.

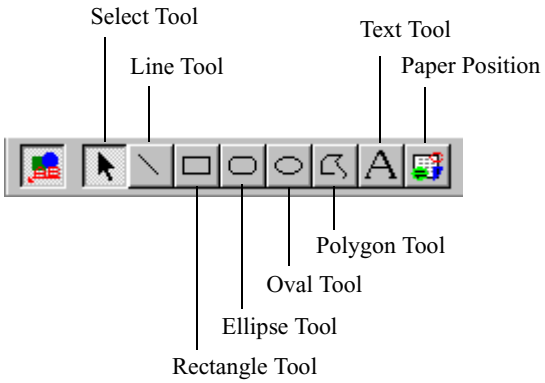


The text box is rotated clockwise through the angle.

4. Position the text box as desired.

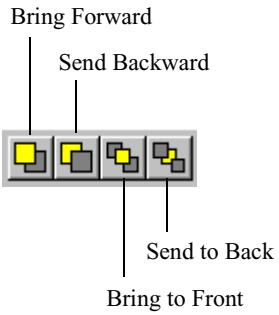

GammaSTR Loci
D18
D7
D13
D5

Draw Tools

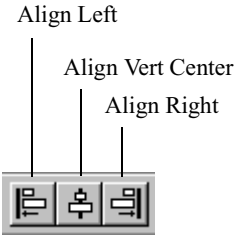


Tool	Draw 1 Function
Select Tool	Select a figure. To select multiple figures, click while pressing the Shift key.
Line Tool	Draws lines.
Rect Tool	Draws rectangles.
Ellipse Tool	Draws an ellipse (rectangle with rounded corners).
Oval Tool	Draws ovals.
Polygon Tool	Any shape bordered by straight lines. Click to establish the point for the beginning of each straight line. Double-click at the end of the last point.
Text Tool	Drag to create a text box, and then type text.
Paper Position	Set the width of the top, bottom, left, and right margins around the image.

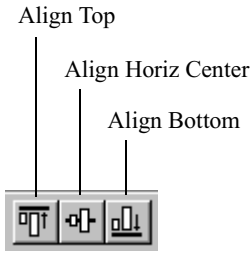
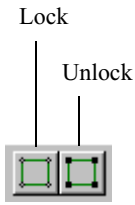
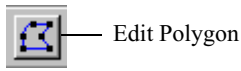
Tool	Draw 2 Function



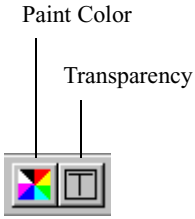
Bring Forward	Moves selected figure forward one layer.
Send Backward	Moves selected figure back one layer.
Bring to Front	Brings selected figure to top layer.
Send to Back	Sends selected figure to back layer.



Align Left	Aligns selected figures left.
Align Vert Center	Centers selected figures vertically.
Align Right	Aligns selected figures right.

Tool	Draw 2 Function
	
Align Top	Aligns selected figures along top edges.
Align Horiz Center	Centers selected figures horizontally.
Align Bottom	Aligns selected figures along bottom edges.
	
Lock	Locks selected figure in position.
Unlock	Unlocks selected figure.
	
Edit Polygon	Drag apex handles to change polygon shape.

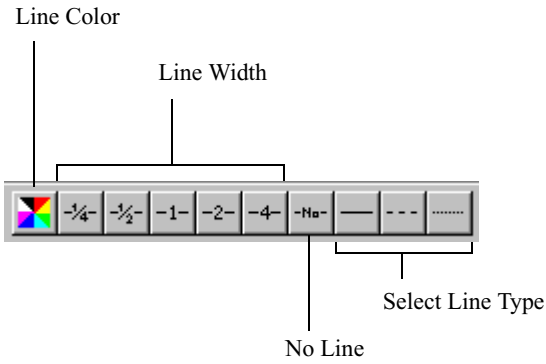
Tool	Draw 3 Function



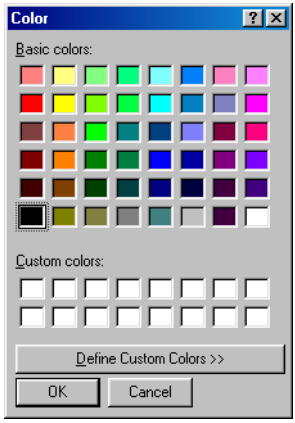
Paint Color — Set color of shape fill.

Transparency — Makes paint color transparent.

Tool	Draw 3 Function

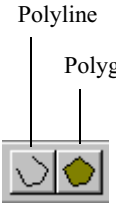


Line Color	Set line color.

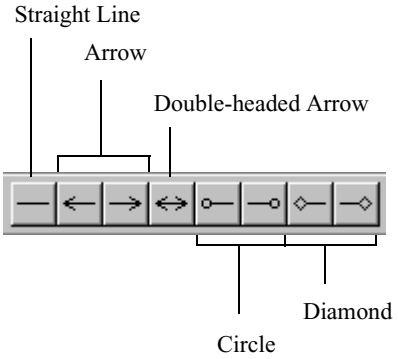


Line Width	Set thickness of line.
No Line	Makes line is transparent.
Line Type	Choose solid line, dashed line, or dotted line.

Tool	Draw 3 Function

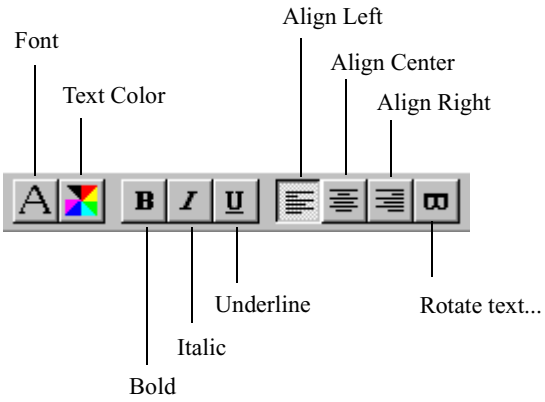


Polyline	Draw lines connected at different angles.
Polygon	Draw closed polyline.



Line marker styles	Select arrows, circles, or diamonds to mark line endpoints.

Tool	Text Attributes Function



Font	Sets text font and size.
Text Color	Sets color of text.
Bold	Bold text.
Italic	Italic text.
Underline	Underline text.
Align Left	Align text lines left.
Align Center	Center text lines.
Align Right	Align text lines right.
Rotate text...	Rotate text.

Working with Draw Objects

- To select an object, click on it with the cursor to select it. A selected object has rectangles at its four corners.
- To move an selected object, drag it to the new location.
- To change the size of a selected object, click one of the corner handles and drag it. To make the size change proportional, hold the Shift key as you drag the corner.
- To delete a selected object, press the Delete key.

9 Maintenance

Cleaning

WARNING! **Always turn the power switch OFF before performing maintenance.**

To clean the exterior surfaces of the unit, use only a clean cloth or sponge moistened with mild detergent solution. Wipe dry with a cloth. Never use benzene, paint thinner or other organic solvents for cleaning purposes, and never apply adhesives or other chemicals to the exterior surfaces of the unit. Doing so could result in discoloration and deformation of the covers, with consequent loss of laser beam protection.

Never attempt to clean, adjust or perform any other maintenance operation on the laser head, the photomultiplier tube, or any other optical, mechanical or electrical component of the unit.

CAUTION! Accumulation of dust in the interior of the scanning unit has been known to cause fires. Ask your service representative to conduct regular maintenance at least once a year.

Service

In the event that fire, flood, earthquake or other adverse environmental incident affect the instrument, contact your Hitachi Software Engineering representative for inspection and service. Do not attempt to reinstall, relocate, or adjust the FMBIO II instrument.

The FMBIO II unit should be serviced annually by Hitachi Software Engineering service personnel.

Laser Head

Never attempt to replace the laser head or make any other mechanical repairs or adjustments to the equipment.

Photomultiplier Tube

The photomultiplier tube settings are carefully adjusted before shipping. Do not attempt to change or adjust the photomultiplier tube settings. If any defective operation occurs, such as a noticeable decrease in signal power, contact a Hitachi Software Engineering service representative.

Operating Temperature

Do not use the scanning unit in a location where the surrounding air temperature exceeds 30°C (86°F). At ambient room temperatures greater than 30°C, the scanning unit may fail to emit the laser beam, making it impossible to produce an image. Should the laser fail due to excessive room temperature, place the power switch in the OFF position, and allow the scanning unit to cool. If the room temperature is close to 30°C, use an air conditioner to lower the ambient temperature. The minimum operating temperature is 10°C (50°F).

Moving the Scanning Unit

The unit's optical scanning device is a precision instrument. Do not relocate or move the scanning unit without prior authorization from Hitachi Software Engineering, or risk voiding your warranty.

Shipping

The FMBIO II unit is a precision device that weighs approximately 50 kg. When transporting it, you must protect it from impact and torque. Pack it in a sturdy shipping container with a rigid structure and ample internal cushioning. Transport it on a sturdy cart. The equipment must always stay upright; never tip it on its side or on its end.

Shutdown

When the FMBIO II unit is not to be used for an extended time, turn off the Main switch on the rear panel, and disconnect the power cord.

Troubleshooting the Scanning Unit

At the first sign of a problem with the operation of the scanning unit, check the red Error light on the front panel.

Red Error Light Flashing

1. Turn off the Power switch and wait 5 seconds.
2. Turn on the Power switch and wait for the FMBIO II self-diagnostic routine to end.
3. If the Error light continues to flash, call Hitachi Software Engineering service personnel.

Responding to Problems

WARNING! **Shutdown the Scanning Unit at the first sign of trouble. If the Scanning Unit emits an unusual smell, smoke, noise or excessive heat, immediately turn the power switch Off, disconnect the power cord.**

If the Scanning Unit emits an unusual smell, smoke, noise or excessive heat:

- Immediately turn the power switch to OFF.
- Disconnect the power cord.
- Contact your nearest authorized Hitachi Software Engineering Service representative.

If you continue to use the scanning unit under these circumstances, fire or electric shock may result.

Water Spills Inside the Scanning Unit

If there is a spill:

- Immediately turn the power switch to OFF.

- Disconnect the power cord
- And contact your nearest authorized Hitachi Software Engineering Service representative.

If you continue to use the scanning unit under these circumstances, fire or electric shock may result.

Foreign Objects In the Scanning Unit

If foreign objects are discovered or may have dropped inside the equipment:

- Immediately turn the power switch to OFF.
- Disconnect the power cord.
- And contact your nearest authorized Hitachi Software Engineering Service representative.

If you continue to use the scanning unit under these circumstances, fire or electric shock may result.

Equipment or Power Cord is Damaged

If the Scanning Unit or the Power Cord is damaged:

- Immediately turn the power switch to OFF.
- Disconnect the power cord.
- Contact your nearest authorized Hitachi Software Engineering Service representative.

If you continue to use the scanning unit under these circumstances, fire or electric shock may result.

Never under any circumstances repair the equipment by yourself.

Unknown Cause of Damage

If the cause of damage is unknown, and the prompting image fails to appear, then refrain from using the Scanning Unit.

- Immediately turn the power switch to OFF.
- Disconnect the power cord.
- Contact your nearest authorized Hitachi Software Engineering Service representative.

10 Allele Calling with STaR Call

STaR Call™ Genotyping Software converts base-pair values generated by Image Analysis to allele names. STaR Call takes advantage of the quantitation features in Image Analysis to perform automated genotype calling and analysis of artifact (“stutter”) bands. First, the calculated band sizes are imported into STaR Call. STaR Call then compares these values with a selected STR Lookup table that contains size ranges for each allele. Based on comparison of band sizes with allele ranges, bands are assigned a locus name and repeat number. Weak bands occurring in the stutter position are identified as artifacts and are excluded from the final output. Genotype data generated by STaR Call are easily converted to common message format (CMF) files for export to the Combined DNA Index System (CODIS) forensic database.

This chapter discusses how to apply STaR Call to evaluate alleles and stutter-band artifacts. If you are interested in evaluating STaR Call Genotyping Software, or obtaining a quotation, please contact your FMBIO Sales Representative.

Installing the STaR Call Software

The STaR Call software is available for both the PC and Macintosh. Both versions require that Excel be previously installed. The Macintosh version will run with either Excel 5 or Excel 98. The PC version will run under either Windows 95 or Windows 98.

STaR Call Installation - Windows Version

Requirements

- At least 2 MB of free memory to load the STaR Call macro and the data file.
- Microsoft Windows 95 or Windows 98, with Excel 95 or Excel 97. Consult the documentation that came with the Microsoft Excel software for Microsoft Excel's memory requirements.

Installing STaR Call

Note To install and use the STaR Call software, you must have Excel loaded on your computer. If Excel is not already installed on your hard drive, install it before attempting to install STaR Call.

1. If you have just installed Microsoft Office or Excel, run Excel once to allow it to complete some automatic configuration steps.
2. Make sure that Excel is closed.
3. Insert the STaR Call disk into the drive.
4. Select the directory corresponding to the version of Excel that is installed; either Excel 95 or Excel 97.
5. According to which version of Excel you have:
   - If you have Excel 95 installed, double click the INSTALL.xla file.
   - If you have Excel 97 installed, double click the install.xla file.
6. If a dialog box appears asking you if you want to enable or disable macros, click the Enable Macros button. The STaR Call Installer dialog box is displayed. Click the Install button.

Starting STaR Call

STaR Call will automatically start whenever you load Excel, and a STaR Call spreadsheet will open.

If you want use Excel for other than STaR Call, you may unload it by selecting Unload from the STaR Call menu. To use STaR Call again you must restart Excel.

If you do not want STaR Call to start each time you run Excel, you may remove it as described below.

Removing STaR Call

1. Make sure that Excel is not running.
2. Locate the Excel startup folder. The Excel startup folder location depends on which version of Office you are using. If you are having trouble finding the Excel startup folder, use the Find application: Click the Start button, select Find > Files or Folders. Then type 'fmbiostr.xla' in the Named field and click the Find Now button.
3. Remove the files *fmbiostr.xla* and *codisdbf.xls* from the startup folder.

STaR Call Installation - Macintosh Version

Requirements

- At least 2 MB of free memory to load the STaR Call macro and the data file.
- Microsoft Excel 5 or Excel 98.

  Consult the documentation that came with the Microsoft Excel software for Microsoft Excel's memory requirements. If you have less memory than is required by Excel, it will be necessary to have the virtual memory turned on. If you have insufficient memory, you may get various error messages. Many error messages occur due to memory allocation problems.

Installing STaR Call

Note To install and use the STaR Call Software, you must have Excel loaded on your computer. If Excel is not already installed on your hard drive, install it before attempting to install STaR Call.

1. If you have just installed Microsoft Office or Excel, run Excel once to allow it to complete some automatic configuration steps.
2. Make sure that Excel is closed.
3. Insert the STaR Call disk into the drive.
4. Select the folder corresponding to the version of Excel that is installed; either Excel_5 or Excel_98.
5. According to which version of Excel you have:
   - If you have Excel_5 installed, double click the INSTALL.xla file.
   - If you have Excel_98 installed, double click the install.xla file.
6. If a dialog box appears asking you if you want to enable or disable macros, click the Enable Macros button. The STaR Call Installer dialog box is displayed.
7. Click the Install button. If you have previously installed STaR Call, a window will be displayed asking if you would like to replace the codisdbf.xls file.
   - Click the Yes button if you would like to replace it (a copy of the old file will be stored in a backup folder located in the Excel startup folder).
   - Click the No button if you want to keep the old version of codisdbf.xls file and continue installing the STaR Call macro.
   - Click Cancel to cancel the installation.
8. At the end of the installation, the following message box will inform you of a successful installation. Click OK.

9. Restart Excel. STaR Call should automatically load when Excel is started. It will appear as a menu item as shown below and the STaR Call spreadsheet will open.

Starting STaR Call

STaR Call will automatically start whenever you load Excel.

If you want use Excel for other than STaR Call, you may unload it by selecting Unload from the STaR Call menu. To use STaR Call again you must restart Excel.

If you do not want STaR Call to start each time you run Excel, you must remove it as described below.

Removing STaR Call

1. Make sure that Excel is not running.
2. Locate the Excel startup folder. The Excel startup folder location depends on which version of Office you are using.

Note If you are having trouble finding the Excel startup folder, use the Find application: Enter Command+F from the desktop, select ‘Find items on local disks’ and ‘name’ and ‘contains’. Then type ‘fmbiostr.xla’ in the ‘contains’ box and click the Find button.

Remove the files *fmbiostr.xla* and *codisdbf.xls* from the startup folder.

Short Tandem Repeats

Short tandem repeats (STRs) are short, repetitive DNA sequences two to seven base pairs long with alleles differentiated by the number of times a sequence is repeated. STR loci may be detected by polymerase chain reaction amplification using labeled primers. Electrophoretic separation is then used to distinguish alleles by size.

STRs are abundant and widely distributed throughout the human genome. They are well characterized and highly polymorphic, making them ideal for use in individual human identification.

The discriminatory power of STR analysis is greatly enhanced by evaluating samples at more than one locus simultaneously (multiplexing). When comparing forensic samples at eight loci, matching probabilities can exceed 1 in 118,000,000. In parentage investigations, multiple-locus analysis can result in paternity probabilities of 0.9979 and higher.

STR data are efficiently analyzed using a unique combination of instrumentation, non-isotopic chemistry, and software that makes processing DNA samples fast, safe, and easy for human identity applications and databasing.

Preparing for Allele Evaluation

Before you can use STaR Call Genotyping Software, you must use 1D-Gel tools in Image Analysis to analyze a gel. Perform the following steps to generate results for allele evaluation.

1. In a one-dye image, adjust the gray level (see page 5-14). In a multicolor project, perform a color separation (see page 5-52).
2. Define the lanes to be analyzed (see page 6-11). STaR Call can accept up to 51 lanes for analysis.
3. Use the Automatic Band Detection tool to define bands (see page 6-11). Check bands and edit as necessary.
4. Register marker lanes, using the Logarithms curve fit (see page 6-13).
5. In the 1D-Gel menu, choose Preferences.... In the Calculation filecard, choose Normalized Volume and Type 4 (see page 5-81).

Note Excel spreadsheets accommodate up to 256 data columns. If the gel image contains many lanes, you may need to limit the number of result types on the table to assure all data is imported into Excel. You can safely eliminate mm, Rf, and IOD%.

6. Click the Volume Calculation  button to generate results (see page 5-88).

Exporting 1D-Gel Analysis Results

1. Click the Open Spreadsheet button  or choose Open Spreadsheet from the 1D-Gel menu or to display the table of analysis results.

   If the image is a multi-color project, Image Analysis displays results for each channel.
2. In the SpreadSheet menu, choose Export....
3. Indicate where you want to save the tab-delimited text file, name the file, then click Save.
4. Save any changes to the project.

Using STaR Call to Evaluate Alleles

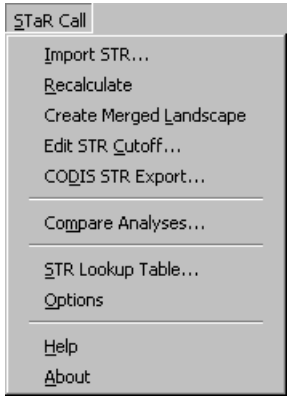
After you analyze a 1D gel and export the results, you are ready to use STaR Call Genotyping Software for allele calling.

1. Open Excel.

   STaR Call appears as a menu on Excel's menu bar, and a table named codisdbf.xls appears.

Important: Do not close the codisdbf.xls table while using STaR Call, because STaR Call uses it for allele calling.

If you do not see the STaR Call menu on the Excel menu bar, choose New from the File menu to make it appear.



Command	Description
Import STR...	Imports up to 6 Image Analysis generated DAT files. You can choose Plain, with OD, or with IOD%.
Recalculate	Recalculates all the DAT files in the selected analysis workbook and assigns alleles to each base pair. Recalculate the DAT files after changing a STR Lookup table.
Edit STR Cutoff...	Allows you to edit the cutoff percentage values for assigning stutter bands.
CODIS STR Export...	Displays two options: • Export v2.0 —exports a STaR Call analysis to a CODIS import file. • Update CODIS values —updates the valid CODIS values using an export file generated from the CODIS system.
Compare Analyses...	Compares the Merged sheets of two analyses workbooks.
STR Lookup Table...	Allows you to create, edit, and delete STR Lookup tables.
Options	Allows you to choose the order of merged allele sorting and whether the phenotype or genotype is listed in the merged landscape worksheet.
Help	Displays the help text.
About	Displays information about the STaR Call program.

Options

To open the options dialog box, choose Options from the STaR Call menu.

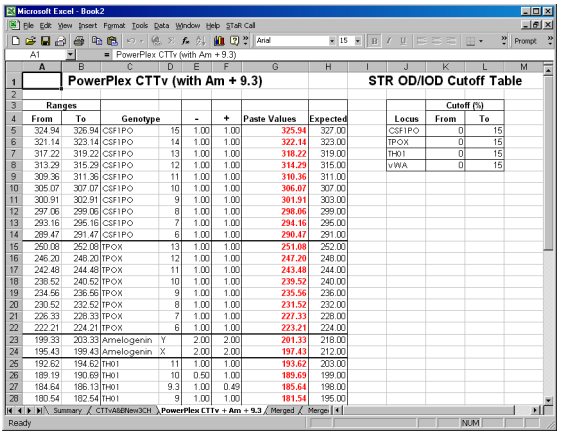
You can choose the sorting order for the alleles in the merged worksheet. The worksheets for the individual DAT files will sort alleles in ascending order.

You can also select whether single values representing phenotypes or duplicate values representing genotypes are displayed in the merged landscape worksheet.

Note If you are exporting data to CODIS, choose Phenotype.

STR Lookup Table

A STR Lookup table is used when evaluating an STR table. The lookup tables are saved in a file called codisdbf.xls.



Title	Description
Ranges	Values derived from the -, +, and Paste Values columns used to assign the allele names.
Genotype	Values determined by the Evaluate STR Data function.
- & +	Variance values used to derive the values in the Ranges column.
Paste Values	Average values for allelic ladders selected in the Evaluate STR Data function are copied to this column and used as the basis for calculating ranges.

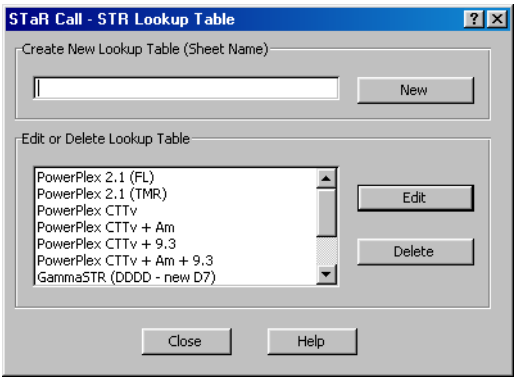
Stutter band reporting. To evaluate stutter, STaR Call calculates the OD (or IOD) ratios of adjacent bands. If a band is in the stutter position (n-4) ***and*** the related ratio falls within a user-defined cutoff range, that ratio is reported in the Percentages column. Information on this band is not exported to CODIS (see page 10-21) and does not appear in the Merged Landscape worksheet (see page 10-20).

- To include a stutter band in either CODIS or the Merged Landscape

worksheet, delete the value in the Percentages column.

- To change the cutoff range, choose Edit STR Cutoff Values from the STaR Call menu.

Creating, editing, or deleting a STR Lookup table.



- In the STaR Call menu, choose STR Lookup Table. The STR Lookup Table dialog box is displayed.

To create a STR Lookup table:

1. Enter a valid sheet name in the Create New Lookup Table field.
2. Click the New button.

To edit a STR Lookup table:

1. In the STaR Call menu, choose STR Lookup Table. The STR Lookup Table dialog box is displayed.
2. Select the STR Lookup Table in the Edit or Delete Lookup Table list box.
3. Click the Edit button.

To delete a STR Lookup table:

1. In the STaR Call menu, choose STR Lookup Table. The STR Lookup Table dialog box is displayed.
2. Select the STR Lookup Table in the Edit or Delete Lookup Table list box.

3. Click the Delete button.

Importing STR files

The Import STR function converts an Image Analysis generated table to a STaR Call table format. It removes all the unnecessary columns such as the migration distance and Rf columns, and adds columns for allele scoring. It also adds rows for CODIS export information as follows:

Row Number	Description
1	The Lane name generated by Image Analysis
2*	Specimen Number
3*	Sample ID
4*	Specimen Category
5*	Tissue Type
6*	Tissue Form
7*	Population Group
8	Include/Exclude lane.
9	Sub Headings
10	Start of bp and Genotype values (OD & Percentages).

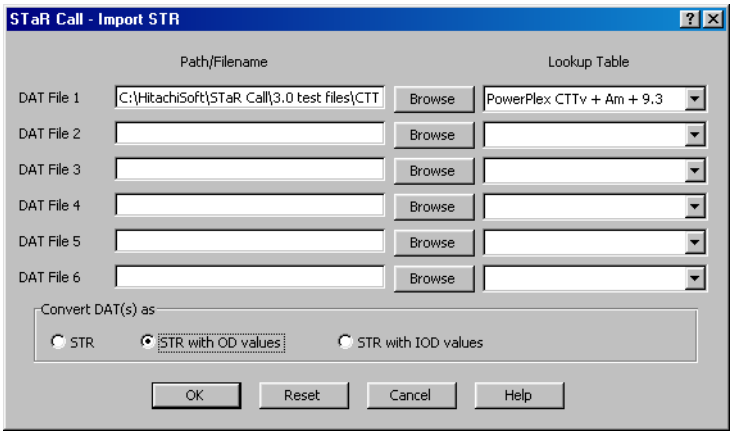
* Actual values are used when exporting to CODIS.

To import an STR file:

1. In the STaR Call menu, choose Import STR....

Note Always use the Import command to open the Image Analysis results file in STaR Call. Errors occur when you use the Open command to open a saved results file.

2. In the Import STR dialog box, click the Browse button.



3. Locate the file you want to evaluate.
4. Select a STR Lookup Table in the drop-down list box.
5. Repeat the process to add more files.
6. Select a conversion method:

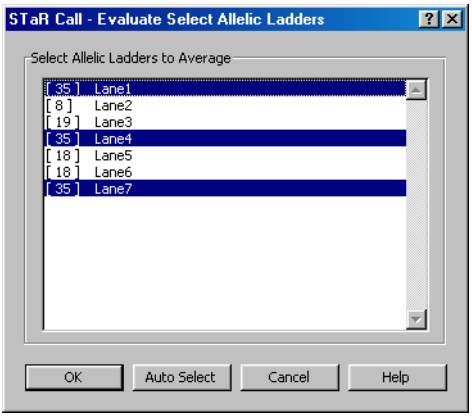
   STR (plain) —Uses base-pair sizes to call alleles. Deletes all columns except for the bp column.

   STR with OD values —Uses base-pair sizes to call alleles, and OD values to calculate n-4 stutter percentages. Deletes all columns except for the bp column and the OD column.

   STR with IOD values —Uses base-pair sizes to calls alleles, and IOD values to calculate n-4 stutter percentages. Deletes all columns except bp column and the OD column.
7. Click OK to import the files. The Evaluate Select Allelic Ladders dialog box is displayed.

Note Do not choose Recalculate. No recalculation of values can occur until the STR files are imported.

A dialog box displays lanes along with the number of bands in each lane. You are prompted to choose the lanes containing allelic ladders for averaging. The default selection is all of the lanes that contain the highest number of bands. The default choices are highlighted.



Note This is a good time to verify that the correct number of bands are in the chosen lanes. The number in parentheses preceding the lane number is the number of bands in the lane.

The allelic ladder dialog box offers three ways to select allele ladders:

a. Click the Auto Select button to let STaR Call choose the lanes with the most bands, as in the example shown here. The average of these ladders becomes the standard for comparison.

b. Click on one allelic ladder to select it as the standard for comparison.

c. Press and hold down the Ctrl key as you click more than one allelic ladder. These ladders will be averaged and the average used as the standard for comparison.

8. Click OK to complete allelic ladder selection.

STaR Call puts the average base-pair values of the allelic-ladder standards into the STR Lookup table. These allelic standard values appear in the Paste Values column. Ranges are then generated using the “Paste Values” for the allelic ladder and the values from the “-” and “+” columns.

After the STR Lookup table is filled, the current table is evaluated. Each base pair value is compared to the ranges in the lookup table.

If the base pair value is within the range for an allele, the corresponding allele is entered into the Genotype column. Otherwise, “Not in range” is displayed.

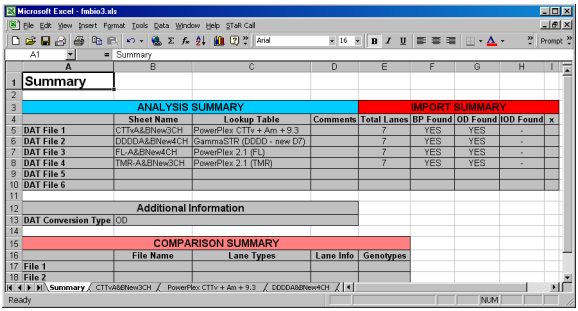
If you evaluate STR data with OD or IOD values, stutter percentages are entered under the Percentages column for all values that fall within the stutter cutoff range assigned for that locus.

Note If a band is “not in range,” it is not included in the stutter calculations.

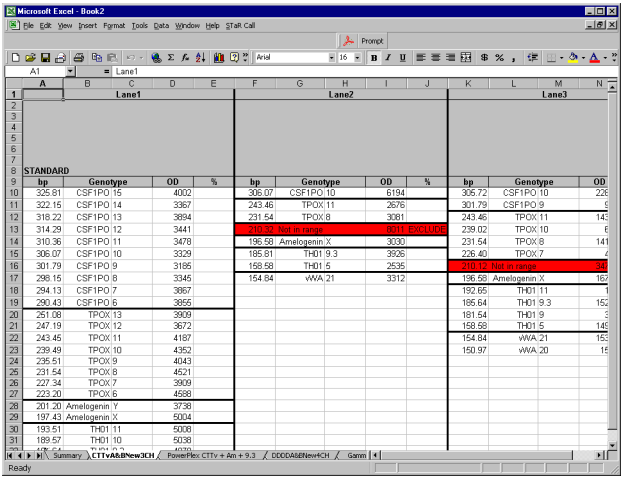
After the evaluation is complete, a workbook is created containing worksheets containing data from the individual DAT files, the corresponding allele evaluations with the selected lookup tables, and merged worksheets containing results from all the DAT file.

To display a worksheet click on its tab at the bottom of the window. If you cannot view the entire worksheet, click on the arrows in the scroll bar at the bottom right of the window. If you cannot see the tab for a worksheet, click on the arrows at the bottom left of the window. The following types of worksheets are created:

Summary Worksheet. Provides a summary of the analysis, STR import, and any comparison performed using the Compare Analysis function.

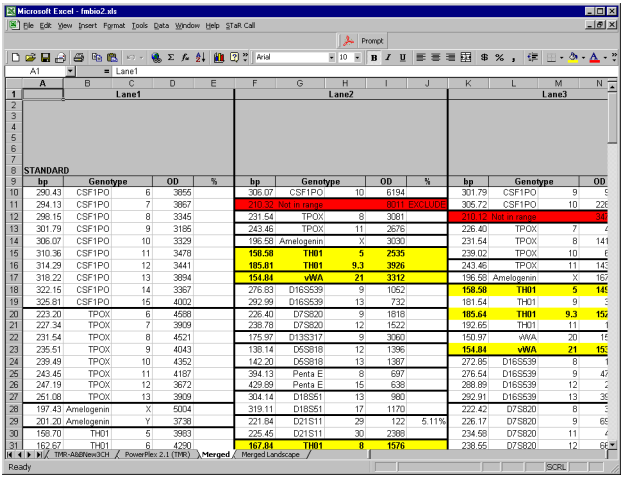


Analysis Worksheet. Provides allele evaluation using the selected STR Lookup table.



Merged Worksheet. Merges data from all analyses. Differences in the data among the different analyses are highlighted.Differences in the data among the different analyses are highlighted. Excluded alleles are highlighted in

red. Non-agreeing allele calls in overlapping loci between the PowerPlex 1.1 and PowerPlex 2.1 systems are highlighted in yellow.



bp	Genotype		OD	%	bp	Genotype		OD	%
306.07	CSF1PO	10	6194		301.79	CSF1PO	9	975	4.27%
210.32	Not in range		8011	EXCLUDE	305.72	CSF1PO	10	22860	
231.54	TPOX	8	3081		210.12	Not in range		34764	EXCLUDE
243.46	TPOX	11	2676		226.40	TPOX	7	486	3.43%
196.58	Amelogenin	X	3030		231.54	TPOX	8	14157	
158.58	**TH01**	**5**	**2535**		239.02	TPOX	10	611	4.25%
185.81	**TH01**	**9.3**	**3926**		243.46	TPOX	11	14361	
154.84	**vWA**	**21**	**3312**		196.58	Amelogenin	X	16734	
276.83	D16S539	9	1052		**158.58**	**TH01**	**5**	**14921**	
292.99	D16S539	13	732		181.54	TH01	9	396	2.60%
226.40	D7S820	9	1818		**185.64**	**TH01**	**9.3**	**15241**	
238.78	D7S820	12	1522		192.65	TH01	11	144	
175.97	D13S317	9	3060		150.97	vWA	20	1530	9.96%
138.14	D5S818	12	1396		**154.84**	**vWA**	**21**	**15358**	

You can edit values for “Not in range” alleles, e.g., microvariants, in the merged worksheet.

1. Click on the cell containing the data you want to edit.
2. Type the value.

3. Click on another cell in the sheet.

After you have finished editing the worksheets, choose Created Merged Landscape from the STaR Call menu. The new merged landscape worksheet will reflect the current values in each worksheet.

Merged Landscape. Shows a summary of allele calls. It does not display the following:

- Lanes labeled as allelic ladders.
- Lanes marked Exclude in the CODIS portion of STaR Call.
- Bands with values reported in the Percentages column (stutter bands)
- Bands marked “Not in range”.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1			Population	CSF1PO		TPOX		Amelogenin		TH01		vWA		D16S539		D7S820	
2	Lane No.	Specimen No.	Group	1	2	1	2	1	2	1	2	1	2	1	2	1	2
3	Lane2			10	10	8	11	X	X	5	9.3	21	21	9	13	9	12
4						8	11			8	11	18	19				
5	Lane3			10	10	8	11	X	X	5	9.3	21	21	9	13	9	12
6						8	11			11		18	19				
7										8	11						
8	Lane5			10	10	8	11	X	Y	5	9.3	21	21	9	13	9	12
9						8	11			8	11	18	19				
10	Lane6			11	13	8	8			9	9.3	18	20	9	11	8	10
11						8	8			7	8	15	17				
12																	

4. Carefully evaluate bands that STaR Call has not assigned to a genotype. Some “Not in range” bands may be only slightly outside the allele range. You can correct these calls as needed.

Adding an STR

1. Select the analysis workbook.
2. Choose Import STR... from the STaR Call menu.
3. Click Yes when asked if you want to add a file to the current analysis.
4. In the Import STR dialog box, click any available Browse button to select a file.
5. Select a STR Lookup Table in the drop-down list box.

Recalculating Results

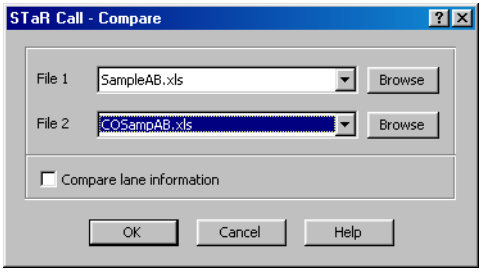
To recalculate results:

- Choose Recalculate from the STaR Call menu. STaR Call copies the STR Lookup tables, evaluates the STR files, and creates the Merged and Merged Landscape worksheets.

Comparing Analyses

To compare two analyses:

1. Choose Compare Analyses... from the STaR Call menu. The Compare dialog box is displayed.



2. Select the two analysis workbooks in the drop-down list boxes. Click the Browse button to open a workbook.
3. Select the Compare lane information option if required.
4. Click OK.

Printing and Saving Results

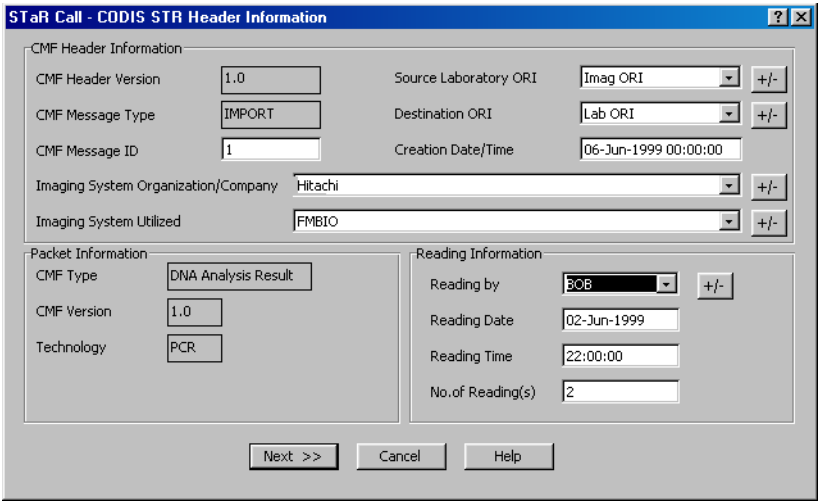
Your evaluation is now complete. You may print and save the STaR Call table of genotypes and stutter percentages. Save the file either as a tab-delimited text file or an Excel workbook.

Exporting Data to CODIS

You can add CODIS information to the table of STaR Call results and prepare a file to upload your allele calls to the CODIS database.

To export to CODIS:

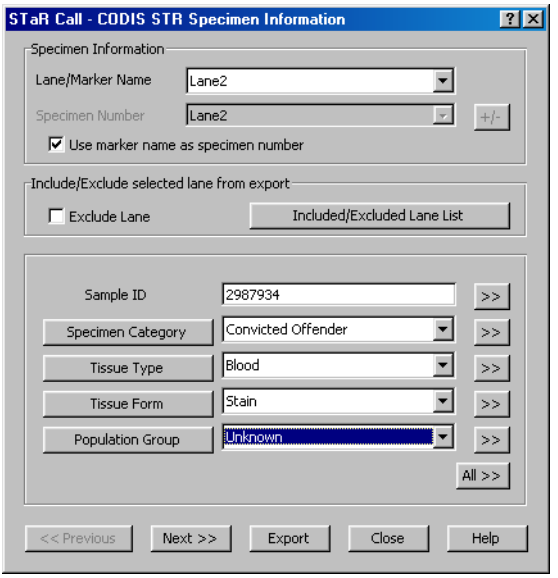
1. In the STaR Call menu, choose CODIS STR Export.... The CODIS STR Header dialog box appears.



Title	Description
CMF Header Version	This is the Common Message Format header version.
CMF Message Type	This is the message type.
CMF Message ID	Accepts numeric values only.
Imaging System Organization/Company	You can enter up to 64 alphanumeric characters.
Imaging System Utilized	You can enter up to 64 alphanumeric characters.
Imaging System ORI	You can enter up to 10 alphanumeric characters.
Laboratory ORI	You can enter up to 10 alphanumeric characters.
Creation Date/Time	Use the format DD-MMM-YYYY HH:MM:SS.
CMF Type	This is the results type.
CMF Version	This is 1.0.
Technology	This is the technique used.

Title	Description
Reading by	You can enter up to 20 alphanumeric characters.
Reading Date	Use the format DD-MMM-YYYY. Reading date must be in 4-digit format.
Reading Time	Use the format HH:MM:SS.
No. of Reading(s)	This accepts numeric values only.

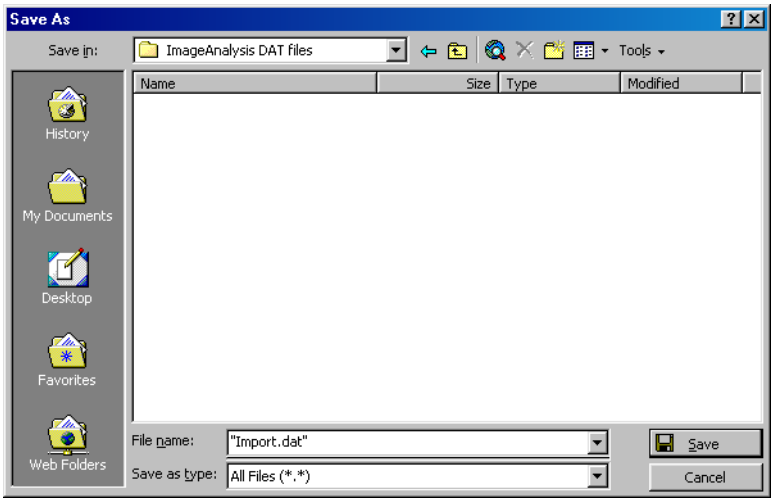
2. Enter the required information. Click the [+/-] button to add or delete list values.
3. Click Next. The CODIS STR Specimen Information Dialog Box is displayed.



Title	Description
Lane/Marker Name	Default is lane number
Specimen Number	You can enter up to 24 alphanumeric characters.
Use marker name as specimen number	Check this box if you want to use the lane/ marker name as the specimen number. Copies row 1 to row 2.
Exclude Lane	Check this box if you want to exclude lane(s) from being exported to CODIS.
Included/Excluded Lane List	Displays a list of lane names where any lanes highlighted will be excluded from export.
Sample ID	You can enter up to 8 alphanumeric characters.
Specimen Category	You must enter a valid CODIS value up to 40 alphanumeric characters long.

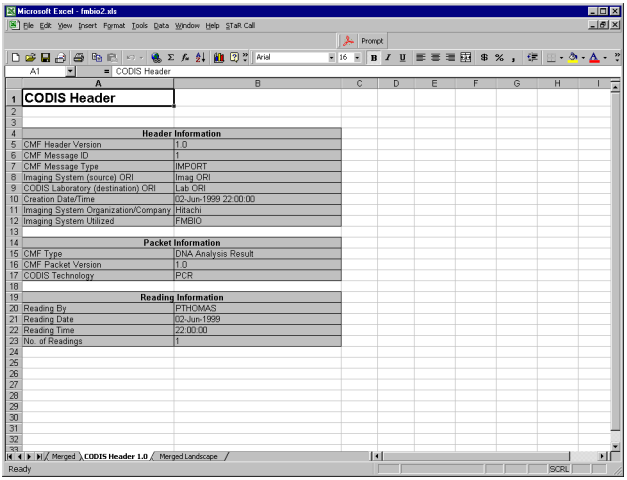
Title	Description
Tissue Type	You must enter a valid CODIS value up to 15 alphanumeric characters long.
Tissue Form	You must enter a valid CODIS value up to 10 alphanumeric characters long.
Population Group	You must enter a valid CODIS value up to 15 alphanumeric characters long.

4. Enter the required information. Click the [+/-] button to add or delete list values.
   - To copy the corresponding control value from the currently selected lane to the remaining lanes, click the [>>] button.
   - To copy the Sample ID, Specimen Category, Tissue Type, Tissue Form and the Population Group values for the currently selected lane to the remaining lanes, click the [All >>] button.
5. Click Export to convert data to a CODIS-compatible data file.



6. Enter a unique name and location.

7. Click Save. A CODIS worksheet is added containing the header information.



CODIS Header	
Header Information	
CMF Header Version	1.0
CMF Message ID	1
CMF Message Type	IMPORT
Imaging System (source) ORI	Imag ORI
CODIS Laboratory (destination) ORI	Lab ORI
Creation Date/Time	02-Jun-1999 22:00:00
Imaging System Organization/Company	Hitachi
Imaging System Utilized	FMBIO
Packet Information	
CMF Type	DNA Analysis Result
CMF Packet Version	1.0
CODIS Technology	PCR
Reading Information	
Reading By	PTHOMAS
Reading Date	02-Jun-1999
Reading Time	22:00:00
No. of Readings	1

The CODIS Specimen Information appears in the Merged worksheet.

Lane2					Lane3				
Lane2					Lane3				
0					0				
Convicted Offender					Convicted Offender				
Blood					Blood				
Stain					Stain				
Unknown					Unknown				
bp	**Genotype**		**OD**	**%**	**bp**	**Genotype**		**OD**	**%**
306.07	CSF1PO	10	6194		301.79	CSF1PO	9	975	4.27%
210.32	Not in range		8011	EXCLUDE	305.72	CSF1PO	10	22860	
231.54	TPOX	8	3081		210.12	Not in range		34764	EXCLUDE
243.46	TPOX	11	2676		226.40	TPOX	7	486	3.43%
196.58	Amelogenin	X	3030		231.54	TPOX	8	14157	
158.58	**TH01**	**5**	**2535**		239.02	TPOX	10	611	4.25%
185.81	**TH01**	**9.3**	**3926**		243.46	TPOX	11	14361	
154.84	**vWA**	**21**	**3312**		196.58	Amelogenin	X	16734	
276.83	D16S539	9	1052		**158.58**	**TH01**	**5**	**14921**	
292.99	D16S539	13	732		181.54	TH01	9	396	2.60%
226.40	D7S820	9	1818		**185.64**	**TH01**	**9.3**	**15241**	
238.78	D7S820	12	1522		192.65	TH01	11	144	
175.97	D13S317	9	3060		150.97	vWA	20	1530	9.96%
138.14	D5S818	12	1396		**154.84**	**vWA**	**21**	**15358**	
142.20	D5S818	13	1387		272.85	D16S539	8	154	3.21%
394.13	Penta E	8	697		276.54	D16S539	9	4792	
429.89	Penta E	15	638		288.89	D16S539	12	266	6.80%
304.14	D18S51	13	980		292.91	D16S539	13	3913	

Appendix A - Software Reference

Keyboard Shortcuts

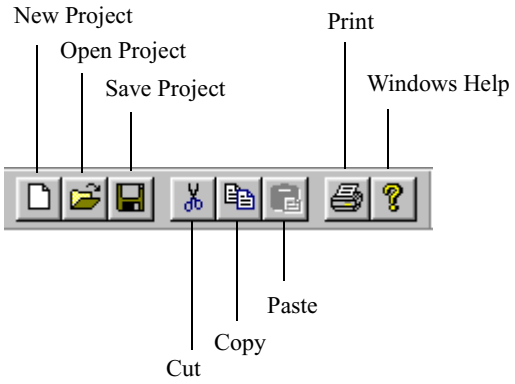
Keyboard shortcuts are a combination of keys that send a command to the computer. Each keyboard shortcut behaves just like one of the commands in the Menu bar. Most keyboard shortcuts use the Control key, represented by the abbreviation Ctrl, and one other key.

For example, to open a project, you can either choose Open Project from the File menu, or you can use the keyboard shortcut. To use the keyboard shortcut to open a project, hold down the Control key and then press the O key. This shortcut is represented as Ctrl+O.

Menu Command	Keyboard Shortcut
Close	Ctrl+W
Copy	Ctrl+C
Cut	Ctrl+X
Duplicate	Ctrl+D
New Project	Ctrl+N
Open Project	Ctrl+O
Paste	Ctrl+V
Print	Ctrl+P
Save Project	Ctrl+S
Select All	Ctrl+A

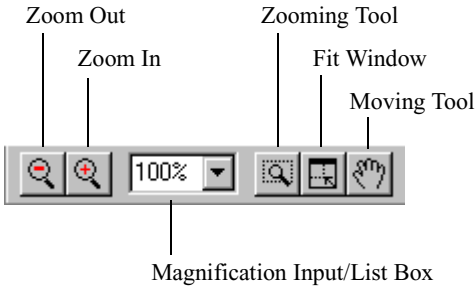
Icons - Image Analysis Tools

File and Edit Tools

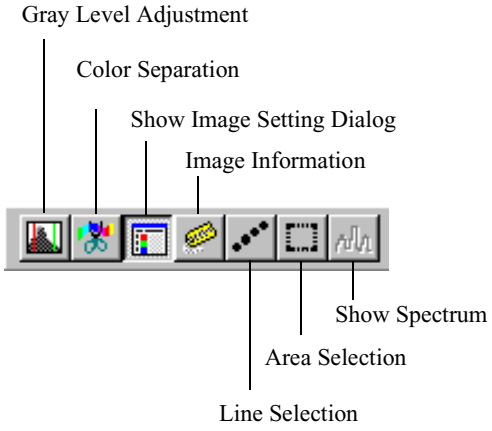


Name	Usage
New Project	Create a new project.
Open Project	Open an existing project.
Save Project	Save a project.
Cut	Remove selected object and store in the clipboard.
Copy	Copy selected object and store in the clipboard.
Paste	Place contents of the clipboard at the cursor.
Print	Print the contents of the active window.
Windows Help	Place the What's This? cursor on an Windows element and click to see Help topic.

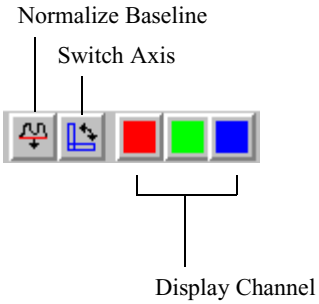
Image Tools



Tool	Description
Zoom Out	Each click reduces the image.
Zoom In	Each click to enlarges the image.
Magnification Input/List Box	Displays current magnification. Enter the desired magnification or select a magnification from the drop-down list.
Zooming Tool	1. Click the button. The cursor transforms into a magnifying glass with a "+" sign. 2. Drag the magnifying glass cursor to create a rectangle surrounding a region of the image. The image is magnified to show the region.
Fit Window	Click to fill the entire window with the image.
Moving Tool	Click the button, and then drag the image.

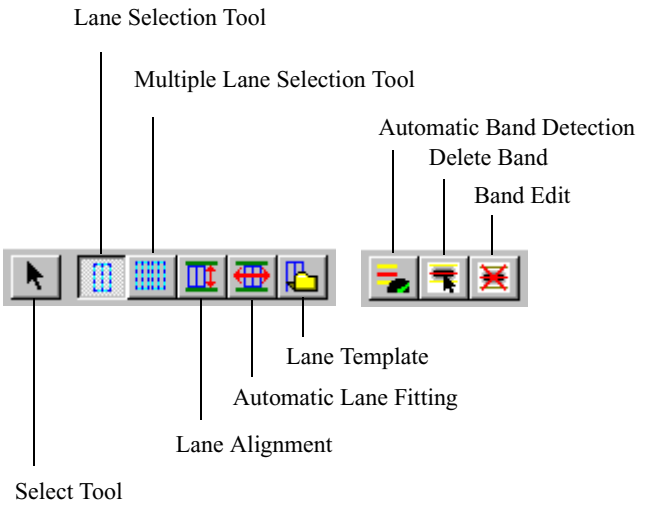


Command	Description
Gray Level Adjustment	See *Gray Level Adjustment* on page 5-14.
Color Separation	See *Color Separation* on page 5-30
Show Image Setting Dialog	See *Image Setting Dialog Box* on page 5-11.
Image Information	View sample, image and scanning information from the FMBIO II ReadImage file.
Export Image	See *Saving an Image* on page 5-7
Transparent Background	See *Transparent Background* on page 5-36
Line Selection	Draw a line to select a location on the image
Area Selection	Draw a rectangle to select an area on the image.
Show Spectrum	See *Spectrum* on page 5-26

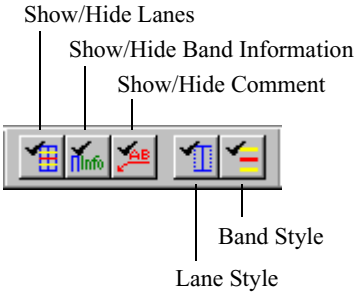


Tool	Description
Normalize Baseline	Normalizes vertical range.
Switch Axis	Switches between the horizontal and vertical spectrum views.
Display Channel	Toggles display of spectrum for individual channels.

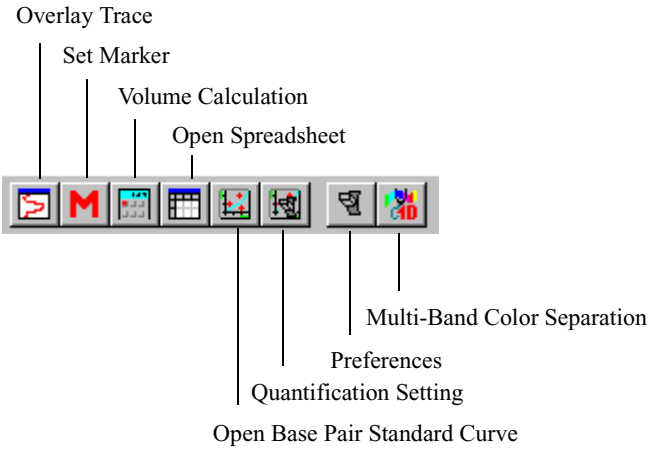
1D-Gel Tools



Tool	Description
Select	Select objects.
Lane Selection	Define a single lane.
Multiple Lane Selection	Define multiple adjacent lanes.
Lane Alignment	Aligns lanes with migration lines.
Automatic Lane Fitting	Automatically adjusts lane boundaries.
Lane Template	Saves all lanes on image.

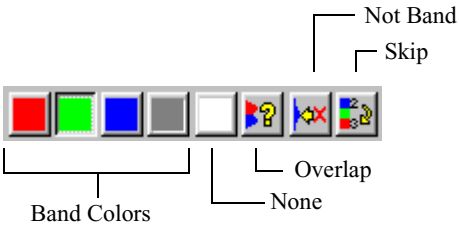


Tool	Description
Show/Hide Lanes	Toggle display of lane boundary lines.
Show/Hide Band Information	Toggle display of band information.
Show/Hide Comments	Toggle display of label information entered in spreadsheet.
Lane Style	Toggle among different display options: center line with lane borders, center lines only, lane boundaries only, and lane display off.
Band Style	Toggle among the different band styles: band display off, peak and range bands displayed, and only peak bands displayed.



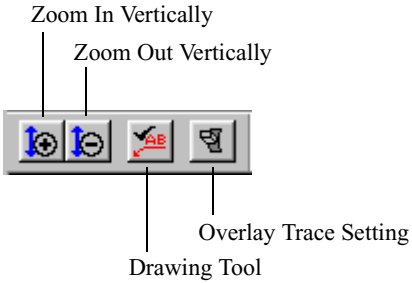
Tool	Description
Overlay Trace	Displays new overlay trace of selected lanes.
Set Marker	Displays Marker Setting window.
Volume Calculation	Performs a volume calculation.
Open Spreadsheet	Displays spreadsheet.
Preferences	Displays Preferences window.
Open Base Pair Standard Curve	Displays BP Standard Curve window.
Quantification Setting	Displays Quantification Setting window.
Preferences	Display Preferences dialog box.
Multi-Band Color Separation	Displays Multi-Band Color Separation window.

Multi-Band Color Separation Tools



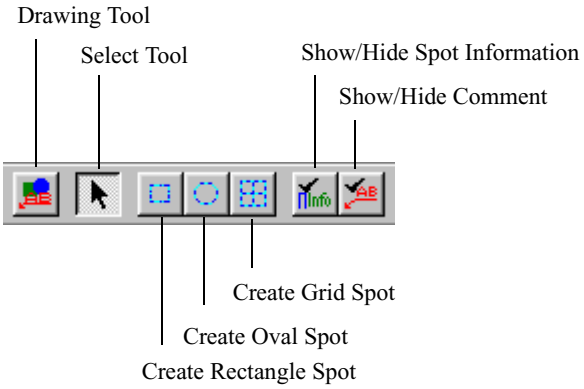
Tool	Description
Band Colors	Select a band color for the selected band.
None	No color is assigned. Does not use band for multi-band color separation.
Overlap	Overlapped band. Does not use band for multi-band color separation.
Not Band	Band is not listed in template. Color list is shifted down.
Skip	The Detected Band numbering skips the band.

Trace Overlay Tools

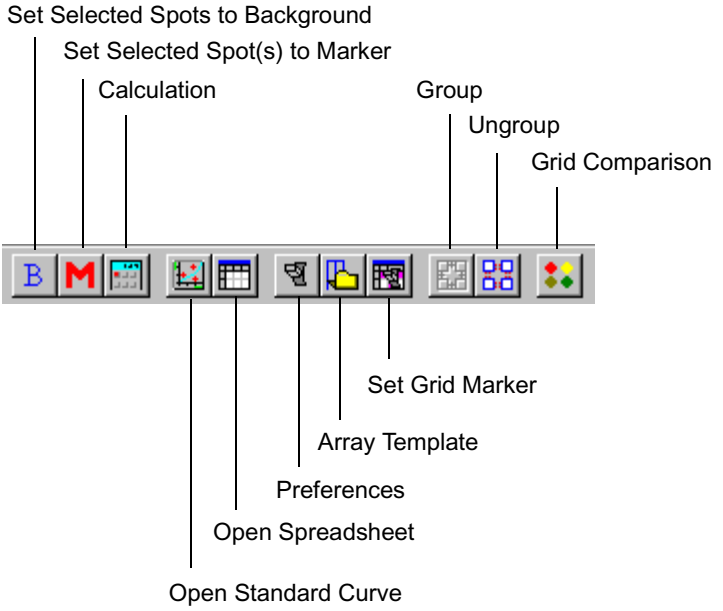


Tool	Description
Zoom In Vertically	Magnify in vertical direction.
Zoom Out Vertically	Reduce in vertical direction.
Drawing	Use drawing tools for Overlay Trace window.
Overlay Trace Setting	Displays Overlay Trace Setting window.

Array Tools



Tool	Description
Drawing	Use to draw text and graphics.
Select	Select objects.
Create Rectangle Spot	Define a rectangular shaped spot.
Create Oval Spot	Define an oval shaped spot.
Create Grid Spot	Define a grid over an array of spots.
Show/Hide Spot Information	Toggle display of spot information.
Show/Hide Comment	Toggle display of label information.



Command	Description
Set Selected Spots to Background	Used selected spots to represent the background value.
Set Selected Spots to Marker	Uses selected spots as the markers.
Calculation	Performs a calculation.
Open Standard Curve	Displays the Standard Curve window.
Open Spreadsheet	Displays the Spreadsheet window.
Preferences	Displays the Preferences window.
Array Template	Save or apply spots and grids.
Set Grid Marker	Displays the Grid Marker Setting window.

Command	Description
Group/Ungroup	Group the selected spots/grids.
Grid Comparison	Opens the Grid Comparison module.

Drawing Tools

For a description of the Drawing tool icons, see Chapter 8, *Drawing Tools*.

Tools Index

This an index of all tool buttons in Image Analysis.

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