Doc-It® Life Science Software

Installation and User Instructions

Doc-It®LS Image Acquisition Software
Doc-It®LS Image Analysis Software

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Introduction

Welcome

Life Science (LS) software from UVP lets users acquire, enhance, and analyze images in a simple and efficient way.

The software is designed to image electrophoresis gels (DNA, RNA, Protein), blots, membranes, plates, plants, and animals. Once the image has been captured with an application-specific camera, it can be saved for record keeping purposes, manipulated for analysis, and annotated to point out key features.

If a software function is grayed out, the function is not available with the version of software loaded on the user's computer.

Getting Started

- Minimum System Requirements
- Registering and Activating the Software

Capturing Images

- Cameras

Performing Analysis Functions on Images

- 1D Analysis
- Molecular Weight Calibrations
- Concentration Calibrations
- Dendrogram Analysis
- Counting Colonies

Minimum System Requirements

- Windows XP Professional with Service Pack-2 or higher (32 bit only), Vista (32 bit only), or Windows 7 (32 bit only)

- Internet Explorer 6.0 or higher [To determine the version of Internet Explorer, open Internet Explorer and click on **Help > About**]

- Intel Pentium Processor or equivalent, 1.6 GHz or higher

- 1 GB of RAM or greater (2 GB recommended)

- 200 MB of available hard disk space for the program, more for data

- To avail the functionality of 21 CFR Part 11, the partition must be formatted with NTFS.

- CD-ROM drive

- Color monitor, supporting at least 1024 x 768 resolution and 16-bit or better colors; 24-bit or 32-bit color is strongly recommended

Registering and Activating the Software

- Overview
- Single-User License
- User Administration

Overview

Use of LS software requires activation of a security code from UVP. Registration and activation of the software can be accomplished by phone, email or by the Internet. Two types of licenses are available, single-user or network-user with a five-user license. Additional licenses are available.

Registering the Software

Activate the software by entering an activation code provided by UVP in order to gain full access rights to the software.

Once the LS software is installed, it will operate in full-future trial mode for 14 days. Within the 14-day trial mode, the LS software must be registered with UVP. Otherwise the software will only operate in demonstration mode after the 14-day trail period. The demonstration mode limits the software to only open and use the demonstration images provided by UVP.

The License Wizard window will appear. There are three ways to retrieve an activation code: phone, email or Internet and selecting one of the options from the License Wizard window.

- **On the Fly Activation**: Choose this option to activate the software immediately through the Internet.

- **Already Have an Activation ID**: This option is useful when reloading (or upgrading) the software after receiving an initial activation code.

- **Offline Activation**: This option is used if the computer is not connected to the Internet. This allows the user to obtain the activation code and enter it at another time

On the Fly Activation

To immediately activate the software through the Internet:

- Choose **On the fly activation.** If the computer is not connected to the Internet, follow the instructions for **Offline activation** or call UVP to register the software.

- Click **Next** to continue.
- Complete all required information on the form.

- Fill out the **Serial Number** located on the CD. The number should be four sets of six numbers.
- Click the **Get Activation No.** button and then click onto **Activate** when the **Activation Number** appears in the box.

Already Have an Activation ID

- Select **Already have an activation ID** from the buttons. This activation function is useful when reloading the software after receiving an initial activation code.
- Click **Next** to continue.
- Click the link provided and complete the form to obtain instructions. Click **Finish**.

Offline Activation

- Select **Offline Activation** if the computer is not connected to the Internet. This allows the user to obtain the activation code and enter it at another time.
- Click **Next** to continue.
- Click the link provided and complete the form to obtain instructions. Click **Finish**.

Single-User License

Single-user license allows the software to be used on a single computer.

A Welcome to the Licensing Wizard window will appear. Select the Single client access license option, and select Next.

User Administration

- Secure User Accounts
- Usernames and Passwords
- User Rights

Secure User Accounts

The concept of User Accounts for individual users is central to the software by providing security of user's data from being tampered with by other users, accidentally or otherwise.

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- This system of usernames and passwords is separate from the one that is used to login to the computer.
- Setting up user accounts is mandatory if support for CFR 21 Part 11 is required from the software.
- If individual accounts are not required, create just one account for all users and give full permissions to that account.

Enable secure user accounts

- Request a **System Administrator** to log into the Windows computer.
- Navigate to C:\Documents and Settings\All Users
- Locate the **Application Data** folder.
- f the **Application Data** folder is not present in the **All Users** window, go to the **Tools** menu and select **Folder Options**.
- A **Folder Options** window appears. On the **View** tab, select **Show hidden files and folders**. Select **Apply** then **OK**.
- Go to **the UVPSettings** folder inside the **Application Data** folder.
- Have the System Administrator set **Read** and **Write** permissions for either the **UVPSettings** folder, or for each file within the folder (depending on the Network Security Policy where the LS software will reside).

User Administration History Definitions

The description of the settings for each column of the **History** section is shown below. To see all of the columns in History, move the scroll bar to right.

Column Heading	Description
User Name	Unique Identification name for a particular user. This could be a name or a word that makes it easier to identify the user.
Date Created	Date the user name was created.
Last Login	Date of last login.
Login Count	Number of times the user has logged in.
Idle Time Lock	Indicates the maximum idle time. To change the idle time, click **Tasks > Set Idle Time.** Zero means no idle time.
Password Expiration Date	Date the password expires.
View	Enables user to view images. To change this setting, click **Tasks > Edit Rights**. Click or unclick the **View** option.
Change	Enables user to change images. To change this setting, click **Tasks > Edit Rights**. Click or unclick the **Change** option.
Has Admin. Rights	Gives user administration permissions. To change this setting, click **Tasks > Edit Rights**. Click or unclick the **Has Administration Rights** option.

Usernames and Passwords

- Start the software. It will bring up a **Login** window.
- The administrator user name will show.

- Enter a password. On initial installation, a screen will pop up to request a password and password confirmation. A password is required.
- Click **Login**.

Adding a New User

- From the menus, click Tools > User Administration to open the User Administration window.
- Click the **New User** button, type in the user name and password.
- Type in the new user name and password. Each time the new user logs in, use that new user name and password.

Editing a User

- Edit the User by highlighting the user name in the **User Administration** window.
- Click the **Tasks > Edit Rights** and select from the **Define User Permissions** screen to allow users to view images, modify images, change templates, or assign administrative rights.
- Click **OK** when changes to the user are complete.

Changing a Password or Other Settings

- To change a password, from the menu go to **Tools > User Administration** to open the **User Administration** window.
- Click on the appropriate user to change.
- Select the **Tasks > Reset Password** and enter the new password. Enter the password again to confirm the change.
- Click **OK**. The change in password will be noted in the **User Administration > History** box.

Deactivating/Reactivating a User

- From the menu, select Tools > User Administration to open the User Administration window.
- Select that user name and click **Tasks > Deactivate**. A red **X** will indicate the user is deactivated. To reactivate, click **Tasks > Activate.**
- Click **OK** to close the window.

NOTE: Never disable the Administration Account.

Viewing the Login History of a User

- Select a user name in the **User Administration** table. The lower half of the window displays the login history associated with the selected user.
- Click **OK** to close the window.

Users Rights

Depending on the privileges for the user that has logged onto windows, the following rights are available to that user for the software:

Login Privileges	Rights				
	Install	Un-Install	Open and Run	User Administration	Use the Camera
Restricted			X*		X
Standard/Power			X*		X
Admin	X	X	X	X	X

X = Supported rights.

Note that even though a user may be able to do things with the software which are outside of this matrix, UVP neither recommends it, nor supports it. For example, the user may try (successfully or otherwise) to uninstall the software as a Power User, but UVP does not provide support for problems arising during or due to that action.

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If located in North America, South America, East Asia or Australia:

- **Call (800) 452-6788 or (909) 946-3197**, and ask for **Technical Support** during regular business days, between 7:00 am and 5:00 pm, PST.
- **E-mail** your message to: info@uvp.com or techsupport@uvp.com
- **Fax** Technical Support at (**909) 946-3597**
- **Write to:** UVP, LLC 2066 W. 11th Street, Upland, CA 91786 USA

If located in Europe, Africa, the Middle East or Western Asia:

- **Call +44(0) 1223-420022**, and ask for **Customer Service** during regular business days between 8:30 am and 5:30 pm.
- **E-mail** your message to: uvp@uvp.co.uk
- **Fax** Customer Service at +44(0) 1223-420561
- **Write to**: Ultra-Violet Products Ltd. Unit 1, Trinity Hall Farm Estate, Nuffield Road, Cambridge CB4 1TG UK

License Agreement

End User License Agreement

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Navigating the Software

- [Main Window](#) - Shows the full software screen
- [Menus](#) - Discusses the menus available
- [Toolbars](#) - Discusses the tollbars and the ability to rearrange the tool buttons
- [Plug-In Modules](#) - Several plug-in modules are provided as user-friendly interfaces for specific software tasks; this section discusses the various modules and the ability to position the modules
- [Image Windows](#) - Discusses the use of image windows in the workspace

Main Window

After the LS software is opened for the first time, the screen will look similar to the one shown below. The LS Main Window contains the application's menu bar, toolbars, image windows, plug-in modules and status bar. Some parts of the window, such as the toolbars, plug-in modules and status bar, can be hidden or shown if preferred.
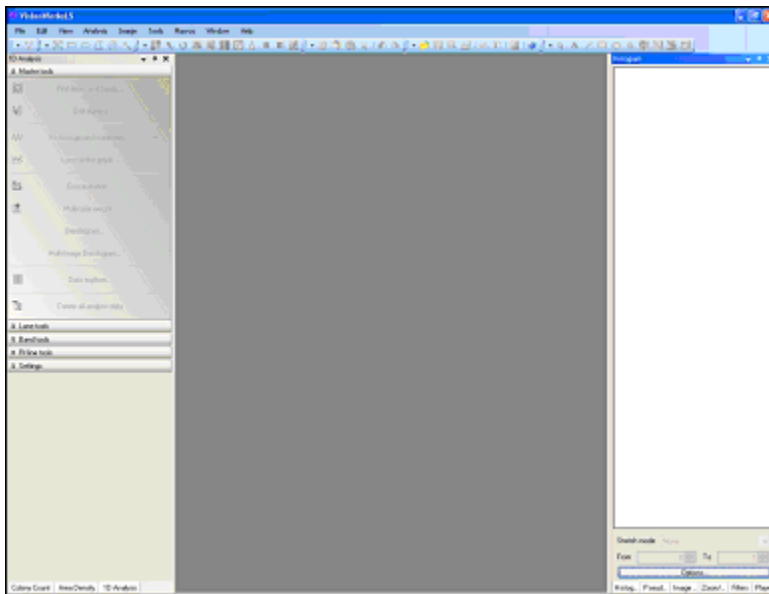
Menus

LS Software offers the following menus:

- *File Menu*: Contains commands to load and to save files, to select profiles, adjust preferences and to print reports.
- *Edit Menu*: Contains the Undo/Redo commands and the clipboard commands: **Cut**, **Copy**, **Paste**, **Paste Special** and **Paste Special Options**, plus the **Crop** function.
- *View Menu*: Contains commands that show and hide various main-window features and commands that affect how the current sub window is displayed.

NOTE: Although most commands appear on the menus, some features are only available through the Plug-in Modules. If the Plug-in Modules are hidden, they can be shown by selecting the modules from **View > Plug-ins** whenever needed.

- *Analysis Menu*: Contains modules to perform Colony Counting, 1D Analysis and Area Density.

- *Tools Menu*: Contains a list of the tools to enhance the image or software environment including seeing Loaded Plug-ins, User Configuration, pre-configured image Reports, plus Region of Interest, Spatial Calibration and Annotation tools.

- *Macros Menu*: Contains commands to work with Macros. A Macro is a collection of commands that can be saved and replayed with special user-defined keys.

- *Window Menu*: Contains commands to organize Image windows. Shows the arrangement of the image window and shows the name of the images currently open.

- *Help Menu*: Contains access to the software help, user license wizard and software version.

Toolbars

The toolbars in LS allow the user to select most commands with a single button click. The toolbars are customizable, so the user can include the commands used most and remove commands rarely used.

Toolbar Buttons
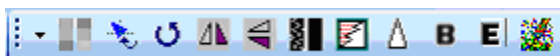
- *Reduce to Mono*: Converts the image to a single-channel (monochrome) image from a multi-channel (color) image

- *Align:* Aligns the image to a grid

- *Rotate*: Brings up the dialog box to rotate image by a specific degree or by manual rotation

- *Flip Horizontal*: Flips image horizontally

- *Flip Vertical*: Flips image vertically

- *Despeckle:* Applies the despeckle filter to the image

- *Remove Noise:* Applies the remove noise filter to the image

- *Sharpen:* Applies the sharpen filter to the image

- *Blur:* Applies the blur filter to the image

- *Emboss:* Embosses the image with selection of the direction of the emboss filter

- *Starfield Subtraction:* Applies the starfield subtraction filter to the image

- *Copy:* Copies selected text, selected portions of the current image or the entire image to the clipboard

- *Paste:* Pastes the current clipboard item onto the screen

- *Paste Special:* Pastes an overlay of the current item in the clipboard

- *Cut:* Cuts a specific area
- *Undo:* Reverses the last command
- *Redo:* Repeats the last command

- *Arrow:* Selects an annotation to edit
- *Text Tool:* Text annotation
- Line, Rectangle, Ellipse and Highlighter Tools: Annotation tools
- *Define Image Scale:* Allows users to set the scale for images
- *Measure Length:* Measures the distance between any two points on the image, units depend on spatial calibration
- *Measure Angle:* Measures an angle
- *Measure Area:* Measures an area on the image

- *New ROI:* Removes the active Area of Interest and prepares for a new one of the current type
- *Rectangular ROI*: Changes the current mouse tool to the select Rectangular Area of Interest and brings up one if already present on the current image
- *Elliptical ROI*: Changes the current mouse tool to the select Elliptical Area of Interest and brings up one if already present on the current image
- *Polygonal ROI:* Changes the current mouse tool to the select Polygonal Area of Interest and brings up one if already present on the current image
- *Freeform ROI:* Changes the current mouse tool to the select a user defined Area of Interest
- *Magic Wand ROI:* Selects a consistently colored area (for example, a red flower) without having to trace its outline
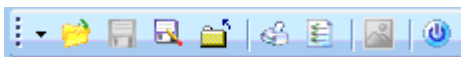
- *Open*: Opens an image file on disk
- *Save*: Saves the current image to disk
- *Save As*: Saves the current image to a different name
- *Close*: Closes the file
- *Print*: Prints the current image
- *Preferences*: Opens the preferences window to set defaults in the following tabs: Main Settings, Log Settings, 1D Analysis, Colony Count settings
- *External Application*: Launches a Qwik-Link external application selected in Preferences
- *Exit*: Closes the software program

Rearranging Toolbars

The order of the individual toolbar sections can be rearranged by placing the mouse arrow over the dotted line at the edge of each toolbar section and moving the toolbar. The default position of the toolbars is located horizontally below the menus. The toolbars may be moved to the bottom of the screen or vertically on the left or right side. Each section can be deleted or added back by clicking **View > Toolbars** and select the toolbars to display on the screen.

Image Windows

Each open image in LS workspace will appear in a separate Image Window. Several Image Windows can be opened at one time. The window below shows several images open, layered with tabs (tabbed interface turned ON in Preferences) for selection of images.
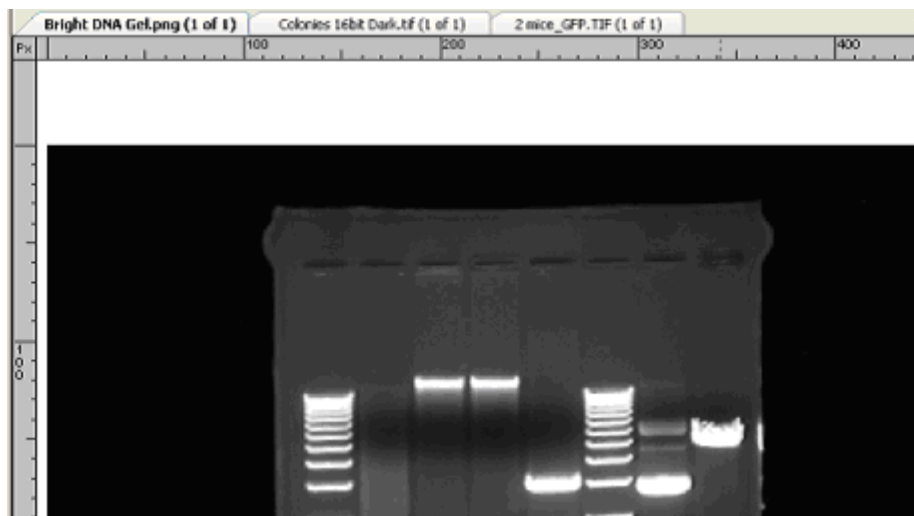
Organizing Image Windows

Images can be visible in the workspace area with either tabbed layout, shown above or cascaded images.

- To change from tabbed layout to cascaded layout, on the menu, go to **File > Preferences > Main Settings** and uncheck the **Tabbed Interface** checkbox.

- To *bring an Image window to the forefront*, either click on the window's title bar or find and select the image's filename from the list in the **Windows** menu.

- To close an image window, click onto the **X** next to the image tabs.

- To *resize a Cascaded Image window*, drag the lower right corner (or an edge) to the desired size.

Information Provided by the Image Window

Besides displaying an image, the Image tab includes the filename of the image. A caption of "Untitled" means that the image has not been saved.

Showing the Image in Actual Size

To show the image in actual size (no scaling), choose View > Zoom. Set the zoom factor to 100%. Or click the right mouse button on the image and select View Original Size. Images taken at high resolution will look large on the screen.

Fitting the Image to the Window

To show the entire image in the window (scaled up or down as required to make it fit), click the right mouse button and click View Best Fit.

Context Menu Commands

A context (shortcut) menu appears when the user clicks on the image itself with the right mouse button. It is a shortcut menu that lets the user sidestep the MENUS OR TOOLBARS. Once brought up, treat it as a regular menu by selecting features from the list.

Click on the image with the right mouse button, a menu with the following commands opens:

- Copy
- Paste
- Paste Special
- Print Selection
- View Best Fit
- View Original Size
- Image Information

TIP: Both the View Best Fit and View Original Size commands are also available from a shortcut menu on the Image window itself. The shortcut menu can be displayed by right clicking on the image.

Status Bar

The Status Bar shows the User Name (Profile), current mouse position in an image, the intensity of the image at that position and status messages during operations. Current date and time is display in the right corner.
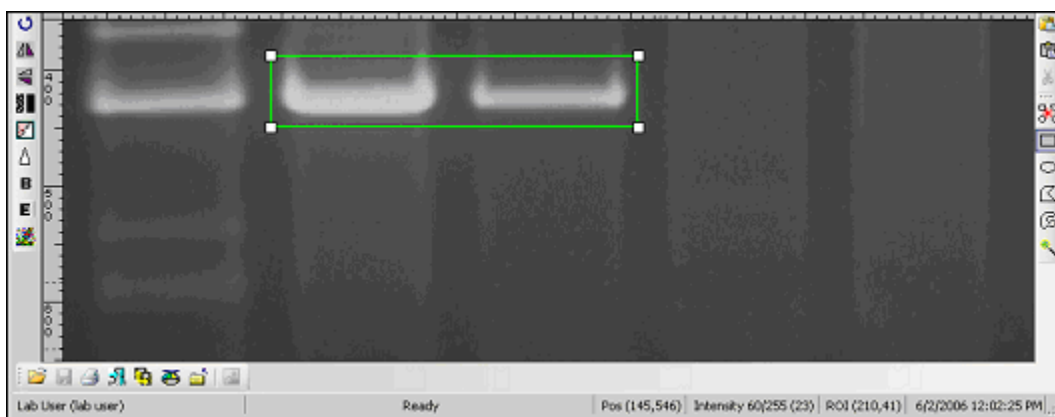
The mouse position (POS) is displayed in pixels (X and Y). The Intensity is displayed as a single value if the image is monochrome and has three values (Red, Green and Blue) if the image is colored. In both cases, the value is reported as a percentage value of the maximum intensity. The ROI (Region of Interest), date, and time is also reported.

Using Plug-In Modules

Overview

The plug-in modules in LS allow the user to position modules on the work area for easy access to the plug-in functions. Position of the modules is customizable, so plug-ins can be organized to include the plug-ins used most and remove plug-ins rarely used.
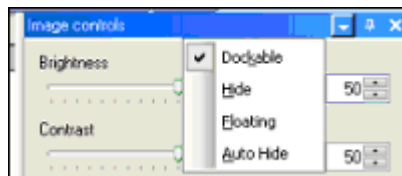
To open the plug-in modules, select **View > Plugins** and select the plug-in to appear in the LS software workspace.

The plugins automatically loaded on the left side of the screen are Colony Count, Area Density, 1D Analysis. The plug-ins loaded on the right side of the screen are Histogram, Pseudocolors, Image controls, Zoom/Pan, Filters, and Player.

Link below for information on using specific plug-in modules:

- Cameras
- Log Viewer
- Digital Video Player
- 3D Plot
- Zoom/Pan
- Image Controls
- Filters
- Pseudocolors
- Histogram Controls
- File transfer protocol
- 1D Analysis
- Counting Colonies

Using the Plug-in Modules

The LS software plug-in modules are essentially tool boxes for many of the commonly used features. Plug-in modules open function-specific windows that can be viewed and placed in virtually any position on the screen. To open the plug-in modules, select **View > Plugins** and select the plug-in to appear in the LS software workspace.

The plugins automatically loaded on the left side of the screen are Colony Count and 1D Analysis. The plugins loaded on the right side of the screen are Histogram, Pseudocolors, Image controls, Zoom/Pan, Filters, and Player.

Positioning Plug-in Modules

Rearranging Plug-in Modules

Plug-in Modules can positioned anywhere on the screen. Plug-ins can be docked, hidden, and floating.

- *Dockable*: Locks the module in a specific position.
- *Hide*: Closes the module. To reopen, go to **View > Plugins** and select the plug-in module.
- *Floating*: Allows module to be moved around on the screen. To move a module, click the top bar of the module and, while holding down the left mouse button, drag the module to the desired location. Arrows will display. Move the module to one of the arrows to dock the module.
- *Auto Hide*: Automatically hides the module when not in use. These tabs will be displayed in the same order as they were selected to Auto Hide. To show the full module, roll the mouse over the tab. To disable the auto hide function, unclick Auto Hide from the module drop down menu or click the pushpin icon.

Moving a Floating Plug-in Module

When a plug-in module is floating, it can be docked to the top, bottom, left or right on the workspace. A floating plug-in module can be placed into another plug-in module's window.

To move a floating plug-in module, click on the title bar of the module and drag it to any of the plug-in module position icons of choice. The following are descriptions of the plug-in module position icons.

Position the plug-in module at the *top* of the workspace

Position the plug-in module at the *bottom* of the workspace

Position the plug-in module at the *left* of the workspace

Position the plug-in module at the *right* of the workspace
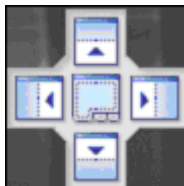
The center arrows console allows positioning of the plug-in module at the *top, bottom, left or right of an empty* workspace by using the exterior arrows
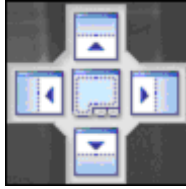
The center arrows console middle button allows positioning the plug-in module at the *inside* of another plug-in module

Histogram Controls

The Histogram Controls plug-in offers options for viewing tonal and color information about an image. By default, the histogram displays the tonal range of the entire image.

Go to **View > Plugins** to load the Histogram Controls plug-in.

Apply a Histogram

- If the Histogram plug-in is not showing, choose **Plugins > Histogram plug-in**.
- From the stretch mode drop-down list, select **None**, **Automatic** or **Manual**.
- The **Options** button offers the choice to select the Y-axis, reset zoom or copy graph.

Digital Video Player

- Purpose of the Digital Video Player
- Using the Player
- Player Features
- Player Options
- Extract or Delete Individual Image Files (Frames)
- Create a Sequence (.sqv) File by Merging Images
- Saving .AVI Files

Purpose of the Digital Video Player

Digital Video Player (DVP) in LS software clubs multiple images in a single file and shifts through the images, allowing users to select one (or more) of the clubbed images to analyze later.
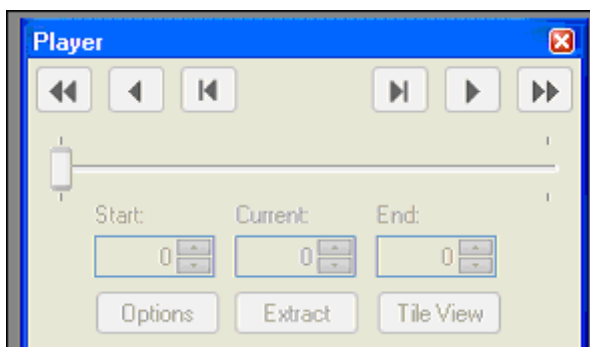
If a sample is to be observed over a period of time it is required to snap multiple pictures at regular and definite time intervals, maybe with progressively higher integration times.

Using the Player
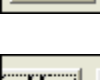
To access the DVP function:

- Go to View > Plugins and click on the Digital Video Player plug-in.
- The DVP comes up automatically, as soon as the image-capture process for Sequential and Dynamic Integration is complete.

Use the SQV whenever possible. AVI file types reduce the image to 8-bit.

Player Features

Below is the description for each of the Player's buttons:

BUTTON	FUNCTIONALITY
	Views the next image.
	Views the previous image.
	Plays the sequence of images. Playing will show the images one after another.
	This button changes to a **Pause**/**Stop** button, which stops any kind of playing.
	Plays the sequence of images in the **reverse order**.
	This button changes to a **Pause**/**Stop** button, which stops any kind of playing.
	Plays the sequence with a number of images to **skip**. The number of images to skip can be set in the Options dialog.
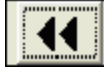
Plays the sequence with a number of images to **skip** in **reverse order**. The number of images to skip can be set in the Options dialog.
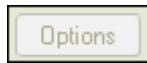
The Options button displays the Options dialog window to set Player properties.
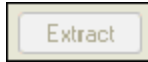
This option lets the user work with individual frames (images) from a single .sqv file. Extract a single frame if required. A detailed description follows in this chapter.
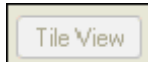
Displays thumbnails of images in a tiled fashion. This can be useful in extracting specific images. When the player is active, this option also shows active scrolling and the currently active frame is highlighted red.

Player Options

Click the **Options** button in the player to set individual options for going through frames in a .sqv or .avi file. Once **Options** is selected the following window appears:

Frame Rate	This is the interval (in milliseconds) between two consecutive images that are displayed. For example, a value of 500 means that a new image will be displayed a half second after the previous image.
	'Frames per minute' is automatically calculated based on this value.
Frames per minute	This is the number of images that will play during a period of one minute.
Fast Frame Skip	This is the number of images to be skipped when playing with {bmc Doci0075.BMP} or {bmc Doci0076.BMP} button.
Repeat	When the Player reaches the end of the sequence of images, it starts again with the first image.
Play to end	When this option is selected, the player will stop when it reaches the end of the sequence of images.
Auto reverse	When this option is selected, the player goes back in reverse order after it reaches the end of the sequence of images.

Extract or Delete individual Image files (frames)

Extracting an image from a .sqv or .avi file creates new images in the workspace and **DOES NOT** remove them from the sequence. Deletion **deletes** the images them from the sequence.

The easiest way to initiate this action is by clicking on **Tile View** checkbox in the player, which brings up the following window. Just right click on any image to extract or delete.

The other two ways this action can be initiated is:

- If the player is open, click on **Extract** from the player.
- If the player is not open, but the .sqv file is open, click on **Images > Extract Frames.**

The following window is brought up, that lists all the frames inside the .sqv in question:
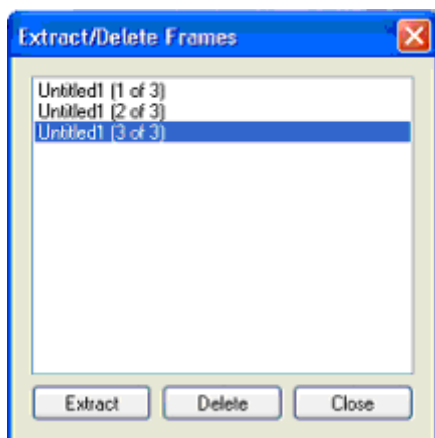
All the images contained in the active sequence are in the list. The user can **Extract** (open the specified image in a new window) images or **Delete** (remove) images from the active sequence.

Create A Sequence (.sqv) file by Merging images

It is possible to merge existing files (or open images) and create a single .sqv file. This is a good way to keep all images in one single file. To create a sequence, select **Image > Merge files**. This feature can be used to merge images open in the LS workspace.
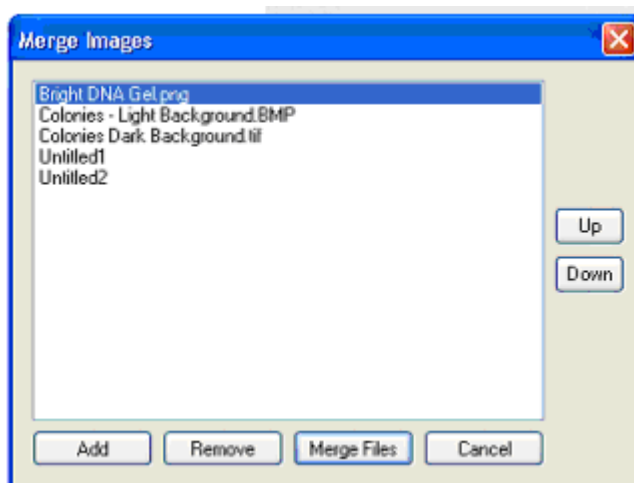
Controls:

- **Add**: Click on 'Add' button to find files from the hard-disk to merge. Existing files in the workspace are automatically listed.
- **Remove**: Select a file and click on **Remove** to remove it from the list.
- **Merge Files**: Merges files in the list into a single .sqv.

- **Cancel**: Closes the window and cancels the process.

Saving .AVI Files

If files need to be shared with another researcher or opened in another place on another computer export the .sqv files to a standard universal format. (SQV is a custom format used by UVP LLC).

Audio Video Interleaved (AVI) is one such broadly acceptable format. (It is a simplistic Windows format that caters to the needs of slow animation, audio and video.)

To import an .AVI file, no special steps are required to be taken since it is one of the standard formats supported by LS Software.

To export a .SQV file to .AVI, use the **Save As** functionality provided by UVP Software.

Depending on the requirement of the target player, AVI files may need to be compressed/ encoded using a specific codec. Before exporting, LS software allows users to choose from one of the pre-installed codecs.

Note: Use the SQV whenever possible. AVI file types reduce the image to 8-bit.

Image Controls Plug-In

The Image Control plug-in offers features to control how an image looks. Changes made to images using this plug-in will not be permanent changes to the image. All the changes can be reversed with the **Reset** button.

Go to **View > Plugins** to load the Image Controls plug-in.

Specific features available on the Image Control module are:

- *Brightness*: affects the overall brightness or dimness of the image. Brightness level of 50 means the image is displayed in its original brightness (i.e. unchanged). Changing the brightness level can make features near the top or the bottom of the intensity scale easier to see.

- *Contrast*: affects the difference between light and dark parts of the image. A contrast level of 50 means that the image is displayed in its original contrast. A level higher than 50 means that contrast has been increased (lights are lighter, darks are darker). A level lower than 50 means that contrast has been decreased (lights and darks are both closer to middle values). *Increasing* the contrast tends to highlight differences in intensity level; *decreasing* it can make patterns that cross intensities more clear.

- *Gamma*: also affects the difference between light and dark parts of the image, but it does so by using a "gamma correction curve." The gamma correction curve affects middle values more quickly than values at either the darkest or the lightest ends of the spectrum. Gamma contrast values range from 0.1 to 5.0. A value of 1.0 means that no gamma correction curve is in effect (the image is displayed at its original levels). Gamma contrast changes have similar results to regular contrast changes.

- *Invert*: reverses all intensities, light for dark and dark for light. This also will have the effect of complementing colors (e.g. red to turquoise, yellow to blue). Inverting the image can make certain features easier to see.

Change Brightness, Contrast, Gamma Or Invert

Change Brightness

- Slide the Brightness control either left or right, or type a desired brightness value into the Brightness text box to the right of the slider.
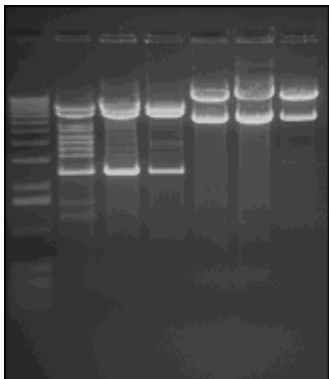
Image Before Effects Applied
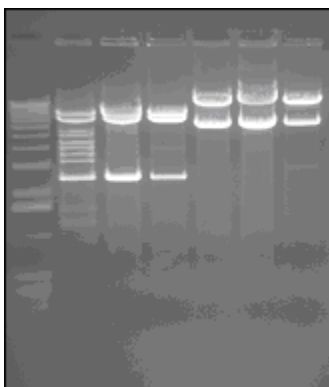
Brightness Applied

Change Contrast

- Slide the Contrast control either left or right, or type a desired contrast value into the Contrast text box to the right of the slider.
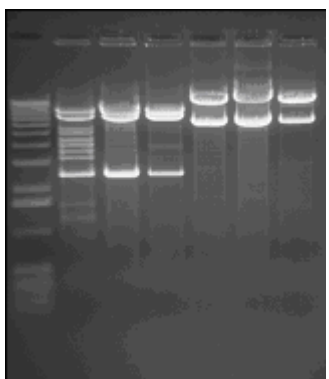
Contrast Enhanced

Change Gamma

- Slide the Gamma control either left or right, or type a desired Gamma value into the Gamma text box to the right of the slider.

Invert Image

- Select the **Invert** check box. *To stop inverting the image*: clear the **Invert** check box
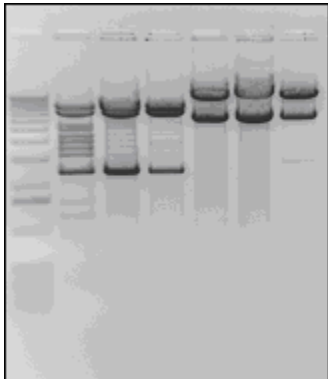
Inverted Image

Return to Default Values

After changing any of the Image Control options, click **Reset** to return all settings to the default settings.

3D Plots

The 3D Plots plug-in allows the user to see a three dimensional view of the sample. For example, if two bands look equally bright in an image, or with naked eye, a 3D plot can actually show if there is a quantitative difference in intensity.

A 3D plot can also be used as a great presentation tool.

The uniformity of the light source in conjunction with the camera response can be checked using 3D plots.

Using the 3D Plot Function

LS software 3D plots can be used for static images as well as live preview and integration.

- Load an image into LS workspace. (It could be an image captured from the camera or an image loaded from the disk.)

- Click on **View > Plugins > 3D Plot Plug-in**. This will bring up a separate window that shows the 3D plot.

Controls on the 3D plot window

Viewpoint tab

Controls on this tab let the user set the correct angle of view.

Rotation Controls:

One can rotate the image in Y as well as Z axes, using the 'Y' rotation and 'Z' rotation handles. The axes are as follows:

- Z axis: The vertical axis.

- Y axis: The horizontal axis.
- X axis: The axis coming out of the plot, towards you.

The plot can also be rotated by dragging it with the mouse in desired direction.

Zoom Controls:

Zooming in and out of the plot is possible in two ways:

- Using the buttons labeled 'Zoom In' and 'Zoom Out'
- Using the spin-box and adjusting the percentage.

Output tab

Three controls in this tab let you export the 3D plot information for various uses:

- **New Image:** Pressing this button creates a new image in the LS workspace with what is visible on the surface plot. This new image must be saved.
- **Clipboard**: Pressing this button copies the 3D plot onto clipboard, so that it can be pasted to any other software (eg. MSWord, Excel, Paint, Photoshop etc.)
- **Printer**: Pressing this button prints the 3D plot on a desired printer. Pressing it brings up the list of available printers.

FTP Transfer

The FTP Transfer plug-in allows the user to transfer files from one computer to another.

- Click on **View > Plugins > Ftp Transfer**. This will bring up a separate window that shows the Ftp Transfer plug-in.
- Enter the IP address of the system acquiring the image and the TS login and password in the FTP plug-in preferences window.
- Start the software and take pictures.
- Press Connect button on the FTP plug-in after starting the software.

Log Viewer

The Log Viewer displays a log of user actions and presents the action list to the user in a log box.

- Go to **View > Plugins** to load the Log Viewer plug-in.

Zoom/Pan

The Zoom/Pan plug-in allows the user to magnify a part of the image. Once the user zooms in on the image, the entire image will no longer be visible in the window. The user may easily change the portion of the image that is visible by panning.

Go to **View > Plugins** to load the Zoom/Pan plug-in.
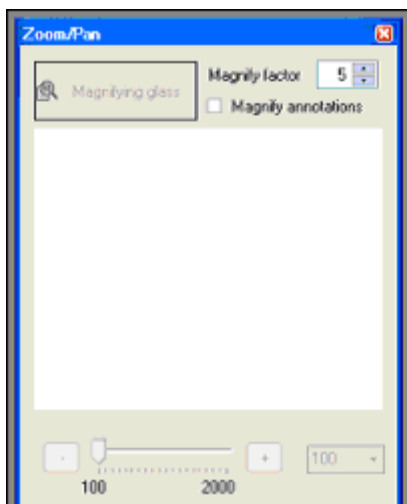
Using Zoom In or Out

- Click on **Zoom In** or **Zoom Out** (located below the thumbnail version of the image on the Effects tab, on either side of the slider).

OR

- Slide the **zoom slider** to the left to zoom out or to the right to zoom in.

OR

- Select the desired zoom factor from the drop-down list to the right of the slider and buttons.

TIPS: There is no need to "turn off" the Magnify tool -- it will be turned off automatically by selecting any other tool (such as a selection tool or an annotation tool).

The image can also be magnified selecting View > Zoom and clicking on the desired zoom percentage.

In the drop-down box, enter a number and press TAB. (The drop down box is particularly useful if a zoom factor is needed between the pre-defined choices in the list.)

Pan to a Different Part of the Image

- In the thumbnail image, drag the **Pan** rectangle to the desired location.

- If the desired location is outside the **Pan** rectangle, simply click the desired location to "jump" the pan rectangle there.

Magnify Part of an Image

- Click the **Magnifying Glass** button.

- The mouse becomes a magnifying glass. Click anywhere on the image to magnify an area. Adjust the Magnify factor number to increase or decrease the magnification.

- To show annotations under the magnifying glass, click the Magnifying Annotations option.

Camera Plug-In

The camera capture plugin provides camera controls for all Canon digital color cameras.

To open the capture tab, go to **View > Plugins > Camera Plug-in.** The module offers the following settings and controls in automatic mode (turning the dial of the camera to **A-DEP**).
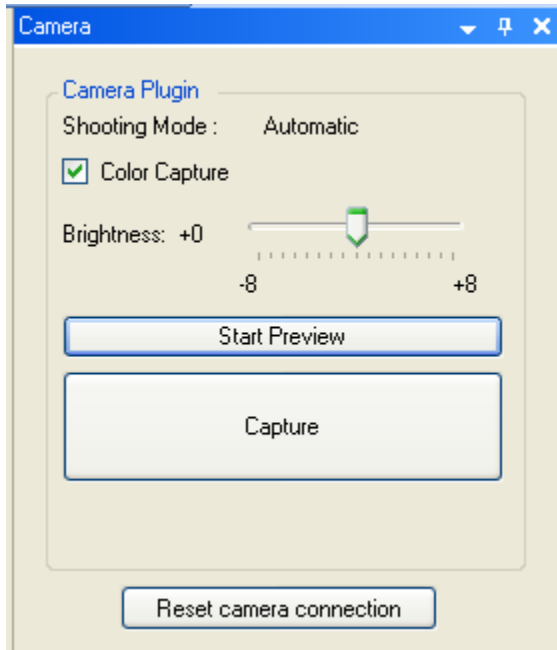
- **Color Capture**: Determines whether the camera captures in color or monochrome.
- **Brightness**: Adjusts the brightness of the image.
- **Start Preview:** Preview a live image.
- **Capture**: To capture an image.
- **Reset Camera Connection**: Reestablish the connection of the camera to the software.

In Manual mode (turning the dial on the camera to **M**) users may adjust the following parameters:
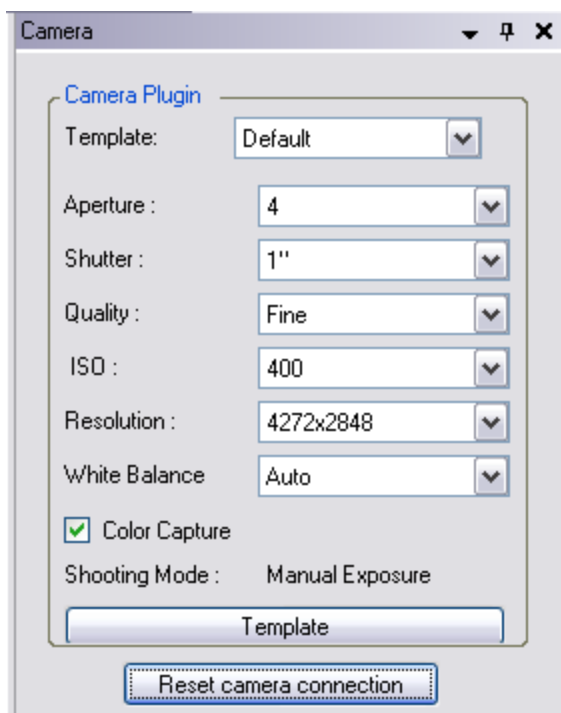
- **Template**: This button allows users to define templates to save customized setting and displayed features. Set-Up Canon Capture Templates

- **Aperture**: The degree to which the shutter will open.

- **Shutter**: The speed at which the shutter will be snapped.

- **Quality**: Consists of Image Resolution and Compression – extra fine, fine, normal. Select extra fine for the least compression and highest quality.

- **Resolution**: Select from the drop down menu.

- **White Balance:** Select from the drop down menu.

The following image is the **Previewed** image. The focus can be automatically or manually adjusted.
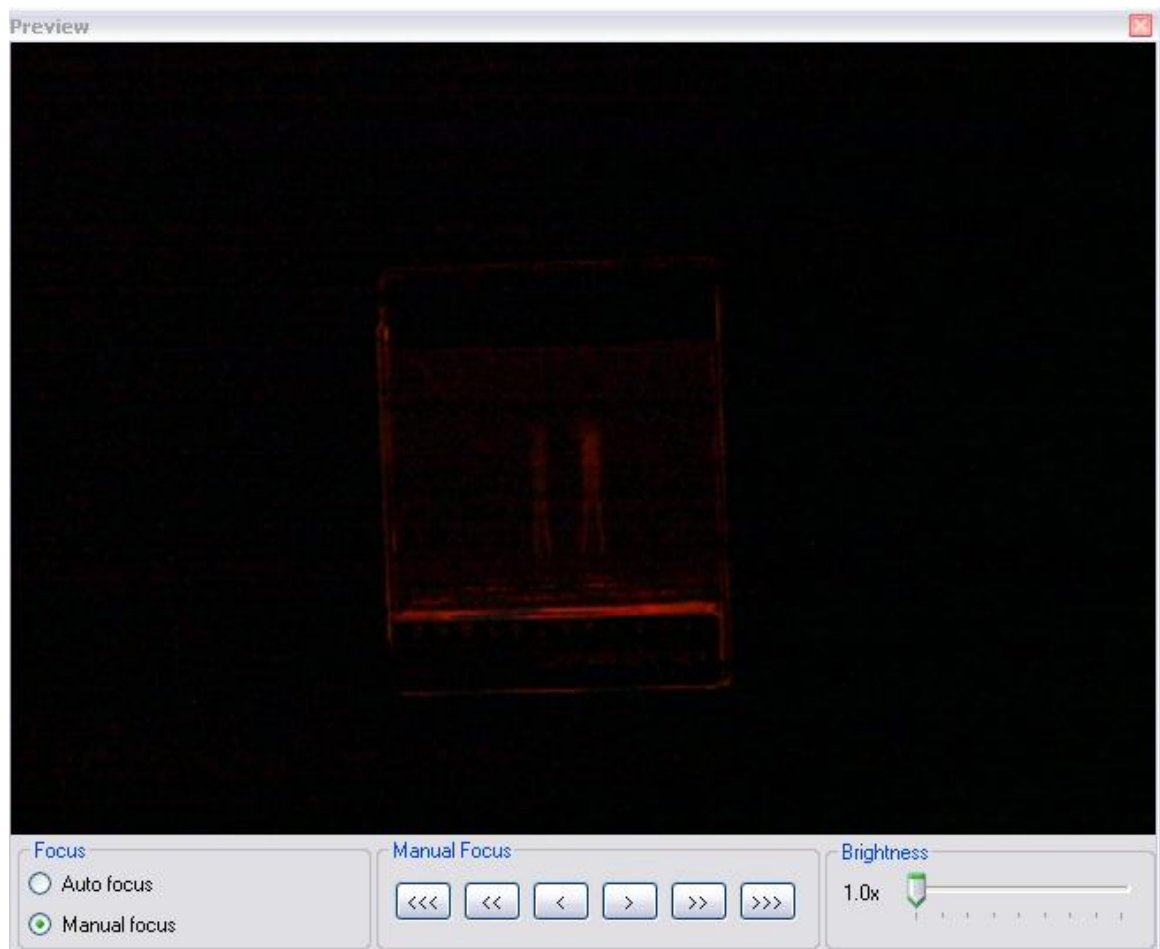
Capturing Images

This topic discusses the process of capturing images using LS software. The following sections explain the process in more detail:

To capture an image with the camera:

- If the camera plug-in is not showing in the software interface, select **View > Plugins** and load the camera plug-in for the camera.

- Place the gel (object to be captured) into the darkroom

- Turn the camera to **A-DEP**.

- Select **Preview** in the camera plug-in**.**

- If the settings are not correct, change the camera dial to M to manually control the settings of the camera.

- When the optimal settings have been found, click onto **Capture**. .

Creating Templates

Set-Up Templates

Templates enable users to configure specific settings along with unique designator name to camera and darkroom/lens plug-ins. Templates allow users to then select the defined settings as needed.

- [Set-Up a Canon Capture Template](#)
- [Set-Up a Colony Template](#)

Edit and Delete Templates

Edit a Template

- While in the Plugin click on the **Templates** drop down menu.
- Select the template to edit from the drop-down list. The default values and will be set to the values used for that template.
- To edit the name, click **Edit Name** to the right of the window. Type a new name and then click **OK**.
- To change the template settings, click **Sync**. This allows experimentation with changes to settings and then allows those settings to be saved.
- To change any setting default, select the new value for that setting.
- Click **Save** (or **OK**) to save the changes.

Delete a Template

- While in the Plugin click on the **Templates** drop down menu.
- Select the template to be deleted from the drop-down list.
- Click **Delete**.
- Confirm the deletion of this template by clicking **Yes**.

Tip: Make many template changes at once by using Save instead of OK.

Set-Up Canon Capture Templates

Capture Templates are groups of preset camera settings. They are used on the Canon Camera Plug-in either to return the camera quickly to a group of settings used often or to default some settings while making others available for alteration.

Each template has a name, a default value for every camera setting and a list of flags indicating which settings will be excluded and which will be shown from the Canon Camera Plug-in when this template is

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selected. If a setting (or group of settings) is not excluded, it can be overridden on the Canon Camera plug-in before each capture.
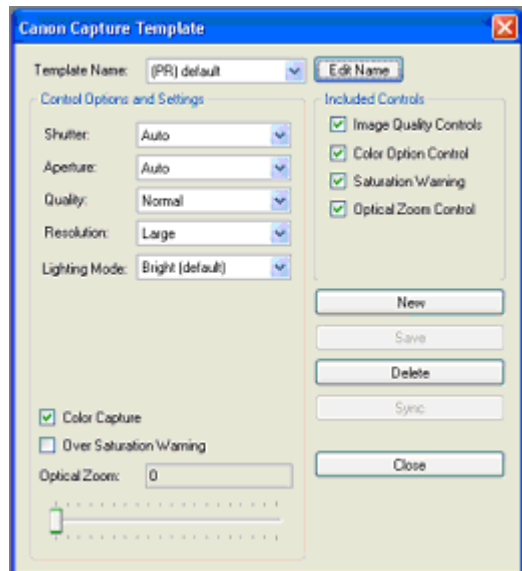
Create a New Template

- On the Canon Camera Plug-in click on the three dots next to the drop-down **Templates** menu. The Capture Templates window will appear.

- Click **New**. The New Template window will appear.

- Type a name for the new template and click **OK**. A new template will be created and then defaulted to the current settings in the Capture Templates window.

- Set each camera setting to the desired value.

- On the right hand side, there is a list of features that can be included in the controls. Check the features (controls) to use in the template. Only those checked will appear in the main capture-panel.

- Click **Save** (or **OK**) to save the new template.

Tip: To copy a template, select the template to copy first, then click **New**. The new template will use the one selected for initial values.

Creating Colony Templates

Templates allow the user to set parameters for colonies most frequently counted and provides a quick count of Petri dishes commonly used by the lab.

- To save a template in **Step 1 of 2: Select Classes,** add the desired points and classes if not already selected) and click the **New** button in the Template section of the window.

- A new pop-up window will ask for a **new Template name**.

- Type in the new name and select **OK**.

- The Template name window will close and direct back to the **Step 1 of 2: Select classes** tab.

- Click on the **Count** button to proceed to the **Step 2 of 2: Finish** tab.
- If the shape and size sliders are changed to capture additional colonies, click **Save filters** in the Templates section of the window to save the new settings.
- Select **Finish** to complete the count and save the template.
- A new pop-up window will ask for a **new Template name**.
- Type in the new name and select **OK**.
- The Template name window will close and direct back to the **Step 2 of 2: Finish** tab.
- Click the **Finish** button to complete the count process and save the template.

Related Topics:

- [Manual Counting](#)
- [User Defined Template Counting](#)
- [Zone Counting](#)
- [Spiral Counting](#)
- [Renumbering Colony/Zone Values](#)

Deleting Colony Counting Templates

To delete a **Template**, from the **Colony Count** module:

- Click Start Colony Count.
- Click Manual Count.
- Click **OK.**
- Click on the **Step 2** tab.
- Under the **Template** section, click the drop down menu.
- Select the Template name to delete.
- Click **Delete**.

Editing Images

- Overview
- Undo and Redo
- Copy
- Paste and Paste Special
- Using Selection Tools (Region of Interest Tools)

Overview

The image editing features provided in LS Software allow users to undo changes to images, to copy images or parts of images, to paste images from the clipboard as new images and to merge clipboard images with existing images.

Most editing features are found on the **Edit** menu. However, to be able to use all editing features, you will also need to use the **Selection Rectangle** and **Selection Ellipse** tools from the **Tools** menu.

Note: The **Edit** menu also offers a **Cut** feature that can be used while editing text in LS Software. Cut does not work on images.

Copy

Used on an image, the **Copy** command copies all or part of the image to the clipboard.

Copying an Entire Image

- If there is a ROI present on the iamge, click away from the ROI once to cancel it.
- Choose **Copy** from the **Edit** menu.

Copying a Selected Region Within an Image

- Select a ROI on the image using one of the selection tools.
- Choose **Copy** from the **Edit** menu.

Note: **Copy** can also be used on text, in which case it acts in the standard Windows fashion.

Copied images or sections of images will include annotations if the annotations were displayed when **Copy** was used. If annotations were hidden, they will not be included.

Tip: **Cop**y also is available by using the Ctrl-C accelerator key, by using the **Copy** button on the Toolbar and by using the shortcut menu on the Image window itself.

Paste

This command takes an image from the clipboard and imports it into the software, displaying it in a new Image window.

Note: **Paste** can also be used on text, in which case it acts in the standard Windows fashion.

Paste an Image

- From the **Edit** menu choose **Paste**. The image will be displayed in a new Image window.

  Note: **Paste** is only available if there is an image on the clipboard.

Paste Special

This command allows an image on the clipboard to be merged into the current image. It is useful for adding comparison or reference information into an image, for making composite images and for testing two images against one another for motion.

- To modify the Paste Special options, go to **Edit > Paste Special Options**:

The following merge modes are available in all the Software:

- *Blend*: mixes the incoming image with the current image in a selected proportion. If the Source proportion (Src%) is set to 100%, pixels in the incoming image replace those in the existing image without mixing (i.e. the incoming image is copied entirely over the existing image wherever it lands). Blend is used primarily to place comparison information into an existing image, especially when using high proportions.

- *Add*: adds pixels in the incoming image to those in the existing image up to maximum intensity. Add is used primarily to build composite images with little or no overlap. This feature requires no additional settings.

- *Subtract*: subtracts pixels in the incoming image from those in the existing image. Subtract is used primarily to test for differences in or motion between two otherwise similar images. This feature requires no additional settings.

Undo and Redo

The Undo command will undo the last material change made to an image. Material changes include all manipulations and the Paste Special command. The Redo command reverses the last Undo. To see what the last material change did in detail, alternate between Undo and Redo. Changes made through the plugins do not permanently change the image.

Undo the Last Change to an Image

With the image open from the **Edit** menu, click **Undo**. The former version of the image will be restored.

Redo the Last Change to an Image

With the image open from the **Edit** menu, click **Redo**. The last change will reappear. The Redo command will be unavailable until Undo is used for the first time.

Using the Region of Interest Tools

Using the Selection Tools

These tools allow users to mark part of the image for use in other operations. LS software provides several Region of Interest (ROI) selection tools:

ROI tools (left to right): New ROI, Rectangular ROI, Elliptical ROI, Polygonal ROI, FreeForm ROI and Magic Wand ROI. Select the tools from the toolbar or from Tools > Region of Interest (ROI).

- **Rectangular, Elliptical, Polygonal ROI:** To define a rectangular, elliptical or polygonal type of ROI, start with the upper-left corner of the desired region and drag the mouse downward and to the right until the desired area is marked.

- **FreeForm ROI**: To define a ROI with the mouse pointer, keep the left mouse button pressed and draw around the region of interest. Lift the mouse button to automatically complete and enclose the area.

- **Magic Wand ROI**: To mark the ROI automatically on the image, click just once inside the region of interest and the software marks that area by identifying the edges. Zoom in on the image to get a better outline of the ROI. The spin-box 'Range' is the sensitivity of finding the edges, and is dependent on the dynamic range of the image. Lower the number, smaller is the area.

  Apart from Analysis features, two operations in the software use an area of interest: **Copy** and **Crop**. **Copy** will copy the selected region to the clipboard. If there is no selected region, the entire image will be copied. **Crop** will remove (crop away) all parts of the image outside of the selected region. Either operation will work with either selection tool.

Select a Region

- Choose the desired selection tool either from the **Tools > Region of Interest (ROI)** or from the toolbar.

- Starting with the upper-left corner of the desired region, drag the mouse downward and to the right until the desired area is marked.

Adjust the Region

If the selection is not quite right, move it or resize it without having to start over:

- To make the selection wider or narrower: drag the left or right dotted lines that bind the selection to the desired size.

- To change both height and width at one time: drag any corner control point to the desired size.

- To move the selection: click on and drag the interior of the selection to the new location.

Cancel the Region

- Click once anywhere on the image away from the current selection. The selection markers will disappear.

Using Image Filters

- Overview
-

Overview

LS Software offers several types image filters. Each filter makes a substantial and material change to the image itself. In general, the results of an applied filter are *not* reversible. Image filters can be used to correct for problems in preparing for and in acquiring the image. They also can be used to expose new information in an image by removing or de-emphasizing other information. Any changes to the image are logged in the image history data, which also offers a notes sections.

Tip: A record of image filters applied to an image is kept in the image's Image History.

To get the most from image filters, it is important to understand the following features first:

- *Undo*: Undo reverses the last filter performed on an image.
- *Saving*: Saving stores an image on disk. Saving as another file name allows users to return to a former version of an image.

Tip: See information on **Undo** in the **Editing Images** section. For additional information on **Saving** images, see **Loading and Saving**.

Filter Navigation

The software provides three different ways to access the Image Filter tools:

- From the toolbar menu
- **Image > Filters** drop-down menu
- View > Plugins > Filters plug-in

Filter Menu

The **Image > Filters** menu, allows users to perform the same commands as in the Filter Plugin. The Filters plug-in offers several types of image filters to make changes to an image. Filters can be used to adjust the resulting image. In addition to accessing the filters from the plug-in module, select filters from the **Image > Filters** menu.

Filter Plugins

The Filter plug-in will display with the following functions. Go to **View > Plugins** to load the Filters plug-in.

Geometry Filters

- Rotate: Rotates the image around its center, useful for aligning images taken with a crooked gel.
- Align: Aligns the image to an adjustable grid.
- Flip Horizontally: Mirrors the image right for left, correcting for an upside-down gel.
- Flip Vertically: Mirrors the image top for bottom, also correcting for upside-down gels in the other direction.
- Resize: Enlarges or reduces the image in size, uses less space and memory, and enables the image to be seen on the entire screen.

Color Filters

- Reduce to Mono: Removes color from the image, making it monochrome.
- Convert: Allows user to change the bit depth of the image

Image Enhancement Filters

- Remove Noise: Removes periodic (patterned) noise in the image
- Despeckle: Removes single-pixel flaws in the image (called artifacts)
- Sharpen: Sharpens edges in the image, making them more pronounced
- Blur: Dulls edges in the image, making them less visible
- Emboss: Gives the image a "chiseled in stone" look
- Starfield subtraction: Removes the star-like single shot noisy spots in dark images taken with long exposures
- Duplicate Image: Creates a copy of the image
- Burn Changes onto a New Image: Creates a copy of the original image and makes all the changes made to the image permanent.

Background Filter

Background Correction

This command allows users to subtract a flat level of intensity off the current image.

Background Subtraction

This command allows users to subtract one image from another.

Burn Changes to a New Image

The Burn Changes to a New Image command takes all the changes made to the image and creates a duplicate copy in a new file. This process is called "burn" because once the changes are made, they are not reversible. Burning changes to a new image is useful when creating a document or presentation.

- Click onto Burn changes to a new image from the Filters plug-in or use the Image > Filters > Burn changes to a new image command.

- A second copy of the image will appear in a new image window as an untitled image.

Convert Image

The Convert filter converts an image bit and color depth when needed.

- Click onto **Convert** from the **Filters plug-in** or use the **Image > Filters > Convert** command. Reducing a large image to monochrome may take a few seconds. Select new depth and click **OK**.

Duplicating Images

The Duplicating Image command creates an identical copy of the foremost image. The new copy will appear in its own Image window.

Note: If the image to be duplicated has been previously saved, the new copy will *not* inherit the old copy's filename. Instead, the new copy will be an unsaved image.

- Click onto Duplicate image from the Filters plug-in or use the Image > Filters > Duplicate image command.

- A second copy of the image will appear in a new image window as an untitled image.

Flip Image

Flip Horizontally

This filter provides a right to left mirror-image. Two clicks of the flip button will return the image to its starting orientation.

Click onto **Flip horizontally** from the Filters plugin or use the **Image > Filters > Flip horizontally** command.

Flip Vertically

This image filter mirror-images an image, top for bottom. Unlike most image filters, it does not degrade the image and may be used repeatedly with no ill effect. Two uses of the filter will return the image to its starting orientation.

Click onto **Flip vertically** from the Filters plug-in or use the **Image > Filters > Flip vertically** command.

Image Enhancement Filters

Image enhancement tools allow users to highlight important features and remove unwanted signal in the image.

- Remove Noise
- Despeckle
- Sharpen
- Blur
- Emboss
- Starfield Subtraction

Remove Noise

This filter removes periodic (patterned) noise from an image. Patterned noise is removed by creating a frequency-space mapping (Fourier transform) of an image and removing frequency spikes away from the graph's origin.

There are two issues to be aware of with noise removal. First, if the image has actual (desired) pattern information, the operation will not be able tell these from noise and it will remove them. Second, the mathematics of the operation can cause some edges pixels to be identified as patterns, resulting in blurring on some images.

- Click onto **Remove noise** from the **Filters plug-in** or use the **Image > Filters > Remove noise** command. Removing noise a large image may take ten or fifteen seconds.

Despeckle

This filter removes single-pixel flaws (called artifacts) from an image. For many reasons, the image capturing process and hardware might misread isolated individual pixels as either white or black. The Despeckle filter mathematically identifies and removes these "loner" pixels.

There are two issues to be aware of with the Despeckle filter. First, if the image has actual (desired) single-pixel bright or dark areas, the operation will not be able tell these from artifacts and it will remove them. Second, the mathematics of the operation can cause some edge pixels to be identified as "speckles," resulting in slight blurring on some images.

- Click onto **Despeckle** from the **Filters plug-in** or use the **Image > Filters > Despeckle** command. Despeckling a large image may take a few seconds.

Sharpen

This filter enhances edges in an image, making them more visible. It is easier to see fine detail after an image has been sharpened.

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- Click onto **Sharpen** from the **Filters plug-in** or use the **Image > Filters > Sharpen** command. Sharpening a large image may take a few seconds.

Blur

This filter blurs edges in an image, making them less prominent. You can see gross (large-scale) detail more easily after the edges have been blurred because details that may have been obscuring it are removed.

- Click onto **Blur** from the **Filters plug-in** or use the **Image > Filters > Blur** command. Blurring a large image may take a few seconds.

Emboss

This filter gives an image a "chiseled in stone" look. Edges take on a three-dimensional (3D) appearance, making them stand out vividly.

Emboss can be performed from any of eight cardinal directions. Conventionally, these are referred to as North (up), Northeast, East (right), Southeast, South (down), Southwest, West (left) and Northwest.

The easiest way to think of the "direction" of the embossment is as the location of a strong light source around the image. For example, if North is selected, the image will appear as if it is illuminated with a strong light from the top. Therefore, horizontal edges will be strongly lighted on the top edge and shadowed on the bottom, and vertical edges will tend to disappear. Diagonal edges will be shadowed in direct proportion to how closely they are horizontal. Alternatively, if East is selected, vertical lines will be shadowed on the left side and lighted on the right side. Horizontal lines would become harder to distinguish.

- Click onto **Emboss** from the **Filters plug-in** or use the **Image > Filters > Emboss** command. The Emboss window will appear.

- Select the desired **Emboss Direction**. The Preview window will show a thumbnail sample of what the image would look like embossed from this direction.

 Tip: Use the arrow keys to cycle through all eight directions while watching the
               Preview.

- Click **OK**.

Starfield Subtraction

This filter eliminates starfield shaped pixel flaws from an image. When long exposures are necessary to capture an image, bright pixels in the shape of a star may appear in the image.

To subtract the starfield:

- Choose Filters > Starfield Subtraction.

Image History

Each material change to an image is tracked in the software's **Image History** feature. Material changes include use of any filters applied and use of the **Paste Special** feature. Changes to **Effects** and **Annotations** are not tracked in the **Image History**.

Viewing Image History

Each material change to an image is tracked in LS software's Image History feature. Material changes include use of any filters applied and use of the Paste Special feature. Changes to Effects and Annotations are not tracked in the Image History.

Entries in the Image History may be of three types:

- *Creation*: Describes how an image was created (captured or scanned) and provides some details.

- *Change*: Describes use of image filters or Paste Special.

- *Error*: Describes an error that occurred while reloading the image from a saved file. The main use of an Error entry is to track the times when the image was changed in another software package, which is important for some kinds of laboratory practice.

The Image History includes information on:

- What type of entry it is, from among the types described above.

- When the change occurred.

- What the change was and details about it.

- Any notes that you add to explain the entry.

Adding Notes in the Image History

- Right click on the image, choose **Image Information**. The Image Information window will be displayed.

- Click the History tab at the top of the window.

- Click on any history entry in the list on the left side to display details about the entry.

- To Add Notes to a History Entry.

- Display image History as described above.

- Click on the history entry to which to add notes.

- Type the notes in the Notes field.

- Repeat steps for any other history entries.

- Click **OK**.

Note: Image History is useful in <u>Supporting 21 CFR Part 11 Compliance</u>

Measurement Tools

Measure Length

- Choose Tools > Spatial Calibration > Measure Length

- Click one edge of the feature.

- Move to the other edge of the feature. As the mouse is moved, the scale tool will show how many pixels (unless the calibration process has been performed) are contained in the straight line drawn with the tool.

- Click the other edge of the feature. The image will now display the total length

measurement value.

- Right click on the new measurement annotation displayed to change the appearance of the annotation. If desired, change the line thickness, line style, font size, or general formatting of the line and value displayed.

Measure Angle

The measure angle tool requires three points to be placed on the image to define an angle.

- Choose Tools > Spatial Calibration > Measure Angle

- Form a line by clicking on one edge of the feature and then click again to finish the line.

- To measure the distance between the line just drawn and second line, move the mouse away from the line. Notice how as the mouse is moved, the scale tool shows how many degrees are contained in the angle drawn with the tool.

- Click to freeze the angle in the desired position. The new angle measurement annotation now appears.

- Right click on the new measurement annotation displayed to change the appearance of the annotation. If desired, change the line thickness, line style, font size, or general formatting of the line and value displayed.

Measure Area

- Choose Tools > Spatial Calibration > Measure Area

- Form a rectangle by clicking onto the image and drawing the rectangle on the image.

- Click again to finish changing the shape of the rectangle.

- The new area measurement annotation now appears in pixels (unless the calibration process has been performed).

- Right click on the new measurement annotation displayed to change the appearance of the annotation. If desired, change the line thickness, line style, font size, or general formatting of the line and value displayed.

  NOTE: Length Measure and Area Measure annotations use the image's scale in calculations. If these annotations display information in pixels ("px"), no scale has been set. See "Changing Sample Width" for instructions on setting the image's scale.

Merging Two Images

Merging results in a new image that includes the existing image plus all of the area of the incoming image, even if that area is off of the edge of the existing image. This allows the composition of "mosaic" images (usually with **Add**) by placing the incoming image at the edge of the existing image.

- Place one of the images on the clipboard using the **Copy** command. If desired, a sub-region of the image can be used.

- From the **Edit** menu, choose **Paste Special**. The Paste Special window will appear.

Tip: **Paste Special** also is available from the **Toolbar** and the **Image window's** shortcut menu.

- If the incoming image is large, select the **Preview Frame Only** check box to allow repositioning of the incoming image without the lag caused by redrawing a large image. Otherwise, clear the check box to preview the result.

- Select the desired merge operation: **Blend**, **Add** or **Subtract**.

- If using **Blend**, set the desired blend percentage.

- Click **OK**.

- Moving the mouse over the existing image, position the merge as desired. The merge results (or positioning frame, for **Preview Frame Only**) will be previewed as the mouse is moved. Position the upper-left corner of the incoming image. Once positioned, the selection can be moved.

- When the desired position is reached, click the right mouse button once. Merges that involve large images may take a few seconds to complete.

Obtaining Image Information

Overview

LS Software keeps various sorts of information about an image. This Image Information includes:

- *Image Scale*: Described as the number of metric units in the image's width, this information is used to calibrate Rulers and Measurement Annotations. Image Scale is also described under Spatial Calibration.

- *Image Resolution*: The width and height of the image in pixels.

- *Image Depth*: The number of bits used to represent intensity. LS Software supports 8-bit, 12-bit and 16-bit image depth.

- *Notes*: Enter notes about an image.

- *File Properties*: The file name, path, create date and size. All will be "N/A" if the image has not yet been saved.

- *Image History*: A list of material changes to the image, when they occurred and any notes to add about why or how the change was made are shown in the Image History.

  To be more compact, the Image Information window is organized into two tabs. All information except Image History is on the first tab; Image History is on the second tab.

To Display Image Information

- If the image is not the foremost image, select it from the **Windows** menu or click its title bar.

- Right click onto **Image Information**. The Image Information window will appear.

- To switch between **Image History** and **Properties**, click the appropriate tab at the top of the window.

To Enter Notes

- Display the **Image Information** window as described above.
- In the **Properties** tab, type information into the **Notes** text box.
- Click **OK**.

Calibrating Image Scale

Each image in LS software can have a scale associated with it. Scaling information is used to display rulers, measure length and measure area annotations. Refer to Spatial Calibration for information on using this tool.

Images scanned into the system from a scanner or imported from another program are not calibrated. In these two cases, therefore, you may wish to adjust or to set the image's scale.

Note: An uncalibrated image will have "Pixels" as the unit type. If the unit type is Pixels, the number of units is the number of pixels in the image width and cannot be changed.

Pseudocolor

The Pseudocolor applies a false-color spectrum to a monochrome or colored image. This process is sometimes called "colorizing." There are two primary reasons for using pseudocolor:

- To make the image look more like what might be seen under a microscope with various kinds of lighting, primarily for comparison purposes.
- To highlight specific intensities for analysis purposes. For example, one of the pseudocolor spectrums highlights black (intensity 0) pixels with blue and white (maximum intensity) pixels with red. This identifies the undersaturated and oversaturated parts of the image.

Go to **View > Plugins** to load the **Pseudocolor** plug-in.

Pseudocolor Spectrums

LS software supplies built-in pseudocolor spectrums:

- *Over-Undersaturation*: Colors pixels in top 5% of dynamic range red and next 5% as yellow. For example, in a 12-bit image, pixels with intensities 4095 to 3891 (5% range) will be colored red and the next 5% range 3890 to 3685 will be colored yellow. There is no indication for undersaturated pixels.
- *Ethidium Bromide*: Mimics the colors used in Ethidium Bromide gel preparation.
- *Fluorescein*: Mimics the colors used in the Fluorescein process.
- *Green Fluorescent Protein*: Mimics the green fluorescent protein colors.
- *Texas Red*: Mimics the colors that appear with a Texas Red stain.
- *SYBR Gold*: Mimics the colors that appear with a SYBR Gold stain.
- *SYBR Green*: Mimics the colors that appear with a SYBR Green stain.

- *SYPRO Orange*: Mimics the colors that appear with a SYPRO Orange stain.
- *SYPRO Red*: Mimics the colors that appear with a SYPRO Red stain.
- *Coomassie Blue*: Mimics the colors that appear with a Coomassie Blue stain.
- *Silver*: Mimics the colors that appear with a Silver stain.
- *Blue to Red*: Colors all intensities from blue at the low end to red at the high end using a natural light spectrum.
- *Red to Blue*: Colors all intensities from red at the low end to blue at the high end using a natural light spectrum.
- *Blue*: Colors all intensities from black to bright blue.
- *Cyan*: Colors all intensities from black to bright cyan.
- *Green*: Colors all intensities from black to bright green.
- *Magenta*: Colors all intensities from black to bright magenta.
- *Red*: Colors all intensities from black to bright red.
- *Yellow*: Colors all intensities from black to bright yellow.

Apply or Remove a Pseudocolor

- If the Pseudocolor plug-in is not showing, choose **Plugins > Pseudocolor plug-in**.
- From the Pseudocolor drop-down list, select the desired pseudocolor. The image will be colorized as desired.
- To remove a Pseudocolor, from the Pseudocolor drop-down list, select **None**.

Reduce Image to Mono

The Reduce to Mono filter reduces a color image to monochrome. This is primarily useful when colors in an image are distracting rather than informative. For example, if light strikes certain surfaces from some angles, a "rainbow effect" (prism) will appear. Another use is to adapt for some software packages and techniques that require monochrome images or which are less reliable on color images.

The **Reduce To Mono** filter uses a weighted mix of colors to arrive at each pixel's monochrome intensity. Green is very heavily weighted while blue is almost disregarded.

- Click onto **Reduce to mono** from the **Filters plug-in** or use the **Image > Filters > Reduce to mono** command. Reducing a large image to monochrome may take a few seconds.

Resize Image

The **Resize** filter allows the image to be changed in size. It replicates or merges pixels as appropriate to arrive at the new size. Resize most commonly would be used to create a smaller version of a very large image to allow users to increase response time when applying filters or to import the image into another software package that does not accept large images.

Tip: There is little point to increasing an image's size, although the filter does support it. Such an image would have more physical pixels after the operation, but it does not gain any new information content.

- Click onto **Resize** from the **Filters plug-in** or use the **Image > Filters > Resize** command. The **Resize** window will appear.

- Select the desired new size from the drop-down list of suggested sizes.

OR

- Select the **Enter Desired Dimensions** option and type either the desired new width or the desired new height.

Note: To distort the image, clear the **Maintain Aspect Ratio** check box and type the new width and height. This should be used only to reverse a similar distortion created in the image capture process.

- Click **OK**. Resizing a large image may take a few seconds.

Rotate and Align Image

Rotate an image by an arbitrary number of degrees. Rotate is helpful to correct for a misaligned gel. Graphically select the degree to align the image based on an internal image feature.

Rotate an Image

- Click onto **Rotate** from the **Filters plug-in** or use the **Image > Filters > Rotate** command. The **Rotate** window will appear and a grid will be overlaid on the image.

- Drag the grid so the yellow arrow moves in the direction you would like the image rotated.

- Once the grid is oriented to the desired position, click **OK** on the **Rotate** window.

Rotate an Image by an Exact Number of Degrees

- Rotate the image by an exact number of degrees. For example, correct for an upside-down gel by rotating it by 180 degrees.

- Click onto **Rotate** from the **Filters plug-in** or use the **Image > Filters > Rotate** command. The **Rotate** window will appear and a grid will be overlaid on the image. For this operation, you will ignore the grid.

- On the **Rotate** window, type the desired number of degrees into the **Angle** text box.

- Click **OK**.

Tip: Rotations by 90, 180 or 270 degrees do not degrade the image. These operations can be completely reversed by a rotation of the same amount in the opposite direction.

Align an Image

Align tool rotates the grid overlaid on the image so that it can be aligned with vertical/horizontal features of the image to make it easy for the user to select the # of degrees rotation.

Rulers

Rulers are located at the top and left of each Image window.

The rulers show the width and height of the visible portion of the image either in metric units (if calibrated) or in pixels (if un-calibrated). When zooming in and out of the image the, software updates the rulers to show the actual size and position of the visible portion of the image. As the user moves the mouse over the image, markers show the position on each ruler.

The units are shown in the upper-left corner (see the image above). Pixels (uncalibrated) are abbreviated "px;" all other values use standard abbreviations. When the rulers are calibrated to a standard measure, they may change units as zoom is applied to the image detail. For example, an image calibrated in centimeters may switch to millimeters when zoom is used. The measurements are still completely accurate; the rulers switch units because they are designed to show a useful number of units at every point.

Note: The scale information used by the rulers also is used by measurement annotations. Length measure annotations can be used to see the length of a feature that is not square to the rulers.

Showing or Hiding the Rulers

- Rulers may be hidden or shown individually for each Image window. Hiding the rulers provides slightly more space in which to view the image.
- On the **View** menu, click **View Rulers**.

Scanning Images

LS software can acquire images from scanners that supply a TWAIN interface. (Almost all scanners support TWAIN.) TWAIN helps scanners provide their own software interface during scanning.

Select the Desired Scanner

- Choose **Image > Scan > Select Scanning Source**. The Select Source window will appear.
- Select the desired device from the list.
- Click **Select**.

Tip: The device you select will remain the default until it is changed, even after rebooting. Generally, if you have only one TWAIN device, it will already be the default.

Scan an Image from a Scanner

- Select Image > Scan > Start Scanning.

- Change any settings offered by the device as desired. Consult Help for the scanner to learn about the **Scan** dialog window for your device.

- Click **Scan** (this button may have different names, including OK or Acquire, for different devices). The image will appear in a new Image window inside the workspace.

Tip: Most scanners offer a "fast preview" mode. You can use this to adjust brightness, contrast and other settings appropriately before scanning.

Spatial Calibration

Using the Image Scale Tool

There are two ways to set the image's scale:

- If the size of a single feature anywhere in the image is known, use the Scale Tool to set the scale of the entire image from that one feature.

- If the size of the entire image is known, change the Sample Width through the Image Information window.

Calibrate the Image Using the Scale Tool

- Choose Tools > Spatial Calibration > Define Image Scale.

- Find the feature in the image for which the size is known. Click one edge of the feature. (It may be useful to position a small ruler with the Petri dish and capture the image of the ruler and Petri dish together so that the ruler can be used for reference)

- Move to the other edge of the feature. As the mouse is moved, the scale tool will show how many pixels are marked with the tool.

- Click the other edge of the feature. The Set Scale window will appear.

- In the **Set Scale** window, select the metric unit for the marked area (e.g. centimeters) and enter the number of units (e.g. 12).

    NOTE: The marked area must be selected first and then the number of units second.

- Click **OK**. The rulers will reflect the new scale.

Changing the Sample Width for an Image

Sample Width is calculated from the width of the image. It is the distance of the width of the scene in the image. For reference, a ruler may be placed across the top of the image so that the entire distance of the scene can be determined.

- Right click onto the image and select **Image Information** for the image.

- There are two tabs present in the Image Information window. Ensure that the **Properties** tab is selected.

- In the **Overall Sample Width** area, select the unit measurement (the right-most drop-

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down list) first. For example, if the sample width of the image is 500 cm, select centimeters.

- Type the number of metric units in the text box immediately following **Overall Sample Width**. For example, if the sample width of the image is 500 cm, type "500."

- Click **OK**.

Using Spatial Calibration for Annotation

Special types of annotations are available from Tools > Spatial Calibration option:

- *Define Image Scale*: Allows the user to spatially calibrate to a known measurement.

- *Measure Length*: Length Measure annotations look somewhat like Line annotations except that they show the length of the line in *image scale units* (see "Rulers"). Position Length Measure annotations from one point to another point to see how long any image feature is in real units. Select the *color*, *line thickness*, *line style* (solid, dotted or dashed), *font size* and *formatting* (bold, italic or underline) of a Length Measure annotation.

- *Measure Angle*: Angle Measure annotations consist of three points. They display the angle between the line formed by the first and second points and the line formed by the second and third points. Use Angle Measure annotations to determine slants in lanes or in related bands across lanes in an electrophoresis gel. Select the *color*, *line thickness*, *line style* (solid, dotted or dashed), *font size* and *formatting* (bold, italic or underline) of an Angle Measure annotation.

- *Measure Area*: Area Measure annotations look somewhat like Rectangle annotations except that they show the area of the rectangle in *image scale units* (see "Rulers"). Select the *color*, *line thickness, line style* (solid, dotted or dashed), *font size* and *formatting* (bold, italic or underline) of an Area Measure annotation.

Note: **Length Measure** and **Area Measure** annotations use the image's scale in calculations. If these annotations display information in pixels ("px"), no scale has been set. See **Changing Sample Width** in this chapter for instructions on setting the image's scale.

Annotations

Overview

Annotations allow users to notate areas of an image without changing the image itself. This means that areas in the image that need more study, are particularly interesting or that support a particular scientific interpretation can be identified. At any time, annotations can be turned on or off to see the image with or without annotations. Users may add and edit annotations through the Tools menu at right or add annotations though the toolbar commands.

Topics explain the annotation system in more detail:

- *Spatial Calibration*: Allows the user to calibrate distance measurements in the image relative to a known measurement.
- *Image Rulers*: Default to pixel units and can be spatially calibrated for image measurements.
- *Creating Annotations*: Explains how to create annotations.
- *Deleting Annotations*: Explains how to remove annotations no longer needed.
- *Formatting Annotations*: Explains how to use the Formatting menu when creating or editing annotations.
- *Moving and Resizing Annotations*: Explains how to move and resize annotations graphically.
- *Selecting Annotations*: Explains how to select annotations.
- *The Text Edit Window*: Explains how to use the Text Edit window for entering and editing text annotations.
- *Types of Annotations*: Explains the annotation tools provided in LS Software.
- *Viewing and Hiding Annotations*: Explains how to show or to hide all annotations on an image.

Types Of Annotation

LS software offers five different types of annotation:

- **Text**: Text annotations consist of written information. Use them to label a particular part of an image. Users have the ability to change the *font size*, *color* and *formatting* (bold, italic or underline) of a Text annotation.

 Note: Users can increase/decrease the size of annotations while zooming in or out. See section **Synchronize Size with Image Zoom** in this chapter of the manual for additional information.

- **Line**: Line annotations permit users to draw lines with optional arrowheads at one or both ends. Use them to associate other annotations such as text with a particular image feature or to draw attention to an image feature. Select the *color*, *line thickness, line style* (solid, dotted or dashed) and *arrowheads* (none, at start, at end, or both) of a Line annotation.
- **Rectangle**: Rectangle annotations allow users to draw a rectangular frame around part of the image. Use them to show the boundaries of an image feature. Select the *color*, *line thickness* and *line style* (solid, dotted or dashed) of a Rectangle annotation.

- *Ellipse*: Ellipse annotations are very similar to Rectangle annotations except that they are oval rather than rectangular. Select the *color*, *line thickness* and *line style* (solid, dotted or dashed) of an Ellipse annotation.

- *Highlighter:* Highlighter annotations work like a highlighting pen by altering the color of the underlying image to draw attention to an area. Select the *color* of a Highlighter annotation.

Viewing And Hiding Annotations

Although annotations are useful for drawing attention to important features, they may cover-up important features. LS software allows annotations to be hidden on an image with a single command.

Each Image window keeps track of whether the annotations are hidden or shown for that image, allowing annotations to be present on one image while hidden on another image.

Note: Annotations are shown automatically when an annotation tool is selected.

To Show or Hide Annotations for an Image choose **View > View Annotations**. If this menu option is checked but no annotations appear, no annotations exist for this image.

Note: To **edit an annotation**, select the annotation in the image, right mouse click on the annotation, then select from the menu options to change the annotation. Also refer to the **Formatting Annotations** section of this chapter for more information.

Synchronize Size with Image Zoom

The default value for LS is Synchronize size with image zoom making the annotations smaller if the user zooms out. To keep the annotations the same size regardless of the size of the image, check the Don't synchronize size with image zoom.

- To change this setting go to **File > Preferences > Annotations**. A **Preferences – Annotations** window appears. Select **Don't synchronize size with image zoom** to enable the text to remain at the same size.

New Annotation Window

Text annotations allow users to place labels on an image to describe or to name a feature of the image. Use the New Annotation window to enter the text.

The New Annotation window appears whenever a new text annotation is added. It also appears when a text annotation is edited, which can be accomplished by double-clicking the text annotation or by choosing Edit from the shortcut menu associated with it.

The New Annotation window saves the last text entered, making it the default for the next text annotation to be created. To enter new text, simply start typing and the existing text will be replaced.

Multiple lines of text can be entered as a text annotation. To have the text automatically word-wrapped, paragraph style, select the Word Wrap check box. To control when a new line is created manually, clear the check box.

To Edit the Text of a Text Annotation

- Double-click the text annotation.

- In the **New Annotation** window, make any changes to text.

- Click **OK**.

Selecting Annotations

After creating an annotation, select it either to change formatting properties or to graphically move, stretch or resize it. Since selecting and editing an annotation are different from selecting part of the image, there is a specific tool -- the Edit Existing Annotation tool -- to select and edit annotations. Every time an annotation is added, the tool switches to the Edit Existing Annotation tool automatically so that the new annotation can be edited immediately.

- Choose **Tools** > **Edit Existing Annotation**. This step is unnecessary immediately following adding an annotation.

- Click on any part of the annotation. Control handles will appear to mark the selected annotation. If there are several annotations in the same area, click on a portion of the annotation to reformat that does not intersect any other annotation. The control handles will show if the wrong annotation is selected.

Tip: The cursor will change from an arrow to a hand when the cursor is over an annotation.

Moving And Resizing Annotations

Once an annotation is selected, the annotation can be moved and resized with the mouse.

- To move an annotation, select **Tools > Edit Existing Annotation** if not already checked; then click on any part of the annotation.

If the annotation is not near any other annotations, drag its interior immediately. There is no need to select it first.

Dragging from the interior of the annotation or on a line, move it to the new position.

To Change the Points of an Existing Line, Length Measure or Angle Measure Annotation

- Select the annotation as described above.

- Drag the control handle for the point to change to the new position. The annotation will stretch appropriately as it is moved.

To Resize an Existing Rectangle, Ellipse, Highlighter or Area Measure Annotation

- Select the annotation as described above.

- Drag a control handle inward or outward to define the new size. The point on the opposite corner will remain fixed and the annotation will resize as it is moved.

To Rotate a Text Annotation

- Select the annotation as described above.

- Drag any control point around the center of the annotation until the desired location of rotation is reached.

  Tip: If it is difficult to align the text, move the mouse away from the center of the text annotation while dragging.

Formatting Annotations

The Annotation Formatting menu contains options to format a selected annotation.

Change the formatting of an annotation by selecting the Annotation Formating (with the Edit existing Annotation tool checked).

The Annotation Formatting menu contains the following formatting options:

- *Color*: Pick a color from the list or choose Custom Color at the bottom to pick any existing color. All annotation types support color options.

- *Line Style*: Choose from solid, dashed or dotted lines. Only the following line annotations support line style: Line, Rectangle, Ellipse, Length Measure, Angle Measure and Area Measure.

- *Line Thickness*: Choose a line thickness from the offered choices. Only select Line Thickness if the line style is Solid. Otherwise the line thickness must be 1 and the Line Thickness menu will be unavailable. The same annotation types that support Line Style support Line Thickness.

- *Arrow Style*: Choose whether a Line annotation (only) has:
    - No arrowheads.
    - An arrow at the start (first point) of the line.
    - An arrow at the end (second point) of the line.
    - Arrows at both ends of the line.

- *Font Size*: Choose the font size of Text, Measure Length, Measure Angle and Measure Area annotations from among the listed values.

- *Bold*: Choose whether Text, Measure Length, Measure Angle and Area Measure annotations should be boldfaced.

- *Italic*: Choose whether Text, Measure Length, Measure Angle and Measure Area annotations should be italicized.

- *Underline*: Choose whether Text, Measure Length, Measure Angle and Measure Area annotations should be underlined.

To Change the Format of an Annotation

- Select the annotation by choosing **Tools > Edit Existing Annotation** and then click on any part of the annotation.

- Change the formatting options using the **Tools > Annotation Formatting** menu.

Deleting Annotations

Deleting an annotation removes it permanently from the image.

To Remove an Annotation:

- Select the annotation by choosing **Tools > Edit Existing Annotation**; then click on any part of the annotation.

- Press the DELETE key.

Note: Select **Delete Annotation** from the Shortcut menu that pops up by right-clicking on the annotation.

Creating Annotations

Creating a Text Annotation

- Select Tools > Add Annotation > Text.

- Click the position on the image to add text annotation. The **New Annotation** window will appear.

- Type the desired text in the text box.

- Using the **Format Text** dropdown menu, change the Font Size and Font Style of the text as needed.

- Click **OK**. The text annotation will be displayed at the position indicated.

- To change the color, font size and formatting (bold, italic, underline), right click once on the text to change or go to **Tools > Annotation Formatting**.

Creating a Line Annotation

- Select Tools > Add Annotation > Line.

- Click the position on the image to begin the annotation. A line will follow the mouse as it is moved. To stop the process of adding an annotation, simply press the **ESC** key.

- Click the position on the image to end the annotation. The new annotation will be drawn.

- To change the color, font size and formatting (bold, italic, underline) of the line, right click once on the line to change or go to **Tools > Annotation Formatting**.

Creating a Rectangle, Ellipse, Highlighter Annotation

- Select **Tools > Add Annotation** and choose the appropriate tool (Rectangle, Ellipse, Highlighter).

- Click the position on the image to place the upper-left corner of the annotation. A view of the new annotation will follow the mouse it is moved. To stop the process of adding an annotation, simply press the **ESC** key.

- Click the position on the image to place the lower-right corner of the annotation. The annotation will be drawn.

- To change the color, font size and formatting (bold, italic, underline) of the annotation, right click once on the annotation to change or go to **Tools > Annotation Formatting**.

-
  NOTE: With **Highlighter** annotations users may only select color.

Related Topics:

Measurement Tool

Loading and Saving Images

Loading Images

LS Software will load images in popular formats, including JPEG, TIFF, GIF, PNG, TGA and BMP. If the image was previously saved using one of the LS software packages, then other image details such as the image's scale, history and annotations will be loaded as well.

Many demo images are included with LS software, to increase user familiarity with the software.

Note: For a discussion of exactly how image information is stored and how to move image files around on disk, or to send them to another LS user, see "Image Files."

To Load an Image

- From the **File** menu, choose **Open**.

Tip: The **Open** button on the Toolbar can also be used. The software opens TIF, JPG, AVI, BMP, TGA, GIF, PNG and SQV files.

- Select the type of file to open, if unsure of the file type, select "All Files."
- Navigate through the disk drives to the file folder in which the image is stored. LS software keeps a folder structure of its own that it will show by default. Most images will probably be in this folder.
- Select the desired image file.
- Click **Open**. An Image window containing the desired image will appear.

To Load a Demo Image

- Go to File > Open Demo.
- Select the desired demo image file from the list of available files.
- Click **Open**. An Image window containing the desired image will appear.

Saving Images

Save images acquired in LS so that they can be used in later sessions. To Save a New Image:

- From the **File** menu choose **Save** or **Save As**. The Save window will appear. The Save button on the Toolbar can also be used.
- Select the file type that to use from the drop-down list near the bottom of the window.
- Navigate through the drive and folder structure to the location to save the image.
- Enter a filename for the image.
- Click **Save**.

To Save Changes to an Image

- From the **File** menu choose **Save** (or use the **Save** button on the Toolbar). Changes will be saved to the existing file, overwriting the previous image data.

To Save Using a Different File Folder, Name or Type

- From the **File** menu choose **Save As**. The Save window will appear.
- Select the file type that to use from the drop-down list near the bottom of the window.
- Navigate through the drive and folder structure to the location to save the image.
- Enter a filename for the image.
- Click **Save**.

Burn in Changes to a New Image

The Burn Changes to a New Image command takes all the changes made to the image and creates a duplicate copy in a new file. This process is called "burn" because once the changes are made, they are not reversible. Burning changes to a new image is useful when creating a document or presentation.

- Click onto Burn changes to a new image from the Filters plug-in or use the Image > Filters > Burn changes to a new image command.
- A second copy of the image will appear in a new image window as an untitled image.

Image File Types

Images are saved as two separate files. While the two files have the same name, they have different extensions.

The Image File

The more important of the two files is the Image File, as it contains the actual image. It is saved in the image file format (file type) selected when saving, which is JPEG by default. The file extension will reflect the file format (e.g. ".JPG" for a JPEG file).

LS Software supports the following formats:

- **JPEG**: Joint Photographic Experts Group. A common lossy compression image format used to store images on disk. JPEG files generally have JPG or JPEG extensions.
- **TIFF**: Tagged Image File Format, a common image format. Depending on settings, TIFF can be either a lossy or a lossless compression format. In LS software, it is used in the lossless mode to reduce image file size without losing integrity. TIFF files generally have TIF or TIFF extensions.
- **TGA**: Truevision Targa image format. TGA is a lossless compression format that reduces file size somewhat. TGA files generally have a TGA extension.
- **BMP**: Microsoft Bitmap image file format. BMP is a lossless format which provides some compression to reduce file size. BMP files generally have a BMP extension.
- **PNG**: Portable Network Graphics, a common image format. PNG is a lossy compression format that results in very small files. Files stored in PNG usually have a PNG extension.
- **GIF**: Graphic Interchange Format, a proprietary Xerox image compression format. GIF is a lossy compression format that results in very small files. Files stored in GIF usually have a GIF extension.

JPEG, PNG and GIF are lossy compression formats. TIFF, TGA and BMP are lossless compression formats (at least, as used by this software; TIFF can actually be either lossy or lossless). Lossy compression makes small, usually non-visible changes to an image in order to make it store more compactly on disk. Typically, formats that use lossy compression store in much less space than lossless

compression formats. By comparison, a lossless format does not store as compactly, but also does not change the image in any way.

The Extended Attributes File

The image will also have a second file that ends with the extension ".EXT." This file contains the image's effect settings, history, scale and annotations. It will appear in the Archive folder and will have the same name with the image's extension added to the end. For example, if the Image File is named "MyImage.JPG," the matching extension file would be "MyImageJPG.EXT".

To copy an image, move an image or send an image to another LS software user, copy, move or send both files.

Performing 1D Analysis

Navigating 1D Analysis

Access the 1D Analysis functions via the **1D Analysis plug-in module, tools** or **menus**. The 1D Analysis plug-in module offers easy access to the analysis functions instead of using the menus.

1D Analysis Menus

LS Software offers the following 1D Analysis Menu from the main menu:

- **Master Tools**: Finds and edits lanes and bands.
- **Lane Tools**: Change the lanes in multiple ways.
- **Band Tools**: Change bands in multiple ways.
- **Background Correction:** Subtracts noise from the image.
- **Rf Line Tools**: Create and change Retardation factor (Rf) Lines in multiple ways.
- **Settings**: Opens the analysis settings window

Commands can be found and utilized from Toolbars in LS software. This allows users to customize most commonly used commands with a single button click. The toolbar can be hidden or shown based on user preference.

1D Analysis Plugin Module

- **Find Lanes and Bands**: Searches for lanes and bands in the image.

- **Edit Objects**: Select, move and resize lanes, bands, Rf lines, and the Region of interest (ROI).

- **No Background Correction**: Corrects the background with methods: No Correction, Straight Line, Joined Valleys, Rolling Disc and Area Between Lines.

- **Lane Profile Graph**: Displays a line graph of intensity or concentration value verses position in the lane for the lane or lanes selected.

- **Molecular Weight**: Calibrates molecular weight with Retardation factor (Rf) lines and by applying standards to lanes.

- **Dendrogram:** Compares the statistics of bands in one lane to bands in other lane for a single image.

- **Multi-Image Dendrogram:** Compares the statistics of bands in one lane to bands in other lane for multiple images.

- **Data Explorer**: Brings up datasheets showing the results of calculations on lanes and bands.

- **Delete all analysis data:** Deletes all recorded analysis data.

- **Lane Tools**: View Lane Properties, Add lane(s), Delete selected lane(s), Find bands in selected lane(s), Straighten selected lane(s), Auto curve selected lane(s).

- **Band Tools:** View Band properties, Add bands(s), Delete selected band(s)

- **Rf Line Tools:** Add Rf line, Delete selected Rf line(s), Delete all Rf lines, Select standard lane

- **Settings:** Sets image analysis settings.
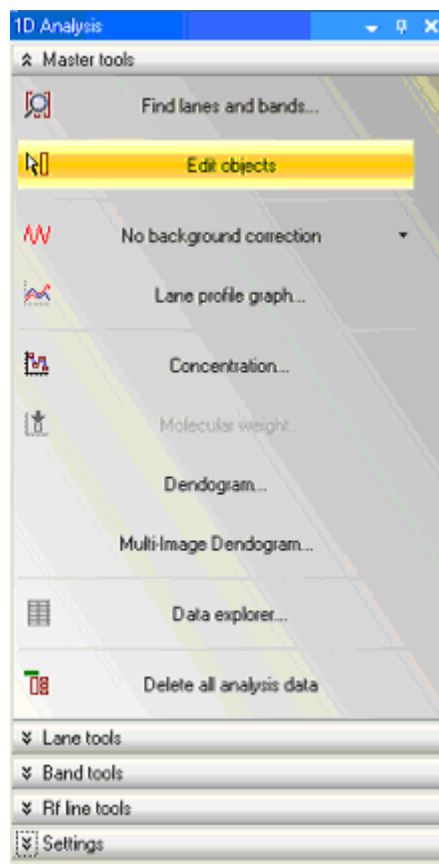
1D Analysis Context Menu Commands

The 1D Analysis context menu appears when 1D objects (lane, band, etc) are selected in the image.

- First, click onto Edit objects from the 1D Analysis Plug-in then right click onto the desired object. The shortcut menu will appear.

The **1D Analysis context menu** contains the following functions which also can be selected from the 1D Analysis toolbar

- Copy
- Lane profile graph
- Concentration calibration
- Molecular weight calibration
- Straighten
- Automatically curve
- Find Bands … Move slider bar to set band sensitivity
- Delete
- Properties shows the lane or band properties

NOTE: If some functions appear to be inactive in the 1D Analysis context menu box, they may have been pre-selected by default at the factory.

Finding Lanes and Bands

Finding 1D Gel Lanes and Bands

In this section, are the step-by-step processes for automatically finding lanes and bands, identifying Region of Interest and performing both automatic and manual searches for lanes and bands.

Identifying Region of Interest (ROI)

By selecting a Region of Interest, the software will analyze only the lane and band information within that area. This typically improves the accuracy of the automatic lane and band finding when the image background and gel background intensities vary.

Selecting the Region of Interest

- From the **Tools** menu, select **Region of Interest**. Select the rectangular ROI. In the Image Window, the selected shape is reflected.
- Outline the lanes of interest with the ROI tool by positioning the mouse at one of the corners to move that corner of the ROI. Then drag the corner to the desired location.

Perform an Automatic Basic Lanes and Bands Search

By selecting a Region of Interest, the software will analyze only the lane and band information within that area. This typically improves the accuracy of the automatic lane and band finding when the image background and gel background intensities vary.

- From the **1D Analysis** toolbar menu or plug-in, click on **Find Lanes and Bands**. The Lanes/Bands dialog window appears and a basic search is automatically performed using determined parameters.

- If the results are not satisfactory, keep the window open and adjust the search parameters as described in the following section, or click **OK** and use the manual **Add Lane** and **Add Band** functions to identify all lanes and bands correctly. Clicking **Cancel** returns the image to the previous the state being opened the Lanes/Bands dialog (for instance, if there were no lanes or bands before opening the window, **Cancel** will return to an image with no lanes or bands.)

Note: Typically the options for "Constant lane width across all lanes: and "Force all lanes straight" are turned on for 1D lane/band analysis.

Adjust Lanes and Bands Search Parameters

If the basic search did not find all lanes, adjust the parameters until the results are satisfactory.

To adjust the parameters:

After selecting Find lanes and bands in the 1D Analysis plug-in or toolbar, a Find lanes and bands window will appear that allows the user to adjust the search parameters.

Ensure that Lanes and Bands is selected in the Search mode at the top of the window; otherwise, the system will only search for bands within the existing lanes previously identified.

Under Search options, the user must choose from several options.

- **Constant lane width across all lanes:** If selected, the width of all the lanes will be consistent. Use this option if most of the lanes in the image are of the same width

- **Force lanes straight:** Use this option if the lanes curved. The software automatically adjusts the lane values so that they are straight in the analysis.

- **Use auto-detected parameter values:** If selected, this function returns the finds lanes and bands search options to its default values.

- Under **Search Parameters**, check that the **Image Background** is correctly set. If not, change it to match the background.

Having the wrong background setting is the cause of most problems in finding lanes and bands. Images with extraneous effects (writing, light leaks) on the edges of the image sometimes cause background color to be detected incorrectly due to poor band to background contrast.

Adjust the Lane sensitivity using the slider control. In general, to detect more lanes, drag the slider to the right; to detect fewer lanes, drag it to the left. Sometimes, however, if the sensitivity is very low, moving the slider to the right may find more and better-defined lanes. As the sensitivity is changing, the image adjusts automatically. Values can also be typed into the text box next to the Lane Sensitivity slider.

Note: The up and down arrows can also be used for incremental adjustment of the lane and band sensitivity slider bars. The LS Software will automatically search after any adjustments are made.

Adjust the Band sensitivity in the same manner as the lane sensitivity. However, it is usually premature to adjust band sensitivity if lanes are not correctly detected.

Directly below the OK button the count of lanes and bands the software will be shown. The image now reflects these lanes and bands with colored lines and rectangles.

To Return to the Automatically Detected Parameters

To return to the automatically-detected parameters:

On the Find Lanes and Bands window click Use auto-detected parameter values. The original parameters will be restored and the image will display lanes and bands as originally detected in Basic Search.

Tip: If the search mode is **Bands only**, the lane sensitivity value will be reset but the system will not search for lanes with the new value. To search for lanes and bands both, ensure that the search mode at the top of the window is **Lanes and bands**.

Searching Existing Lanes for Bands

- Choose 1D Analysis > Master Tools > Find Lanes and Bands.

- Ensure that the search mode at the top of the window is for **Bands Only**.

- Adjust the **Band sensitivity** using the slider control. In general, to detect more bands, drag the slider to the right; to detect fewer bands, drag it to the left. Sometimes, however, if the sensitivity is very low, moving the slider to the right may find more and better-defined bands. As the sensitivity is changing, the image adjusts automatically. Values can also be typed into the text box next to the Band Sensitivity slider.

- Click **OK.** The image now reflects the new information.

Note: Select Edit objects and then right-click on any lane to set the band sensitivity.

Related Topics:

- [Performing Dendrogram Analysis Calculations](#)

- [Performing Molecular Weight Calibration](#)

- [Performing Concentration Calibrations](#)

1D Analysis Image Window Features

In addition to displaying the image, the Image Window also displays various 1D gel objects and allows editing with the mouse.

- **Lane Curve Lines** (vertical lines down the center of each lane that control lane curving)

- **Bands** (marked by horizontal lines at the band peak)

- **Rf Lines** (white lines interrupted by small white circles at each intersection of the Rf line and a lane)

In addition to the objects above, there are also some 1D gel text labels that help identify data in the image. The labels cannot be directly manipulated. These labels are:

- **Lane IDs:** Letter codes at the top of each lane. Calibrated lanes are indicated by showing the lane ID in brackets (e.g. "[A]" as opposed to "A").

- **Lane Names:** Names entered in the Lane Information window for each lane, also shown at the top of the lane.

- **Band IDs:** Letter-and-number combinations showing the lane (letter) and band position (number) that uniquely identifies each band.

1D Analysis Settings

To access analysis settings, go to Analysis > 1D Analysis > Settings > 1D Analysis tab. This tab sets the following defaults:

- **Visible 1D analysis objects:** Use this window to identify lanes and bands.
- **Calibration settings**: At the request of the user, deletes calibration when changing background correction.
- **Mass Units**: This feature is for concentration units. The unit of mass that appears is "ng" (nanograms). Note that by clicking **Customize**, users may add additional units. Mass units are weight numbers, relative units or any relational units defined by the user.
- **Font size**: By clicking the buttons for **Lane Font Size** and **Band Font Size**, font size can be changed for the Lane ID, Lane Name and Band ID labels. The font range is from 6 - 20.

Manually Modifying Lanes and Bands

Lanes and bands can be added and edited manually. The following topics can be found in this section.

- Add
- Move
- Resize
- Delete
- Clear Information

Add Lanes and Bands Manually

LS software allows the user to add lanes and bands manually.

To Add Lanes Manually

- On the Analysis > 1D Analysis > Lanes tools > Add lanes or use the 1D Analysis plug-in > Lane tools then select Add Lane.
- Move the cursor over the image. A movable box will appear when the cursor is moved. Simply move the mouse over the image to the spot to add a lane. If the color of the box is green, a lane can be placed. If, however, the color is red, there is already a lane at this position and another lane cannot be placed there. Move the mouse until the box appears green.
- Click the left mouse button to place the new lane.
- When finished placing the new lanes, click on **1D Analysis > Master Tools > Edit Objects** to disable the **Add Lanes** tool.

To Add Bands Manually

- On the **Analysis > 1D Analysis > Band tools > Add bands** or use the 1D Analysis plug-in > Bands tools then select Add Band.

- Move the cursor over the image. A movable horizontal line will appear whenever the cursor is moved over a lane. Simply move the mouse over the image to the spot where to place the new band. If the color of the horizontal line is green, a new band can be placed where the cursor is. If, however, the color is red, there is already a band at this position and a new band cannot placed there. Move the mouse until the line appears green or adjust the other bands extents before adding the band.

- Click the left mouse button to place the new band.

- When finished placing band(s), click on **1D Analysis > Master Tools > Edit Objects** to disable the **Add Bands** tool.

Move and Resize Lanes and Bands

LS software allows the user to move and resize lanes and bands.

To Move Lanes or Bands Using the Image Window

- Click **Analysis > 1D Analysis > Master Tools > Edit Objects** or use the 1D Analysis plugin > Master Tools > Edit Objects.

- Select the lane or band to move by clicking on it. Control handles will appear at the four corners of the lane as white boxes.

- Drag the box side to side (or up and down) until it is moved to the desired location. Note that lanes can only be moved between other lanes, not beyond other lanes. Bands can only be moved between the bands above and below it, not beyond other bands.

To Move Bands Using the Lane Profile Graph

- Click **Analysis > 1D Analysis > Master Tools > Lane Profile Graph**, or use the 1D Analysis plug-in > Master Tools > Lane Profile Graph. The graph appears in a new window.

- Ensure that **Band Peaks** and **Band Extents** are both selected. Under the graph find the band markers. Drag the peak marker of the band (the large marker in the middle of two smaller markers) to the desired location for that band. Note that the markers on the graph change position as well. Note that bands can only be moved between the bands above and below it, not beyond other bands.

- Click on **OK**. The Lane Profile window closes and bands are now in their new positions.

Note: When editing bands or lanes, ensure that **1D Analysis > Master Tools > Edit Objects** is selected prior to clicking on a band or lane.

Resize Band Extents

To Resize Bands using the Image Window

- To turn on and view the Band Extents, go to **Analysis > 1D Analysis > Settings** to open the Preferences Window. Select the 1D Analysis tab and click the **Band Extents** check box. Click **Apply**. Close the **Preferences** Window.

- Click **Edit Objects** from the **1D Analysis** plugin. Place the mouse hand pointer over the band to resize, and click on the band. Control handles will appear at the four corners of the band as red boxes.

- Place the mouse hand over the top or bottom of the band's extents and click and drag the box border to increase or to decrease the size of the band until the size matches the band seen on the image

To Resize Bands Using the Lane Profile Graph

- Click **Analysis > 1D Analysis > Master Tools > Lane Profile Graph**, or use the 1D Analysis plugin > Master Tools > Lane Profile Graph for the graph to appear in a new window.

- Ensure that **Band Extents** is turned on. Under the graph, find the band markers. Drag the markers to the left or the right to the desired location. Note that the markers on the graph change position as well.

- Click on **OK**. The Lane Profile Graph closes and the bands are now in their new positions.

To Place Bands Exactly

- Click **Analysis > 1D Analysis > Master Tools > Edit Objects** or 1D Analysis plug-in > Master Tools > Edit Objects.

- Select the band to resize. Control handles will appear at the four corners of the band as red boxes.

- Under the **Analysis > 1D Analysis > Bands** and then **Band Properties**. The Band Information window appears.

- In the section of the window labeled **Geometry**, the numerical values of the **Top**, **Peak** and **Bottom** of the band may be changed.

- Enter the new numbers, click on **OK**. Now the location and dimensions of the band reflect precisely the values entered.

Delete Lanes and Bands

Delete Lanes

- Click **Analysis > 1D Analysis > Master Tools > Edit Objects** or 1D Analysis plug-in > Master Tools > Edit Objects.

- Click on the lane to delete. A box will appear around the band with white boxes at the corners.

- On the **Analysis > 1D Analysis** menu, select **Lane tools > Delete** selected lane(s). Or go to the **1D Analysis plug-in > Lane tools > Delete selected lane(s).** A window will pop up to request confirmation. Click **Yes** to delete the lane.

Delete Bands

- Click **Analysis > 1D Analysis > Master Tools > Edit Objects** or 1D Analysis plug-in > Master Tools > Edit Objects.

- Click on the band to delete. A box will appear around the band with red boxes at the corners.

- On the **Analysis > 1D Analysis** menu, select **Band tools > Delete** selected band(s). Or go to the **1D Analysis plug-in > Band tools > Delete selected band(s)**. A window will pop up to request confirmation. Click **Yes** to delete the band.

Clear All Lane and Band Information

- To begin a new analysis of your image, save the existing image with its lane and band information. Then perform a **Save As** for the new image, filing it under a new name, and proceed as follows:

- From the **Analysis > 1D Analysis > Master Tools**, click on **Delete All Analysis Data** or go to the 1D Analysis plugin > Master Tools, click on Delete all analysis data.

- All lane and band information will be deleted. To restore analysis information in the image, click the **Undo** button on the **Edit Menu**. Also **Redo** may return to the cleared image. Once the file is saved, however, undo or redo will not work to restore information.

Lane and Band Information

View Lane and Band Information

LS software allows users to view lane information in the following formats as listed below:

- Lane Properties
- Lane Profile Graph
- Data Explorer
- Printing Data Explorer Tabular Reports
- 1D Analysis Settings

To View and Use Lane Properties

Click on a lane of interest to select a lane. The lane will appear with four white boxes one in each corner of the selected lane. From Analysis > 1D Analysis > Lane tools, select Lane properties or click on Lane properties from the 1D Analysis plug-in. A new window appears.

In this window, the software allows users to perform various changes to the lane including:

- Name
- Current Color
- Current Font
- Geometry
- Molecular Weight Standard
- Mass

Lane Properties also offers the following information:

- Lane ID
- Unit Name of mass
- Intensity Maximum
- Intensity Volume
- Concentration Maximum
- Concentration Volume.

To View and Use Band Properties

Click on a band of interest to select a band. The band will appear with four red boxes one in each corner of the selected lane. From Analysis > 1D Analysis > Band tools, select Band properties or click on Band properties from the 1D Analysis plugin. A new window appears.

In this window, the software allows users to perform various changes to the lane including:

- Name
- Geometry
- Band Properties also offers the following information:
- Band ID
- Left and Right band positions
- Calculated Peak Values (Rf value and Molecular Weight);
- The Intensity of the band, including its Maximum, Volume, % of lane, and Mass; and
- The Concentration of the band, including its Maximum, Volume, % of lane, and Mass.

Lane Profile Graph

LS software allows users to view profile graphs (intensity vs. position) of one or more of the lanes in the image in the Lane Profile Graph. To access this function, select **Lane Profile Graph** from the **Analysis > 1D Analysis > Master tools > Lane Profile Graph** or click on Lane Profile Graph from the 1D Analysis plugin. A new window appears.

Underneath the graph, the software displays an image of the graphed lane (or of the lane last selected to be graphed).

In this section, the following topics are presented:

- How to display a single lane
- How to display multiple lanes at one time
- How to change variables for the x-axis and the y-axis
- How to change colors of the graph
- How to display specifics including band extents, band peaks, and background correction

Display a Single Lane

There are two options for displaying a single lane.

To Display a Single Lane by Selecting a Lane

- Select the lane of interest.
- From the **Analysis > 1D Analysis > Master Tools** select **Lane Profile Graph** or click on **Lane Profile Graph** from the **1D Analysis plug-in**. A new window appears with a graph of the selected lane.

To Display a Single Lane from the Lane Profile Graph

- From the Analysis > 1D Analysis > Master Tools, select Lane Profile Graph or click on Lane Profile Graph from the 1D Analysis plug-in. A new window appears with an empty graph.
- From the **Lanes** section of the Lane Profile Graph window and to the right of the graph, click on the lane to graph (Lane A is selected in the example on the right). The software automatically displays the graph of that lane.

Display Multiple Lanes

The software has two options for displaying a multiple lanes.

To Display Multiple Lanes by Selecting Lanes

- Click on the lanes to see graphed while holding down the **Ctrl** key.

- From the **Analysis > 1D Analysis > Master Tools**, select **Lane Profile Graph** or click on **Lane Profile Graph** from the **1D Analysis plug-in**. A new window appears with the selected lanes graphed.

To Display Multiple Lanes from the Lane Profile Graph

- From the Analysis > 1D Analysis > Master Tools, select Lane Profile Graph or click on Lane Profile Graph from the 1D Analysis plug-in. A new window appears with an empty graph.

- From the **Lanes** section of the Lane Profile Graph window and to the right of the graph, select all the lanes to graph (Lane A through Lane H are selected in the example on the right). The software automatically displays the graph of that lane. After selecting more than one lane to display, the **Multiple selection** box will contain a check.

Change the Selected Lane

- Click on the desired lane to de-select or re-select the desired lane in the **Lanes** section of the graph.

- The lane will be removed from or added to the line graph.

Axis Options

By default, the x-axis displays **Pixels** and the y-axis displays **Intensity**. However, after calibrating molecular weight, **Rf** values or **Molecular Weights (MW Standard)** may be a better naming option for the x-axis. Similarly, after calibrating concentration, **Concentration** may be a better naming option for the y-axis.

Change Axis Variables

To change axis variables after performing either molecular weight calculations or concentration calibrations, simply go to the **Y Axis** and **X Axis** options under the bottom left of the graph. Use the dropdown menus to select the desired variable.

Effects of Selecting Other Axis Values

If Retardation factor (Rf) or Molecular Weight (MW) is selected to be displayed on the x-axis, then the graph takes into account Rf effects. This means that other lanes may appear to be stretched or compressed horizontally relative to the selected lane.

Once Concentration is selected to be displayed on the y-axis, the curve adjusts the intensities of the lane, and the relative differences in the graph may change.

Display Options

In the Lane Profile graph, LS software allows users to choose what details to show in the graph. The program also allows users to change the colors of the background, graph, and axes.

Display Options for Details

Underneath the graph and the lane image, in the **Display** options section, the following are available:

- *Band Peaks*: Selecting this option means LS software will display an arrow labeled with the band's position at the top of the band on the graph, and a small rectangular control under the graph that can be used to move the band peak.

- *Band Extents*: Selecting this option means it will display parentheses showing the width of each band, and two small rectangular controls under the graph that can be used to adjust the band's extent.

- *Raw or Corrected Values*: Selecting this option means it will change the graph to reflect the new values after the correction.

- *Background Correction*: Selecting this option means it also will place on the *original* graph the graphed line of whatever background correction chosen.

Note: For more information on changing Band Peaks, Band Extents, and Background Correction, see Modifying Lanes and Bands.

The background correction can be displayed either on the graph with corrected values or the original graph with the correction line. It is not possible to display both at once.

Selecting Background Color

To change the background color of the graph for easier viewing:

- Underneath the Graph color options in **Lane Profile Graph**, click on the down arrow of **Background Color**.

- Select the color to display. The software automatically changes the color.

Change the Color of the Graph's Axes

- Underneath the Graph color options in **Lane Profile Graph**, click on the down arrow of **Axis Color**.

- ●elect the color to display. The software automatically changes the color.

Background Correction Options

To change the background correction option from the Lane Profile Graph:

- Underneath the Background Correction options in **Lane Profile Graph**, click on the down arrow of **No Background Correction**.

- Select the correction to display. The software automatically changes the graph. The user may also select to see a graph that takes into account the corrected values.

Viewing and Printing 1D Gel Analysis

- Overview
- Lane and Band Information
- Lane Profile Graph

- Viewing Data Explorer Results
- Printing Data Explorer Reports
- Exporting Data
- Fixed Image and Analysis Reports

Overview

LS software simplifies viewing and printing information about the image. This chapter will describe how to:

- View lane and band information
- Use the Lane Profile Graph, including displaying multiple lanes in a graph, changing the variables on the axes, and changing the display options
- Manage and print tabular reports
- Use the Data Explorer
- Export data
- View and print fixed reports of analysis settings, analysis lanes, analysis bands and the lane profile

Viewing Data Explorer Results

Aside from viewing graphs and information windows about the lanes and bands, the software also offers the option of seeing the data in a spreadsheet format that is user configurable.

- To access this **Analysis > 1D Analysis > Master Tools**, select **Data Explorer** or click on Data Explorer from the 1D Analysis plug-in.
- Data Explorer opens a tabular format with the ability to include or exclude certain data fields from the Data Explorer Report. Predefined report configurations are included to quickly select/deselect data fields appropriate to certain experiments.
- The Data Explorer window also offers Report Printing and Data Export options.
- The top left corner of the Data Explorer window offers a drop-down menu for quickly selecting preconfigured reports rather than having to manually filter report data for commonly reported analysis data. When selecting these reports, notice the various fields being selected/deselected from the list of data fields.

Filtering Data

Accessible when creating tabular reports or export data, the drop-down menu allows users to choose what specific data to show in reports and in exported files.

To Select Filter Data Fields

The top left corner of the Data Explorer window offers a drop-down menu for quickly selected preconfigured reports rather than having to manually filter report data for commonly reported analysis data.

When selecting theses reports notice the various fields being selected/deselected from the list of data fields. Select from:

- Image Information
- Lanes
- Bands for each lane
- Concentrations
- Molecular weights

To Show Filtered Data

To the right of the data fields is the actual analysis data, which includes the fields currently selected. Next to the image name there is a + symbol which indicates all of the analysis data is minimized under the image name.

- To show the analysis data, click the + symbol.
- To further expand the data views for the individual categories, select the + symbol to the left of each category.
- To filter the data further, select/deselect the appropriate data fields on the left side of the Data Explorer window.

Note: Any data column can be removed from the report by clicking and dragging that data column back to the data field on the left side.

Definition of Fields

Image information Report

- Image Name: Name of the opened image.
- Number of lanes: Number of lanes found.
- Background Color: Background color of the image.
- Background Correction: Type of background correction applied.
- Disk Radius: Radius of the Rolling Disk background correction type when is applied.
- Lanes Constant: Shows if the lanes have the same width.
- Lane Sum: Shows if the bands' mass sum up to the lane's value.
- Lanes Straight: Shows if the lanes are forced made vertical.

Lanes Report

- Lane Id: ID of the current lane.
- Name: User defined name for the current lane.
- Start: Starting pixel (in offset from top) of the current lane.
- End: Ending pixel (in offset from the top) of the current lane.
- Width: Width of the current lane.
- Mass: Mass for a specific lane as defined by the user in the lane properties window. Default is 100.

- I-Max: Maximum intensity value reached within a lane. It actually shows the brightest (for black background) or darkest (for white background) pixel value in the specific lane, with values accordingly with the image's bit depth.

- I-Vol: Sum of all intensities found in every pixel of the current lane.

- C-Max: Highest concentration value for a pixel achieved in this lane. It is computed accordingly with user-selected concentration.

- C-Vol: Sum of each pixel's concentration from the selected lane.

- LanesBands: Number of bands found in the current lane.

Bands for Each Lane Report

- Lane ID: ID of the referenced lane.

- Band ID: ID of the current band.

- Name: Given name by editing.

- MW: Computed MW value after a MW calibration has been applied.

- Rf: Computed Rf value obtained either by default assigning 0 to top and 1 to bottom, user-defining Rf lines, or applying MW calibration.

- Top: Intensity of the higher extent.

- Peak: Max intensity of the extent.

- Bottom: Intensity of the lower extent.

- Left: Position of the left extent.

- Right: Position of the right extent

- I-Max: Maximum intensity value reached within a band. It actually shows the brightest (for black background) or darkest (for white background) pixel value in the specific band, with values accordingly with the image's bit depth.

- I-Vol: Sum of all intensities found in every pixel of the current band.

- I-%: Lane's volume percentage occupied by this band's volume.

NOTE: I% of bands will sum up to 100% only if when the option "Lane volume is sum of bands" is checked. Otherwise, the lane volume value will include all pixels, including those outside the bands.

- I-Mass: How much of the user-calibrated mass for the lane this band is accounted for.

- C-Max: Concentration represented of the maximum value in a band.

- C-Vol: Sum of concentration in one band.

- C%: Percentage concentration in one band.

- C-Mass: Amount of reagent in one band with respect to the amount in the entire lane.

- Background Correction: Value that defines the background correction applied to the current band as follows: for white backgrounds, the higher the value, the lesser the correction applied; for black backgrounds, the lower the value, the lesser the correction.

Concentrations Report

Each row represents the number of the band and each column represents the ID of the lane.

- Reading the chart: The chart is read like a matrix, each cell value representing the computed/calibrated value for that band. A dash shows that the lane/column combination has no band representation on the image. (values assigned while performing concentration calculations)

- Calibrated Concentration Intensities: Represents the amount of intensity for which this calibration point was created. If this is a point from a band, it represents the total intensity of that band (I-Vol).

- Calibrated Concentration Ranges: User defined value for the amount of light specified in the previous column. (values assigned while performing concentration calculations)

- Source: Method in which this point has been added to the collection.

- N/A: Assigned to the Origin (0, 0) point.

- User: User adds both the Amount, and the Intensity values, in the concentration dialogue.

- A letter-number combination: Name of the band, used for setting the intensity. The value is added by the user.

- File: Values are loaded from a concentration file (*.conc).

Molecular Weight Report

Each row represents the number of the band and each column represents the ID of the lane.

- Reading the chart: The chart is read like a matrix, each cell value representing the computed/calibrated value for that band. A dash shows that the lane/column combination has no band representation on the image. (values assigned while performing molecular weight calculations)

Printing Data Explorer Tabular Reports

In Tabular Reports, print the data selected about the image. In the bottom left corner of the Data Explorer Tabular Reports window there are several print options:

- **Page header**: Displays as the page title on the top of the report

- **Page footer**: Displays as the page information at the bottom of the report

- **Page setup**: Displays the page setup options as offered by your specific printer

- **Print preview**: Displays a preview of what will be print on the report.

- Enter the header and footer information, set the page format, and click **Print**. When the print window opens, click **OK**.

Export Data Explorer Reports

LS software enables exporting of data to Microsoft Excel® or to other software packages for further analysis or documentation.

To Export Data Explorer Tabular Reports

To export data:

- Select the data fields you wish to export.

- In the bottom right corner of the Data Explorer Tabular window, select from two options and select whether to export the data by **Comma**, **Semicolon**, **Space**, **Tab** (where the delimiting character(s) are typed):

       ○ To Excel

       ○ To CSV

- Click the To Excel or To CSV button.
- Name the file and click Save.

Select a Report Type

- Summary
- Bands
- Lanes
- Molecular Weight
- Calibrated Concentrations
- All information

Fixed Image and Analysis Reports

Fixed Reports

LS software offers several standard reports that cannot be altered but that provide valuable reference information:

The Analysis Settings Reports:

- Background color
- Background correction
- Disk radius
- Lane width constancy across all lanes
- Lanes forced straight
- Lane volume is sum of all bands

The Analysis Lanes Reports:

- Lane ID and name
- Location of lane start and end
- Band count
- Mass
- Unit of mass
- Intensity maximum
- Intensity volume
- Molecular weight information
- Concentration maximum
- Concentration volume

The Analysis Bands Report:

- Band Name and ID
- Calculated peak values (Rf value and molecular weight);
- Intensity of the band, including its maximum, volume, percentage of the lane, and mass; and
- Concentration of the band, including its maximum, volume, percentage of the lane, and mass.

The Lane Profile Report: Gives a graphical representation of lane data.

To Print the Reports

Under the Tools Menu, select Reports. Select the report needed by clicking into the checkbox. For example, if the Analysis Settings report was needed, the user would click onto the Analysis Settings check box.

- Select **Print**, **OK**. Click **Exit** to close the **Reports** window.

To Change General Layout Before Printing

To change the margins, click on the Page Setup then make the margins larger or smaller by varying degrees. To view the margin lines, select Show Margins in the Preview Mode in the Reports window.

Enter information in Header Text or Footer Text. Note the following abbreviations for header and footer text:

- %p puts the page number at the top or bottom of the page;
- %c puts the page count at the top or bottom of the page;
- %d puts the date at the top or bottom of the page; and
- %t puts the time at the top or bottom of the page.

Performing Concentration Calibrations

- Overview
- Background Correction
- Concentration Calibration

Overview

Having sized and moved lanes and bands, concentration calculations can be performed. This section explains how to:

- Correct background for overexposure or uneven exposure of light or chemicals;
- Show the concentration graph for calibration;
- Select data points to plot on the graph;
- Select the type of curve to fit to the data points; and
- Remove all concentration calibration data.

Background correction is necessary to account for possible variable illumination or overexposure during image capturing, LS software offers options to apply mathematical background correction. These options generally remove background "noise" and elevated levels of pixel intensity due to excess exposure, highlighting data. There are four corrections options that are explained in the Specific Correction Options section of this chapter.

From the **Analysis > 1D Analysis > Master Tools > Background Correction** or the 1D Analysis plugin dropdown menu a new list appears.

Using the 1D Analysis Plugin

- From the list select which type of background correction, if any, that the software should perform.

Viewing Background Correction in the Lane Profile Graph

Choose the **Analysis > 1D Analysis > Master Tools > Lane Profile Graph** or click on Lane profile graph from the 1D Analysis Plugin.

On the graph, in the second box below Background Correction, click the down arrow to show the drop-down menu of the background correction options. Click the appropriate correction type. The graph will now display the background correction in the selected lane as a dotted line.

Specific Correction Options

LS Software offers background correction options.

- No background correction
- Straight line
- Joined valleys
- Area between the lines
- Rolling disc

No Background Correction

Selecting this option on the menu leaves the image uncorrected for overexposure, "as is."

Straight Line

Selecting this option tells LS Software to place a straight (but not necessarily horizontal) line under the lowest points at the beginning and end of each lane. LS removes the area of the graph under the straight line, so that all remaining values are emphasized. Straight line correction tends to correct well for overexposure, and for variable illumination that is focused on an edge or corner of an image.

Note: Straighten lines by using right-clicking the mouse button to open the
              shortcut menu.

Joined Valleys

Selecting this option accentuates the data by telling LS software to join lines between the lowest point, or "valley", before the first band, between each pair of bands, and after the last band. Intensities above the valleys (band data) are emphasized. Joined valleys can perform well in a variable illumination condition where the "bright spot" is somewhere in the middle of the image, and where bands are sharply defined

and quite distinct. Joined Valleys requires a sensitivity value to be entered. A higher value of sensitivity starts "eating" into the bands, which may not be accurate.

To Use Joined Valleys:

- Select **Analysis > 1D Analysis > Background Correction > Joined valleys** or use the 1D Analysis Plugin to select Joined valleys in the dropdown menu.

- A pop-up window appears that allows the user to set a sensitivity value from 0 to 100. Change the size either by typing in the sensitivity value, or by using the up and down arrow signs. Click **OK** after entering the radius

Rolling Disc

Picture turning the lane profile graph upside and then rolling a ball over the new top. Everything the ball is able to roll over is eliminated by the software; whatever the ball cannot roll over remains in the graph for analysis. Rolling disc performs well in all background conditions providing the size of the disc is carefully chosen. An excessively small disc will "roll into" bands, eliminating the band data almost entirely. An excessively large disc rolls across the lowest valleys, acting much like Straight Line correction.

To Use Rolling disk:

- Select **Analysis > 1D Analysis > Background Correction > Rolling disk** or use the 1D Analysis Plug-in to select Rolling disk from the dropdown menu.

- A pop-up window appears that allows the user to set a **Rolling Disk Radius** value from 1 to 1000. Change the size either by typing in the sensitivity value, or by using the up and down arrow signs. Click **OK** after entering the radius

Area Between Lanes

Part of the image may be overexposed, and there may be patterns of deformity between the lanes. This correction takes cross-sections between lanes and subtracts those "inter-lane" profiles. Area Between Lanes performs well in all variable illumination situations, providing lanes are distinct and there are clear gaps between them. It performs badly if bands in different lanes "bleed together" or touch, because it will tend to eliminate almost all band data at such a point.

Tip: For better concentration calibration accuracy, it is recommended the band boundaries/ extents be reviewed and adjusted if necessary. Related topic: Band adjustment. See Finding Lanes and Bands.

Concentration Calibration

Showing the Concentration Graph

With the background of the image corrected, LS software is now ready to graph intensity versus concentration and to fit curves or lines on the graph. It also allows the user to change the Unit Type plotted on the y-axis.

To Show the Concentration Graph:

- From **the Analysis > 1D Analysis > Master Tools > Concentration** or click on Concentration in the 1D Analysis Plug-in. A new window appears with a blank graph.

Graph Options

Graph display and units are easily modifiable by the user. The user may change the unit type or change the colors of the background, data points, and graph line. The user may also choose to display the anchor lines, point names, and point values.

Changing Unit Type

In the Calibration Graph, the Unit Type plotted along the y-axis is given as Concentration.

Selecting Unit Type

To plot a different type of unit along the y-axis, do the following:

- Select the **Graph Options** tab in the Concentration window. Click on the **Y-axis unit** drop down menu.

- Select the unit type to display. The y-axis reflects the new unit name.

  Note: If unit type required is not in the drop-down menu, LS software allows the user to add it in.

Adding Unit Type

To add a unit type, click the Add button. A New Unit field will appear and be highlighted. Type in the name of the unit to appear in the Y-axis unit drop down menu.

Click **OK**.

Editing Unit Type

To edit a unit type, click on the unit name to edit (Concentration cannot be edited). Edit the name of the unit to change.

Click **OK**.

Deleting Unit Type

To delete the unit type, click on the unit name to delete (Concentration cannot be deleted). Then click on Delete. The unit name is removed.

Click **OK**.

Selecting Data Points

LS asks the user to select data points to plot on the graph.

In the Concentration Window, note that there are three tabs, Graph, Data Points and Graph Options. Any of these tabs may be selected when clicking on bands to calibrate to data points on the graph.

- Click on a band in the image that has a known amount to calibrate. The **Edit calibrated intensity** window opens. Enter the "known" amount (standard) in the **Concentration** field.

- Click **OK**. Continue to select the remainder (individually) of the "known" concentrations and enter the "known" concentrations in the Concentration Window. The data point entered is now plotted on the graph under the **Graph** tab. Under the **Data Points** tab, LS shows the exact position of the data points and where they will be plotted.

  Select as many data points as desired following the steps above. Note that as data points are added, LS will fit a curve to the points using the method selected by the user.

Editing Data Points

To edit the data points that already selected:

- Click on the **Data Points** tab of the Concentration window

- Click on the value that to edit.

- Click **Edit.** The **Edit** window pops up.

- Change the concentration value to the desired number.

- Click **OK.**

Deleting Data Points

- Click on the **Data Points** tab of the Concentration window.

- Click on the concentration that to delete.

- Click **Delete**.

- Click **OK**.

Note: The Concentration window can be accessed from the 1D Analysis Toolbar.

Selecting Curve Type

Once the data points are selected to graph, the software allows the user to select the type of curve or line to fit to the data points.

In the Graph tab of the Concentration window, select the curve model desired from the drop down menu.

LS software has several possibilities for curve models:

- *Least square line:* a straight line (polynomial degree 1);

- *Least square quadratic*: a binomial curve (polynomial degree 2);

- *Polynomial 3rd degree*: a polynomial curve of degree 3;

- *Polynomial 4th degree*: a polynomial curve of degree 4;

- *Best Fit:* Selects the curve with the highest 'Goodness of Fit' value.

Note: In using polynomial curve types, make sure that there is at least one more data point selected than the degree of the curve e.g., if a Polynomial 3rd degree is selected, there needed to be at least four data points.

LS software automatically and immediately fits the curve model chosen to the data points as the various models are selected. In the curve model list, it shows the Best Fit for the curves as they are graphed. The goodness of fit is found from the coefficient of determination (also known as "r-squared"). The goodness-of-fit value ranges between 0.0 and 1.0. A value of 1.0 for the goodness of fit indicates a perfect fit.

Removing Concentration Calibration

To remove all calibration information including data points plotted on the graph and curve lines, simply go to Analysis > 1D Analysis > Master Tools > Concentration or go to Concentration in the 1D Analysis Plugin. The Concentration Window pops up.

- Select the **Data points** tab in the Concentration Window then select **Delete all**. A window will pop up asking the user to confirm the removal all calibration data.

- Click **Yes** or **No**. By clicking on **Yes** all calibration data is removed and a new analysis can be started.

Note: Changing the background correction method changes net intensity values and therefore invalidates concentration calibration. If the user changes the background correction method, LS software will ask the user to confirm deletion of all concentration data. Answering **Yes** is the same as selecting **Delete all** in the **Data points** tab.

Note: Moving the bands or resizing them also changes their net intensity values. As a result, the user will see the word "Custom" appear in the data-source column (Concentration Calibration Window), instead of the name of the specific band.

When all lanes and bands information is deleted, the user will be asked if all corresponding Concentration Curve data should be removed.

Performing Molecular Weight Calibration

- Overview
- [Retardation factor Rf Lines](#)
- [Applying a Molecular Weight Standard to a Lane](#)
- [Removing a Molecular Weight Standard from a Lane](#)

Overview

Molecules in an electric field migrate through a gel matrix at rates inversely proportional to the log10 of the number of base pairs. Large molecules migrate more slowly due to large frictional force from the pore of the matrix while small molecules migrate faster due to less frictional force.

There are many experimental conditions affecting the migration rate: gel concentration; conformation of the DNA; applied voltage; direction of electric field; base composition and temperature; presence of intercalating dyes; and electrophoresis buffer. It is therefore desirable to use a known molecular weight standard as a reference to unknown samples. This marker is used to calibrate the resulting molecular weight for each unknown bands.

Using a molecular weight (MW) marker results in a band encompassing the whole gel horizontally. This band can be thought of as the distance traveled of a band relative to its front (Retardation factor - Rf) or starting position. This Rf line exists for each band in the molecular weight standard. Any bands in the unknown samples that migrate to any of these Rf lines are then compared to the Rf lines.

This section will explain how to:

- Manage molecular weight standards and calibrate lanes to these standards
- Determine Rf values automatically
- Add Rf lines manually

Note: Rf line functions are only required if there are less than two calibrated lanes

Molecular Weight Calibration

Calibration of molecular weight (MW) involves associating a known standard with one or more lanes in the image. This allows Rf values to be calibrated to molecular weight values. LS Software allows the user to employ several different standards per gel.

To help with analysis, LS provides a library of molecular weight standards. Standards can be added, edited, and deleted from the library using the following instructions.

Adding a Molecular Weight Standard to the Library

- Select the lane to calibrate by clicking on it.

- Select **Analysis > 1D Analysis > Master Tools > Molecular Weight** or select Molecular Weight in the 1D Analysis Plugin. A new window appears.

- In the **Step 1: Select MW standard** tab, click **Add**. A new **Create MW standard** window opens. In this window, provide the new **Name** of the standard, **Group**, and **Unit Type**.

- Click **Add**. Now the numerical values of the standard can be entered. After entering the numerical value, click on **Add** again for as many values as desired

- Click **OK** on the right side. The first window appears again with the new standard entered.

Editing a Molecular Weight Standard in the Library

- Select the Lane to calibrate by clicking on it.

- Select **Analysis > 1D Analysis > Master Tools > Molecular Weight** or select Molecular Weight in the 1D Analysis Plug-in. A new window appears.

- In the **Step 1: Select MW standard** tab select the standard to edit. (NOTE: This applies only to standards created by users. The MW Standards included with the software cannot be edited or deleted.) Then click on **Edit**.

- The information previously entered into this window (including group, name, units, or most commonly, weight) can now be changed.

- Click **Edit** to reflect the new change, and then click **OK**.

Deleting a Molecular Weight Standard in the Library

- Select the Lane to calibrate by clicking on it.

- Select **Analysis > 1D Analysis > Master Tools > Molecular Weight** or select Molecular Weight in the 1D Analysis Plugin. A new window appears

- In the **Step 1: Select MW standard** tab select the standard to delete. (NOTE: This applies only to standards created by users. The MW Standards included with the software cannot be edited or deleted.) Then click on **Delete**. LS software then confirms that the standard will be deleted. Select **Yes** to confirm.

- Click on **Cancel** to return to the image window.

Copying a Molecular Weight Standard in the Library

- Select the Lane to calibrate by clicking on it.

- Select **Analysis > 1D Analysis > Master Tools > Molecular Weight** or select Molecular Weight in the 1D Analysis Plug-in. A new window appears.

- In the **Step 1: Select MW standard** tab select the standard to copy. Then click on **Copy**.

- The **Edit** window will appear. Change the Name of the new MW Standard. Click on **Add to enter a new Value** or click on a value and click **Edit** to make changes in the copy. Then click on **OK**.

Applying a Molecular Weight Standard to a Lane

Calibrating a Lane

- Select the lane to calibrate.

- From the **Analysis > 1D Analysis > Master Tools > Molecular Weight** or select Molecular Weight from the 1D Analysis Plugin.

- A new **Molecular Weight** window opens. On tab **Step 1: Select MW standard** choose the standard to use. If the standard is not on the list, add the standard to the list.

- Click Next. A new screen appears **Step 2: Calibrate MW standard**.

- In the Step 2 screen, the weights can be adjusted to match the bands that actually appear. *Complete instructions for this appear below.*

- Click **OK** to save the calibration.

Using the Stretch Factor

The stretch factor establishes a mathematical relationship between the weights to describe their relative movement. The larger the stretch factor, the lighter weights move in relationship to heavy ones. The smaller the stretch factor, the less the lighter weights move. A stretch factor of 1.0 indicates linear movement (weights move in direct proportion to their relative weights).

To adjust the weights to match the bands, choose to use the stretch factor:

- In the **Step 2** screen of the **Molecular Weight** tool, ensure that **Proportional** calibration mode is selected.

- Drag a known weight up or down with the mouse until it matches the appropriate band. Alternatively, select the weight with the mouse (or TAB key) and move the weight up and down with the UP and DOWN arrows on the keyboard (this helps adjust the weight by small amounts). When there is a match of the weight with the band, the line color changes from blue to yellow (default colors).

- Adjust the stretch factor (scaling) between weights until the other weights match their appropriate bands. Adjust it using the mouse wheel (rolling up increases the value, rolling down decreases it), or enter a new value into the text box and click **Set**.

- Click **OK** to save the calibration.

Note: Using the Stretch Factor gives a weight match on the band up to 0.5% of the assigned weight. To obtain *exact* placement on the band, use the manual mode, described in the **Using Manual Placement of Weights** below.

Using Manual Placement of Weights

If the user does not want to use the Stretch Factor, the weights can be adjusted individually using manual mode:

- In the **Step 2** screen of the **Molecular Weight** tool, select **Independent** calibration mode.

- Move each weight separately, with either the mouse or the keyboard arrows, to position them exactly on the band. Weights cannot pass one another, so it is usually best to start with either the lightest or heaviest weight and work toward the other end.

Exact Placement of Bands

In the Independent calibration mode, as soon as the weight is exactly on the band, the color of the line changes from blue to yellow (default colors). This means *exact* placement is achieved-- the weight will be exactly on the band peak. Exact placement only occurs when the color changes from unmatched to matched; further movement of the weight may alter the exact positioning. When in doubt, move the weight completely away from the band and reposition it with the arrow keys until the color changes.

The Independent > Tag All button reduces some manual work by aligning the weights of the ladder starting with first band in the lane. Stretch factor is not taken into account – only simple matching is done.

Note: On the second window of the calibration operation (where users adjust weights to bands), the colors of both the unmatched and the matched lines can be changed by using the controls in the upper right corner.

Note: After calibration of two or more lanes, Retardation factor lines will be automatically calculated and will replace any previous Rf line work.

Removing a Molecular Weight Standard from a Lane

If a Molecular Weight (MW) Standard has already been calibrated to a lane, the calibration can be removed from a lane by doing the following:

- Select the **Analysis > 1D Analysis > Master Tools > Molecular Weight** or select Molecular Weight from the 1D Analysis Plugin.

- A new Molecular Weight window appears. Go to the **Step 1: Select Molecular Weight Standard** tab.

- Choose Uncalibrate lane.

- Click **Yes** to confirm the removal of the MW calibrated lane.

Retardation factor (Rf) Lines

Automatic Rf Line Determination

When two or more lines are calibrated with molecular weight standards, LS Software creates Retardation factor (Rf) Lines automatically. These lines express any differences in horizontal alignment between bands (or points on a lane) of equal molecular weight. Ideally, there will be one Rf line for each distinct molecular weight used in a calibration. Since users may utilize more than one standard on a single image, and each standard may contain several weights, automatic generation can result in a large number of Rf lines.

An automatic RF line can be moved by re-calibrating the lanes. An automatic Rf line can be adjusted by dragging the lane-intercept marker up or down, but only in lanes that are not calibrated.

To Adjust an Automatic Rf Line

- If Rf lines are not visible, turn them on through **Analysis > 1D Analysis > Settings.** When the **Preferences** window opens, select the **1D Analysis** tab and click on the **Rf line** box (so that a check appears in the box) in the Visible 1D analysis objects area.

- Select the Rf line to adjust. Control handles will appear.

- Any lane that has a white control handle *without* an "X" is an uncalibrated lane, and it is possible to move the intercept point up or down by dragging it. If a lane has a gray control handle *with* an "X," it is calibrated and cannot be moved.

- After adjusting the Rf lines, hide them to view the other 1D objects more easily. To hide the Rf lines, go to **Analysis > 1D Analysis > Settings.** When the **Preferences** window opens, select the **1D Analysis Settings** and unclick **Rf line** in the Visible 1D analysis objects area.

Adding Rf Lines Manually

LS Software allows users to add Rf lines to an image with less than two lanes that are calibrated to molecular weight standards. (On images with two or more calibrated lanes, Rf lines are created automatically and the software will not allow new lines to be added, although the automatically added lines can be adjusted as described in the Automatic Rf Line Determination section.)

To add an Rf line manually

- On the **Analysis > 1D Analysis > Rf lines**, click **Add Rf Line** or click on **Add RF line** in the **1D Analysis Plugin**. Rf lines will be made visible if they were formerly hidden, and a window will pop up entitled **Add Rf Line**.

- The cursor will now appear as a square cross. Select the first band that is part of the Rf relationship in the captured image.

- Click on a second and subsequent bands in other different lanes. Click on as many bands as preferred to draw the line (up to one per lane), but at least two must be chosen.

- To place an Rf line anchor (circle) on a non-band location, hold down the CONTROL (CTRL) key as the circle is placed.

- When finished selecting the points that will make up the line, click **Confirm Add** in the **Add Rf Line** dialog window. The green line will now appear white, and a new Rf line will appear.

- The software will require the user to select a standard lane. Identify the standard lane by clicking on the standard lane in the image. The standard lane will be outlined in green when selected.

- To place more Rf lines, follow the same process. Note, however, that new Rf lines are allowed as long as they do not cross another Rf line. If the line appears green, the Rf line is allowed. If there

are red marks on the Rf line created, the Rf line will not be created when **Confirm Add** is selected. Ensure that the entire line is green before clicking **Confirm Add**.

- Click **Close** when finished adding Rf lines.

Moving Rf Lines

Existing Rf lines can be adjusted, whether they were created automatically or manually. To move Rf lines to match bands of the same molecular weight:

- Click Analysis > 1D Analysis > Master Tools > Edit Objects or select Edit objects in the 1D Analysis Plug-in.

- Select the Rf line to adjust. Control handles will appear.

- Any lane that has a white control handle *without* an "X" is an uncalibrated lane, and it is possible to move the intercept point up or down by dragging it. If the lane has a gray control handle *with* an "X," it is already calibrated and cannot be moved.

- After Rf lines have been adjusted hide them so other 1D objects can be seen more easily. From the Analysis menu, select 1D Analysis Settings, then turn off Rf Lines.

Deleting Rf Lines

Rf lines that were added manually can also be removed (automatic Rf lines cannot be removed).

To Remove One Rf Line

- Click Analysis > 1D Analysis > Master Tools > Edit Objects or select Edit objects in the 1D Analysis Plugin.

- Select the Rf line to delete in the image.

- From the Analysis > 1D Analysis > Rf lines and click Delete selected Rf line(s) or select Delete selected Rf line(s) from the 1D Analysis Plugin.

Tip: Rf lines can also be deleted by selecting it and pressing the DELETE key.

To Remove All Rf Lines

- From the **Analysis > 1D Analysis > Rf lines** and click Delete all Rf lines or select Delete all Rf line(s) from the 1D Analysis Plugin.

Performing Dendrogram Analysis

- Overview

- Dendrogram Calculation

- Multi-Image Dendrogram Calculation

- Modify Graphical Display

Overview

Dendrograms are graphical/numerical displays used to show the similarity bands. The similarity is grouped into clusters. Each lane in an image has its own cluster. Then, repeatedly, a linkage rule (selected by the user in the software) is employed to merge smaller groups into larger clusters, until all the clusters have been combined into a single cluster. The result is a hierarchy of clusters. When moving up the hierarchy, each cluster contains more but less similar lanes. Lanes that are very similar to each other will appear together in clusters near the bottom of hierarchy.

This section will explain how to:

- Automatically generate dendrogram information
- Automatically generate multi-image dendrogram information
- Modify dendrogram graphs

Generating Dendrogram Graphs

After finding the lanes and bands in the image the user may begin the process of generating dendrogram graphs.

- Finding lanes and bands must be performed first.
- Go to **Analysis > 1D Analysis > Dendrograms** or select Dendrograms from the 1D Analysis Plugin.
- A **Dendrogram View** window will appear.
- The user may select from Band Formula: Rf Values or Weight Values, Lane Formula: Dice Similarity or Jaccard Similarity and the Linkage Formula.
- A **Dendrogram View Graph** will appear detailing the relationships between lanes and bands.

Generate Multi-Image Dendrogram Graphs

- Finding lanes and bands must be performed first on all images to be used in the graph. Related topic: Band adjustment.
- Go to Analysis > 1D Analysis > Multi-Image Dendrograms or select Multi-Image Dendrograms from the 1D Analysis Plugin.
- A **Dendrogram MultiImage** will window will appear. Click **Add** to select the images on which to perform the multi-image dendrogram calculation.
- Click **OK**.
- A **Dendrogramv View** window will appear.
- The user may select from **Band Formula**: Rf Values or Weight Values, **Lane Formula**: Dice Similarity or Jaccard Similarity and the **Linkage Formula**.

Modify Dendrogram Graphs

The dendrogram graph allows users to change the Background color, Line Color, Node Color, and Text Font. The user may also select to change the lane identification type to Names or Names and IDs. Simply use the dropdown menu in each category to select the desired graphical display option.
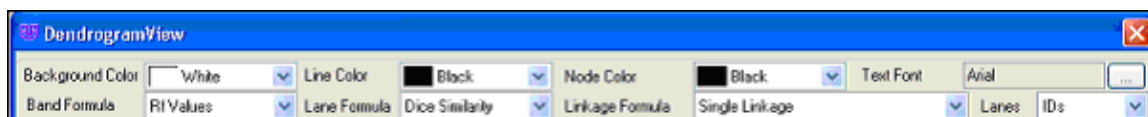
Creating clusters

Initially, each lane has its own cluster. Then, repeatedly, a linkage rule (see below) is used to merge smaller groups into larger clusters, until all the clusters have been combined into a single cluster. The result is a hierarchy of clusters. Moving up the hierarchy contains clusters with more but less similar lanes. Lanes that are very similar to each other will appear together in clusters near the bottom of hierarchy.

The dendrogram shows the links that have been made between the clusters to form larger clusters – the shorter the distance between items in the dendrogram, the more similar they are.

Linkage rules

A linkage rule offers a method to calculate a measure of the distance between two clusters.

- **Single Linkage (nearest neighbor)**: The distance between two clusters is given by the distance between the two closest items (lanes) in the different clusters.

  Using this method often causes *the chaining phenomenon*, which is a direct consequence of the single linkage method tending to force clusters together due to single entities being close to each other regardless of the positions of other entities in that cluster.

- **Complete Linkage (furthest neighbor)**: The distance between two clusters is given by the greatest distance between two items in the different clusters.

  This method should not be used if there is a lot of noise expected to be present in the dataset, because outliers are given more weight in the cluster decision. It also produces very compact clusters. This method is useful if one is expecting entities of the same cluster to be far apart in multi-dimensional space (provided there is no noise).

- **Unweighted pair-group method average (UPGMA)**: The distance between two clusters is calculated as the arithmetic mean of the distances between all possible pairs of entities of the two clusters in question.

  This method is a halfway choice between single and complete linkage. The chaining problem is not observed for this method and outliers are not given any special favor in the cluster decision, which makes this method the most popular.

- **Weighted pair-group method average (WPGMA)**: This is identical to UPGMA except that the number of items in a cluster is taken into account – this may be useful when there is a large variation in the number of items in the clusters.

- **Unweighted pair-group method centroid (UPGMC)**: The distance between two clusters is the distance between the centroids of each cluster (the centroid of a cluster is the average point in the multidimensional space of the cluster). The resulting trees are not right-aligned and branches can have negative values.

- **Weighted pair-group method centroid (WPGMC)**: This is identical to UPGMC except that the number of items in a cluster is taken into account – this may be useful when there is a large variation in the number of items in the clusters.

- **Ward's method**: This method differs from the others in that it uses an analysis of the variance to calculate distances between clusters. An item is joined to a cluster if the joining results in a minimum degree of variation within the cluster. This means that items will not get grouped into a cluster simply because they do not belong anywhere else. As a consequence, Ward's method can lead to a large number of small clusters. Details of the method can be found at

http://marketing.byu.edu/htmlpages/books/pcmds/WARD.html

- **Neighbor joining**: At each stage of clustering the total branch length is minimized. The distance between two items is approximately the sum of the branch lengths between them. The trees are not right-aligned and branches can have negative values. Details of the method can be found at

http://www.icp.ucl.ac.be/~opperd/private/neighbor.html

Performing Colony Counting

The software offers several functions for counting colonies.

- [Automatic Counting](#)
- [Manual Counting](#)
- [Zone Analysis](#)
- [Spiral Counting](#)
- [Renumbering Colony/Zone Values](#)

Related Topics:

- [User Define Template Counting](#)

Colony Count Module

The Colony Count module contains options that allows users to perform analysis on the captured image

- **Main Tools** – Allows users to perform the colony count function and gather the colony count results

  - o **Start Colony Count**…- Allows the user to count colonies in the captured image

  - o **Show Results Window** – Allows users to view colony count results in a tabular format

  - o **Clear Analysis** – Allows users to clear the colony count analysis

- **Post-Processing Tools –** (column header-no actual functions performed) contains options that allows users to perform edits to the original colony count

  - o **Edit Colonies –** (column header-no actual functions performed)

  - o **Add Colonies –** Allows users to add colonies to the original count

  - o **Delete Colonies –** Allows users to delete colonies in the original count

  - o **Manual Split Colonies –** Allows users to split colonies by drawing
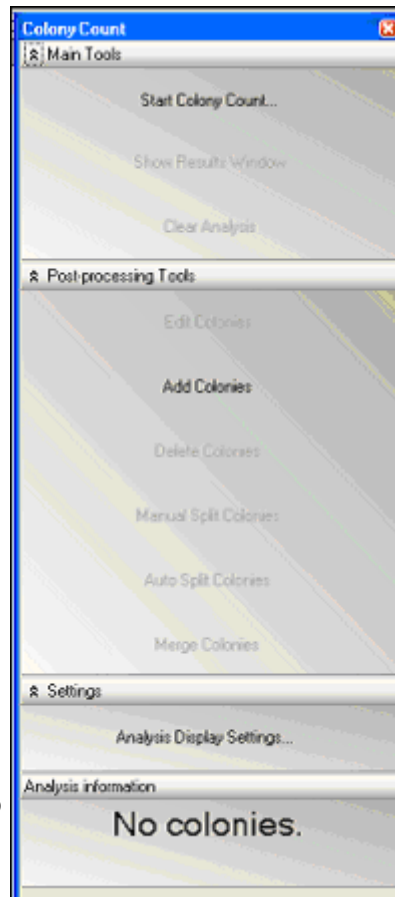
a line through the colony to be split

- o **Auto Split Colonies** – Allows users to split colonies by clicking on a colony to split

- o **Merge Colonies** – Allows users to merge colonies that are less than four pixels apart

- **Spiral Plate Analysis –** Controls the settings specifically for spiral plates

  - o **Spiral Plate Analysis** - Brings up a spiral plate analysis window to define measures and function

  - o **Align Spiral Plate Overlay** – Allows users to reposition the grid overlay on the counted plate

  - o **Clear Spiral Plate Analysis** – Clears the grid and all analysis from the plate and reports

- **Settings** - (column header-no actual functions performed) contains options that allow the user to set more appealing display options

  - o **Set Display Settings –** Allows the user to set more appealing display options such as color annotations and fill type

- Grid Analysis Tools –

- **Recompute Colony Labels** – Renumbers colonies or zones found by the counting process

- **Analysis Information** – Displays the number of colonies counted

Preference Settings for Colony Counting

Open the Preferences window from **File > Preferences**. This window allows users to set new defaults for the display options, label color, annotation, camera template settings and miscellaneous functions such as annotation.

Label Type

To change the **Label Type**, from the **Analysis > Colony Count** preferences window, click the drop down arrow and select from:

- **None**: Assigns no number to the colony or zone counted.

- **Class**: Assigns the class number to colony or zone counted.

- **Number (general):** Assigns an increasing value to the colony or zone counted.

- **Number (in class):** Assigns an increasing value to the colony or zone counted and starts from a value of one for each class represented.

Colony Marking

To change the **Colony Marking**, click the drop down arrow to select from:

- **None**: Shows no pictorial indication of the count value.

- **Outline**: Draws a bounding line around the colony or zone.

- **Fill**: Shows the entire colony or zone filled in with color.

Label Color

To change the **Label Color**, click **Auto** or click the drop down arrow and select from the colors listed.

Circle Radius

To change the **circle radius**, click the drop down arrow and select from the numbers to change circle pixel radius.

Text Annotation

To change the **text annotation behavior**, select from:

- **Synchronize size with image zoom**: Increases/decreases the size of the annotation if the size of the image changes using the zoom feature.

- **Don't synchronize with image zoom**: Does not increase/decrease the size of the annotation if the size of the image changes while using the zoom feature.

Log Preferences

The **Logging** preferences window allows users to define the log file path and the log level.

- To change the **Log File Path**, click … and locate a new directory.

- To change the **Log Level**, click the drop down arrow and select from the list.

Automatic Counting

- Open the Colony Counting Plug-in by selecting View > Plug-in > Colony Counting Plug-in and select **Start Colony Count.**

- Select Automated Count.

- Select **OK**.

- The automated count will be displayed.

- Colonies can be filled in with red or outlined. Colonies can also be numbered or annotated using multiple colors.

- If desired, colonies can added, split, deleted, merged.

Manual Counting

- Open the Colony Counting Plug-in by selecting View > Plug-in > Colony Counting Plug-in and select **Start Colony Count.**

- Select Manual Count.

- Click **OK**.

- The **Manual Count Colony Wizard** module will open.

- Proceed to Step 1 and Step 2 of the manual counting process.

Manual Counting Step 1: Select Classes
Manual Counting Step 2: Finish

Manual Counting Step 1: Select Classes

The window that appears after choosing Manual Count is the manual count window. It contains two tabs. The first tab is Step 1 of 2: Select Classes and the second tab is Step 2 of 2: Finish.

Defining the Counting Region

This tab allows the user to define the region of interest. The region of interest is identified by a green circle that should include all the colonies (or zones) of interest in the Petri dish. The software automatically selects a region of interest but by clicking the **Define Counting Region** button, but the user can increase or decrease the circle size.

If a smaller or larger region of interest is desired, click the **Define Counting Regio**n button and hold and drag the pointer over one of the corners of the circle to change the size.

Select Classes

The **Select classe**s window allows users to select the number of classes and colonies (or zones) in the Petri dish sample.

- To select the desired colonies (or zones), ensure that the **Add Poin**ts button is highlighted in yellow and **click on a colony (or zone**) in the original image of the Petri dish. (Not the image shown in Step 2 of 2: Select classes window.)

- Classes are defined as different types of bacteria, yeast, or mold present on the same Petri dish sample. The software can detect various types of classes in one dish. To add a class, click onto the **Add** button in the **Classes** section of the window.

Once the first desired colony (or zone) is selected, the new image in the **Step 1 of 2: Select classes** window will show a black background along with all of the colonies that contain the same color as the point selected.

The color of the point selected will also appear in the **Analysis Details** box (for example: rgb (202,183,151).

- Continue adding points and classes as necessary until the black and white image shows all the colonies of interest in white.

To remove a class or a point, highlight the class or point in the **Analysis Details** box and click the **Remove Point** button.

NOTE: The Step 1 of 2: Select classes tab allows the settings created in this window to be saved in a user defined template. If there are no templates created, the drop down menu will list Default as the only template option.

Manual Counting Step 2: Finish

To filter the colonies (or zones), adjust the **shape** or **colony size** (or add and subtract zones) in the **Step 2 of 2: Finish**, **Filter colonies** section:

- Change slider buttons for **shape** (from circular to irregular) and **size** (1 pixel to 200 pixels) to capture additional colonies or eliminate colonies (or until all of the zones of interest are highlighted).

- This window is a second means to save settings into templates. Go to the Templates

section of this manual for instructions on creating templates.

- Click **Finish** to exit the manual count mode.

The software will redirect to the initial screen and display the total number of colonies (or zones) identified.

To view the analysis data for the zones click onto **Show Results Window** from the Main Tools section of the Colony Count module. A window appears that provides information relating to the classes, zones (listed as colonies), statistics, and distribution of the zones. In the report, users may view the area and perimeter of the zone.

Related Topics:
[Creating Colony Templates](#)

Edit Colonies

Once a colony has been counted, users may edit colonies using several functions:

- [Add Colonies](#)
- [Delete Colonies](#)
- [Split Colonies](#)
- [Merge Colonies](#)

Add Colonies

Users may add colonies after the initial count.

- To add colonies, ensure that the **Add Colonies** tab is highlighted in yellow.
- Click on a colony to add.
- The colony selected will now have a circle (filled in or outlined) around the point selected.
- The **Total colonies** count will change to include the colonies added.
- If the colony is hard to see, zoom in on the colony.
- A rectangular object will appear in the **Zoom/Pan** module. Move it over the area of interest.
- The central Petri dish image will appear larger in the area of interest identified.
- To use a larger circle to highlight the colony, go to the **Analysis Display Settings**. A new window will open. Browse to **Main Settings > Analysis > Colony Count.**
- In the **Add Tool** section of the window, use the drop down menu to select between a **Circle Radius** of 5, 10, 15, or 20.

Delete Colonies

Users may delete colonies after performing the initial count.

- To delete colonies, ensure that the **Delete Colonies** tab is highlighted in yellow.
- Click on a colony to delete.
- The colony circle (filled in or outlined) will now disappear. The **Total colonies count** will change to remove the colonies indicated.
- If the colony is hard to see, zoom in on the colony.
- A rectangular object will appear. Move it over the region of interest.
- The central sample image will appear larger in the region of interest identified.

Split Colonies

Manual Split Colonies

Users may split colonies after performing the initial count.

- To split colonies, ensure that the Manual Split Colonies tab is highlighted in yellow.
- A new window will ask the user to "Draw lines through the colonies you want to split then release".
- Draw lines through two or more colonies that are close together and treated as one. Use the pointer and, while holding the left mouse button down, move from one edge of the colony to the other.
- The Total colonies count will change to add new colonies identified by the split.
- A rectangular object will appear. Move it over the region of interest.
- The central sample image will appear larger in the area of interest identified.
- If the colony is hard to see, zoom in on the colony.

Auto Split Colonies

Users may split colonies after performing the initial count. Colonies should be split when two or more colonies are very close together and are counted as one colony by the software.

- To split colonies, ensure that the Auto Split Colonies tab is highlighted in yellow.
- Click onto the colony to split.
- If the colony is hard to see, zoom in on the colony.
- The Total colonies count will change to add new colonies identified by the split.

Merging Colonies

Users may merge colonies after performing the initial count. Colonies should be merged when one colony is treated as two separate colonies by the software. Colonies will only be merged if they are less than 4 pixels apart.

- To merge colonies, ensure that the **Merge Colonies** tab is highlighted in yellow.
- A new window will ask the user to Select the desired colonies and then press **Merge** button to join them.
- Click onto the colonies to be merged.
- The **Total colonies count** will change to reflect the changed due to using the merge function.
- A rectangular object will appear. Move it over the area of interest.
- The central sample image will appear larger in the area of interest identified.
- If the colony is hard to see, zoom in on the colony.

User Defined Template Counting

Before proceeding through the next steps, a template must have been saved during the Manual Counting process.

- Select **Start Colony Count**, select **User Defined Template Counting**, and then select the desired template from the dropdown menu.
- Click **OK**.
- The new window will display the total count.

Spiral Counting

Perform Spiral Count

To perform a spiral plate count:

- Select the **Spiral** checkbox from the **Main Tools** section of the Colony Count module.
- Select Start Colony Count.
- Count the colonies.

  NOTE: If using the software for the first time, no templates are created and the **User Defined Template Count** option appears grey. To create a template, first proceed through the **Manual Count** functions to store settings.

Adjust Grid Size and Volume

After performing a count, a **Spiral Plate Analysis** window will appear along with a green grid over the counted image.

To change the overlay grid size:

- In the **Spiral Plate Analysis** window move the **Overlay Size** slider bar to the left (to decrease the grid size) or to the right (to increase the grid size).

    To calculate the SPLC/mL:

- Type in or use the up or down arrows in the **Total volume deposited** box provided. The calculated amount will appear immediately below the **Calculate SPLC/mL** button.

View Results

To view the resulting **Data Table** analysis for the spiral plate count:

- Click onto the + sign (expanded report shown here after clicking the + sign).
    The values listed will provide the number of colonies found in each quadrant and section of the counted plate.

To save the spiral plate settings in a template:

- Click onto **Save As** and provide a name for the template.

To print or export the data into Excel:

- Click onto the **Print** or **Export to Excel** button.

User Tips

While in the **Spiral Plate Analysis** window, the user may choose to move the green overlay grid by using the **Align Spiral Plate Overlay.**

To move the grid:

- Click onto Align Spiral Plate Overlay

- Click and drag the green grid until the center of the grid is aligned with the center of the captured image.

To clear the spiral plate analysis:

- Click onto the **Clear Spiral Plate Analysis**. This action will remove the data entered for this plate and remove the spiral plate grid.

Spiral plate analysis may be performed on any counted plate. To perform the spiral plate analysis on the previously counted plate:

- Select the Spiral Plate Analysis option directly above the Align Spiral Plate Overlay option.

Zone Analysis

Zone analysis provides users with zone sizing information useful for applications such as **inhibition zone analysis.**

Before proceeding through the zone analysis process, ensure that an image of the Petri dish has been captured and is ready for analysis.

To obtain zone analysis information for a plate:

- Click on the checkbox for **Zone.**

- Select Start Colony Count.

- Choose to count with the **Manual** or **User Defined** method.

- Click **OK**.
- If using the software for the first time, no templates are created and the **User Defined Template Count** option appears grey. To create a template, first proceed through the **Manual Count** functions to store settings.

Renumbering Colony/Zone Values

To enable easier statistical reporting of each colony or zone, the values assigned to each colony or zone may be renumbered.

To renumber the values assigned to each colony or zone:

- Click **Recompute Colony Labels** under the **Grid Analysis Tools** in the Colony count module.

  NOTE: The plate must have already been counted.

  The **Recompute Colony Labels** function will read the plate from left to right and from top to bottom. So the colony labeled 1 will be seated at the left and towards the top of the plate.

Reporting Functions

Colony Counting Reports

The results of the colony count can be displayed in the results window. To show the results:

- Click the **Show Results** window to bring up the colony count results.
- The colony count results for the Petri dish are displayed with tabs on the upper left hand side of the screen.
- The tabs are Classes, Colonies, Statistics, and Distribution.

Classes

In the **Classes** tab **Show Results** window, information is displayed which shows the:

- Number of classes in the sample
- Number of colonies in that class
- Percent of colonies of that colony classification in the sample
- Total area of the class on the sample (in pixels)
- Percentage of area of the class on the sample
- Mean area of the class (in pixels)
- Standard deviation of the area
- Minimum area (in pixels)
- Maximum area (in pixels)

Statistics

In the **Statistics** tab of the **Show Results** window, information is displayed which shows the Statistical property of the entire sample.

- ▪ Area of the sample (in pixels) of the colony

In the second window, the property is listed alongside the area. Several numerical values are listed which include:

- Minimum area
- Colony with the minimal area
- Maximum area
- Colony with the maximum area
- Range of area values
- Mean of area values
- Standard deviation of values
- Sum of all values
- Number of colonies

  All dimensional values are reported in pixels unless the calibration process is performed on the image.

Distribution

In the **Distribution** tab, colony area information is displayed graphically.

- The dropdown menu allows users to report graphical information about the average diameter, area, perimeter, and circularity of the colonies counted in the Petri dish.
- Users may also change the number of bins that are set to display in the graph.

Export and Print Colony Results

Export to Excel

- To export data, from **Colony count results** window go to **File > Send results to Excel**. Save the file in Excel format to later open the file.
- The first tab shows **Classes**. The second tab shows **Colonies**. The third tab shows **Statistics**.

Print Colony Results

Use Excel to open and print the Colony results reports.

Printing Reports and Images

- [Printing Image Information](#)
- [File Print Command](#)
- [Exporting and Printing Colony Count Results](#)
- [Viewing and Printing 1D Gel Analysis](#)

File Print Command

Go to **File > Print** and select a printer to print from.

Printing Image History

Reports

LS Software provides several types of reports:

- ***Image Report***: Prints the image, using as much of the page as possible while preserving the image's aspect ratio.
- ***Image History***: Prints the image history, as reported in the Image Information window.
- ***Image Notes***: Prints the image notes, as entered in the Image Information window.
- ***Image Properties***: Prints the image's resolution (width and height), depth, scale and file information.
- All reports include a header and footer that are user defined.

  The Report Preview window shows the layout of the data on each page.

To View and Print a Report

- Choose **Tools > Reports** and then choose the desired report from the **Reports** window.
- To change the target printer, paper, paper source (tray) or page layout, click **Page Setup** and make the desired changes.
- To enter the header or footer text, type new text in the text box and click **Preview** the pages. There are some special character combinations in the header and footer:
    - "%p" is the current page number.
    - "%d" is the current date.
    - "%t" is the current time.
    - "%c" is the total count of pages for printing
- To change the margins, choose an alternate margin setting in the **Page Setup** window, click OK after selection.
- In the **Reports > Preview Options**, click Show Margins in Preview Mode to see the margins graphically.
- When the preview looks correct, click **Print**.

Print

Images can be printed to any Windows-supported printer, regardless of it being a file-printer, local printer or a network printer.

  Note: The **Print** command uses exactly the same format as the Image Report in Reports.

To Print an Image

  Note: The **Print** command is also available on the Files toolbar, but does not show the Page Setup dialog window. Instead, it prints an Image Report directly to the default printer.

- Open an Image.
- Choose File > Print.
- If necessary, choose the target printer, paper size, paper source (tray) and page layout.
- Click **OK**.

Related Topics:

Supporting 21 CFR Part 11 Compliance

Supporting 21 CFR Part 11 Compliance

Purpose

US - Food and Drug Administration (US-FDA) created and released Part 11 of Title 21 of Code of Federal Regulations (CFR) in August 1997.

The rules delineate the conditions under which the US-FDA considers electronic records and electronic signatures equivalent to paper records and paper signatures. The instructions for compliance really span the entire organization and its practices. The Software by UVP is one piece that rightly fits into the bigger picture and *supports* compliance.

NOTE: While software from UVP, LLC is an essential tool for assisting an organization to maintain CFR compliance, UVP cannot claim that this is the only tool needed to achieve overall CFR compliance. The organization must establish policies and procedures that work in conjunction with such efficient tools, to ensure total compliance with 21 CFR Part 11 regulations.

Features

UVP provides software support for the following two sections of CFR regulations:

- Section 11.10 (e) – For electronic records, this section requires the use of computer- generated, time-stamped audit-trails to track changes.

- The software keeps track of all changes that affect image-data. Any action in the software that modifies the original data of an image open in the LS workspace, is logged. The log of such changes is individually maintained for each image and is referred to as 'History' in the software.

- Section 11.3 (b) (4) – This section mandates that the system be controlled by users responsible overall for contents of electronic records required to track.

The software provides an elaborate system of maintaining secure user accounts. Assign unique usernames and passwords to all the users who will be using the software. Each account can also be configured to provide read or modify access to other users' data. Events generated in the audit trail (above) are logged with the username.

Usage

Viewing an Audit Trail (History)

- Open the image in question.

- Right click on the image and select Image Information. Open the **History** tab.

- Events are listed in the left column. Click on each event to view the entry details on the right.

- Add notes to each event if required.

Printing an Audit Trail (History)

- Open the image for which to print the Audit Trail.

- Go to **Tools > Reports**. (This option is disabled, if no printer is available.) A window

opens with various types of reports available.

- To print the Audit Trail, click the **Image History** item. To print the image along with the trail, click on **Image Report** and **Image History**. Adjust the header and footer settings or printer settings if necessary, and print the trail.

- Click **Print** to print report.

Glossary

Artifact: In imaging, a flaw caused either by the imaging process or by the hardware itself. For example, dust on the camera lens could cause small bright or dark spots in an image.

Aspect Ratio: The ratio between an image's width and its height. If the aspect ratio is not preserved, the image will appear stretched or squashed.

Bits: The smallest units of computer measurement. A bit is a single binary value (i.e. it can be "on" or "off" only). Bits typically are combined into units of eight, called "bytes." Modern computer processors work with groups of 4 ("32-bit processor") or 8 ("64-bit processor") bytes at a time.

BMP: Microsoft Bitmap image file format. BMP is a lossless format which provides some compression to reduce file size. BMP files generally have a BMP extension.

Control Handle: A small square at the corner (or similar point) of a graphical object that marks its extent and indicates that the object is selected. Usually the object can be resized by dragging the control handle; in some cases, different behavior results.

Electrophoresis: The movement of suspended particles through a fluid or gel through the application of electrical current to the suspension medium.

Fidelity: The degree to which an image is true (i.e. accurate and uncorrupted) to the original scene it represents. Also used in audio technology with the same meaning.

GIF: Graphic Interchange Format, a proprietary Xerox image compression format. GIF is a lossy compression format that results in very small files. Files stored in GIF usually have a GIF extension.

Image Depth: The size (and thus range) of intensity numbers supported per pixel in an image. Doc-It supports two depths: 8-bit (in which intensity numbers range from 0 to 255) and 16-bit (in which intensity number range from 0 to 65535). For a more detailed explanation, see Inside a Pixel.

Intensity: The measure of brightness of a pixel. In a monochrome image, each pixel has a single intensity. In a colored image, each pixel has three intensities: one for red; one for green; and one for blue. The actual intensity values depend on an image's depth.

JPEG: A common lossy compression image format used to store images on disk. JPEG files generally have JPG or JPEG extensions.

Lossless Compression: Compression schemes that preserve the image's integrity in full. Generally, lossless compression results in much larger files than lossy compression on the same image.

Lossy Compression: Compression schemes that tolerate some pixel value changes to make the image compress to a smaller size. Because the changes are irreversible, the image has "lost" some of its original detail after such an operation.

Macro Mode: Close-up mode for a digital camera or web-camera. Macro mode is usually appropriate for imaging microbiology slides.

Microbiology: The branch of biology dealing with microscopic forms of life.

Microscopy: The use of or investigation with a microscope.

Monochrome: Black-and-white, with shades of gray. Doc-It cameras capture 256 shades of gray in monochrome mode.

Pixel: Short for "picture element." A pixel is a single dot in a computer image. The dot has a certain color (for a color image) or an intensity (for a monochrome image). For a more detailed explanation, see Inside a Pixel.

PNG: Portable Network Graphics, a common image format. PNG is a lossy compression format that results in very small files. Files stored in PNG usually have a PNG extension.

Pseudocolor: Artificial application of color to a non-color (monochrome) image, or artificial re-tinting of a colored image. Doc-It provides several built-in pseudocolor sets that mimic certain lighting conditions and reveal specific information in the image.

Resolution: The number of total pixels (width of the image in pixels multiplied by height of the image in pixels). Higher resolution produces a smoother image (especially when zoomed in) but requires more RAM and disk space.

TGA: Truevision Targa image format. TGA is a lossless compression format that reduces file size somewhat. TGA files generally have a TGA extension.

Thumbnail: A reduced-size version of an image. From "thumbnail sketch."

TIFF: Tagged Image File Format, a common image format. Depending on settings, TIFF can be either a lossy or a lossless compression format. In Doc-It, it is used in the lossless mode to reduce image file size without losing integrity. TIFF files generally have TIF or TIFF extensions.

Zoom Factor: The percentage by which the image is scaled. A zoom factor of 100% (1.0) means that each pixel is not scaled; it is its original size. Zoom factors greater than 100% indicate that the image has been scaled up (meaning that several screen pixels are used to show one actual pixel). This generally makes detail easier to see. Zoom factors less than 100% mean that the image has been scaled down. This makes it possible to see more of the image in the Image window.