VisionWorks® Life Science Software

Installation and User Instructions

VisionWorks®LS Image Acquisition Software
VisionWorks®LS Image Acquisition and Analysis Software

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Welcome

Welcome

Life Science (LS) software from UVP allows users to acquire, enhance analyze and document 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 an image has been captured with an application-specific camera, it can be saved for documentation and presentations, manipulated for analysis, and annotated to point out key features in the image.

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

What's New in Version 7.0

VisionWorksLS release 7.0 software new features include:

- Redesigned interface
- Portable analysis data
- Capture presets
- Windows 7 (32-bit) compatibility

Getting Started

- Minimum System Requirements
- Registering the Software

Capturing Images

- Acquisition

Performing Analysis Functions on Images

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

What's New

Interface

- Plug-in modules have been replaced by tabs and menus
- Camera plug-ins, Darkroom and Lens plug-ins etc. have all been replaced by menus organized under tabs
- Five tabs provide access to most functions within the software

The tabs are:

- Acquisition: Offers options to change camera, darkroom, lens etc. to obtain an image
- 1D Analysis: Offers options to perform 1D Analysis on a captured image
- Area Density: Offers options to perform Area Density on a captured image
- Colony Counting: Offers options to perform Colony Counting on a captured image
- Image: Offers options to apply image filters, brightness & contrast, pseudocolors, annotations, and other tools to alter the appearance of the image

Preset Capture Buttons

Common operations for operators include: Fluorescence, White Light, and Chemiluminescence. Based on practical experimentation, each of these buttons has a defined set of camera & lens, darkroom, and exposure time settings.

Click onto each button to find the following presets that accompany the button press: Fluorescence, White Light, Chemiluminescence.

Image Analysis Data and Image linked together

Images saved in TIF/TIFF format stores both image and analysis data in a single file. Note: the file must be opened with VisionWorks version 7.

The stored data includes:

- Annotations
- Image adjustment (pseudocolor, histogram, etc)
- Analysis (1D Analysis, Area Density Analysis, Counting Colonies, calibration)

  Analysis data for JPG/BMP/GIF/PNG/etc images will be stored in the ”Archive” folder in VisionWorks LS installation directory, just as it is in earlier software versions.

Windows 7 Compatibility

- 32-bit Windows 7 only.
- Compatible editions: Enterprise, Professional and Ultimate.
- For Windows 7 installation the setup program must be run by a user with Windows Administrator privileges.
- Important: VWLS is installed on Windows 7 with Compatibility Mode disabled, so that the 210- and 310-series cameras can be used. This configuration disables the Canon controls.

Minimum System Requirements

Operating System Requirements

- Windows XP Professional with Service Pack-2 or higher (Windows 7 and Vista compatible)
- 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 the Software

- Overview
- Types of Registration
    - On the Fly Activation
    - Already Have an Activation ID
    - Register Offline
- Single-user license
- Network-user license
- User Administration

Overview

LS software is activated 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-feature 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. There are three ways to retrieve an activation code: phone, email or Internet. All three methods are accessed via 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

Types of Registration

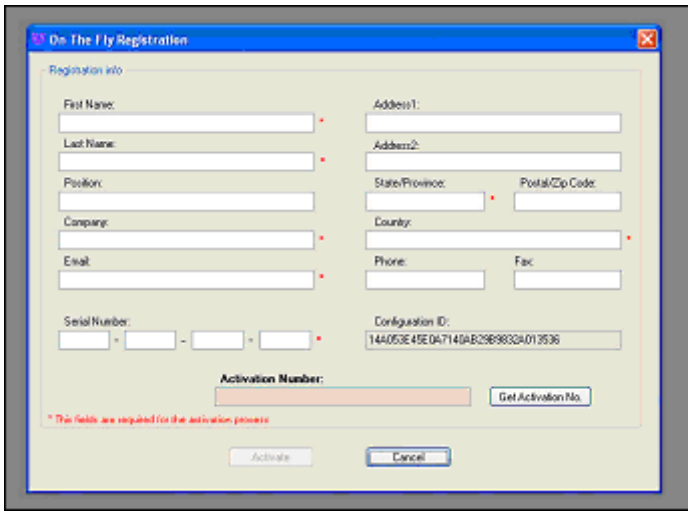
On the Fly Activation

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.



- A new form will appear. 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

Once the software is registered, define the single-user license which 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.
- Select **Next**.

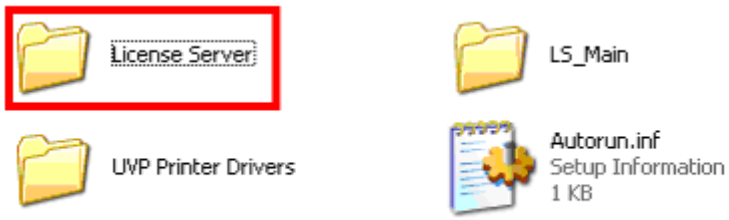


Network-User License

Once the software is registered, define the network-user license which allows five users to operate the software. All licenses must run on the same network. For additional licenses, contact UVP.

Follow these steps to install and use the network license:

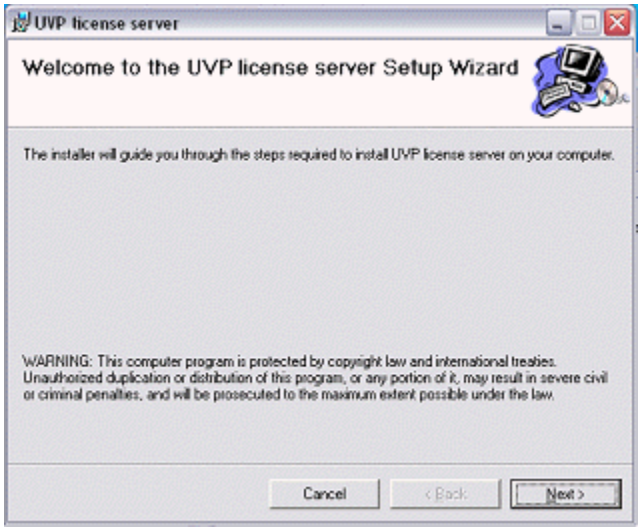
- Ensure that a computer administrator is available to support the installation of the network license. The administrator must provide an administrative login for each local PC installing VisionWorks.
- Identify one computer running a minimum of Windows XP (SP2) on the network to hold the network license. This computer will be called the "Server".
- From the VisionWorksLS software Disk 1, navigate to the License Server folder.



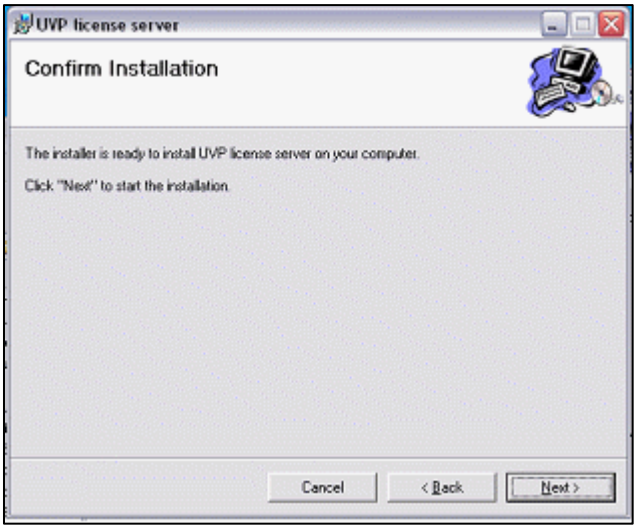
- In the License Server folder, select the **Setup.exe** to begin the Network License configuration.



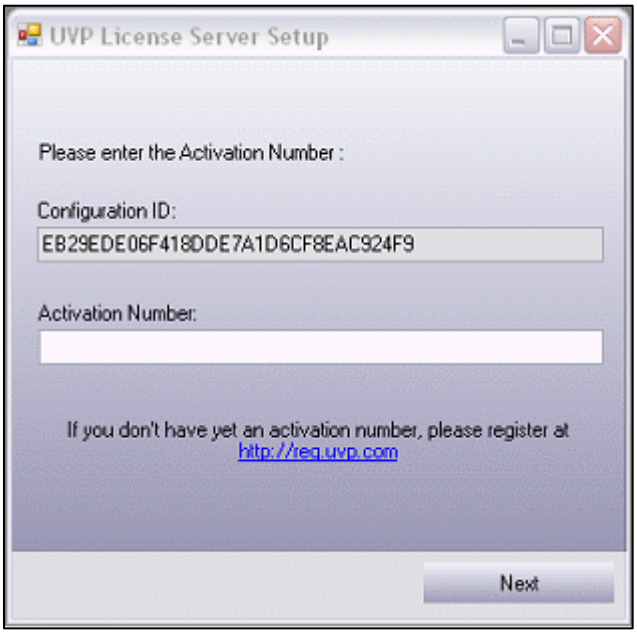
- After selecting the **Setup.exe**, a UVP license server window will enable an Installation Wizard to assist with the installation process. Select **Next** to continue.



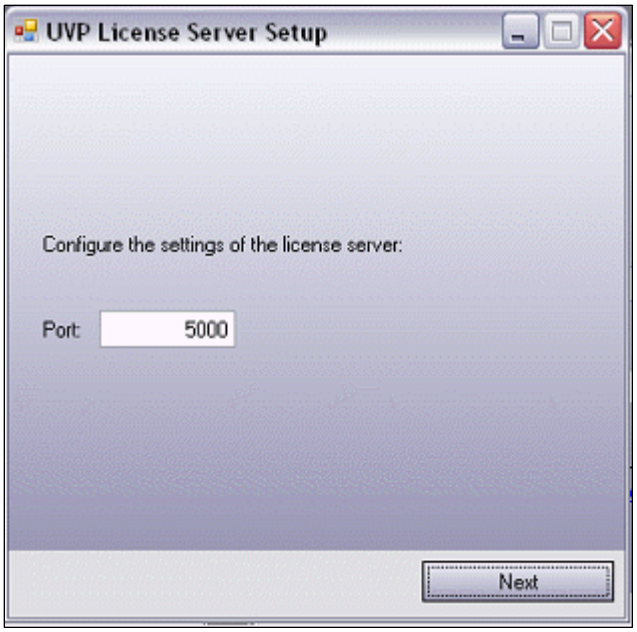
- Click **Next** to confirm the installation of the License Server.



- The next screen will require the user to enter an Activation Number. To get an activation number, register at http://reg.uvp.com or call technical support at (800) 452-6788 (inside USA) or (909) 946-3197 . Enter the provided Activation Number.
- Click **Next**.

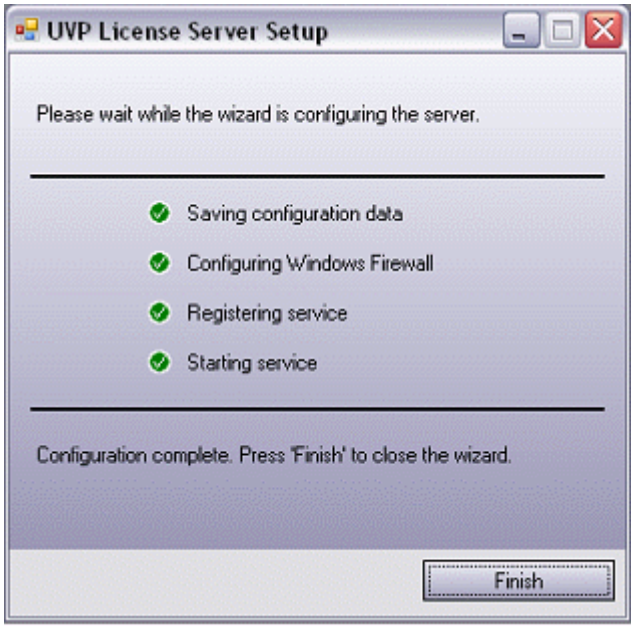


- The next screen will verify the "Port" number to be used. It will be necessary to note this port number, as this is the port that the other/client PC's will use to access the software license. Generally, the port number is automatically provided and will not need to be altered.
- Click **Next** after noting the Port number being used.



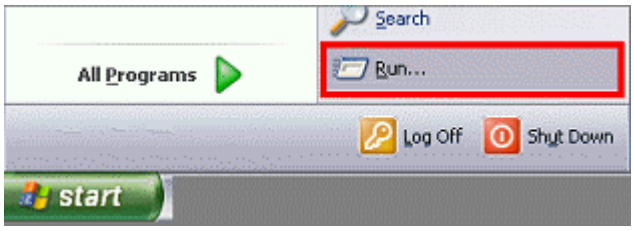
NOTE: Administrative IT support will need to ensure that the port chosen is not blocked on the network. If the network has a restrictive network use policy (blocked websites, no IM clients) port blocking is likely to be implemented.

- The next screen will display the configuration status as it is applied. When the configuration is complete, the text at the bottom portion of the window will read "Configuration complete." Click **Finish** when this message appears.

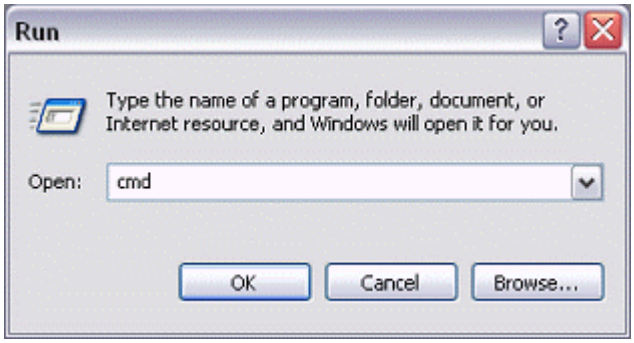


- Next it will be necessary to obtain the "Server" computer's IP Address. This address will be used to set-up the other "client" computers, so that they will know where to locate the VisionWorks LS Network License. Click the Windows Start button and select the Run option.

  NOTE: "Client" computers are computers within the network that will run VisionWorksLS; therefore a "Server" computer may also be a "Client" computer.



- In the Run dialog box, type **cmd** (as pictured). Then click **OK**.

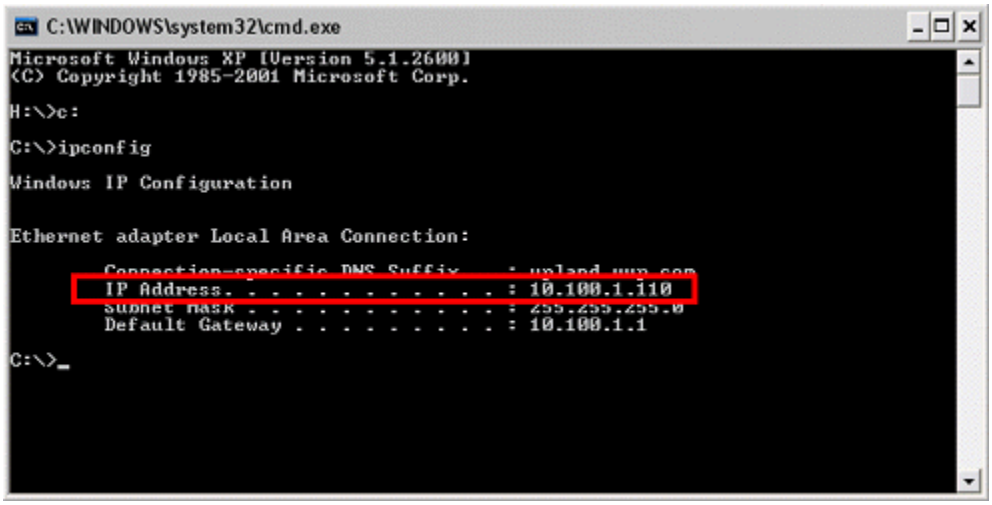


- Type the command to display the IP Address of this PC. Type "ipconfig" (as pictured), and click **Enter**.



- The IP Address is listed and should be noted, as it will be used with every "client" computer's License Manager setup. After noting the IP Address, close the Command Dialog.

  NOTE: In some cases users will notice a list of multiple IP addresses. If the shipped system includes an OptiChemi 600 camera, for example, users will see more than one IP address. To ensure that the correct IP address is recorded, check with the IT Administrator before proceeding. The IT Administrator must verify that the selected IP address corresponds to the network adapter with access to the domain.



Additional Network Users

Repeat the following steps for each client computer.

- Open VisionWorksLS on the client computer. A Welcome to the Licensing Wizard window will appear. Select the **Server based licensing** option, and select **Next**.

  NOTE: VisionWorksLS will not authenticate across network segments so the IT Administrator must

- Ensure that all client PC's are on the same subnet/segment before installing VisionWorks on each PC.
- Enter the previously noted IP Address and Port Number form the "r;Server" computer. Click **Finish** once the two values have been entered.
- UVP window will appear if the License Server installation/ configuration was successful. Click **OK**, and close any other dialogs still open.

  NOTE: During the installation process, a Systems Settings Change window may appear that asks the user to restart the computer. This is not an error. Click No to continue with installation.

  NOTE: The Hardware Installation window suggests that the camera drivers have not passed the Windows Logo Testing suggested by Microsoft, which is not a problem. Click Continue Anyway.
- DO NOT SELECT STOP Installation. Doing so may render the cameras inoperable.

User Administration

- About Secure User Accounts
- User Rights
- Configure User Accounts

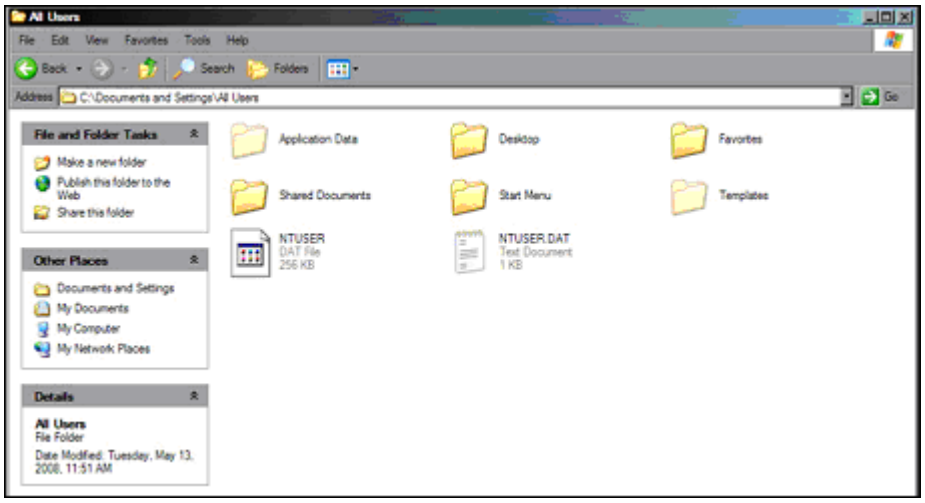
About Secure User Accounts

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.

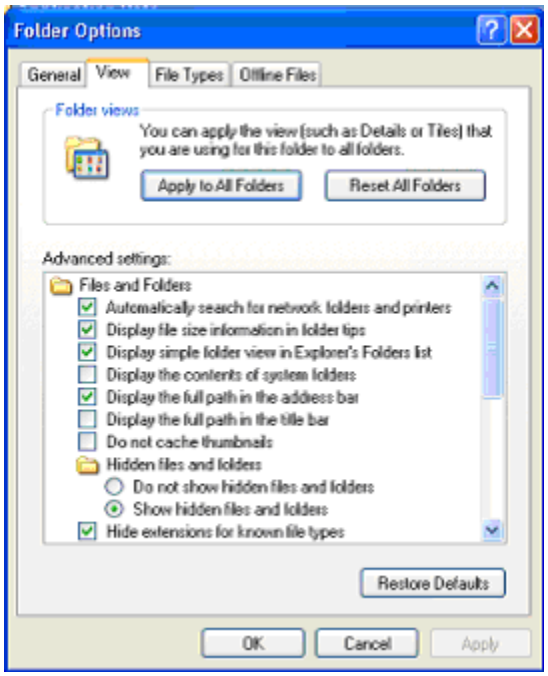
- LS system of usernames and passwords is not to be confused with the login required to open the computer. LS users must provide a separate login and password to enter the LS software.
- Setting up user accounts is mandatory if support for CFR 21 Part 11 is required from the software.
- If individual accounts are not required, create just one account for all users and give full permissions to that account.

Enable Secure User Accounts

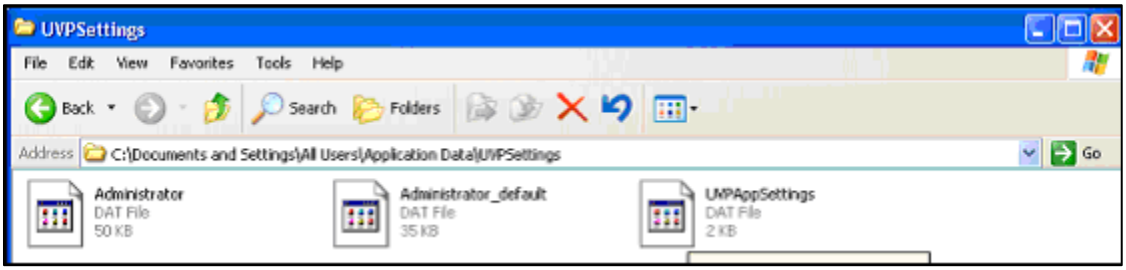
- Request a **System Administrator** to log into the Windows computer.
- Navigate to C:\Documents and Settings\All Users
- Locate the **Application Data** folder.



- If the **Application Data** folder is not present in the **All Users** window, go to the **Tools** menu and select **Folder Options**. (If the Application Data is present, skip the next step.)
- 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 select **Allow** for all permissions listed for each user or group for either the **UVPSettings** folder, or for each file within the folder (depending on the Network Security Policy where the LS software will reside).



Definitions of the User Administration Columns

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.

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.

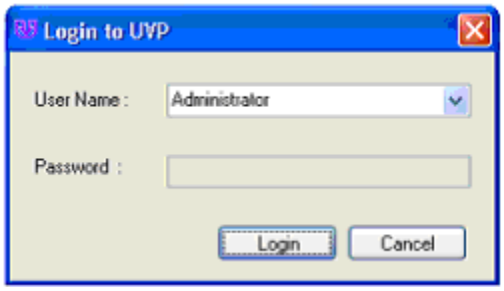
Note that even though a user may be able to do things with the software which are outside of this matrix, UVP neither recommends it, nor supports it. For example, the user may try (successfully or otherwise) to uninstall the software as a Power User, but UVP does not provide support for problems arising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

Configure User Accounts

- User Names and Passwords
- Add a User
- Edit a User
- Change a Password or Other Settings
- Deactivative/Reactivate a User
- View the Login History of a User

User Names and Passwords

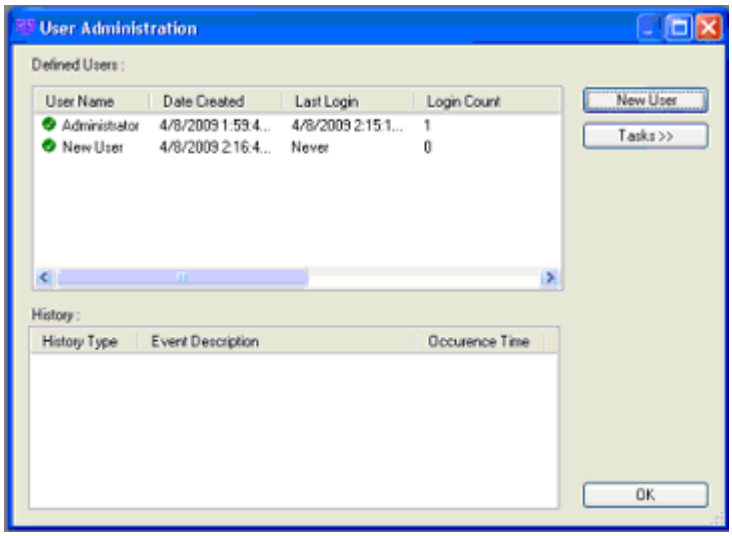
- Start the software. It will bring up a **Login** window.
- The administrator user name will show.
- Click **Login**.



- A Reset password window appears. Enter the new password. Confirm the new password.
- Click **OK**.

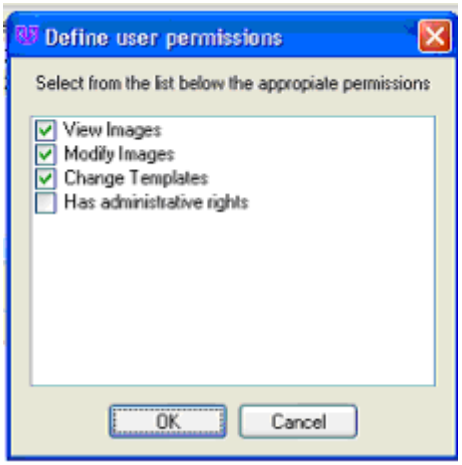
Add a New User

- From the menus, click the **Advanced** menu button then select **Configure user accounts** 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.



Edit a User

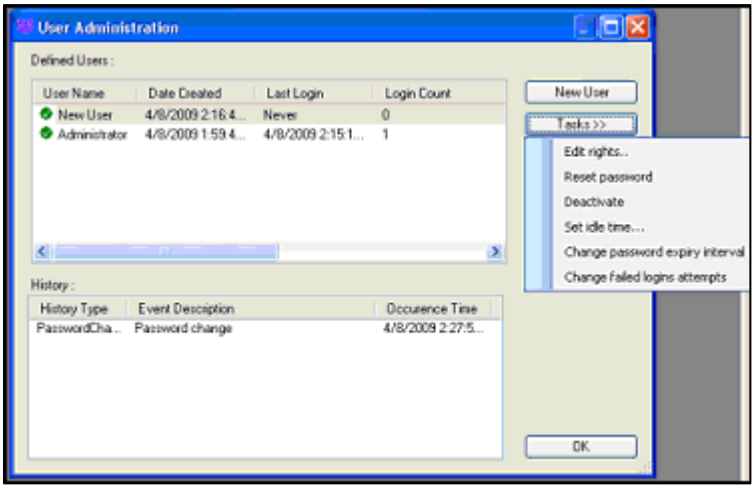
- Highlight the user name in the **User Administration** window.
- Click the **Tasks > Edit Rights** and select from the **Define User Permissions** screen to allow users to view images, modify images, change templates, or assign administrative rights.
- Click **OK** when changes to the user are complete.



Change a Password or Other Settings

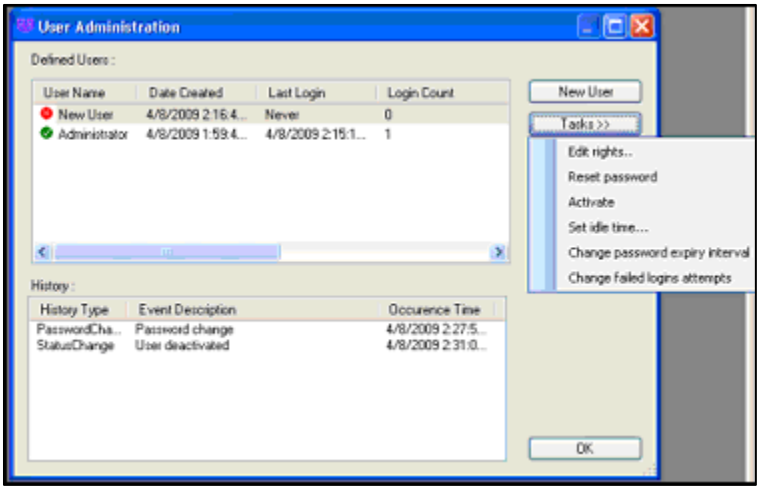
- To change a password, click the **Advanced** menu button then select **Configure user accounts** to open the **User Administration window**.
- Click on the appropriate user to change.
- Select the **Tasks > Reset Password** and enter the new password. Enter the password again to confirm the change.

- Click **OK**. The change in password will be noted in the **User Administration > History** box.



Deactivate/Reactivate a User

- To deactivate/reactivate a user, click the **Advanced** menu button then select **Configure user accounts** 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.



NOTE: Never disable the Administration Account.

View 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.

Technical Support

UVP offers expert technical support on all of our products. If there are questions about the product's use, operation or repair, please contact our offices at the locations below.

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: techsupport@uvp.com or info@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

PLEASE READ THE FOLLOWING AGREEMENT CAREFULLY

This Agreement is between UVP, LLC of 2066 West 11th Street, Upland, California 91786 (hereinafter "Licensor") and the end user of UVP software (hereinafter "Licensee").

Licensor has developed and offers to Licensee on a non-exclusive basis pursuant to the terms and conditions set forth hereinafter, the following software, including related copyrighted instructional materials, (collectively referred to hereafter as "The Software"):

VisionWorks®LS Software

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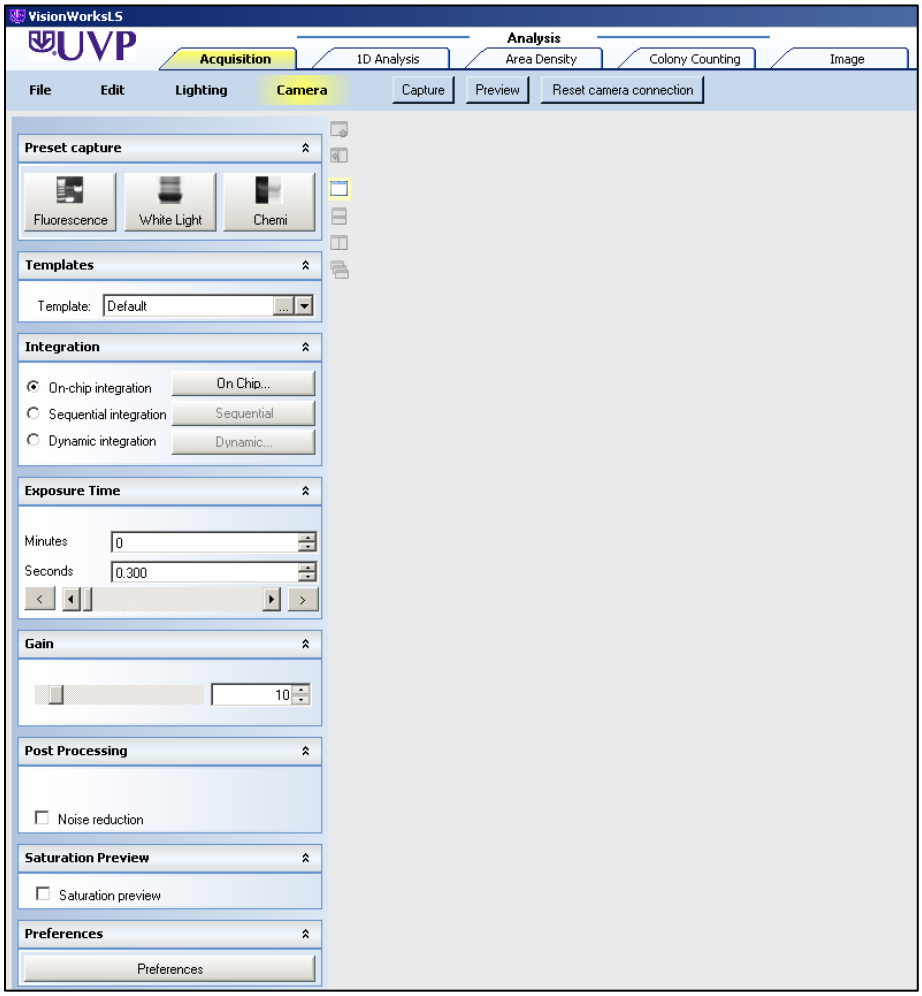
Navigate the Software

Navigating the Software

- Main Window
- Action Tabs
- Menu Buttons

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 Action Tabs, Menu Buttons and modules. Action tabs for functions used the most are shown across the top. Menu buttons are displayed below. Each Action tab has a unique set of menu buttons. The example below shows the Acquisition Action Tab highlighted and its corresponding menu buttons. When select menu buttons are clicked, modules will open to provide additional function options. The example below shows **Acquisitions Action Tab > Camera module**.



Action Tabs

The purpose of the Action Tabs is to enable quick selection of major tasks.

- Acquisition: Options to change camera, darkroom, lens etc. settings to obtain an image
- 1D Analysis: Options to perform 1D Analysis on a captured image
- Area Density: Options to perform Area Density on a captured image
- Colony Counting: Options to perform Colony Counting on a captured image
- Image: Options to apply image filters, brightness & contrast, pseudocolors, annotations, and other tools to alter the appearance of the image

Action Tabs

Action tabs are shown in the top red outlined box. Menu buttons are displayed in the second red box below. Each Action tab has a unique set of menu buttons. The example below shows the Acquisition Action Tab highlighted and its corresponding menu buttons. When select menu buttons are clicked, modules will open to provide function options.

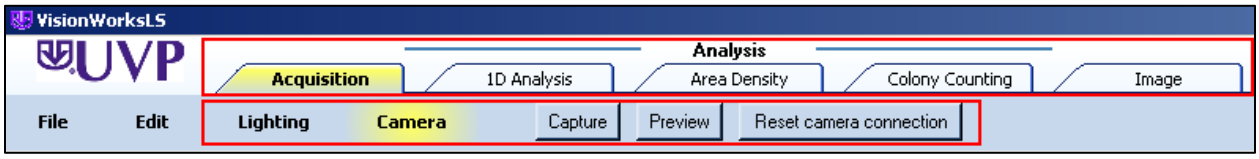
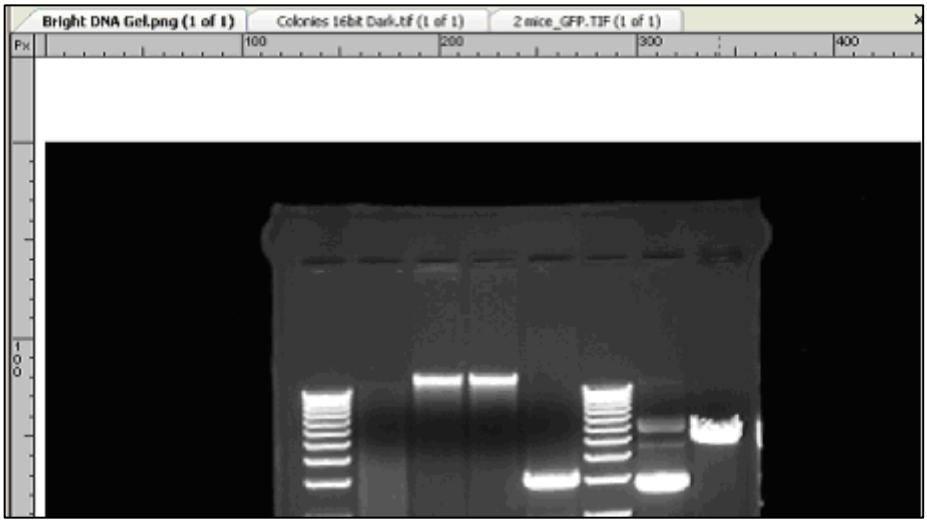


Image Windows

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.

- Organize Image Window
- Information Provided in Image Window
- Show the Image in Actual Size
- Fit the Image to Window
- Context Menu Commands
- Status Bar



Organize 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 **Advanced > Configure Application > 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.

Show the Image in Actual Size

To show the image in actual size (no scaling), right click on the image and select **View Original Size**. Images taken at high resolution will look large on the screen.

Fit the Image to the Window

To show the entire image in the window (scaled up or down as required to make it fit), right click on the image and select **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 menu buttons. 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:

- Undo
- Redo
- Cut
- Copy
- Paste
- Paste Special
- View Best Fit
- View Original Size
- Print
- 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 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) is also reported.

Obtain Image Information

- Overview
- Display Image Information
- Enter Notes
- Calibrating Image Scale
- Image History

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 window.

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.

Display Image Information

- Right click onto the image to obtain its image information. A shortcut menu will appear.
- Click onto the function **Image Information** at the bottom of the shortcut menu. The Image Information window will appear.
- To switch between **Image History** and **Properties**, click the appropriate tab at the top of the window.

Enter Notes

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

Calibrate 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. Images scanned into the system from a scanner or imported from another program are not calibrated. In these two cases, therefore, the image's scale should be set.

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.

Use the Image Scale Tool

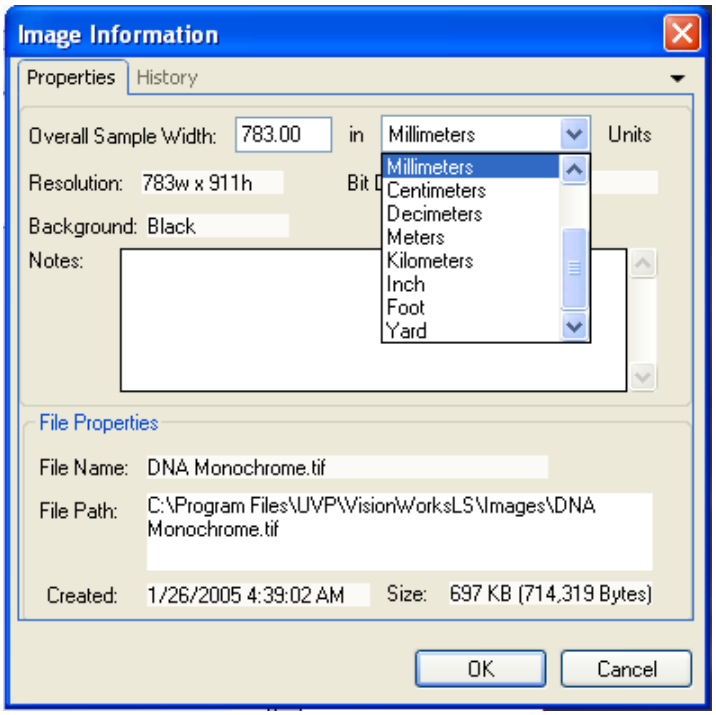
There are two ways to set the image's scale:

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

Change 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 captured 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-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**.

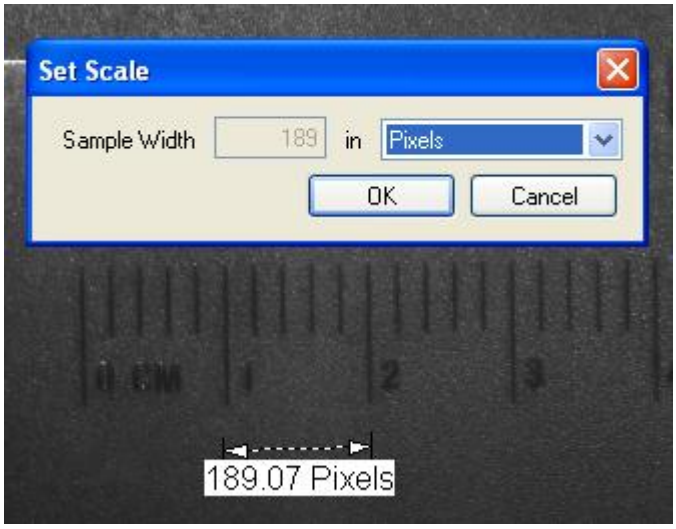


Calibrate the Image Using the Scale Tool

- Click onto **Define image scale** in the Colony Counting Action Tab in the **Results** menu. (A count must be performed before enabling the Results function.)

  OR

- Click onto **Spatial Calibration** from the Area Density Action Tab in the **Calibration** menu.
- 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 or gel sample and capture the image of the ruler and Petri dish/gel sample 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.

Related Topics

Measure Length

Measure Angle

Measure Area

Menu Buttons

Main Menu Buttons

There are only four menu buttons that are always available (not dependent on what Action Tab is selected) these are:

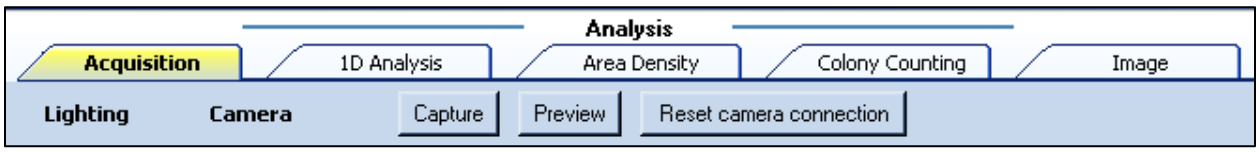
- ***File Menu***: Contains commands to open, save, and print files. Also contains a FTP window to assist in using the FTP function and a function to import an image from a scanner.
- ***Edit Menu***: Contains the Undo/Redo commands and the clipboard commands: **Cut**, **Copy**, **Paste**, **Paste Special** and **Paste Special Options**, plus the **Crop** function.
- ***Advanced Menu:*** Contains option for saving and playing Macros, displaying the log file, configuring user accounts, and viewing options.
- ***Help Menu:*** Contains access to the software help guides, information on the software version and contact numbers, and a link to begin the registration process.

Action Menu Tabs

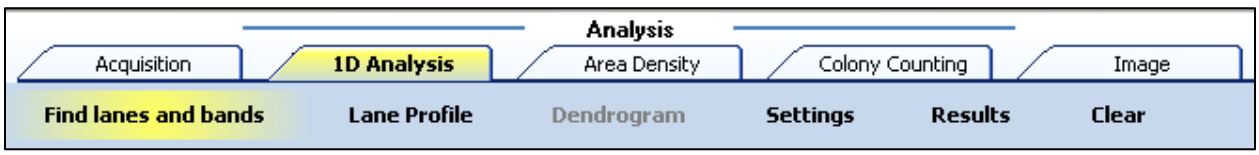
Each Action Tab has an associated menu for providing software functionality. In many cases, the Action Tab must be selected before the menus buttons under the Action Tab can be opened.

- Acquisition Action Tab
- 1D Analysis Action Tab
- Area Density Action Tab
- Colony Counting Action Tab
- Image Action Tab

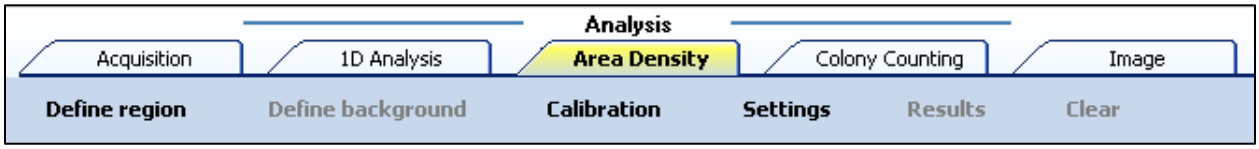
Acquisition Action Tab Menu Buttons



1D Analysis Action Tab Menu Buttons



Area Density Action Tab Menu Buttons



Colony Counting Action Tab Menu Buttons

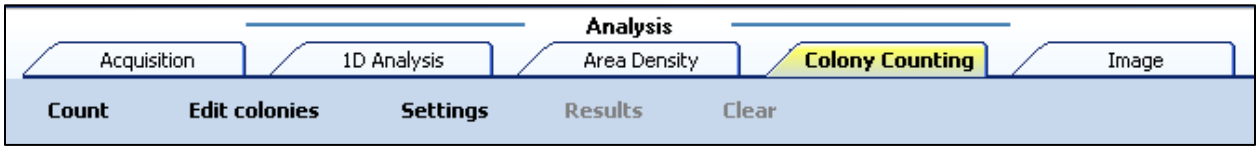


Image Action Tab Menu Buttons



File Menu Button

File options are available at any time. The **File** menu does not need to be accessed through any particular Action Tab.

Select from common file actions:

- Open image from file
- Open demo image
- Save
- Save as
- Close image

Select from printing options:

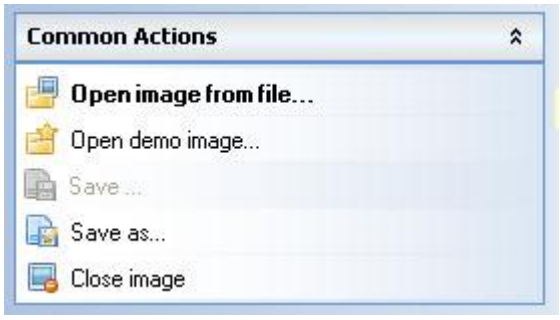
- Print preview
- Print
- Print a report

Use FTP functionality.

Scan an image.

Open and Save Images

- Open Images
- Save Images
- Image File Types



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 the **Image Files** topic below.

Open a Previously Saved Image

- From the **File** menu button, choose **Open image from file**.
- 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.

Open a Demo Image

- Go to **File** menu button and click on **Open Demo Image**.
- Select the desired demo image file from the list of available files.
- Click **Open**. An Image Window containing the desired image will appear.

Save Images

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

- Click on the **File** menu button choose and select **Save** or **Save As**. The Save window will appear.
- Select the file type 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**.

Save Changes to an Image

- From the **File** menu button choose **Save**.
- Changes will be saved to the existing file, overwriting the previous image data.

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**.

Image File Types

When analysis is performed, images are saved as two separate files. While the two files have the same name, they have different extensions. The two files are Image File and the Analysis File (Extended Attributes - TIF/TIFF).

NOTE: Images saved in TIF/TIFF format stores both image and analysis data in a single file. The file must be opened with VisionWorks version 7 or later.

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 TIF/TIFF by default. The file extension will reflect the file format (e.g. ".TIF" or ".TIFF").

NOTE: If the image is not saved as a TIF file, the analysis will be saved in the archive folder.

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.

Extended Attributes File

The image will also have a second file that ends with the extension ".TIF." 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.TIF," the matching extension file would be "MyImageJPG.TIF".

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

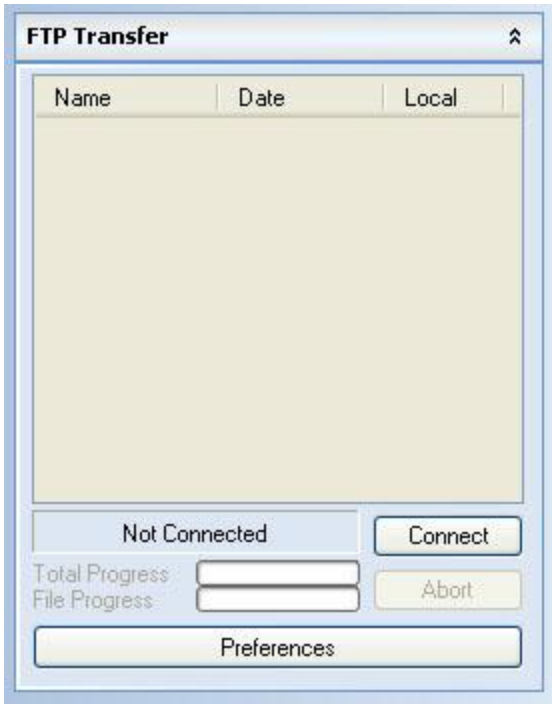
File Print Command

- Go to **File > Print** and select a printer to print from.

FTP Transfer

The FTP Transfer function allows the user to transfer files from one computer to another.

- Click on **File** menu button > **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 login and password in the FTP module preferences window.
- Start the software and take pictures.
- Press the **Connect** button on the FTP module after starting the software.



Scan an Image

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.

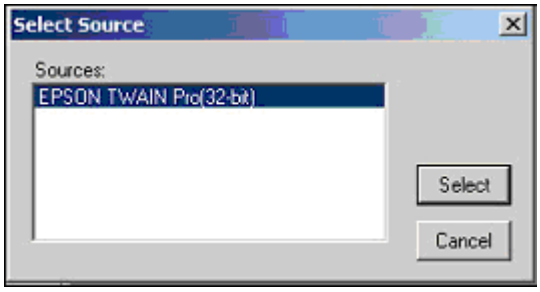
Scan an Image from a Scanner

- Click onto the **File** menu button then **Scan**.
- 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. Adjust the brightness, contrast and other settings appropriately before scanning.

Select the Desired Scanner

- Click onto the **File** menu button then **Select Source**. The Select Source window will appear.



- Select the desired device from the list.
- Click **Select**.

Tip: The device selected 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.

Edit Menu Button

Edit options are available at any time. The **Edit** menu does not need to be accessed through any particular Action Tab.

Copy / Paste

- Copy
- Cut
- Paste, Paste Special and Paste Special Options

Undo and Redo

ROI Tools (Region of Interest)

- New
- Rectangle
- Ellipse
- Polygon
- Freeform
- MagicWand

Filter

- Sharpen
- Blur
- Emboss
- Remove Noise

Adjust

- Rotate
- Align
- Flip Horizontally
- Flip Vertically
- Crop
- Resize
- Reduce to Monochrome
- Change Image Depth

Copy

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



Copy an Entire Image

- If a Region of Interest (ROI) is present on the image, click away from the ROI once to cancel it.
- Choose **Copy** from the **Edit** menu button.

Copy a Selected Region Within an Image

- Select a Region of Interest (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.

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 button 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 three merge modes (Blend, Add, Subtract) are available 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 modules 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.

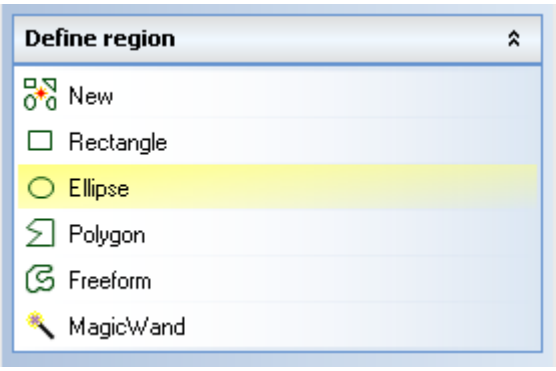
Redo the Last Change to an Image

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.

Region of Interest (ROI)

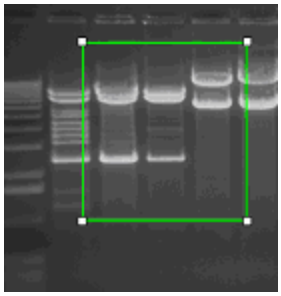
- About the Selection Tools
- Select a Region
- Adjust a Region
- Cancel a Region

About the Selection Tools

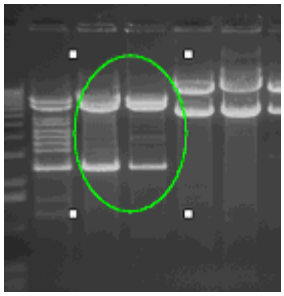


These tools allow users to mark part of the image for use in other operations. LS software provides several Region of Interest (ROI) selection tools:

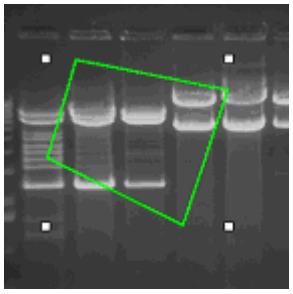
ROI tools (left to right): New ROI, Rectangular ROI, Elliptical ROI, Polygonal ROI, FreeForm ROI and Magic Wand ROI. Select the tools from the **Edit** menu.



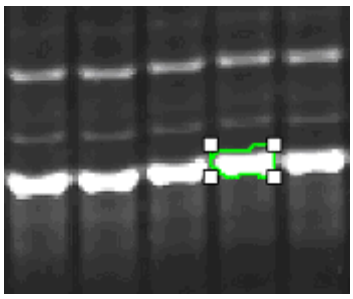
View Rectangular ROI selects rectangular regions.



View Elliptical ROI selects elliptical (oval) regions.



View Polygon ROI selects Polygonal regions.



View FreeForm ROI selects irregular regions.

- **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 'r;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 from the **Edit** menu button.
- 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 a 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 a Region

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

Image Enhancement Filters

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

- Blur
- Sharpen
- Emboss
- Remove Noise



These functions are accessed from the **Edit menu > Filter module.**

Blur

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

- Click onto **Blur** from the **Edit** menu button.
- Blurring a large image may take a few seconds.

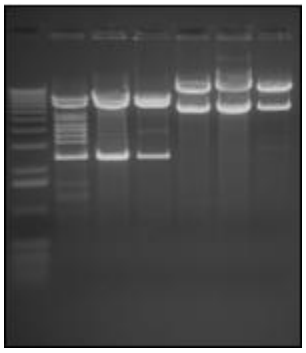


Image before Blur applied

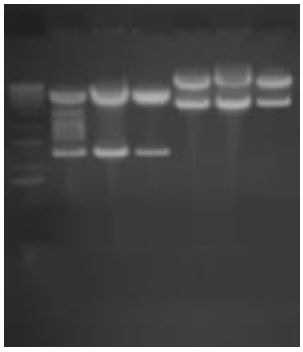


Image after Blur applied

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.

- Click onto **Sharpen** from the **Edit** menu button.
- Sharpening a large image may take a few seconds.

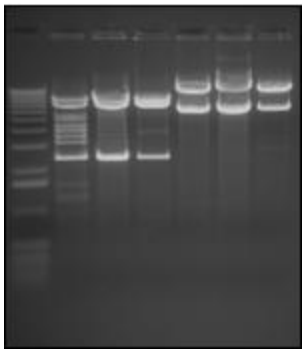


Image before Sharpen applied

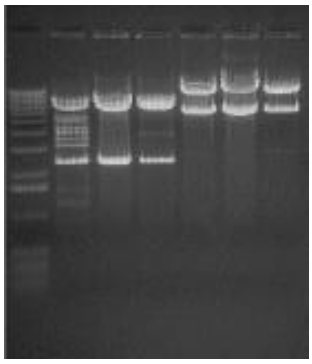


Image after Sharpen applied

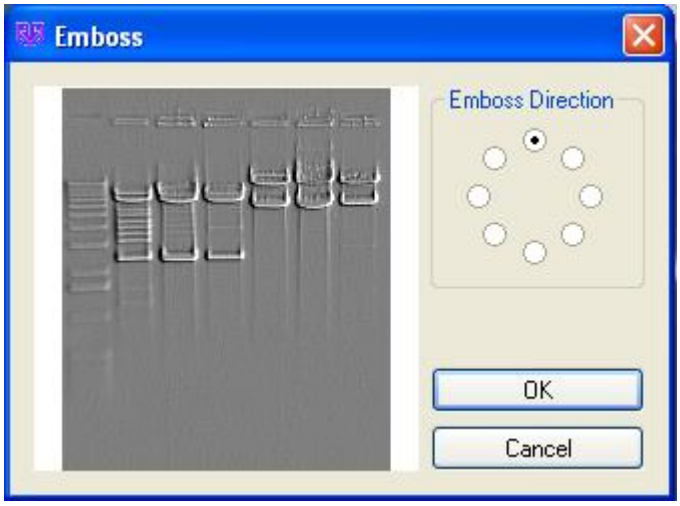
Emboss

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 **Edit** menu button. 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**.

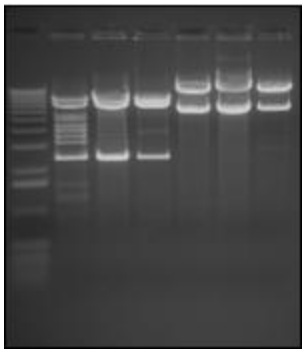


Image before applying Emboss



Image after applying a "North" Emboss

Remove Noise

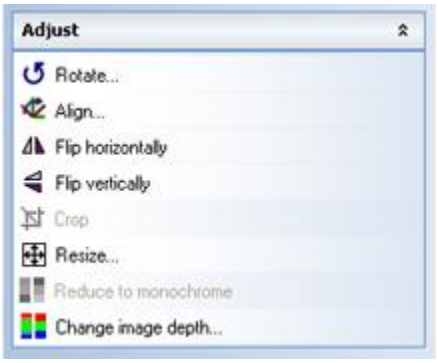
This filter removes periodic (patterned) noise from an image. Patterned noise is removed by creating a frequency-space mapping (Fourier transform) of an image and removing frequency spikes away from the graph's origin.

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 to be identified as patterns, resulting in blurring on some images.

- Click onto **Remove noise** from the **Edit** menu button.
- Removing noise in a large image may take ten or fifteen seconds.

Adjust the Image

- **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.
- Crop: Use the crop tool to select the part of the image you want to keep.
- **Resize**: Enlarges or reduces the image in size, uses less space and memory, and enables the image to be seen on the entire screen.
- **Reduce to monochrome**
- **Change image depth**



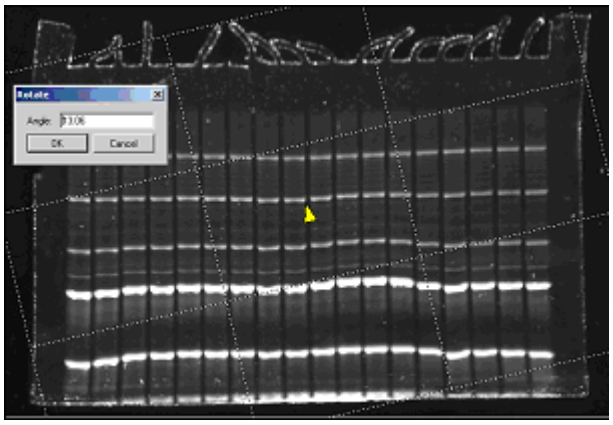
These functions are accessed from the **Edit menu > Adjust module.**

Rotate Function

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 **Edit** menu.
- 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 **Edit** menu. 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**.

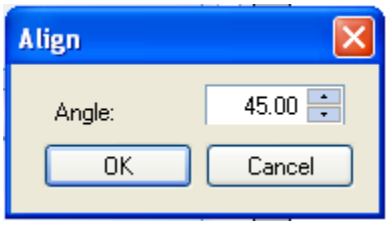
Tip: Rotations by 90, 180 or 270 degrees do not degrade the image. These

operations can be completely reversed by a rotation of the same amount in the opposite direction.

Align the Image

Align the image to a grid by clicking onto the **Edit** Menu then select **Align** from the Adjust category of functions.

An Align Window will appear that will allow manual entry for moving the grid. As an entry is entered the grid moves into position in real time.



Select **OK.**

The image will align to the grid.

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 **Edit** menu.

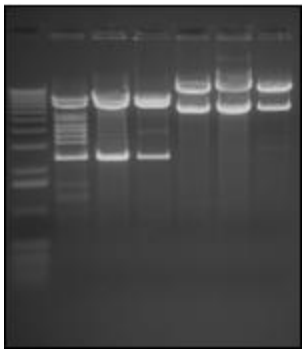


Image before Flip Horizontally

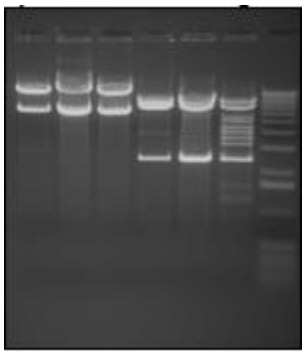


Image after applying Flip Horizontally

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 **Edit** menu.

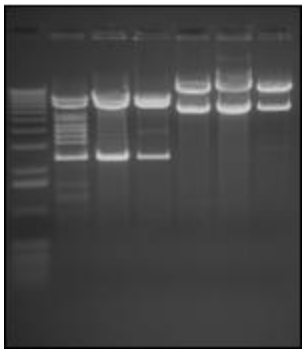


Image before Flip Vertically

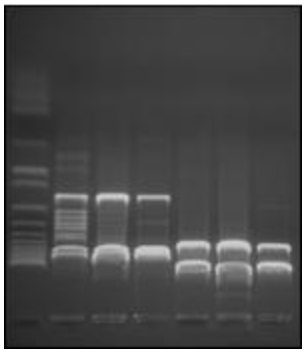


Image after applying Flip Vertically

Crop

Use the crop tool to select the part of the image to keep.

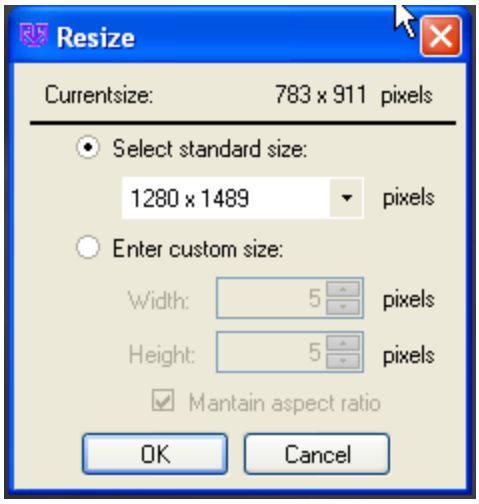
- Click onto **Crop** from the **Edit** menu.
- Move the crop tool over the area of the image to keep.

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 **Edit** menu. The **Resize** window will appear.



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

OR

- Select the **Enter custom size** 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.

Reduce Image to Monochrome

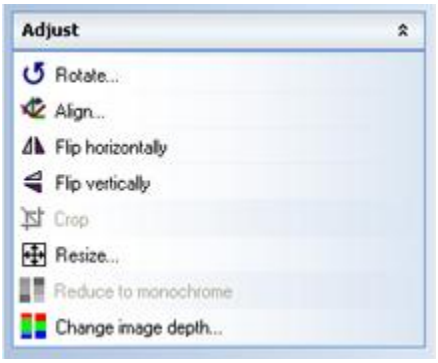
The Reduce to Mono filter reduces a color image to monochrome. This is primarily useful when colors in an image are distracting rather than informative. For example, if light strikes certain surfaces from some angles, a "rainbow effect" (prism) will appear. Another use is to adapt for some software packages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

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

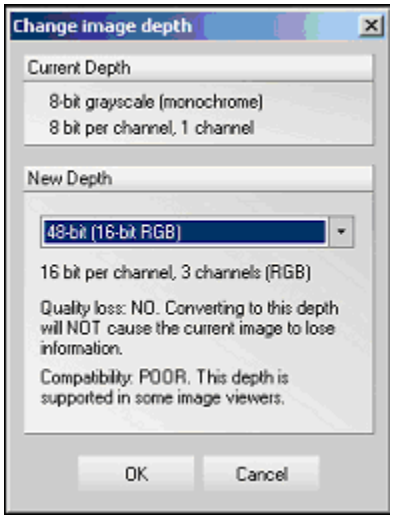
- Click onto **Reduce to monochrome** from the **Edit** menu button.
- Reducing a large image to monochrome may take a few seconds.

Change Image Depth

The Change Image Depth adjustment converts an image bit and color depth when needed.



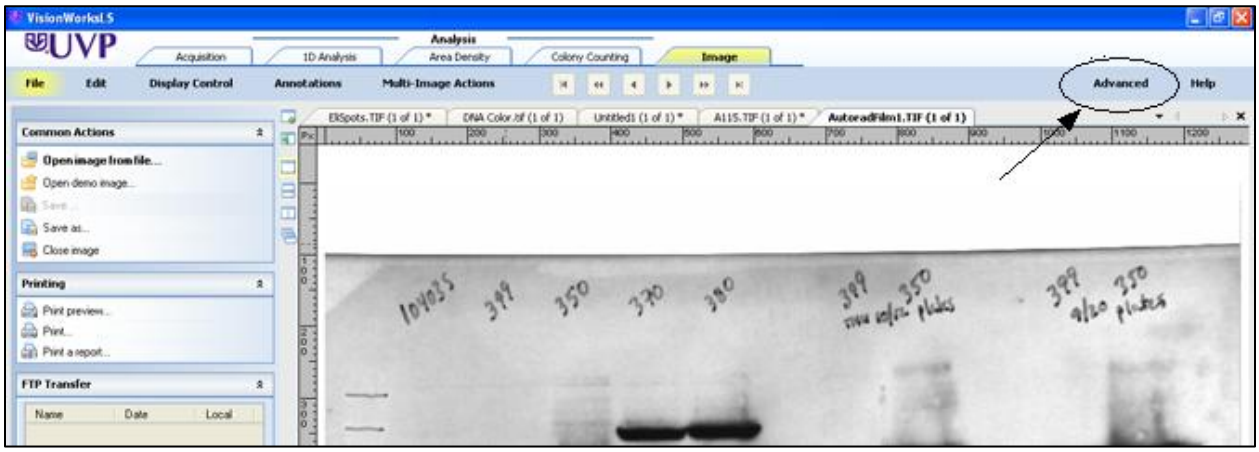
- Click onto the **Edit** menu button and click onto **Change Image Depth**. Reducing a large image to monochrome may take a few seconds.
- Click onto the new depth desired from the drop down menu and select **OK**.



Advanced Menu Button

Advanced Menu Button

This menu button contains advanced features for use by power-users. Access these functions from the Advanced Menu button.



Advanced Options

- Show Log File
- Configure User Accounts
- Display Loaded Modules
- Configure Application

Macros

- Macros
- Record
- Edit

View Options

- View Annotations
- View Rulers

Macros

Creating Macros

- Overview
- Macros Navigation
- Record Macros
- Edit Macros
- Play a UVP Pre-Recorded Macro
- Play a User-Created Macro
- Example 1: Repeating a Sequential Capture
- Example 2: Creating an Area Density Template

Overview

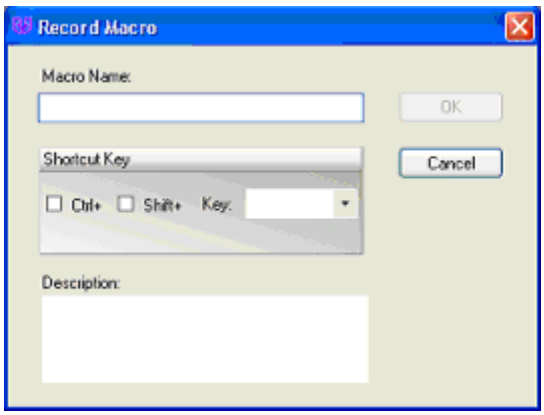
Macros are user-defined functions that group a series of commands into a single command. Macros allow users to replicate image capturing conditions or image analysis functions which require multiple steps. Typically a macro is created by recording steps the first time the user performs a series of actions. Details can be edited or added to the recorded script, saved, and stored to be recalled and executed at a later time.

Macros Navigation

There are three main options **Macros**, **Record**, **Edit**. To access these functions, click the **Advanced** menu button. The Macros option allows users to manage all the information about the created macros. The location of the file containing the macro scripts can be changed and loaded here. The created macros are listed here and can be run, deleted, renamed or edited by accessing the macros script file. The recently ran scripts will be listed under the macros options. As discussed later, the Record option allows users to create macros and Edit allows users to modify a created macro.

Record Macros

- To record a macro, click onto **Record** from the **Advanced menu.**
- A **Record Macro** window appears.



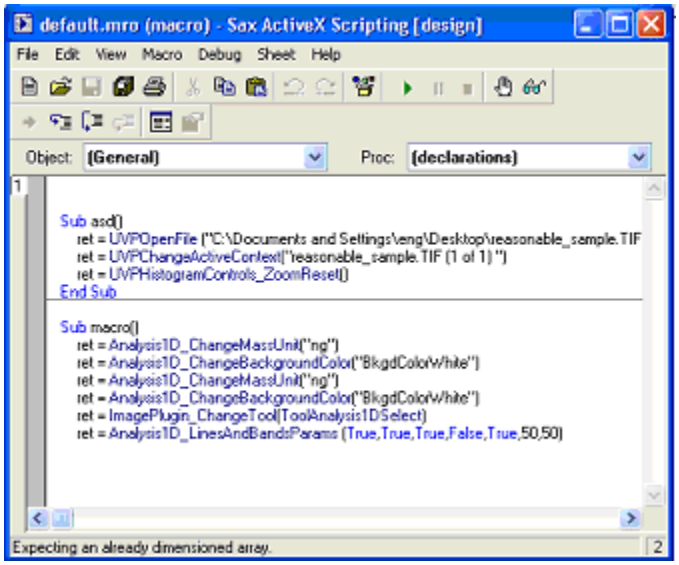
- Provide a **Macro Name** and **Shortcut Key**. NOTE: The macro name must begin with an alpha character.
- Select a **Shortcut Key** to execute the macro and provide a **Description** of the macro, if necessary.

- Click onto **OK.**
- A **Recording** window appears. Select **Template Mode** or **Show Recording**. To ensure the creation of the macro, click onto Show Recording to display the software code of the macro as it is formulated.
- Click onto any available command from the main screen or menu buttons to be saved into the macro.
- Select **Stop Recording** when finished recording the macro.

NOTE: Template Mode allows variable user input from dialog boxes. For example, a user may want to perform analysis on several different captured images. To do this, an image from the "Open" dialog box must be selected by the user. The macro will wait for the user to provide input on which image to open in the "Open" dialog box before continuing to the next step.

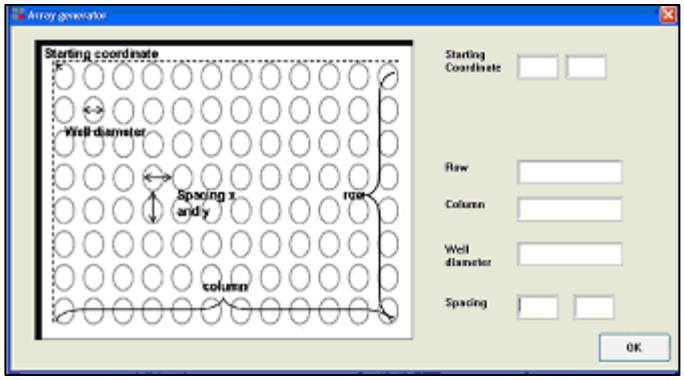
Edit Macros

- To edit a macro previously created, select **Edit** from the **Advanced menu.**
- A **default.mro** window appears with macro programming script. All the macros created will be listed in this window and can be edited manually.
- To edit the script code, click and type the new information into the code.

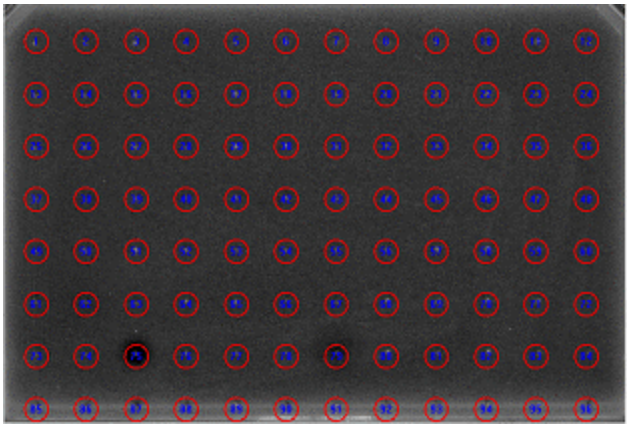


Play a UVP Prerecorded Macro

- UVP has prerecorded an **Area Density Array** Macro for users. To play this Macro click onto **Macros** then highlight the **Area Density Array** macro in the new pop-up window and select **Run**.
- After the macro is played the first time, it will appear under the **Edit** command in the dropdown menu.
- After selecting **Run** a new Array generator window will appear.



- Insert the corresponding values and select **OK.** A grid will now appear on the window for performing Area Density on a well plate of any number.



Play a User-Defined Macro

To playback a macro, select **Macros** from the **Advanced menu** then move the cursor down to the desired macro and click on the macro name.

Macro Example 1

Repeating a Sequential Capture

In this example, automated darkroom and camera templates have been pre-configured to capture white light and fluorescence images. The "white" camera template sets the exposure time to 0.5 seconds, whereas the "fluorescence" camera template sets the exposure time to 10 seconds to capture an image with a weak fluorescence signal. The "white" darkroom template moves the filter wheel to an empty filter slot to project unfiltered white light into the darkroom. The "fluorescence" darkroom template moves the filter wheel to the appropriate wavelength blocking filter and turns on the UV transilluminator for exciting the fluorescent sample.

- Select **Record** in the **Macros** menu.
- Name the recording. In this case the name "sequential_capture" is used.

Note: The name of the macro must begin with an alpha character and cannot start with numbers, or contain blanks or symbols.

- Click OK. The Recording window will appear.
- In the Acquisition Action Tab under the Lighting menu, set the darkroom template to "white".

Note: Even if the template is currently set to white, select the "white" template again. This step ensures that no other template is pre-selected.

- In the Acquisition Action Tab under the Camera menu, change the camera template to "white."
- Click on the Capture button under the Acquisition Action Tab.
- In the Acquisition Action Tab under the Lighting menu, change the darkroom template to "fluorescence."
- In the Acquisition Action Tab under the Camera menu, change the camera template to "fluorescence."
- Click on the Capture button under the Acquisition Action Tab.
- Select Stop Recording.

Edit the Sequential Capture Macro

The recorded macro script looks like the following:

```
Sub seqential_capture()
ret = DarkroomLensCtrl_ChangeFilter ("5")
ret = DarkroomLensCtrl_ChangeEpiLight ("4")
ret = DarkroomLensCtrl_ChangeTransLight ("2")
ret = DarkroomLensCtrl_ChangeTemplate("white")
Do
ret = DarkroomLensCtrl_CheckHWBusy()
Wait 0.5
Loop Until ret = 0
ret = BioChemiHRCam_ChangeExposureTime (0.5)
ret = BioChemiHRCam_ChangeTemplate("white")
ret = BioChemiHRCam_StartCapture()
Do
ret = BioChemiHRCam_CheckCameraCapturing()
Wait 0.5
Loop Until ret = 0
ret = UVPChangeActiveContext("Untitled24 (1 of 1) *")
ret = UVPHistogramControls_ZoomReset()
ret = DarkroomLensCtrl_ChangeFilter ("1")
ret = DarkroomLensCtrl_ChangeEpiLight ("2")
ret = DarkroomLensCtrl_ChangeTransLight ("1")
ret = DarkroomLensCtrl_ChangeTemplate("fluorescence")
Do
ret = DarkroomLensCtrl_CheckHWBusy()
Wait 0.5
Loop Until ret = 0
ret = BioChemiHRCam_ChangeExposureTime (10)
```

ret = BioChemiHRCam_ChangeTemplate("fluorescence")
ret = BioChemiHRCam_StartCapture()
Do
ret = BioChemiHRCam_CheckCameraCapturing()
Wait 0.5
Loop Until ret = 0
*ret = UVPChangeActiveContext("Untitled25 (1 of 1) *")*
ret = UVPHistogramControls_ZoomReset()
End Sub

The macro can be run as is, or, stripped down to the critical functions. For example, the templates automatically change the position of the filter wheel, but when the filter wheel is moved while the darkroom template is executed; the macro doubly records the filter wheel change. To increase the speed of the macro play, remove all unnecessary functions. The recorded script can be trimmed to the text following and still perform the same function:

Sub seqential_capture()
ret = DarkroomLensCtrl_ChangeTemplate("white")
ret = BioChemiHRCam_ChangeTemplate("white")
Wait 2
ret = BioChemiHRCam_StartCapture()
Wait 2
ret = DarkroomLensCtrl_ChangeTemplate("fluorescence")
ret = BioChemiHRCam_ChangeTemplate("fluorescence")
Wait 2
ret = BioChemiHRCam_StartCapture()
Wait 12
End Sub

The "Wait" command pauses the script by the number of seconds indicated in the command. It allows time for the filter wheel to settle. It can also be used to perform time elapsed captures. For example:

Sub seqential_capture()
ret = DarkroomLensCtrl_ChangeTemplate("white")
ret = BioChemiHRCam_ChangeTemplate("white")
Wait 2
ret = BioChemiHRCam_StartCapture()
Wait 2
ret = DarkroomLensCtrl_ChangeTemplate("fluorescence")
ret = BioChemiHRCam_ChangeTemplate("fluorescence")
Wait 2
ret = BioChemiHRCam_StartCapture()
Wait 600
ret = DarkroomLensCtrl_ChangeTemplate("white")
ret = BioChemiHRCam_ChangeTemplate("white")
Wait 2
ret = BioChemiHRCam_StartCapture()
Wait 2
ret = DarkroomLensCtrl_ChangeTemplate("fluorescence")

ret = BioChemiHRCam_ChangeTemplate("fluorescence")
Wait 2
ret = BioChemiHRCam_StartCapture()
End Sub

The above code will repeat the same capturing sequence once after 10 minutes (600 seconds).

Macro Example 2

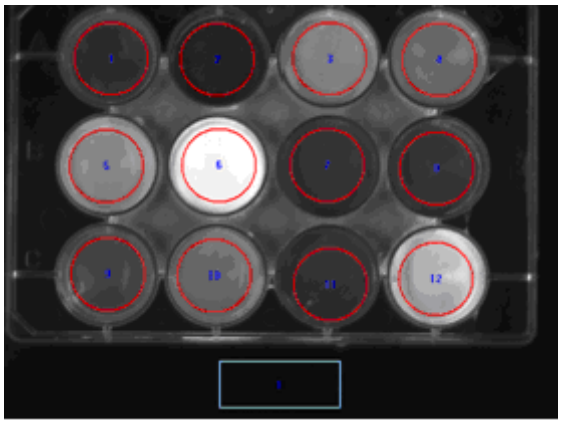
Area Density Template

In this example, the macro is used to assist area density analysis of a 12-well plate. The same format plate can be analyzed using the created macro without having to manually specify the wells each time. Refer to the chapter on Area Density to become familiar with performing Area Density.

- Open the image on which to perform the Area Density function.
- Select **Record** in the **Macros** menu.
- Name the recording. In this case the name "well_analysis" is used.

Note: The name of the macro must begin with an alpha character and cannot start with numbers, or contain blanks or symbols.

- Click **OK**. The Recording window will appear.
- Use the ROI (Region of Interest) selection tools to define the region of interest. In this case, the circular region of interest tool was used to define the location of the wells.



- Define the user background in the image by selecting the ROI tool and drawing a ROI shape around an area without a well.
- Select Stop Recording.

The following recorded script can automatically create areas as shown in the image. No further editing is required.

Sub well_analysis()
ret = UVPAreaDensity_StartAreaDensity ()
ret = ImagePlugin_ChangeTool(ToolAreaDensityEllipse)
ret = AOIPlugin_ImgDrawObject(202, 50, 418, 263, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()

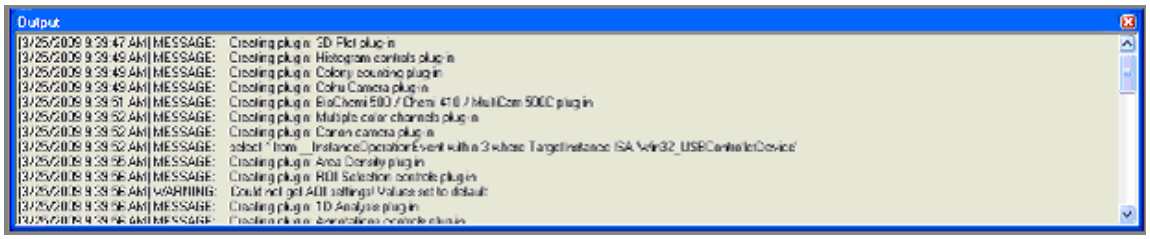
ret = AOIPlugin_ImgDrawObject(509, 52, 725, 265, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()
ret = AOIPlugin_ImgDrawObject(829, 50, 1045, 263, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()
ret = AOIPlugin_ImgDrawObject(1146, 53, 1362, 266, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()
ret = AOIPlugin_ImgDrawObject(187, 361, 403, 574, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()
ret = AOIPlugin_ImgDrawObject(514, 359, 730, 572, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()
ret = AOIPlugin_ImgDrawObject(822, 359, 1038, 572, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()
ret = AOIPlugin_ImgDrawObject(1139, 368, 1355, 581, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()
ret = AOIPlugin_ImgDrawObject(192, 674, 408, 887, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()
ret = AOIPlugin_ImgDrawObject(500, 679, 716, 892, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()
ret = AOIPlugin_ImgDrawObject(831, 703, 1047, 916, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()
ret = AOIPlugin_ImgDrawObject(1136, 687, 1352, 900, 38)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateSelROI()
ret = ImagePlugin_ChangeTool(ToolUserBackgroundRect)
ret = UVPAreaDensity_UserBackground ()
ret = AOIPlugin_ImgDrawObject(624, 1033, 970, 1169, 48)
ret = AOIPlugin_ROICreateRectEllipse()
ret = UVPAreaDensity_AreaDensCreateUserBgndROI()
End Sub

Advanced Options

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 the **Advanced** menu button and click on **Show log file.**

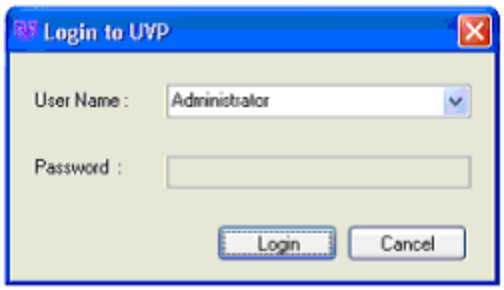


Configure User Accounts

- User Names and Passwords
- Add a User
- Edit a User
- Change a Password or Other Settings
- Deactivative/Reactivate a User
- View the Login History of a User

User Names and Passwords

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

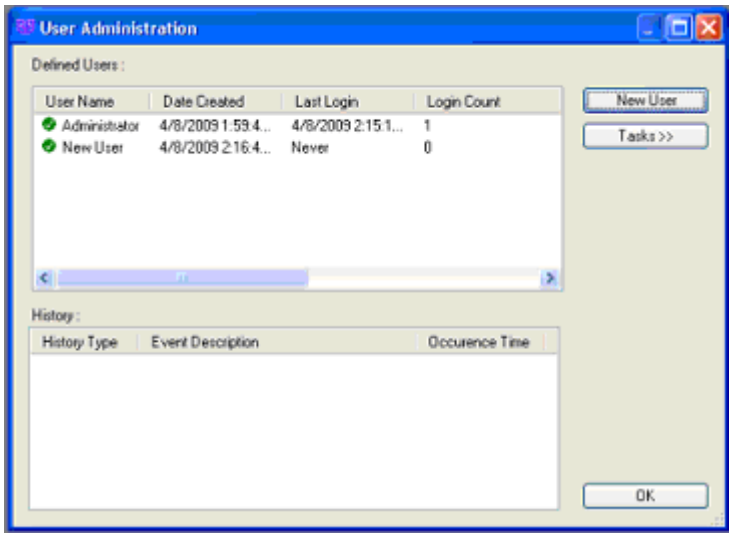


- A Reset password window appears. Enter the new password. Confirm the new password.
- Click **OK**.

Add a New User

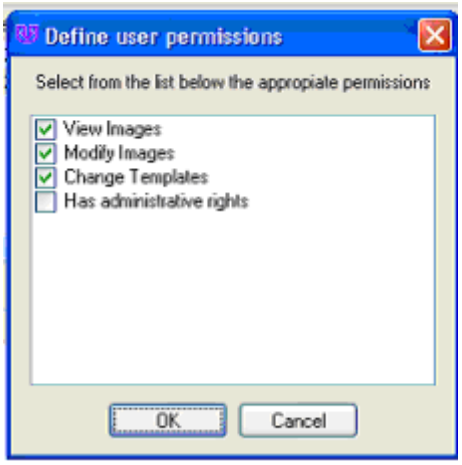
- From the menus, click the **Advanced** menu button then select **Configure user accounts** to open the **User Administration window**.

- 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.



Edit a User

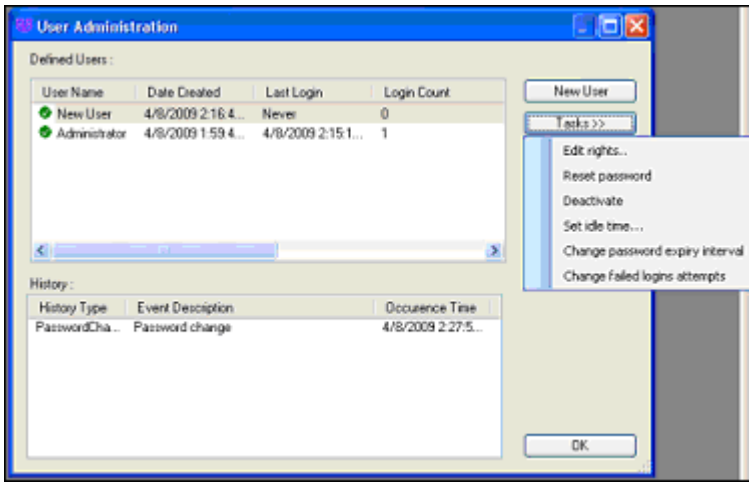
- Highlight the user name in the **User Administration** window.
- Click the **Tasks > Edit Rights** and select from the **Define User Permissions** screen to allow users to view images, modify images, change templates, or assign administrative rights.
- Click **OK** when changes to the user are complete.



Change a Password or Other Settings

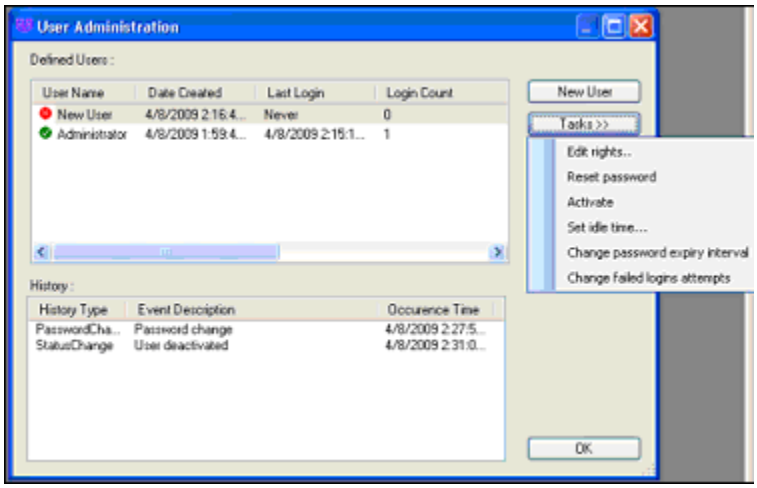
- To change a password, click the **Advanced** menu button then select **Configure user accounts** to open the **User Administration window**.
- Click on the appropriate user to change.

- 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.



Deactivate/Reactivate a User

- To deactivate/reactivate a user, click the **Advanced** menu button then select **Configure user accounts** 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.



NOTE: Never disable the Administration Account.

View the Login History of a User

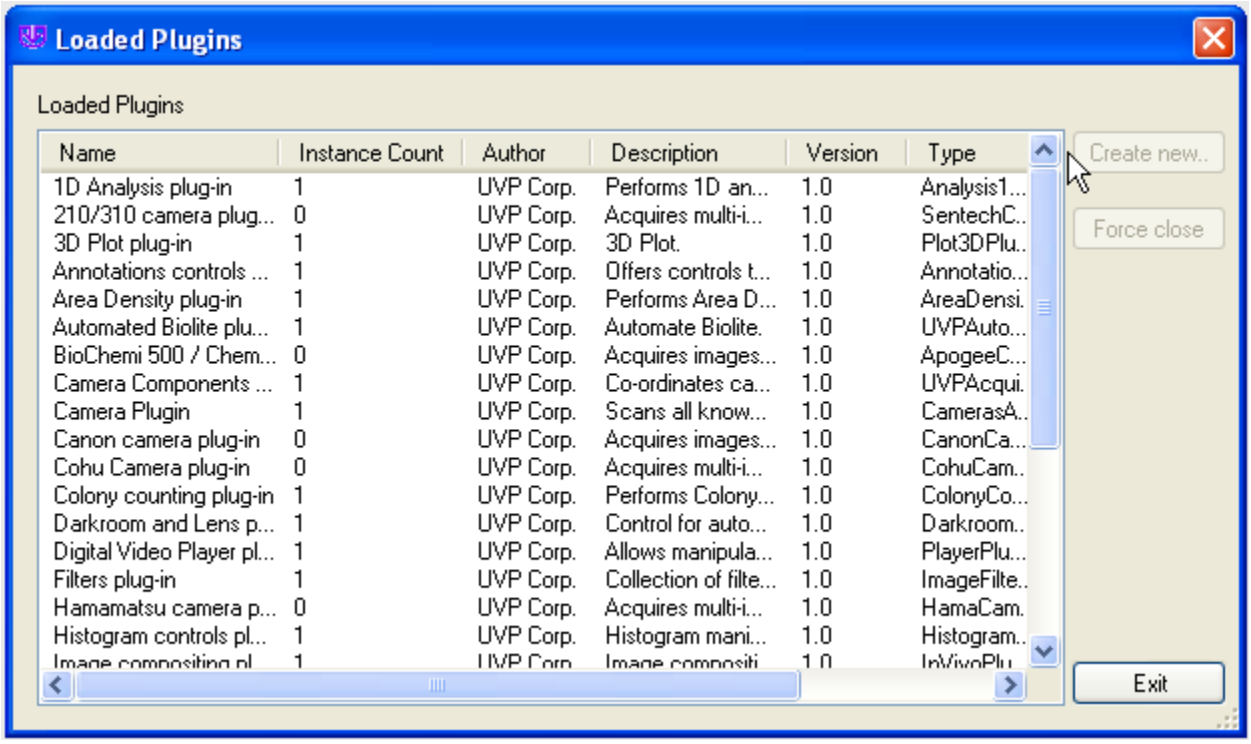
- Select a user name in the **User Administration** table. The lower half of the window displays the login history associated with the selected user.

- Click **OK** to close the window.

Display Loaded Modules

This window is typically used for technical support. Our technical support people can view this window and diagnose and prevent future system failures.

- To access, click onto the Advanced menu button and select **Display Loaded Modules**.

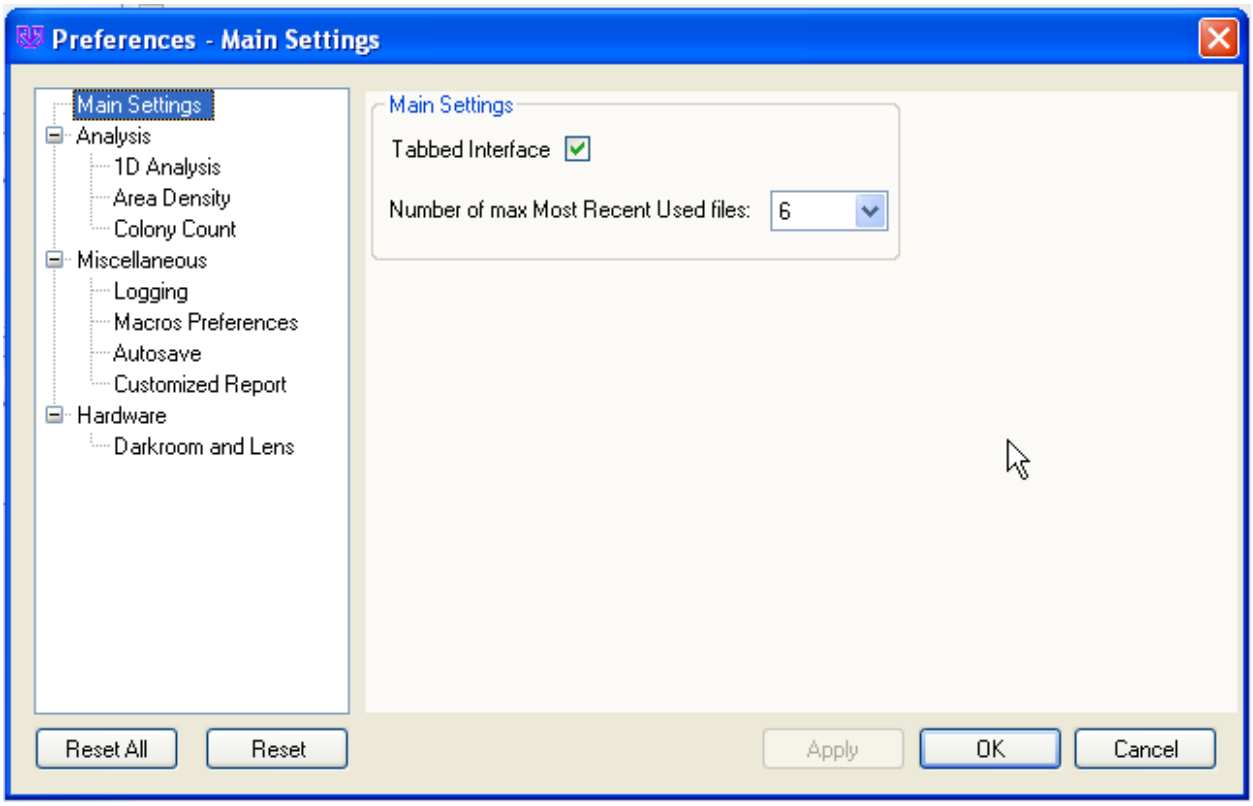


Configure Application

The function Configure Application shows global application settings. Such settings include:

- switching between tabbed/windowed interface for images
- setting the # of recently-opened images to show in the "open" dialog.
- examine/modify the analysis/hardware settings.

To access, click onto the Advanced Menu Button and select **Configure Application.**



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. 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.

Show or Hide 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.
- From the **Advanced menu** click onto **View Rulers**.

View Annotations (Click on this link to get more information on creating and viewing annotations)

Help Menu Button

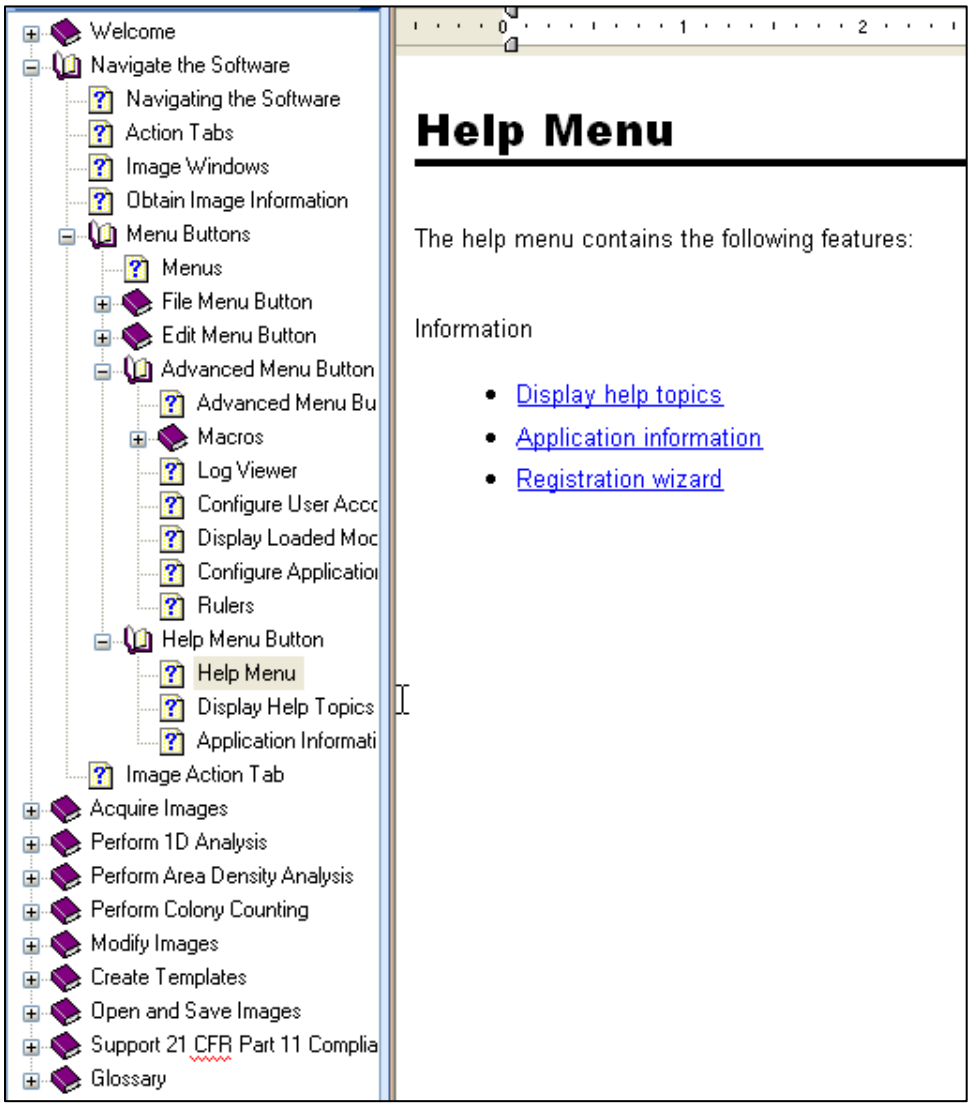
The help menu contains the following features:

- Display help topics
- Application information
- Registration wizard

Display Help Topics

These are some of the help topics available. Simply click through the help topics or search for a word for assistance using the help files.

To access go to the Help Menu Button and select **Display Help Topics**.

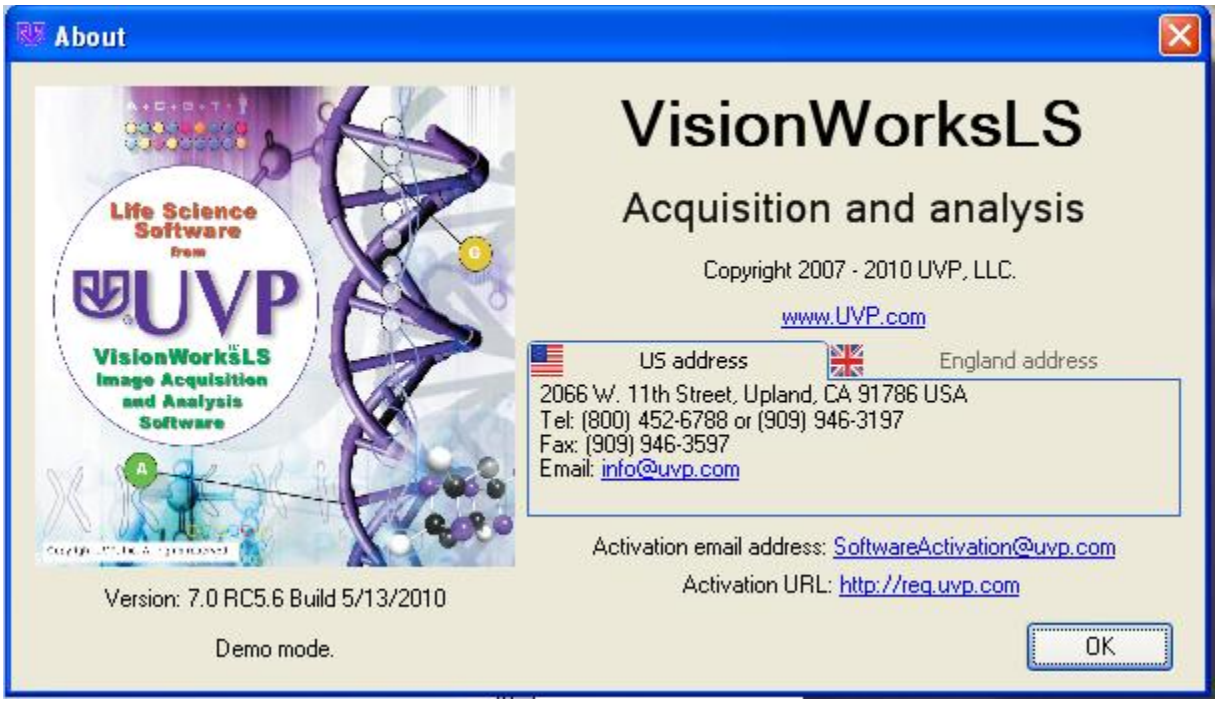


Application Information

The Application Information function brings up a window which describes the:

- Name of the software
- UVP's contact information
- Version of software (usefully when calling UVP's technical services department) and
- Links to activate the software

To access, click onto the Help Menu button and select **Application Information**.



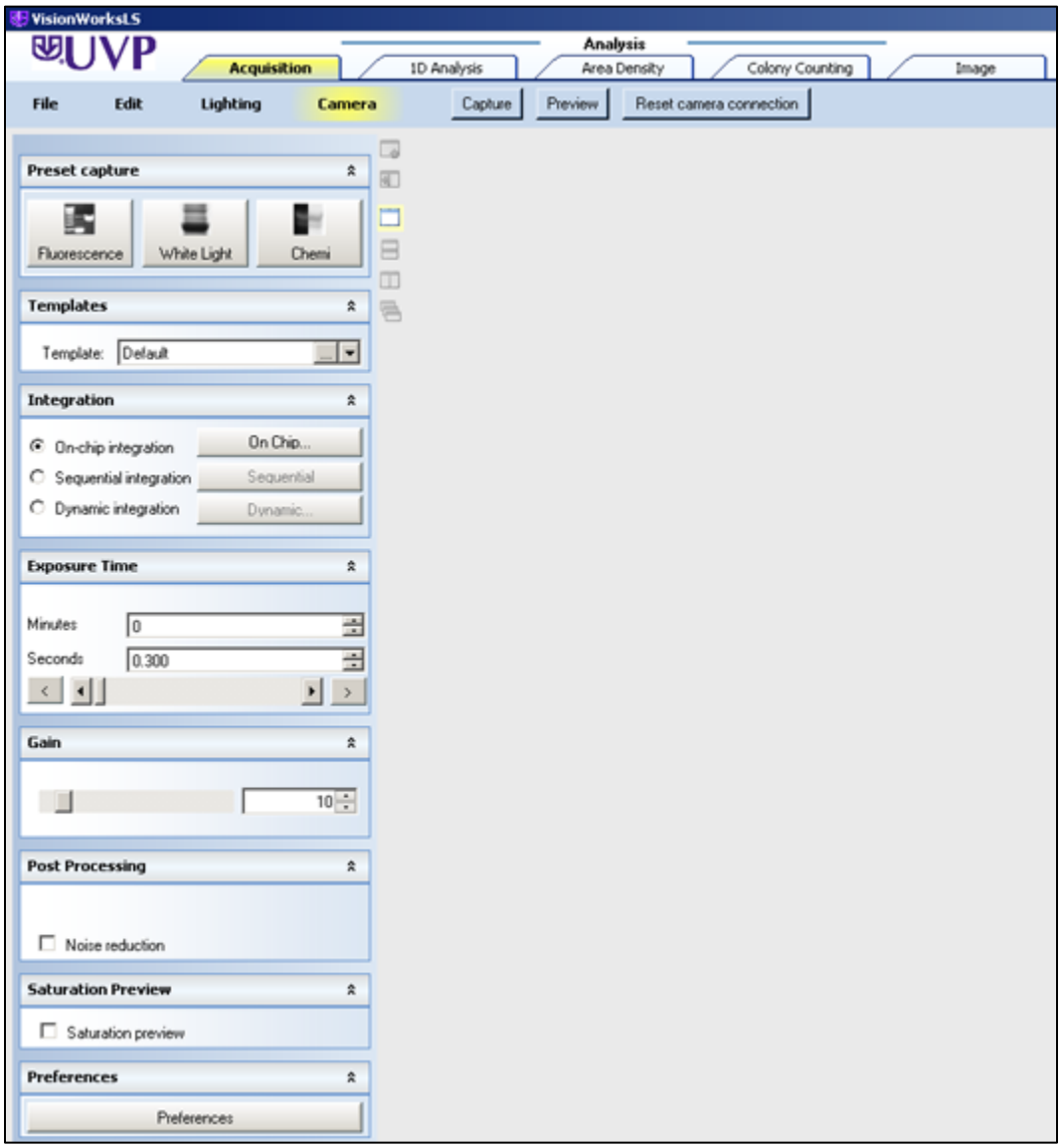
Registration Wizard

(Click on the Link to go to Register the Software)

Acquire Images

Acquisition Action Tab

The **Acquisition** Action Tab provides the means to capture images with the UVP system.



Menu buttons contained in the Acquisition Action Tab are:

Lighting

- Preset Capture Buttons
- Darkroom Templates
- Lens Controls
- Lighting and Filters
- Tray Height
- BioLite

Camera

- Preset Capture Buttons
- Template
- Integration
- Exposure Time
- Binning
- Gain
- Post Processing (some cameras will have this feature)
- Saturation Preview
- Preferences

Capture (button)

Preview (button)

Reset camera connection (button)

Image Acquisition

Capture an Image

To capture an image with the camera:

- Place the gel, blot, plate (object to be captured) into the darkroom.
- Select **Preview** in the **Acquisition** action tab.
- Set the optimal settings (for the lens, camera, BioLite (optional), and darkroom or use the capture preset buttons). Ensure that the **Saturation Warning** checkbox is selected.
- When the optimal settings have been found, click onto **Capture**. Should the captured image appear black increase the exposure time and/or check the **Compensate exposure to binning** checkbox.

Preview and Capture Buttons

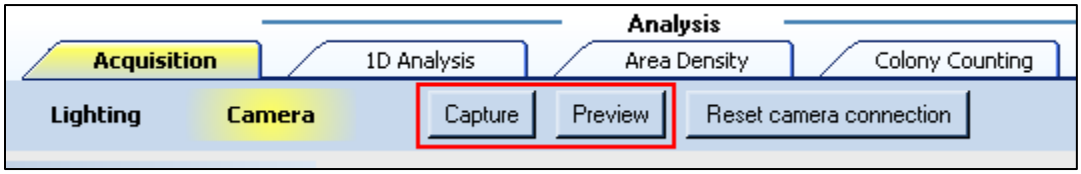
Access the Preview and Capture buttons (outlined below) in red from the Acquisition Action Tab.

Preview

Click on this button to see a preview of the sample, in order to ensure that the camera sees the sample clearly before taking the actual picture.

Capture

Click on this button to take the picture.



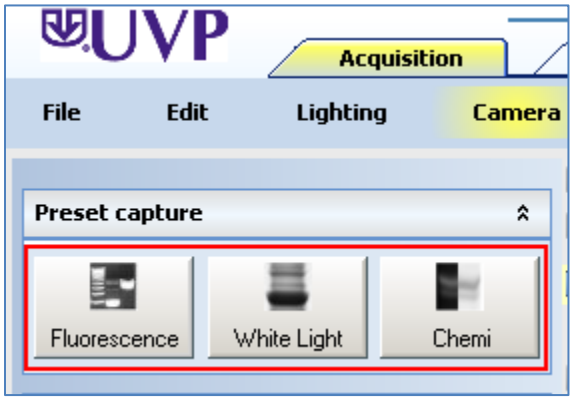
Lighting

Preset Capture Buttons

In order to capture images quickly and easily, VisionWorks software allows users to click onto one of three buttons:

- Fluorescence,
- White Light, and
- Chemiluminescence.

Clicking each of the buttons (outlined in red below) will automatically capture an image with the settings as defined below.



Note: All settings assume the 50 f/1.2 lens is utilized for image capture.

Fluorescence

The settings for capturing images using the **Fluorescence button** assume that the object to be imaged is placed on top of the transilluminator at the bottom of the darkroom:

- Aperture: 1.2
- Filter position: 5
- Binning: 1x1
- Epi lighting: Off
- Trans lighting: UV
- Exposure time: 300 ms

White Light

The settings for capturing images using the **White light button** assume that the object to be imaged is placed on top of the transilluminator at the bottom of the darkroom:

- Aperture: 4.9
- Filter position: 1
- Binning: 1x1
- Epi lighting: white light
- Trans lighting:Off

- Exposure time: 20 ms

Chemiluminescence

The settings for capturing images using the **Chemiluminescence button** will automatically display the image with a dark signal on a white background. (The camera preview window will not be inverted.) The process to automatically adjust the histogram to show a bright sample while minimizing the required exposure time will also be applied to all captured images. This mode can be disabled by clicking the ”Chemi” button again:

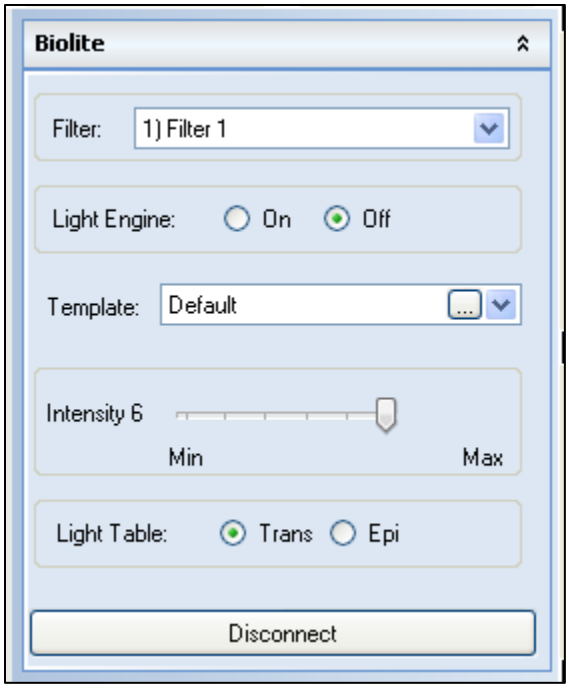
- Aperture: 1.2
- Filter position: 1
- Binning: 2x2
- Epi lighting: Off
- Trans lighting: Off
- Exposure time: 1.2 sec

BioLite MultiSpectral Light Source

The automated BioLite MultiSpectral Light Source is an added accessory to some systems. The Automated BioLite has a fiber optic cable that threads into the darkroom and strictly controls the wavelength of light shining inside the darkroom.

In the Acquisition Action Tab in the **Lighting** menu is a list of functions for the BioLite. These are:

- **Filter**: Drop down list consists of a variety of filters defined by the user.
- **Light Engine**: Turn the BioLite On and Off.
- **Template**: A template is a group of BioLite settings, which can be saved under a common name. Select the Template for the sample, to apply from the drop-down box.
- **Intensity**: Provides the range for light intensity. The intensity ranges from 0 to 100%.
- **Light Table:** Turns the light on for the Transillumination light or for the Epi Illumination light.
- **Connect**: Connects the Automated BioLite device from the software.



Using the BioLite Functions

For additional information on using the automated BioLite MultiSpectral Light Source, refer to the user instruction manual for this unit.

- Turn on the **Light Engine** in the software.
- Set the **Intensity** of the BioLite. Select the highest intensity then move to a lower threshold if desired.
- Turn on the **Light Table** to Trans or Epi. Use the Trans option if the light source is below the sample. Use the Epi option if the light source is above and shining down on the sample.

Tip: Changes made to the BioLite through the mechanical buttons on the device will NOT be reflected in the software. Please double check settings periodically to ensure that the software settings match with those on the device.

Darkroom Control

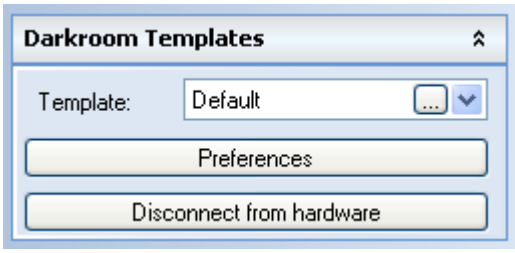
LS Software works with a series of darkrooms offered by UVP. Support software will be installed along with the LS software in a single installation. The software controls the lighting inside the darkroom. If the order came with a motorized darkroom, the lift platform height position will be controlled by the software as well. This software controls the following systems:

- BioSpectrum® Systems
- iBox® Systems

To access these controls click onto the **Acquisition** Action Tab and then click onto the **Lighting** menu button.

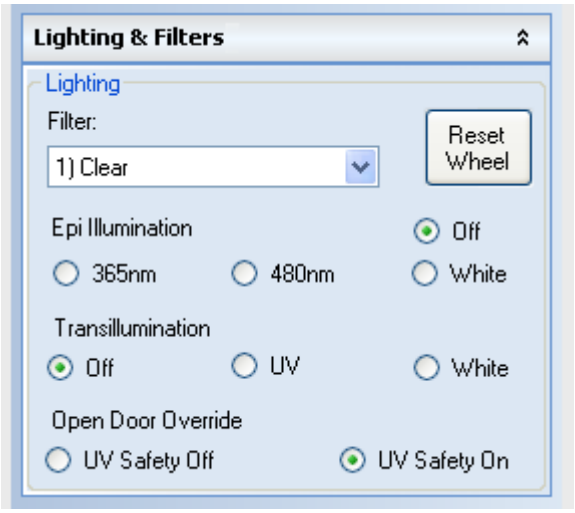
Darkroom Templates

- **Templates:** Templates allow users to set up easy-to use preset functions for their experiments.
- **Preferences:** Users may change the look and feel of the capture functionality.
- **Connect to Hardware:** If the darkroom hardware does not seem to be connected, or the software functions for the darkroom are greyed out in the software interface, clicking Connect to hardware may help to connect the darkroom to the software controls.



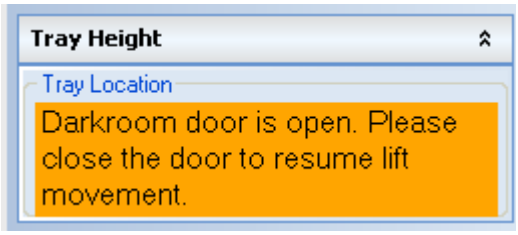
Lighting & Filters

- **Filter:** Turns the darkroom filter wheel to the user-defined filter.
- **Reset Wheel:** Click on this button to realign the filter wheel after accidental movement of the darkroom.
- **Epi Illumination:** Darkroom supplies 365nm, 480nm and white light epi illumination. Click onto the radio button to make a selection.
- **Transillumination:** Darkroom supplies power to the UV transillumination source and also to the white light transillumination source. Click onto a radio button to make a selection.
- **Open Door Override**: Under standard operating conditions, when the darkroom door opens, the UV Light shuts off in the darkroom. When **UV Safety Off** is selected as the door opens, the UV Light stays on.



Tray Height

- **Open Door Override:** Under normal circumstances when the darkroom door is open, the UV light shuts down; to override this occurrence, click onto the Open Door Override switch.
- **Tray Location:** Slidebar adjusts the height of the automated lift platform. (Manual platform systems do no have this option). The warning (as shown below) appears when the door is open to help prevent UV exposure.

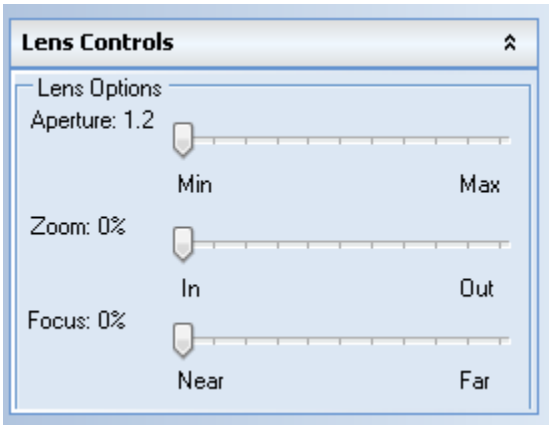


Lens Control

This section contains information on adjusting lens and darkroom parameters to capture an image. To access these functions, click on the **Acquisition** Action Tab and then onto the **Lighting** menu button. The lens purchased must be an software controlled lens.

Lens Control

- **Aperture:** Slidebar adjusts the amount of light that enters the lens.
- **Zoom:** Slidebar controls the object's apparent distance from the observer.
- **Focus:** Slidebar adjusts the clarity of the image.

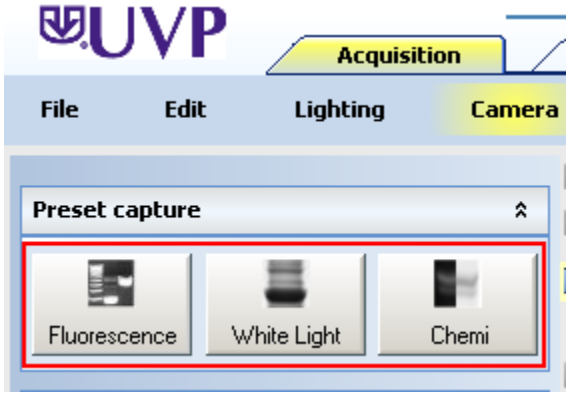


Camera

Functions under the **Camera** menu button include internal camera settings to help acquire an image. To access these functions click onto the **Acquisition** Action Tab and then click onto **Camera.**

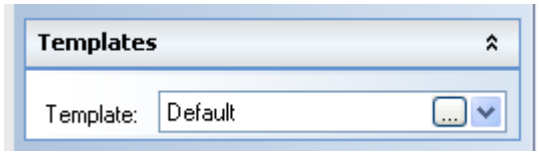
Preset Capture

- Software contains Fluorescence, White Light, and Chemi Capture settings



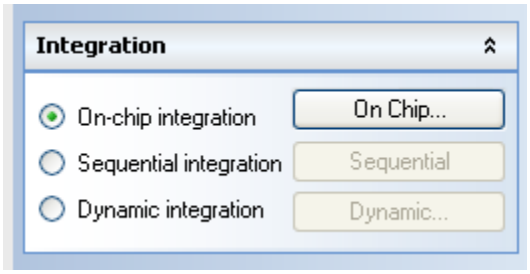
Templates

- **Template:** A template is a group of camera settings, which can be saved under a common name. Select the correct Template for the sample, to apply from the drop-down box.



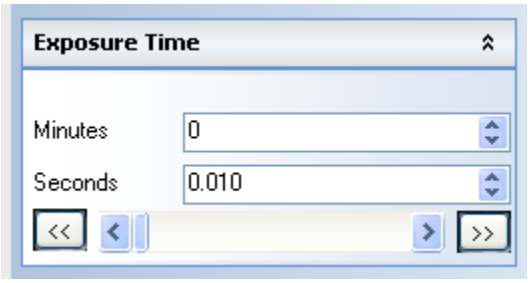
Integration

- **On-chip Integration:** When this radio-button is selected before pressing **Capture**, the software takes only ONE picture, with the current exposure time (below) set.
- **Sequential integration:** When this button is selected before clicking **Capture**, multiple pictures are taken at a uniformly increasing exposure time.
- **Dynamic Integration:** With this option selected before capture, multiple pictures are taken at set uniform intervals.



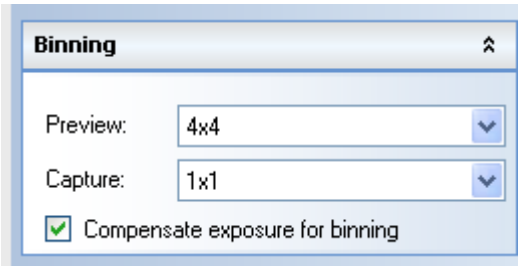
Exposure Time

Adjusts the time for how long the camera should expose the image and collects light from the sample. Various arrows increment the time in a steady manner.



Binning

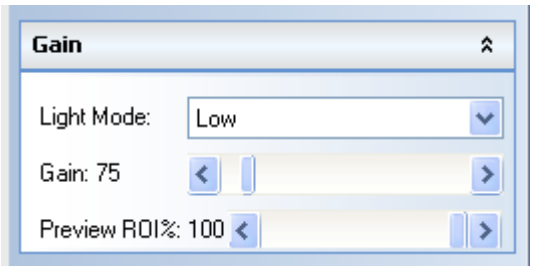
- **Preview**: Sets the binning mode to be used when **Preview** is selected.
- **Capture**: Sets the binning mode to be used when **Capture** is selected.
- **Compensate Exposure for Binning:** When the binning mode is changed (above), it automatically adjusts the exposure time to match the brightness between preview and capture.



Gain

(Only available for some cameras)

- **Light Mode**: When set to 'low', the Anti-Blooming feature of camera is turned off. When set to 'high', the said feature is on.
- **Gain**: Set a high value for gain to get increased sensitivity. That also increases background noise.
- **Preview ROI%:**
- **Post Processing:** Used for longer exposures to produce a high-quality image by removing the noise floor and filtering out pixels that are substantially brighter than their surroundings.



Saturation Preview

- **Saturation Preview:** Click this checkbox during **Preview** to see if any part of the image is over exposed to light. Over exposed pixels are shown in Red color.



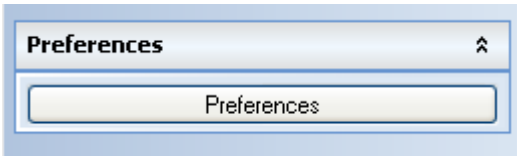
AutoExposure

Only applies to 410 and 500 cameras. Located by the Lighting and Camera menu buttons resides the **AutoExposure** feature which automatically calculates the ideal exposure time for the application to be imaged.



Preferences

- Shows the camera and template settings.

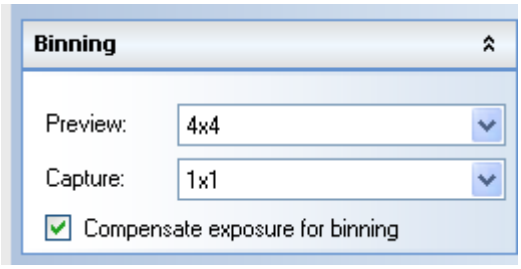


Binning Modes

Binning is an advanced feature provided by most UVP cameras. "Binning" literally means to "bin" or "combine" pixel values. A camera set to the binning of 4 x 4 (read 4 by 4) means, that it "combines" the values from 4 pixels across and 4 pixels down – 16 pixels in all – into one single pixel on the image. Binning is also referred to as creating a "Super Pixel".

Higher binning, hence, increases the sensitivity of the images, at the cost of resolution and image-size (due to combining of pixels). A good strategy is to Focus at higher binning (say 2 X 2) so that the refresh rate is higher and then to snap at a lower binning, to capture full resolution in the resultant image.

To access the Binning feature go to the Acquisition Action Tab and click onto the Camera Menu button and find the Binning feature.

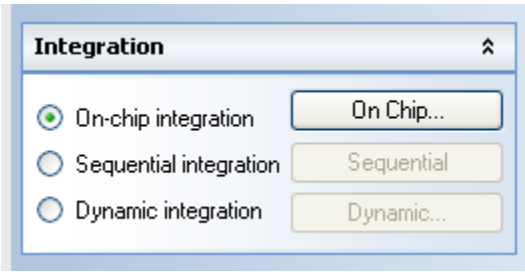


Integration-A Detailed Look

Integration is the term used to mean the length of time the camera is exposed to incoming light. Integration time and Exposure time are sometimes used interchangeably. There are various different types of Integration modes provided by LS software.

- On Chip Integration
- Sequential Integration
- Dynamic Integration

To access the Integration feature go to the Acquisition Action Tab and click onto the Camera Menu button and find the Integration feature.



On Chip Integration

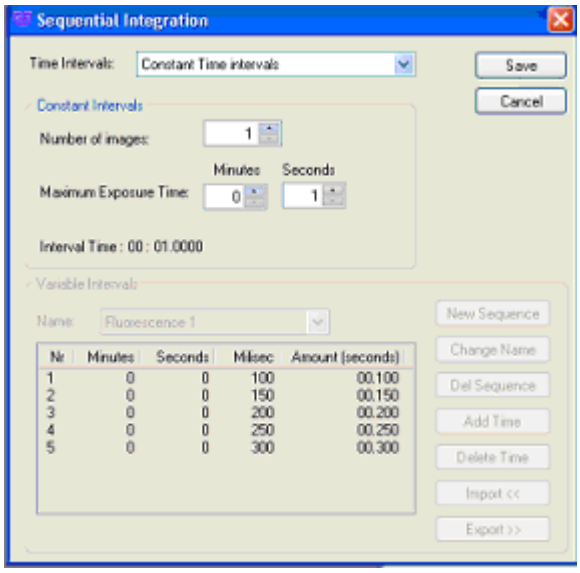
The software takes only ONE picture, with the current exposure time set.

Sequential Integration

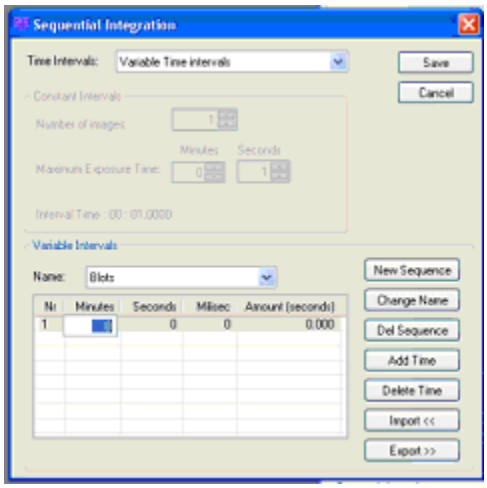
Used when the ideal exposure time for the sample is unknown. A low light Chemiluminescence sample may have an unknown exposure time. This option captures multiple images at either constant or irregular time-intervals. The user may browse through the images taken and select the image that provides the best results.

To use Sequential Integration, click onto the **Sequential Integration** radio button and click on the **Sequential** button.

- **Constant Time Intervals:** Use this when the number of images and the longest exposure time is known. LS software will then calculate the integration time to be used for the first image and use the same as an increment to capture subsequent images. E.g., if five images are to be taken and the maximum integration time is set at 20s, the exposure times calculated will be as follows:
    - First: 4s
    - Second: 8s
    - Third: 12s
    - Fourth: 16s
    - Fifth: 20s



- These five pictures will be placed inside a sequence file, and can be viewed in the Player to select the perfect image.
- **Variable Time Intervals:** Use this setting to take multiple images with different exposure times. No addition of exposures is done as in the 'Constant Time Intervals' case. A large number of images can be captured in sequence. Each image needs a separate line of entry in the table shown.

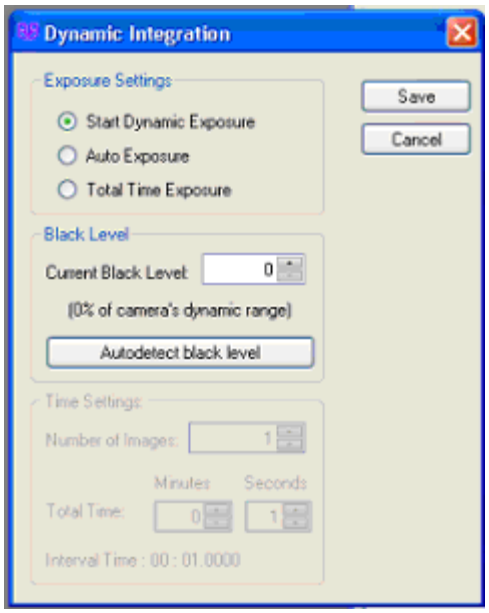


- **New Sequence**: Add a new sequence of images.
- **Change Name**: Enter a new name for the currently displayed sequence.
- **Del Sequence**: Deletes the currently displayed sequence.
- **Add Time:** Adds a new entry to the sequence. Enter time in Minutes, seconds and milliseconds. 'Amount' shows the total time in milliseconds.
- **Delete Time**: Deletes the currently active entry of the sequence.

Dynamic Integration

Used when the integration time offered by the camera is not enough for a long exposure. In this mode, LS software does "stacking" of frames, i.e., it adds the corresponding pixel values of first image-frame to the next. This compensates for a low light limitation, by making dim areas brighter with increasing number of images. [Stacking replaces the first image after the second is captured and pixel values added to it from the first.] UVP cameras typically have a long exposure time capability, which would be more than enough for most of samples. If, however, the application's requirements go beyond what is available, then this feature offers a software solution.

Switch to the '**Dynamic Integration'** option and click on 'Dynamic Integration' button to bring up the following window:

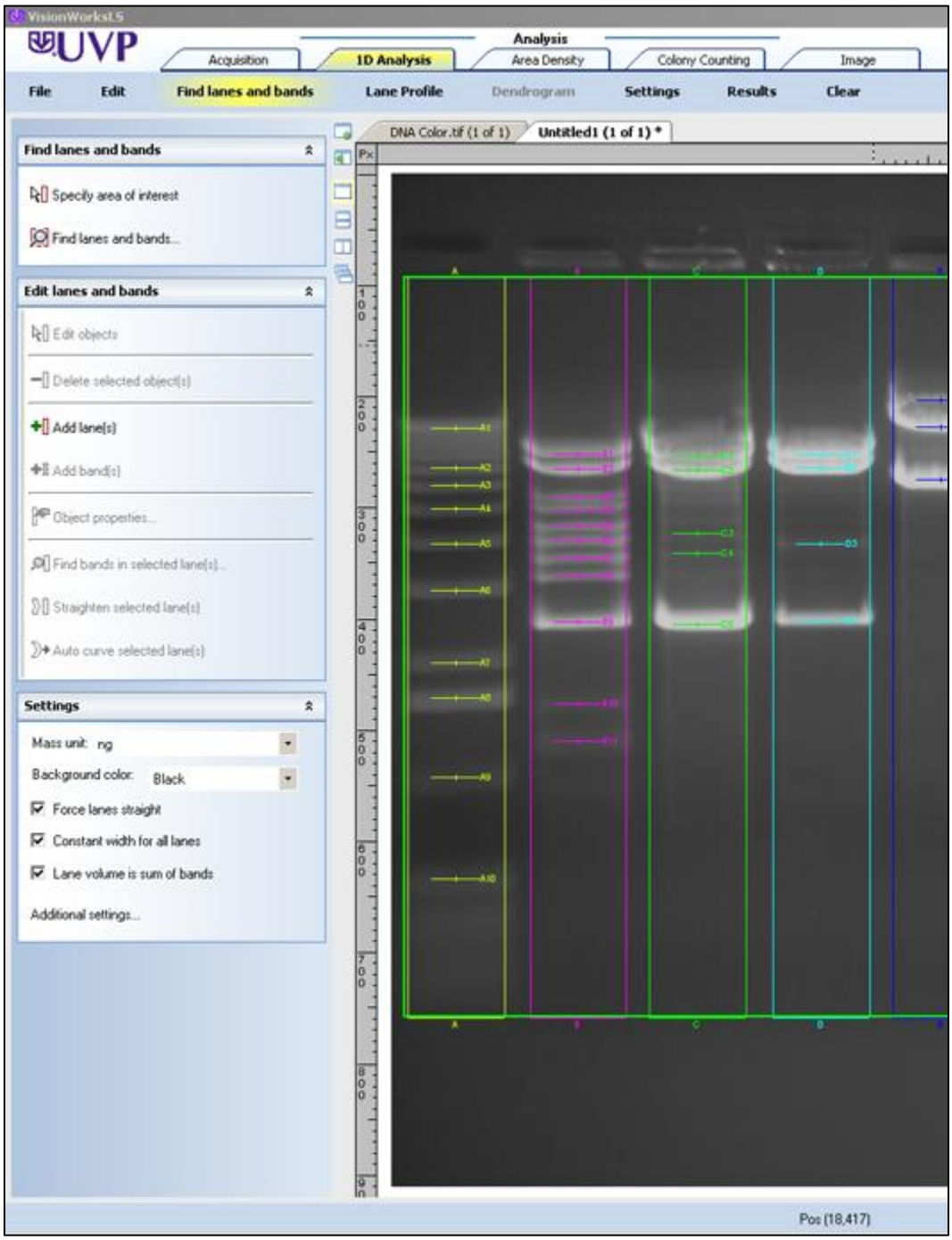


- **Auto Exposure:** With this box checked, the stacking automatically stops once the image gets saturated.
- **Total Time Exposure:** Select this radio button to have a greater control over how many images to capture, and for how long each one should be exposed. Interval Time is simply the division of total time by number of images. Uniformly exposed images are stacked.
- **Current Black Level:** Stacking frames also stacks noise along with it. To clip noise from each image before stacking, it is important to set the black-level. Value of the darkest pixel in the image (which will typically be noise) should be set inside the spin-box. Pixel values less than this value are not recorded or clipped during acquisition and before stacking.

Perform 1D Analysis

1D Analysis Action Tab

The 1D Analysis Action Tab provides the means to perform 1D Analysis on a captured or demo image.



The functions in the 1D Analysis Action Tab include:

Find Lanes and Bands

- Find Lanes and Bands
    - Define Region
    - Find Lanes and Bands

- Edit Lanes and Bands
    - Edit Objects
    - Delete Selected Objects
    - Add Lane
    - Add Band
    - Object Properties
    - Find Bands in Selected Lanes
    - Straighten Selected Lane
    - Auto Curve Selected Lane

- Settings
    - Mass Unit ng
    - Background Color
    - Force Lanes Straight
    - Constant Width for all Lanes
    - Lane Volume is Sum of Bands
    - Additional Settings

Lane Profile

- Molecular Weight
    - Calibrate Lane
    - Uncalibrate Lane

- Concentration
    - Lane Profile Graph
    - Concentration

- Background Correction
    - No Background Correction

- Straight Line
- Rolling Disc
- Joined Valley
- Area Between Lanes

- Rf
  - Edit Objects
  - Add Rf Line
  - Delete Selected Rf Lines
  - Delete All Rf Lines
  - Select Standard Lane

- Dendrogram
  - Dendrogram
    - Single-Image Dendrogram
    - Multi-Image Dendrogram

Settings

- Settings
  - Additional Settings

Results

- Print Reports
  - Page Setup
  - Print Preview
  - Print
- Export Report
  - Export to Excel
  - Export to CSV
- Report Type
  - Report Type (Dropdown)
- Show Report
  - Show Report

Clear

1D Analysis Overview

Overview

The 1D Analysis topics can be accessed by clicking on the following links:

- 1D Analysis Image Windows Features
- Finding 1D Gel Lanes and Bands (described immediately below)
- Edit Lanes and Bands
- 1D Analysis Settings
- Viewing and Printing 1D Gel Analysis

Related Topics:

- Performing Molecular Weight Calibration
- Performing Concentration Calibrations
- Performing Dendrogram Analysis

Finding 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.

- Find Lanes and Bands Automatically (described immediately below)
- Lane and Band Information
- Add Lanes and Bands
- Delete Lanes and Bands
- Edit Lanes and Bands

After finding, adding, deleting, and editing lanes and bands as appropriate, the following steps are available for further analysis of the lanes and bands.

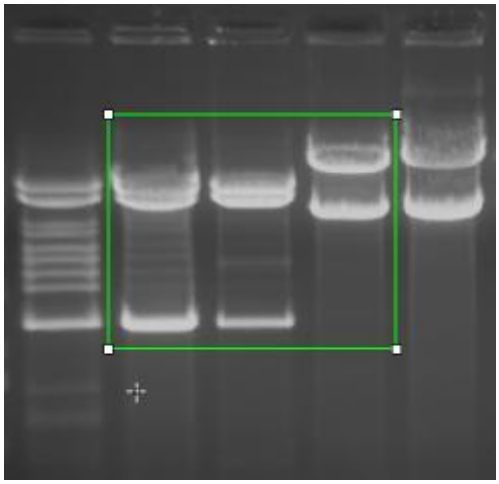
- Performing Molecular Weight Calibration
- Performing Concentration Calibrations
- Performing Dendrogram Analysis

Find Lanes and Bands

Define Region of Interest (ROI)

By defining 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.

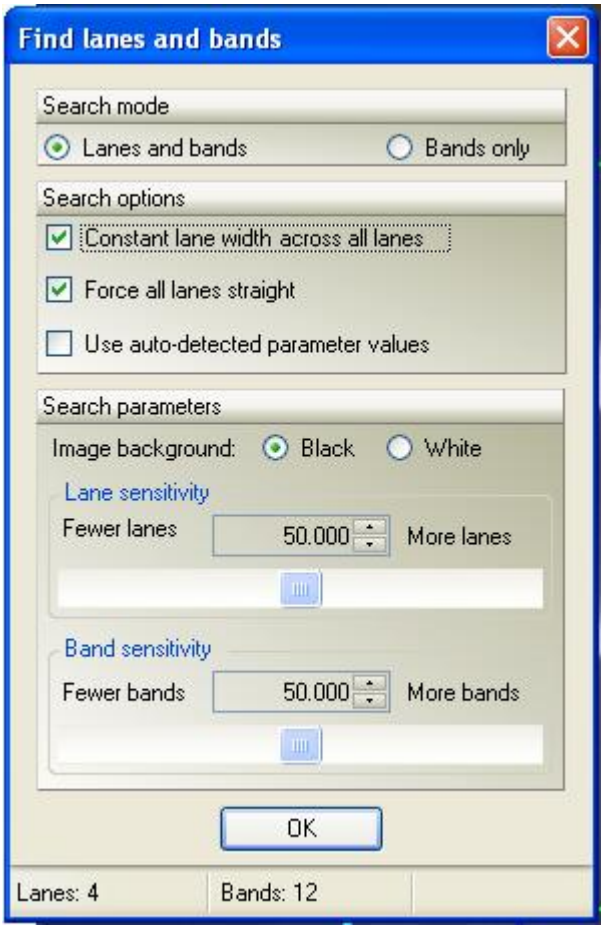
- Select **Define Region** from the **Find Lanes and Bands** menu. (The ROI tool utilized is rectangular by default.)
- Outline the lanes of interest with the ROI tool by positioning the mouse at one of the corners to move that corner of the ROI. Then drag the corner to the desired location. The area defined will be encapsulated in green.
- Next, go through the Perform Automatic Finding of Lanes and Bands steps below.



Perform Automatic Finding of Lanes and Bands

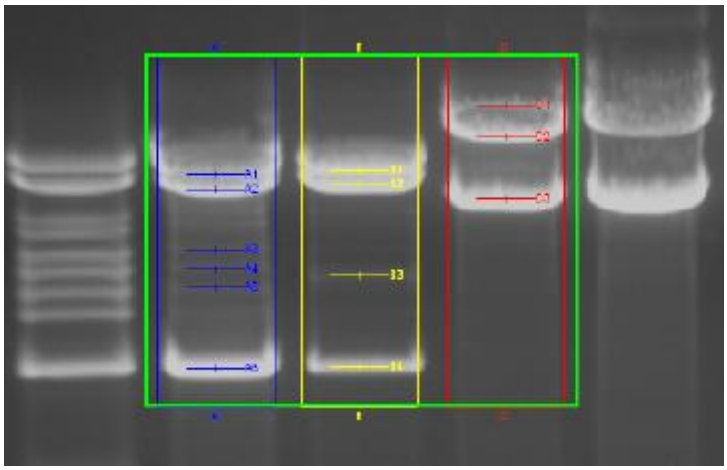
After defining the region of interest, the software will only analyze the lane and band information within that area. This typically improves the accuracy of the automatic lane and band finding when the image background and gel background intensities vary.

- Select **Find Lanes and Bands** from the **Find Lanes and Bands** menu. The Lanes/Bands dialog window appears and a basic search is automatically performed.



- If the results are not satisfactory, keep the window open and adjust the search parameters as described in the following **Adjust Lanes and Bands Search Parameters** 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 state being opened in 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 automated search did not find all lanes, the parameters may be adjusted.

Under Search options in the **Find lanes and bands** dialog window the user may 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 are 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 find lanes and bands search options to its default values.
- Under **Search Parameters**
- Ensure that the **Image Background** is correctly set to white or black.
- To add Lanes and Bands change the **Lane Sensitivity** and/or **Band Sensitivity.**
- **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**.

Lane and Band Information

First Find Lanes and Bands to view information on lanes and bands. LS software allows users to view lane information in the following formats as listed below:

- Lane and Band Properties
- Lane Profile Graph
- Data Explorer

- Printing Data Explorer Tabular Reports
- 1D Analysis Settings

Lane and Band Properties

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 **1D Analysis** Action Tab > select **Object properties**.

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.

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 the **1D Analysis Action Tab** > select **Object properties**. 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.

Information Found in the 1D Analysis Image Window

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.

Add Lanes and Bands

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

Add Lanes Method #1: From the Find Lanes and Bands Dialog Window

- To add lanes, open the **1D Analysis** Action Tab **> Find Lanes and Bands** menu.
- Perform an automated search for lanes and bands.
- While still in the Find Lanes and Bands dialog window (shown below), 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.
- Select OK.
- The updated image will display with the new lanes identified in the image window.

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

Add Bands Method #1: From the Find Lanes and Bands Dialog Window

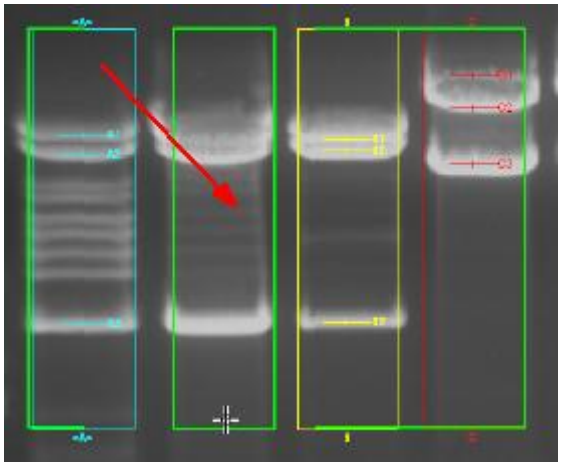
- To add bands, open the **1D Analysis** Action Tab **> Find Lanes and Bands** menu.
- Perform an automated search for lanes and bands.
- While still in the Find Lanes and Bands dialog window (shown above), adjust the Band sensitivity using the slider control. In general, to detect more bands, drag the 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.

- Select OK.
- The updated image will display with the new bands identified in the image window.

Add Lanes Method #2: From the Image Window

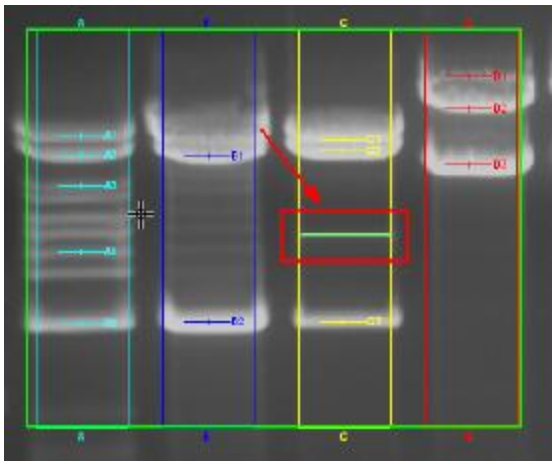
- To add lanes, open the **1D Analysis** Action Tab **> Find Lanes and Bands** menu.
- Perform an automated search for lanes and bands.
- When finished with the automated search the software will direct back to the Find Lanes and Bands menu. Click onto the **Add Lanes** function in the Find Lanes and Bands menu.
- 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 as shown below.



- Click the left mouse button to place the new lane.
- When finished placing the new lanes, click **Edit Objects** to disable the **Add Lanes** tool.

Add Bands Method #2: From the Image Window

- To add bands, open the **1D Analysis Action Tab > Find Lanes and Bands** menu.
- Perform an automated search for lanes and bands.
- When finished with the automated search the software will direct back to the Find Lanes and Bands menu. Click onto **Add Bands** function in the Find Lanes and Bands menu.
- 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 as shown below.



- Click the left mouse button to place the new band.
- When finished placing band(s), click Edit Objects to disable the Add Bands tool.

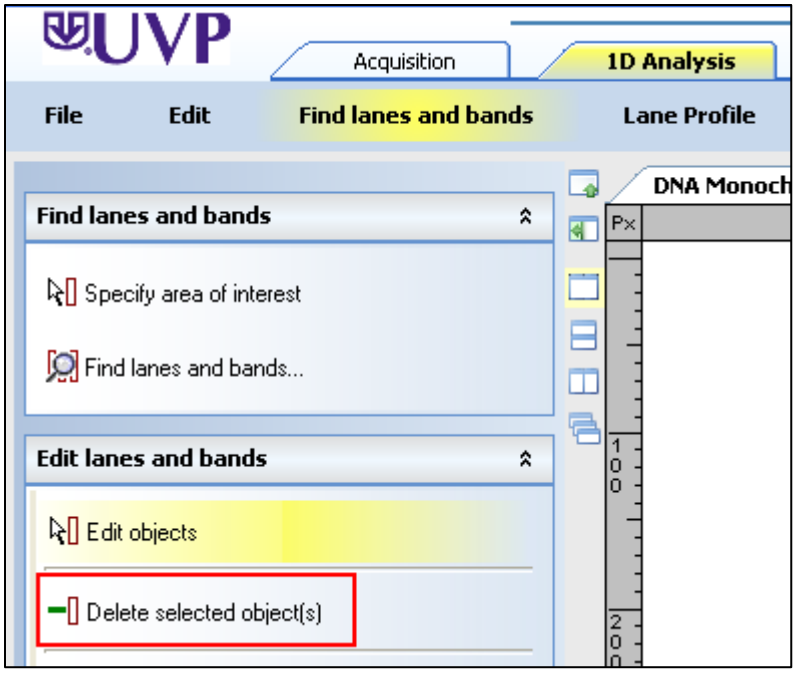
Delete Lanes and Bands

Delete Lanes

- Click **1D Analysis Action Tab > Find Lanes and Bands** menu
- Click on the lane to delete. A box will appear around the band with white boxes at the corners.
- Click **Delete selected objects.**

Delete Bands

- Click **1D Analysis Action Tab > Find Lanes and Bands** menu
- Click on the band to delete. A box will appear around the band with red boxes at the corners.
- Click **Delete selected objects.**



Edit Lanes and Bands

Move Lanes and Bands

LS software allows the user to move lanes and bands using the Image Window function.

- Click **1D Analysis Action Tab > Find Lanes and Bands** menu
- 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.

Move bands using Lane Profile Graph

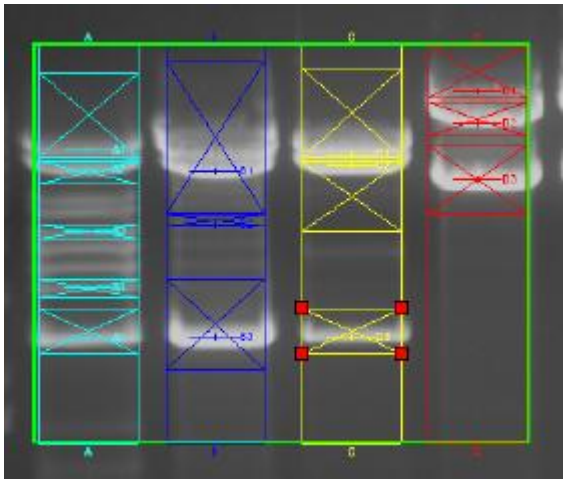
Resize Band Extents

LS software allows the user to resize lanes and bands using the Image Window function.

- To turn on and view the Band Extents, Click **1D Analysis Action Tab > Find Lanes and Bands** menu to open the Settings module.
- Click onto the **Additional Settings** function. A new **Preference-1D Analysis** window appears.
- Select the 1D Analysis tab and click the **Band Extents** check box. Click **Apply** and **OK**. Close the **Preferences** Window.
- Click **Edit Objects** from the **Find Lanes and Bands** menu. 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.

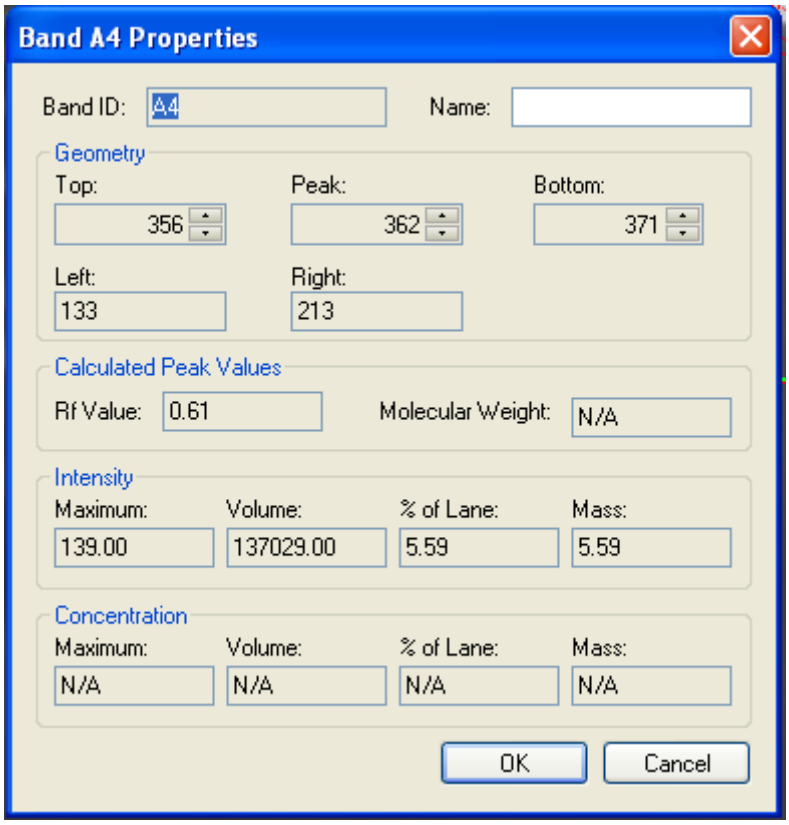
- Place the mouse hand over the top or bottom of the band's extents and click and drag the box border to increase or to decrease the size of the band until the size matches the band seen on the image.

Resize bands using the Lane Profile Graph



Place Bands Exactly

- Click **1D Analysis Action Tab > Find Lanes and Bands** menu.
- Select the band to resize. Control handles will appear at the four corners of the band as red boxes.
- Right click onto the band of interest. **Analysis > 1D Analysis > Bands** and then select **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.



Next Steps:

- Performing Molecular Weight Calibration
- Performing Concentration Calibrations
- Performing Dendrogram Analysis

Lane Profile

Lane Profile Overview

The commands in the Lane Profile help to graphically display lane and band information. Contained in this section includes the following topics:

- Molecular Weight Calibrations
- Concentration Calibrations
- Rf lines

Molecular Weight

Perform Molecular Weight Calibrations

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.

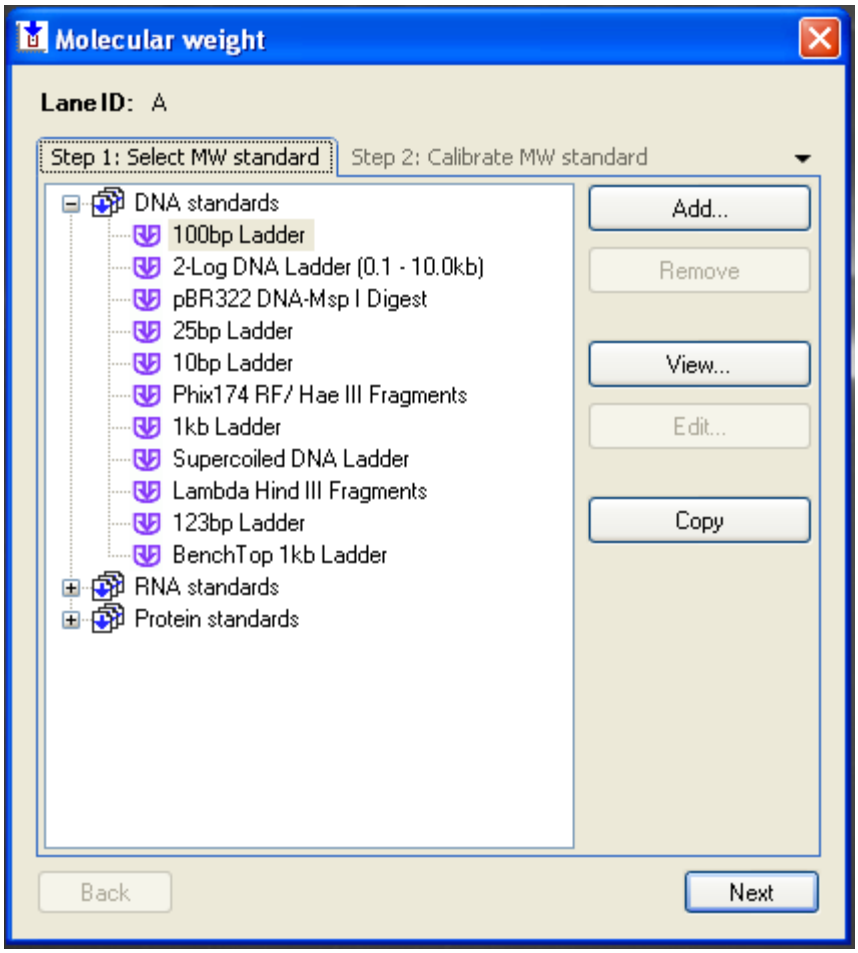
Note: Rf line functions are only required if there are less than two calibrated lanes

Apply a Molecular Weight Standard to a Lane

- Calibrate a Lane
- Stretch Factor
- Manual Placement of Weights
- Exact Placement of Bands

Calibrate a Lane

- First identify all lanes and bands in the image.
- Select the lane to calibrate.
- From the **1D Analysis** Action Tab **> Lane Profile** Menu select **Calibrate Lane**.
- A new **Molecular Weight** window opens. On tab **Step 1: Select MW standard** click on the standard to use and then select **Next.** If the standard is not on the list, click Add to add a standard to the list (see Adding a Molecular Weight Standard to the Library**)**.



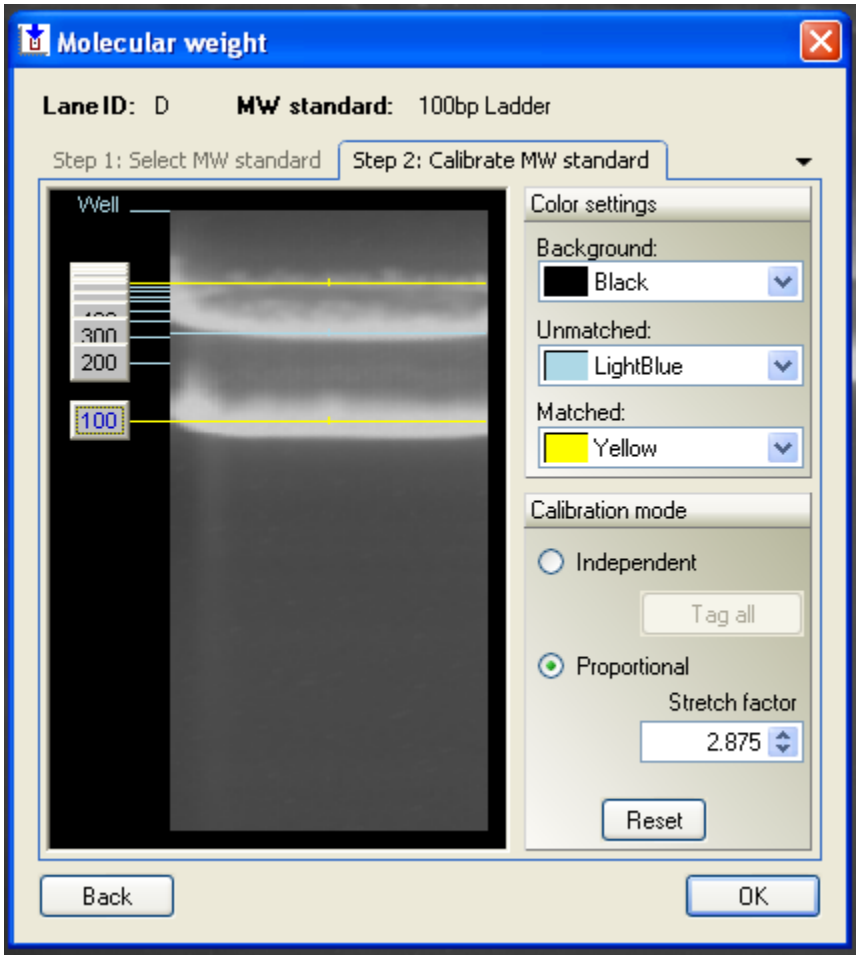
- A new screen appears **Step 2: Calibrate MW standard**.
- In the Step 2 screen, the weights can be adjusted to match the bands that actually appear. *Complete instructions for this appear below-see Stretch Factor.*
- Click **OK** to save the calibration.

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.

Manual Placement of Weights

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

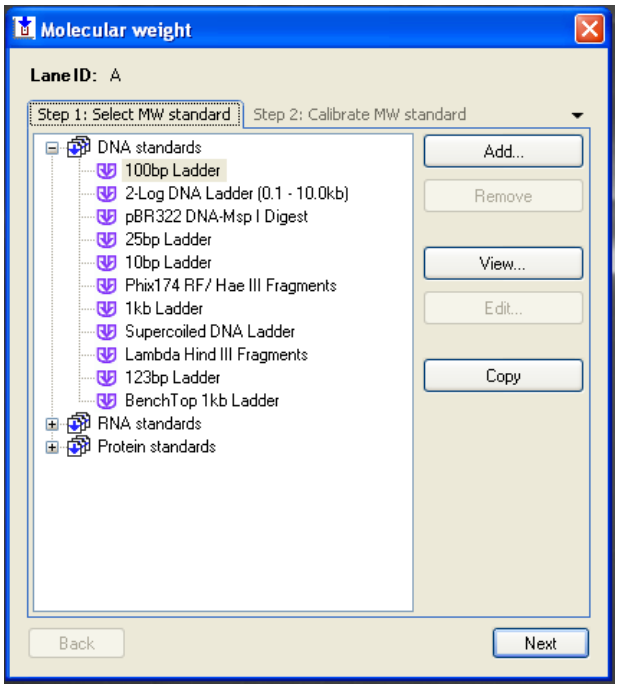
Note: On the second window of the calibration operation (where users adjust weights to bands), the colors of both the unmatched and the matched lines can be changed by using the controls in the upper right corner.

Molecular Weight Calibration

- Add Molecular Weight Standard to a Library
- Edit Molecular Weight Standard to a Library
- Remove Molecular Weight Standard to a Library
- Copy Molecular Weight Standard to a Library

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 listed below. Standards can be added, edited, and deleted from the library using the following instructions.



Add Molecular Weight Standard to the Library

- First identify all lanes and bands in the image.
- Select the lane to calibrate by clicking on it.
- Select **1D Analysis Action Tab > Lane Profile** menu and click **Calibrate lane**.
- In the **Step 1: Select MW standard** window, click **Add**. A new **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.

Edit Molecular Weight Standard in the Library

- First identify all lanes and bands in the image.
- Select the Lane to calibrate by clicking on it.
- Select **1D Analysis Action Tab > Lane Profile** menu and click **Calibrate lane**. A Molecular Weight window opens.
- 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**.

Remove Molecular Weight Standard in the Library

- First identify all lanes and bands in the image.
- Select the Lane to calibrate by clicking on it.
- Select **1D Analysis Action Tab > Molecular Weight** and click **Calibrate lane**.
- In the **Step 1: Select MW standard** tab select the standard to remove. (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.

Copy Molecular Weight Standard in the Library

- First identify all lanes and bands in the image.
- Select the Lane to calibrate by clicking on it.
- Select **1D Analysis Action Tab > Molecular Weight** and click **Calibrate lane**. 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**.

Background Correction

Background Correction Options

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.

Note: Before selecting to correct the background in the image, first find lanes and bands. The **Lane Profile** menu button will open and allow a background to be set for the image.

- Select **1D Analysis > Lane Profile > Background correction options**.

LS Software offers background correction options.

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

***N*o 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 **1D Analysis > Lane Profile > Background correction options** select **Joined valleys** from the drop down 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 **1D Analysis > Lane Profile > Background correction options** and select **Rolling disk** from the drop down 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 topics: Band adjustment. See Finding Lanes and Bands.

View Background Correction in the Lane Profile Graph

Choose the **1D Analysis > Lane Profile > Background correction**.

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.

Concentration

Concentration functionality can be broken up into two main categories, Lane Profile Graph and Concentration Calibration.

Lane Profile Graph

Contained in the Lane Profile Graph are the following graphical options:

- Use the Lane Profile Graph
- Display Lane(s)
- Axis Options
- Display Options
- Move Bands Using the Lane Profile Graph
- Resize Bands Using the Lane Profile Graph

Use the 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.

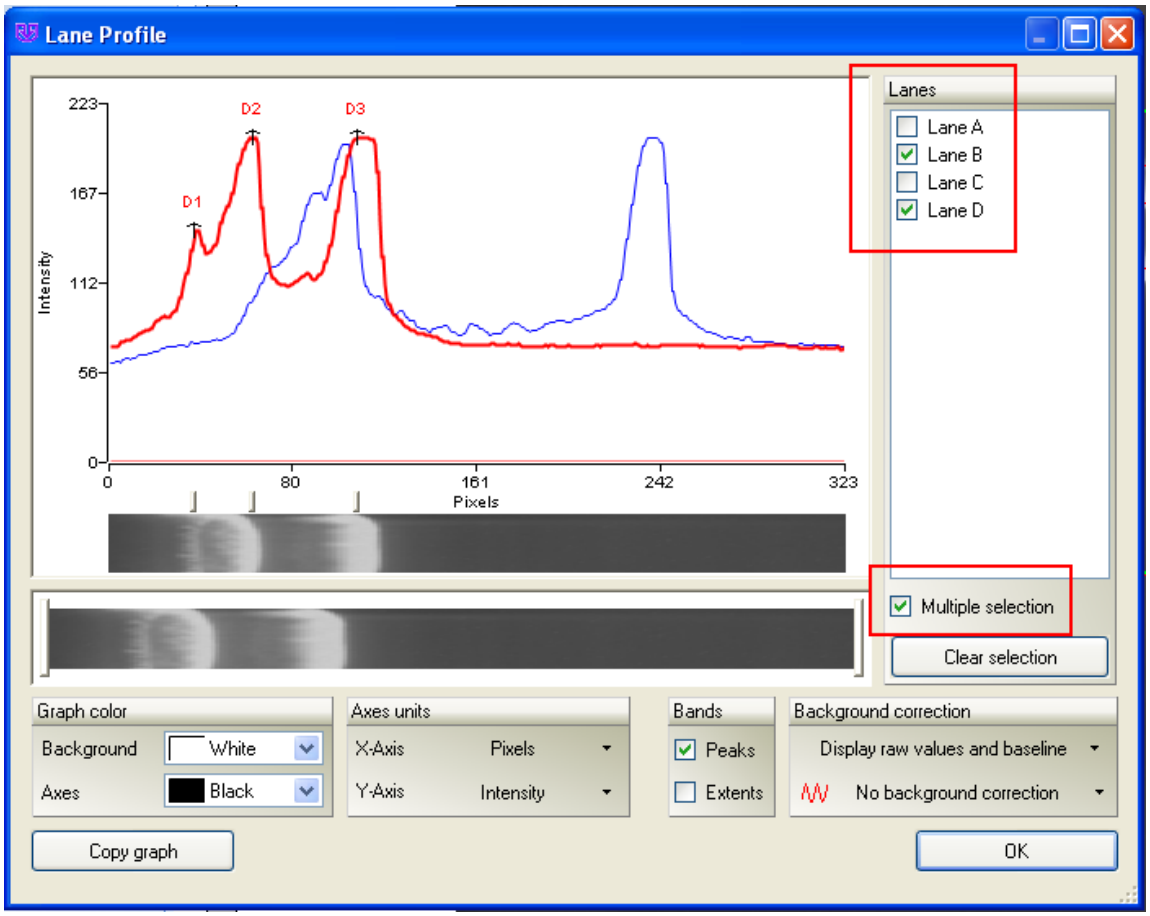
- To access the Lane Profile Graph, select **1D Analysis Action Tab > Lane Profile.**

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 Lane(s)

- Perform an automated search for lanes and bands first before continuing to the next steps.
- From the **1D Analysis > Lane Profile > Lane Profile Graph**. 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 show in the graph. The software automatically displays the graph of that lane.
- To display multiple lanes in the Lane Profile Graph, click the **Multiple selection** check box and also click on each lane to display.
- To de-select or re-select a lane to display, click on the desired lane(s) 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 Units

- To change axis units 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.
- 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.

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 Finding and 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**.
- Select 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.

Move Bands Using the Lane Profile Graph

- Click **1D Analysis Action Tab > Lane Profile > Lane Profile Graph**.
- 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.

Resize Bands Using the Lane Profile Graph

- Click **1D Analysis Action Tab > Lane Profile > Lane Profile Graph.**.
- 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.

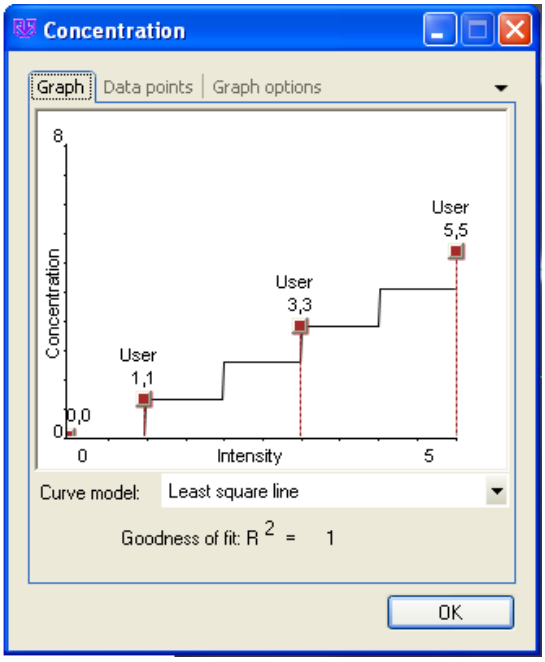
Concentration Calibration

- Graph Options
- Change Unit Type
- Select Unit Type
- Add, Edit or Delete a Unit Type
- Select Data Points
- Select Curve Type
- Remove Concentration Calibrations

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:

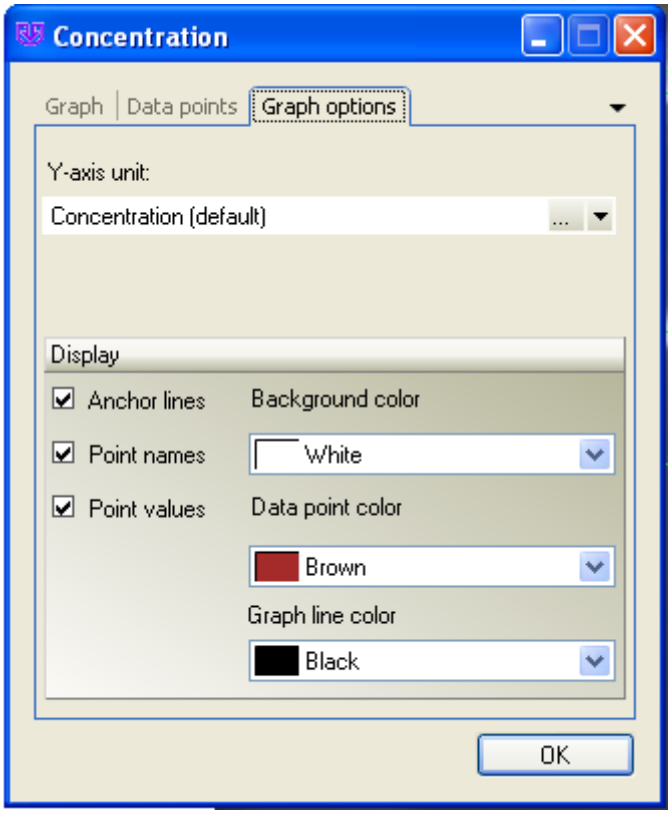
- From **1D Analysis > Lane Profile** click on **Concentration** from the drop down menu. A new window appears with a blank graph. (The image below includes data points that have been added)



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.

Change Unit Type

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



Select 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.

Add, Edit or Delete a Unit Type

- To open the Y-axis unit menu, click the "…" button under the Graph options tab Y-axis unit. Click a button as appropriate to add, delete, edit or set default.

Add 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**.

Edit 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**.

Delete 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**.

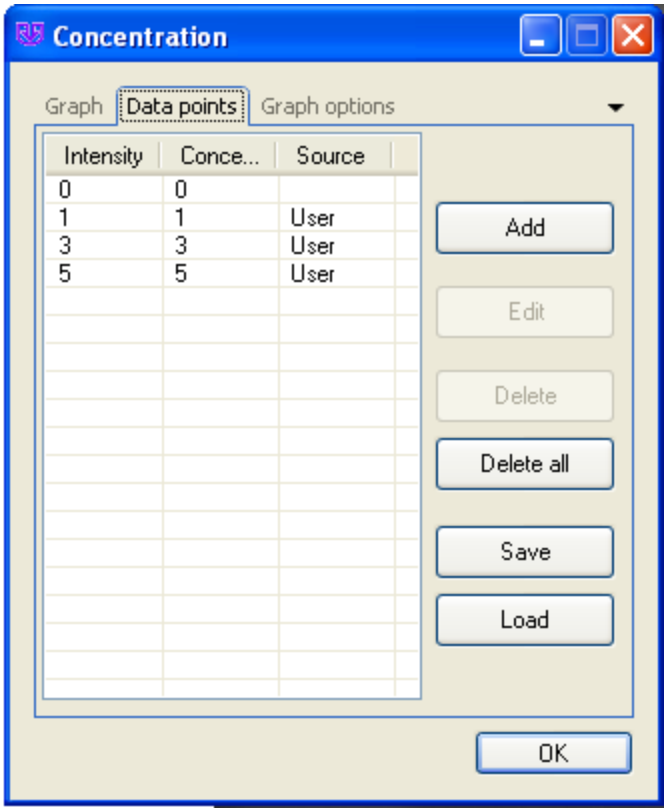
Select Data Points

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.



Edit 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.**

Delete Data Points

To delete data points from the graph:

- 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.

Select 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 'r;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.

Remove Concentration Calibration

To remove all calibration information including data points plotted on the graph and curve lines, go to **1D Analysis Action Tab > Lane Profile** and select **Concentration**. 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.

Retardation factor (Rf) Lines

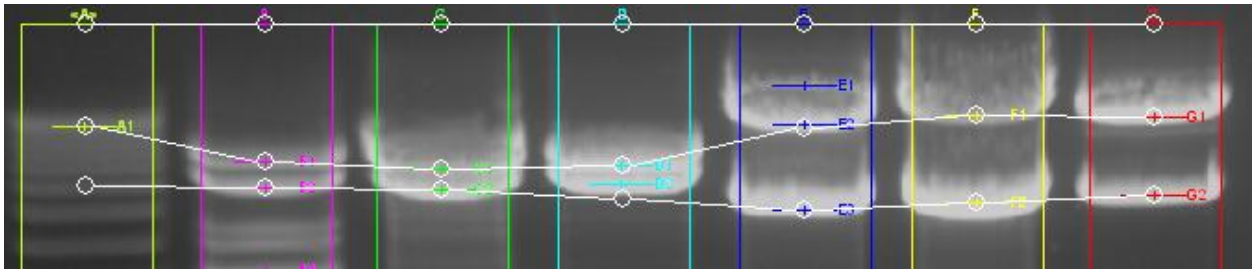
- Automatic Rf Line Determination
- Adjust an Automatic Rf Line
- Add Rf Lines Manually
- Move Rf Lines
- Delete Rf Lines

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.

Image below shows Rf lines in white drawn manually



Adjust an Automatic Rf Line

- If Rf lines are not visible, turn them on through **1D Analysis Action Tab > Settings > Additional 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 **1D Analysis Action Tab > Settings > Additional Settings.** When the **Preferences** window opens, select the **1D Analysis Settings** and unclick **Rf line** in the Visible 1D analysis objects area.

Add 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.)

- On the **1D Analysis** Action Tab **> Lane Profile** menu and click **Add Rf Line**. 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.

Move 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 **1D Analysis** Action Tab **> Lane Profile** menu and click **Edit objects**.
- 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**.

Delete Rf Lines

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

Delete One Rf Line

- Click **1D Analysis** Action Tab **> Lane Profile** menu.
- Select the Rf line to delete in the image.
- Click Delete **selected Rf line(s)**.

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

Remove All Rf Lines

- Click **1D Analysis** Action Tab **> Lane Profile** menu.
- Click **Delete all Rf line(s)**.

Dendrogram Analysis

- Overview
- Generate Dendrogram Graphs
- Modify Dendrogram Graphs
- Dendrogram Standards
- Create Clusters

Overview

Dendrograms are graphical/numerical displays used to show the similarity of 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.

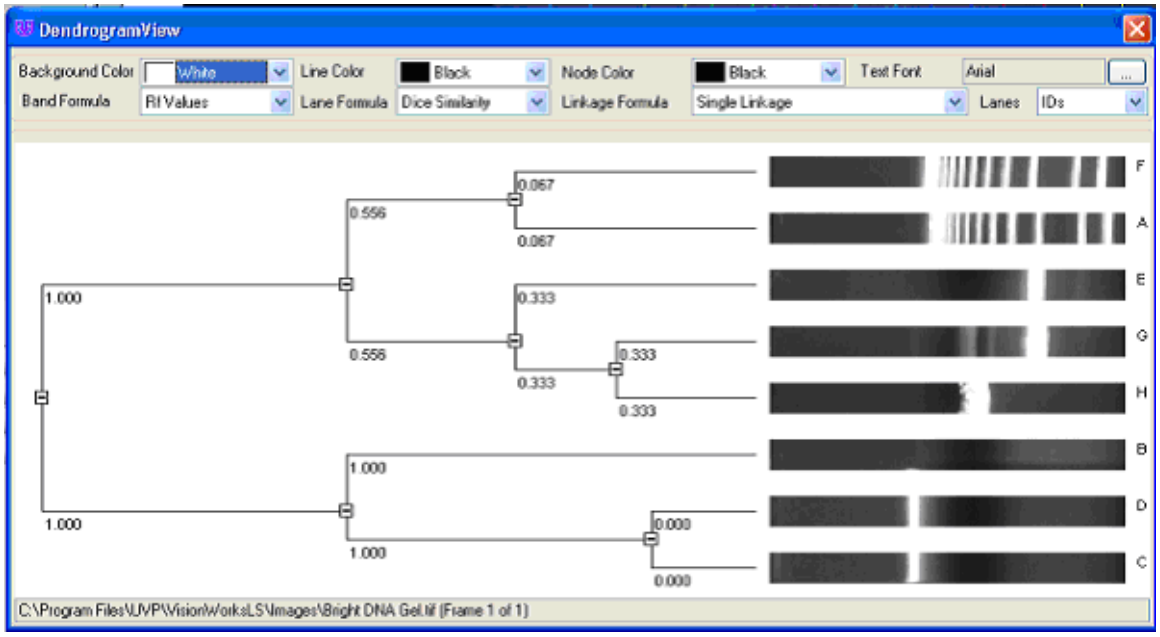
This section will explain how to:

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

Generate 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. (See: Finding and Modifying Lanes and Bands)
- Go to **1D Analysis > Dendrogram > Single-Image dendrogram**.
- 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.

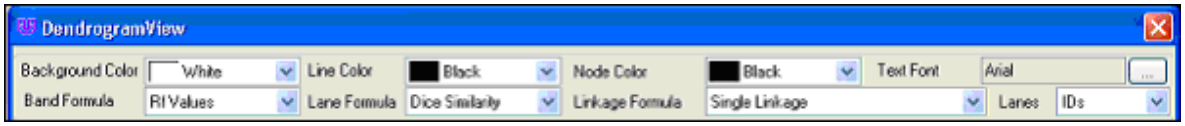


Generate Multi-Image Dendrogram Graphs

- Finding lanes and bands must be performed first on all images to be used in the graph. Related topic: Band adjustment . See: Finding and Modifying Lanes and Bands
- Go to **1D Analysis > Dendrogram > Multi-Image dendrogram**..
- A **Dendrogram Multi-Image** will window will appear. Click **Add** to select the images on which to perform the multi-image dendrogram calculation.
- Click **OK**.
- 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**.

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. Use the drop down menu in each category to select the desired graphical display option.



Dendrogram Standards

Matching bands

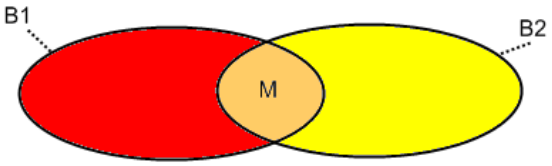
Two bands B1 and B2 match when the absolute difference between their values x1 and x2 of a measure is below the tolerance level:

$$\left|\frac{x_1 - x_2}{\max(x_1, x_2)}\right| < T$$

Similarity and distance between lanes

Based on band matching, the similarity between two lanes L1 and L2 can be evaluated. Notations:

- B1 is the number of bands in lane L1
- B2 is the number of bands in lane L2
- M is the number of matching bands in each lane, therefore $M \leq Min(B_1, B_2)$



The similarity between two lanes can be measured using Dice or Jaccard scores. Dice similarity formula is:

$$DiceSimilarity(L_1, L_2) = \frac{2M}{B_1 + B_2}$$

Jaccard similarity formula is:

$$JaccardSimilarity(L_1, L_2) = \frac{M}{B_1 + B_2 - M}$$

The opposite to the concept of similarity is the concept of distance:

$$Distance = 1 - Similarity$$

Distance values will be used to create the dendrogram.

Create 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.

$$d(C_{R,S}, C_K) = \min(d(C_R, C_K), d(C_S, C_K))$$

  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.

$$d(C_{R,S}, C_K) = \max(d(C_R, C_K), d(C_S, C_K))$$

  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.

$$d(C_{R,S}, C_K) = \frac{d(C_R, C_K) + d(C_S, C_K)}{2}$$

  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.

$$d(C_{R,S}, C_K) = \frac{n_R \cdot d(C_R, C_K) + n_S \cdot d(C_S, C_K)}{n_R + n_S}$$

  , where n_R and n_S are the respective sizes of C_R and C_S

- 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).

$$d(C_{R,S}, C_K) = \frac{1}{2} \cdot d(C_R, C_K) + \frac{1}{2} \cdot d(C_S, C_K) - \frac{1}{4} \cdot d(C_R, C_S)$$

  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.

$$d\left(C_{R,S},C_K\right)=\frac{n_R}{n_R+n_S}\cdot d\left(C_R,C_K\right)+\frac{n_S}{n_R+n_S}\cdot d\left(C_S,C_K\right)-\frac{n_R\cdot n_S}{\left(n_R+n_S\right)^2}\cdot d\left(C_R,C_S\right)$$

- 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

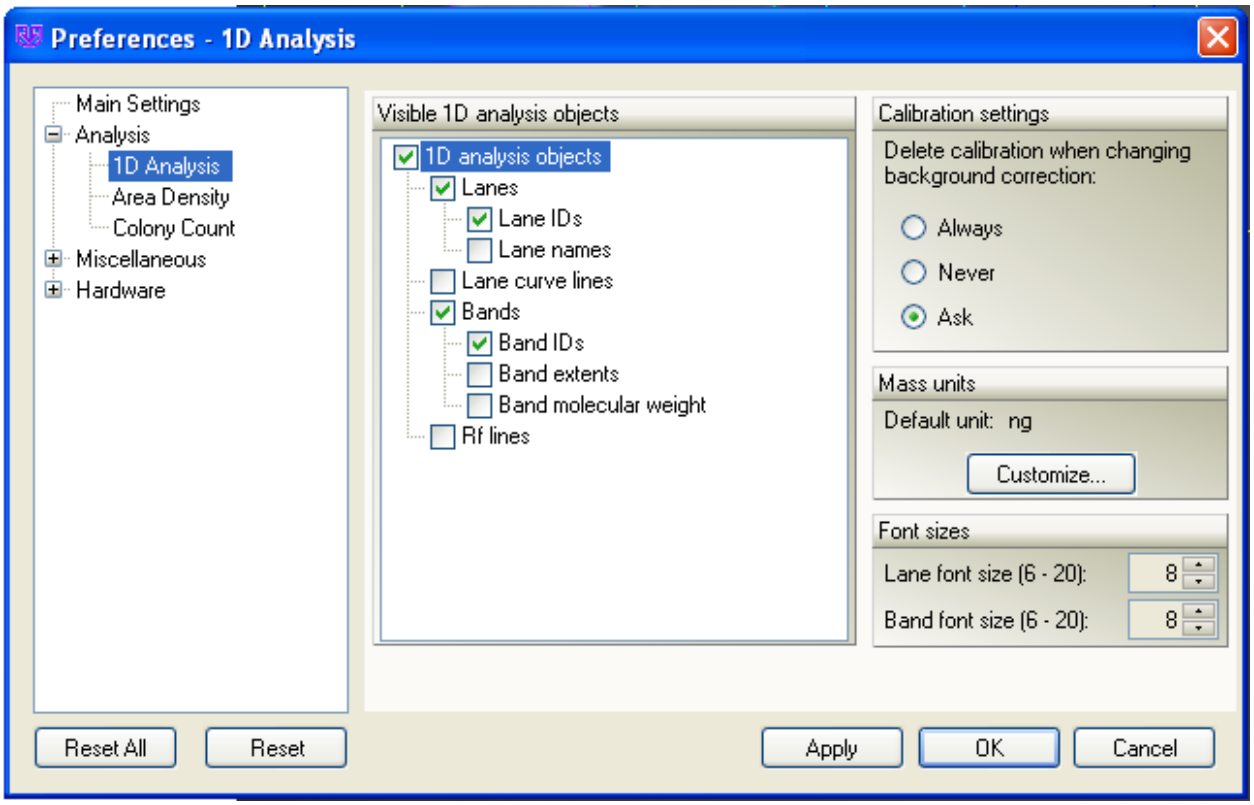
  http://marketing.byu.edu/htmlpages/books/pcmds/WARD.html

- 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

1D Analysis Settings

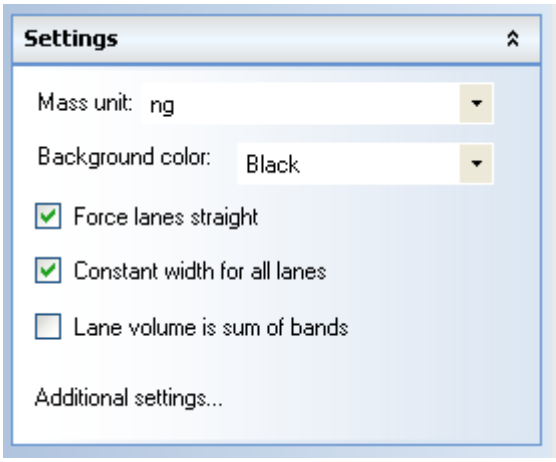
To access analysis settings, go to the **1D Analysis** Action Tab and click on the **Settings** menu button.



Note: Some settings can accessed by clicking on **1D Analysis** Action Tab and clicking on the **Find Lanes and Bands** menu button.

- **Visible 1D analysis objects:** Allows users to view specified values in the Image Window such as Lane ID's, Lane Name, Lane Curve Lines, Band ID's, Band Extents, Band Molecular Weights, and RF Lines.
- **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.

In the **1D Analysis** Action Tab in the **Find Lanes and Bands** menu under **Settings**, are the following options:



- **Mass unit:** Same as above
- **Background color:** Allows the user to choose a background for the image. For example, if the image has white bands with a black background, the software automatically detects the white bands and black background. If the auto-detection does not occur properly, the user may choose "Black" from the dropdown menu.
- **Force lanes straight**: If lanes are curved, the software automatically corrects for the curves.
- **Constant width for all lanes:** Lanes will automatically be assigned the same width unless otherwise noted. For example, when one lane is resized, all lanes are resized.
- **Lane volume is sum of bands**:Software assumes the amount of bands adds up to the amount of material originally deposited in a lane and enters that information.
- **Additional Settings:** Same functions as found in the **1D Analysis** Action Tab > **Settings** menu.

Results (Viewing and Printing 1D Analysis)

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

Results Data Explorer

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 go to **1D Analysis > Results**.
- 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.

Printing Data Explorer Tabular Reports

Page Setup

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**.

Print Reports

Under the Results menu, 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.

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.

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:

- 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.

Report Types

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

Fixed Image and Analysis 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.

Clear (Lane and Band Information)

To clear the 1D Analysis information simply click onto the **Clear** button from the **1D Analysis Action Tab**.

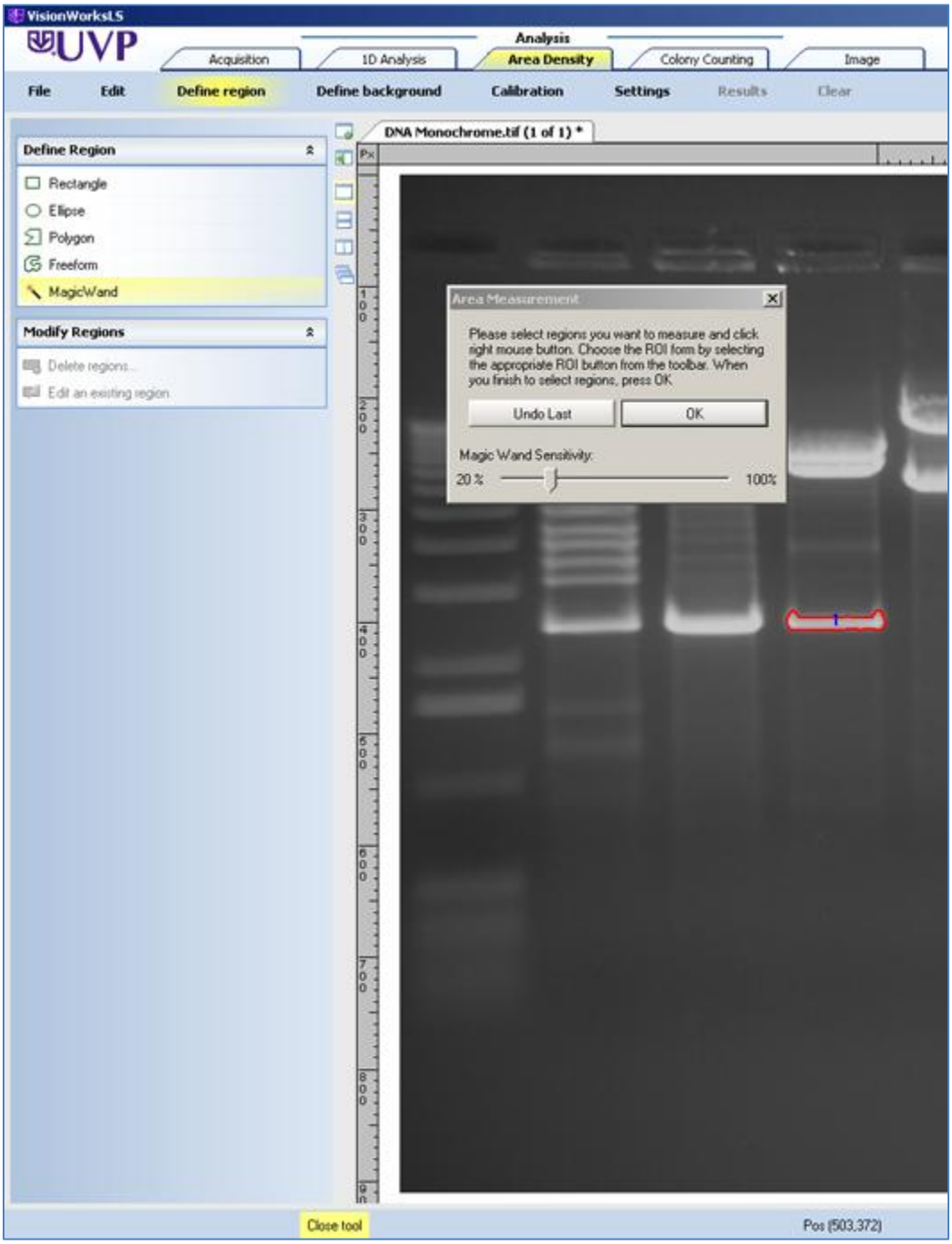
To begin a new analysis of your image, save the existing image with its lane and band information. Then perform a **Save As** for the new image, filing it under a new name, and proceed as follows:

- Click **1D Analysis Action Tab > Find Lanes and Bands** menu.
- 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.

Perform Area Density Analysis

Area Density Action Tab

The Area Density Action Tab provides the means to perform Area Density on a captured or demo image.



The functions in the Area Density Action Tab include:

Define Region

- Define Region
    - Rectangle
    - Ellipse
    - Polygon
    - Freeform
    - MagicWand
- Modify Regions
    - Delete Regions
    - Edit an Existing Region

Define Background

- Define User Background
    - Rectangle
    - Ellipse
    - Polygon
    - Freeform
    - MagicWand
- Modify Background Regions
    - Delete Background Regions
    - Edit an Existing Region

Calibration

- Measure
    - Line Measure
    - Area Measure
    - Angle Measure
- Calibration Tools
    - Intensity Calibration
    - Amount Calibration
    - Spatial Calibration

Settings

- Display Settings
    - Set Display Settings

Results

- Estimate Volume
    - Define Image Scale
    - Calculate Volume
- Results
    - Display Results

Clear

Area Density

Found within the Area Density commands are the following topics:

- Perform Area Density (described immediately below)
- Define Area Density Background
- Estimate Region Volume
- Set Calibration Curves
- Area Density Settings
- Reporting Area Density Information

Perform Area Density

- Overview
- Perform Area Density
- Select Multiple Bands and Regions in the Image

Overview

The Area Density tool can be used to carry out precise quantitative calculations on the regions of interest in the image. It provides the flexibility to carry out calculations based on Optical Density as well as Grey Levels. Additionally, users can calibrate the amount of sample loaded in each spot. Calibration curves, spatial calibration and area density display options can also be selected from the Area Density menu.

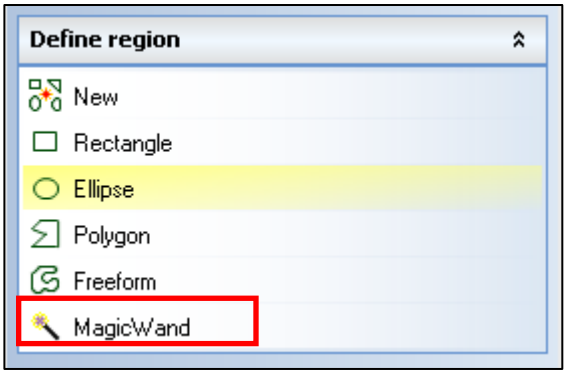
To access the Area Density functions, select the **Area Density Action Tab**. The module will display with the following functions.

- **Define Region**: Select the region of interest (ROI) on the active images with the ROI tools.
- **Define Background**: Defines background intensity on the image for calculations.
- **Calibration**: Converts pixel information into realized measurements information
- **Settings**: Select from display options including which colors to use while marking and displaying areas on the image
- **Results**: Provides results of Area Density calculations

Perform Area Density

Use Magic Wand Tool to Define ROI

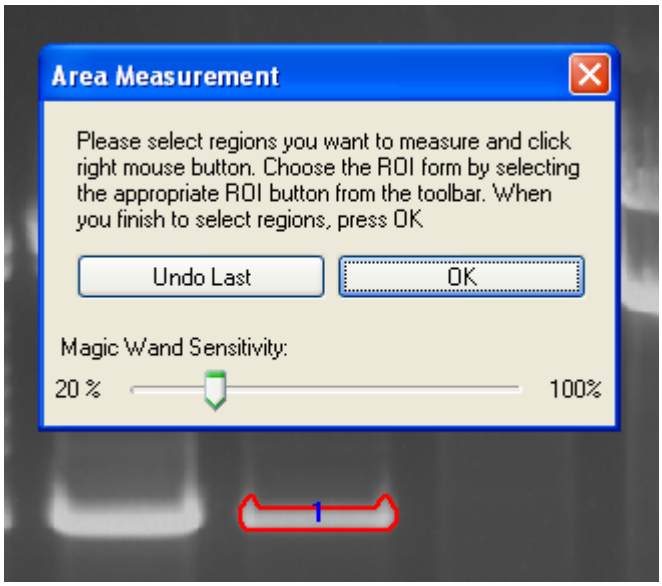
- Click onto **Define Region** to start the ROI identification process.
- Click onto the **Magic Wand ROI** tool.



- Click onto the shape of interest in the captured image to identify the region. A green shape will appear.
- Adjust the **Magic Wand Sensitivity Slider** to outline the entire ROI.
- Right click inside the shape outlined in green. The green shape will turn red and a number will appear inside the red shape.

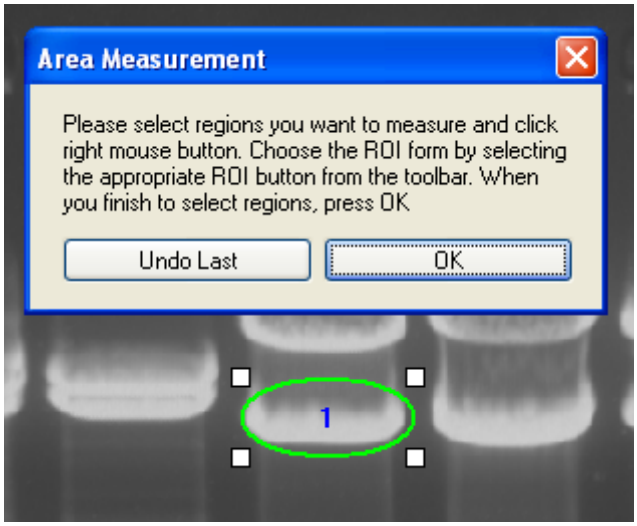
Note: Ensure that the number is set inside the box BEFORE clicking the OK button.

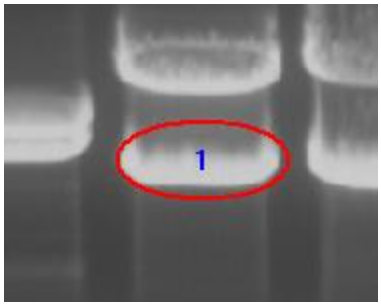
- Click **OK** to complete the identification process.



Define the Region using a secondary ROI tool (rectangle, ellipse)

- Go to **Area Density Action Tab** and click on an option to define the region (ROI) of interest. Select from rectangle, ellipse, polygon, etc.





Green circle turns red

- The region of interest (ROI) drawn on the image using the ROI tool selected will appear green. Adjust the size of the ROI with white bounding boxes.

  Note: The user must right-click inside each area selected to set. Once set, a number will display in the ROI and the bounding box will turn red.

  Note: Ensure that the number is set inside the box BEFORE clicking the OK button.

- Click **OK** in the **Area Measurement** window when done. This will show all the regions/areas marked.

Select Multiple Bands and Regions in the Image

Users may want to determine the area density of more than one band or ROI in a single image.

- To identify more than one similarly shaped band in an image, click on a previously defined ROI and drag that shape to a second or third band.
- When finished, right click onto the area selected to set.
- Once set, a number will display in the ROI and the bounding box will turn red.

Related Topics:

- Estimate Region Volume

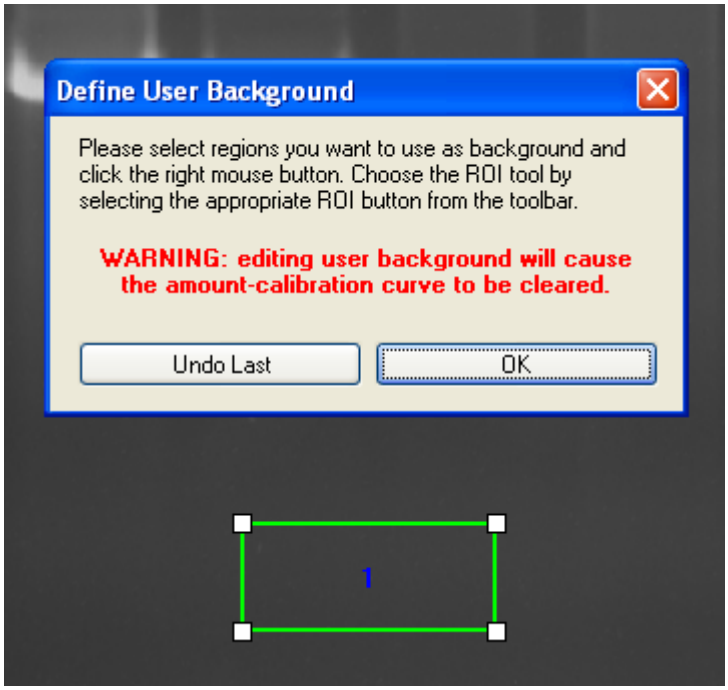
- Calibration Curves
- Reporting and Printing Area Density Results

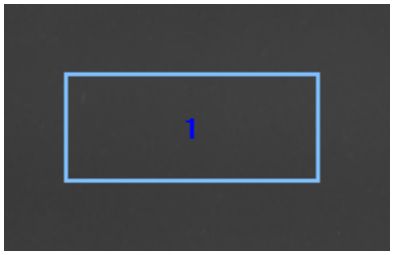
Define Area Density Background

- Define Background
- Delete Background Region
- Modify and Existing Background Region

Define Background

By default, each area defined using the Define Region of Interest tools in the Area Density Action Tab has a separate background. It is equal to the sum total of the perimeter around the region marked, three pixels wide.





Background box turns blue

If a common background for the entire image is needed

- Click on the **Define Background** menu button from the **Area Density** Action Tab.
- Click onto a Region of Interest tool to define the background area.
- Right-click on each area to set. Once set, a number will display in the ROI and the bounding box will turn blue.

Delete Background Region

To delete a background region:

- Click onto **Delete Background Regions** in the **Define Background** menu > **Area Density** Action Tab, then highlight the number of the region to delete.

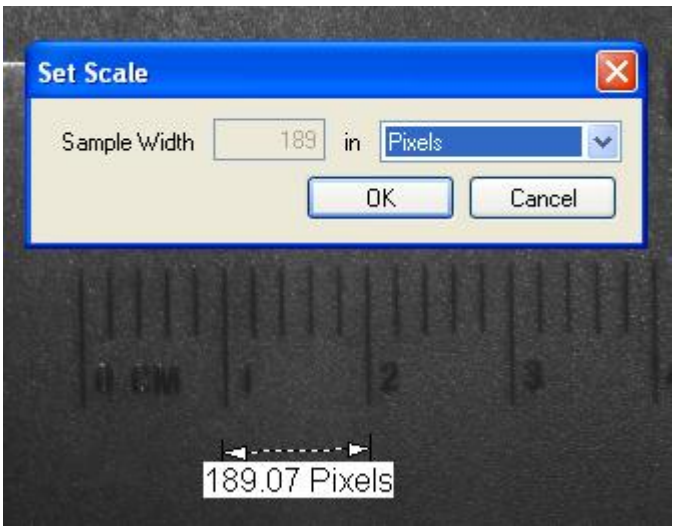
Modify an Existing Background Region

- Click onto **Edit an Existing Region** in the **Define Background** menu > **Area Density** Action Tab, then highlight the number of the region to delete.
- Click onto the region to modify and after changing the size of the region, use the right mouse button to store the changes.

Estimate Region Volume

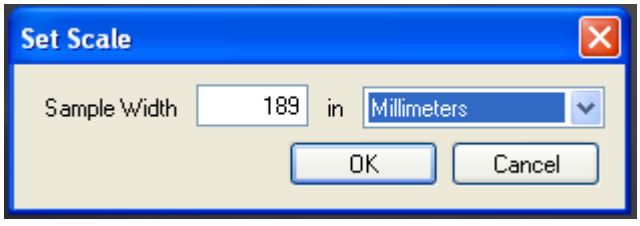
Estimate Region Volume is useful in determining the volume occupied in a cancerous growth in an animal study, for example. The calculations are based on the linear model in J. of Surgical Research, Vol. 113, No. 1, July 2003.studies that connect various factors (such as area and pixel intensity) to volume.

- Go to the **Area Density Action Tab** > **Results**.
- Close the tabular report that opens automatically.
- Open the image to calculate the volume. (For example use a mouse image showing a fluorescent tumor)
- Use an ROI Tool (preferably the Magic Wand tool) to define the region(s) and calculate the Area Density of the area identified by the ROI tool.
- Click inside the region of interest. The outline of the region will change from green to red and will have an identification number listed inside the ROI.
- Use the **Define Image Scale** tool to calibrate the size of the image.
    - 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 gel/blot sample and capture the image of the ruler and the gel sample together so that the ruler can be used for reference)



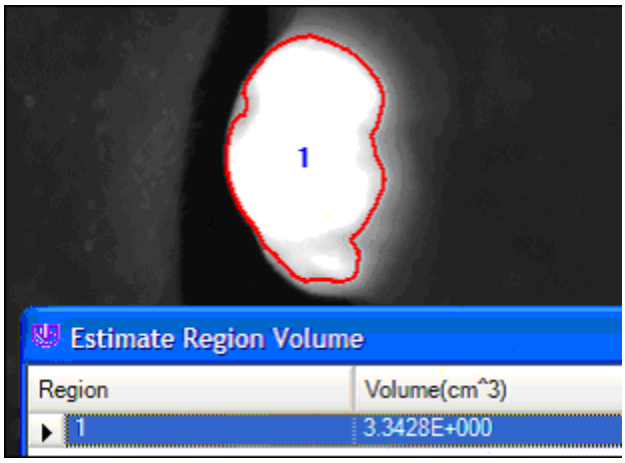
    - Move to the other edge of the feature. As the mouse is moved, the scale tool will show how many pixels are marked with the tool.
    - Click the other edge of the feature. The **Set Scale** window will appear.
    - In the **Set Scale** window, select the metric unit for the marked area (e.g. centimeters) 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.

- Once regions have been defined and calibrated, click the **Estimate volume** in the **Results menu** to see the calculated values.



Calibration Curves

- About Intensity Calibration Curves
- Optical Density
- Grey Levels
- Set Intensity Calibration Curve
- Apply Pre-Defined Calibration Curve
- Add New Intensity Calibration Curve
- Set Amount Calibration Curve
- Change Calibration Curve Graph

About Intensity Calibration Curves

Intensity Calibration is the method of creating a mapping of input intensities to output intensities. The input and output relationship establishes a curve. Ideally, such a mapping would be linear. However, if finely tuned results are required, the mapping process may need to change. E.g. a pixel value of 100 might look slightly brighter, say 120. Or possibly the camera being used has a noise step of 40, which will make all values below 40 look as dark as 0.

Two different metrics can be used in the Area Density tool in LS Software to carry out analysis:

- Optical Density
- Grey Levels

LS software allows users to create curves for both types of calibrations.

Optical Density

Standard Optical Density (OD) is used when the sample of interest is imaged with transmitted light. (i.e. light going thru the sample, into the camera for imaging.) OD value of an area gives an idea of how much light can pass through that area. If the area belongs to a sample in question, OD measures how much of sample might be present in that area. Higher OD means less light can get through, suggesting presence of higher quantity of sample.

Following Beer's Law, the Optical Density of a given pixel P (say at position x,y) is calculated by LS software in the following way:

OD = - Log [(P(x,y) – Black) / (Incident – Black)], if P(x,y) < Black

= - Log [1/ (Incident - Black)], otherwise

Where

White = value of brightest white pixel in the imaging environment

Black = value of darkest black pixel in the imaging environment

Total Optical Density of an area is simply the sum total of OD values of all pixels.

Grey Levels

Grey Level calculations are used when the sample is imaged using reflective light. Grey level of an area is simply the sum of grey levels of all pixels in the area.

Changes are reflected on the curve after the window is closed.

Set Intensity Calibration Curve

Two types of curves options can be defined using the Intensity Calibration Curve button:

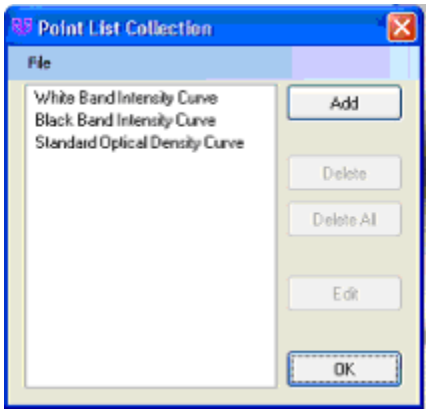
- **Standard Optical Density** - Use this type if the sample is excited through transmitted light (such as the FirstLight uniform UV Illuminator by UVP). Also useful when imaging colorimetric samples.
- **Free Form** - Use this type when fine-tuning input and output intensity values or when imaging fluorescent/luminescent samples to produce results in Grey Levels.

Apply Pre-Defined Intensity Calibration Curve

- Click the Intensity Calibration Curve button from the Area Density Plug-in or use the Analysis > Area Density toolbar menu to select the Intensity Calibration Curve.
- An **Intensity Calibration Curve** window opens. To select a previously defined intensity curve, click onto the dropdown menu next to the **Edit List** in the upper right hand corner of the window. A list of intensity curve options will appear.
- Select the appropriate curve from the list. (For example: choose **Black Band Intensity Curve** if black bands are captured in the image)
- Based on the user selection, **Free Form** or **Std. Optical Density** will be automatically selected.
- Click **OK**.

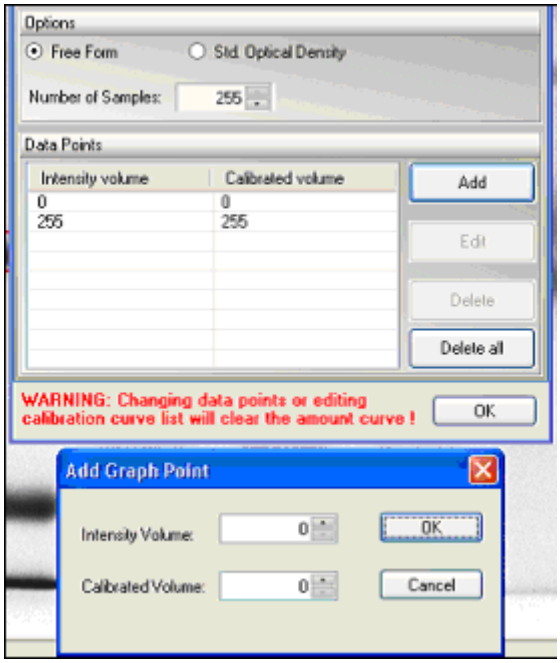
Add New Intensity Calibration Curve

- Click onto Edit List in the Intensity Calibration Curve window.
- A **Point List Collection** window will appear. Click onto **Add** to add a new curve then select **OK.**
- The new user-created curve will appear in the dropdown menu list. This new curve can be deleted and edited in the **Point List Collection** window, if desired.



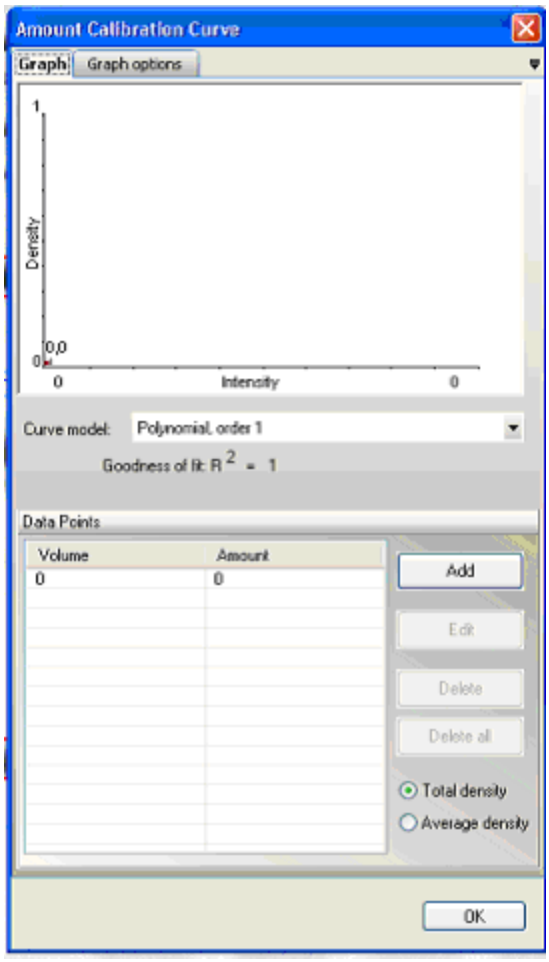
- With the new curve selected, choose **Free Form** or **Std. Optical Density** for this new curve.

- The scroll-box **Number of Samples** lets users select the total numbers of samples to display on the X-axis of the curve. By default, it is set to the highest value of the dynamic range of the image (8 bit=>256, 16bit =>65536).
- If **Free Form** is selected, new **Data Points** can be added to increase the accuracy of the curve.
- Click onto the **Add** button to add points.
- Select **OK** when finished adding data points.

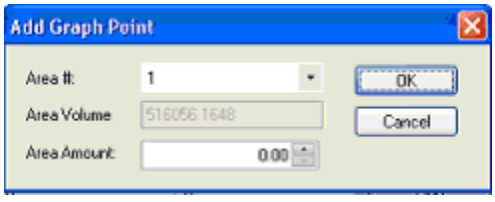


Set Amount Calibration Curve

- Click on Amount Calibration Curve in the Area Density Plug-in or click onto the Analysis > Area Density and select Amount Calibration Curve.
- An Amount Calibration Curve window appears.
- Data points can be added to the amount calibration curve. Select Add to include additional data points.



- The Add Graph Point window appears. Area Amount can be added to each Area # defined in the captured image. Area Volume cannot be changed since the value is automatically calculated from the given area, and depends on the type of Intensity curve calibrated (Freeform or Standard Optical Density).



Note The amount of material loaded for known regions can be assigned. Unknown regions get calibrated from the resultant curve. Click 'Update' after creating the curve, to update these unknown values.

Change Calibration Curve Graph

- While in the Intensity Calibration Curve window or the Amount Calibration Curve window, click onto the second tab, Graph options.
- The Y-axis unit can be changed to other units such as concentration.

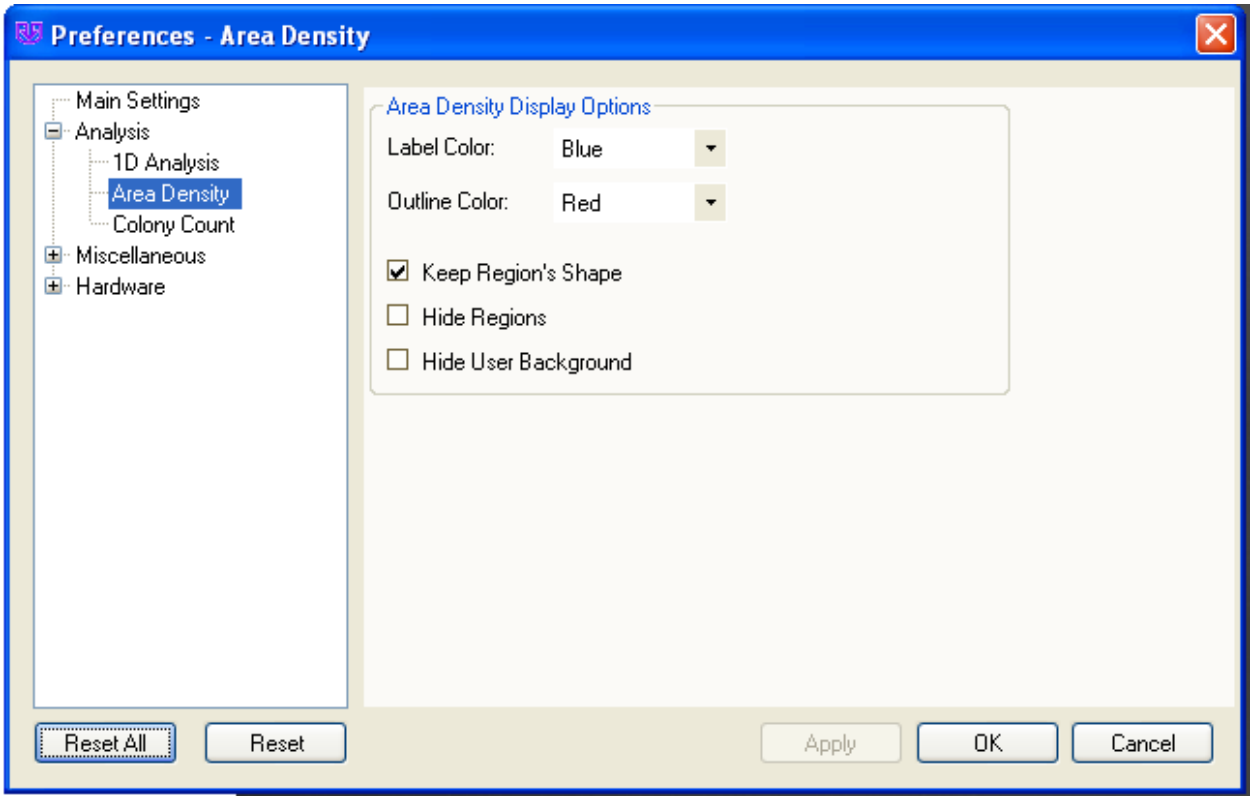
- The Calibration **always positive** checkbox can be checked on both the Intensity Calibration Curve window or the Amount Calibration Curve window. The Monotonic calibration can only be checked in the Intensity Calibration Curve window.
- In the Display users may change that appearance of:
    - Anchor lines
    - Point values
    - Background color
    - Data point color
    - Graph line color

See explanation of Calibration always positive and Monotonic calibration in the Special Cases of Free Form Curves in this section.

Area Density Settings

To change Area Density settings go to the **Area Density** Action Tab > **Settings** menu button and click onto **Set Display Options**.

The **Preferences** window appears which allows users to change the color of the **Label** and **Outline** from the dropdown list.



The **Preferences** window also provides checkboxes that allow users to Keep Region's Shape, Hide Regions, and Hide User Background.

- **Label color:** Select the region's label color from the drop down menu.
- **Outline color:** Select the region's outline color from the drop down menu.
- **Keep region's shape**: When checked, this saves time by preserving the shape (ROI type) of the region across new regions. So if most of your regions are of the same shape and size, it is beneficial to use this option
- **Hide Regions:** Hides the regions selected by the user
- **Hide user background:** Hides the regions identified by the user as background

Reporting and Printing Area Density Results

Area Density Results and Data

Below is a definition of the Area Density Results fields. The Results of Area Density are found in the **Area Density Action Tab > Results** menu button **> Display Results**.

Area Density Results Definitions: Density List (Tab)

Region	User-defined area of interest for Area Density calculations
Total Density	Sum total of intensities of all pixels within the region, minus the background. Intensity is either in terms of Grey-Levels (GL) or Optical Density (OD), depending on the Intensity Calibration curve.
Total Background	Sum total of intensities of all pixels within the region marked as background. Background is calculated in two different ways. Explanation below.
Mean Density	Average of intensities of all pixels of the region, minus average intensity of background pixels.
Mean Background	Average intensity of background pixels.
Total Raw Density	Sum total of intensities of all pixels within the region. No background is subtracted.
Mean Raw Density	Average intensity of intensities of all pixels within the region.
Area (Px)	Total number of pixels in the region.
Minimum Intensity	Minimum intensity value among all pixels.
Maximum Intensity	Maximum intensity value among all pixels.
Calibrated Value	If an Amount Calibration curve exists, this displays the calibrated value based off that curve.

Area Density Results Definitions: Statistics (Tab)

Min	Minimum value of attribute listed in corresponding columns, among all regions. **Region** row below it provides the region for which that value was recorded.
Max	Maximum value of the attribute listed in the corresponding columns, among all regions. **Region** row below it provides the region for which that value was recorded.
Range	Difference between Maximum and Minimum values, among all regions
Mean	Average of the corresponding attribute, among all regions
Std. Dev.	Standard Deviation of corresponding attribute in columns, among all regions
Sum	Total of the particular attribute across all regions
Samples	Total number of regions

Save the Results

- After displaying Results an Area Density Results window appears.
- From the **Area Density Results window > File** menu select from multiple options to save the analysis data:
- **Save Outlines**: Save the file
- **Load Outlines:** Load a previously saved file

Print the Results

- From the **Area Density Results window > File > Print** to print analysis data:

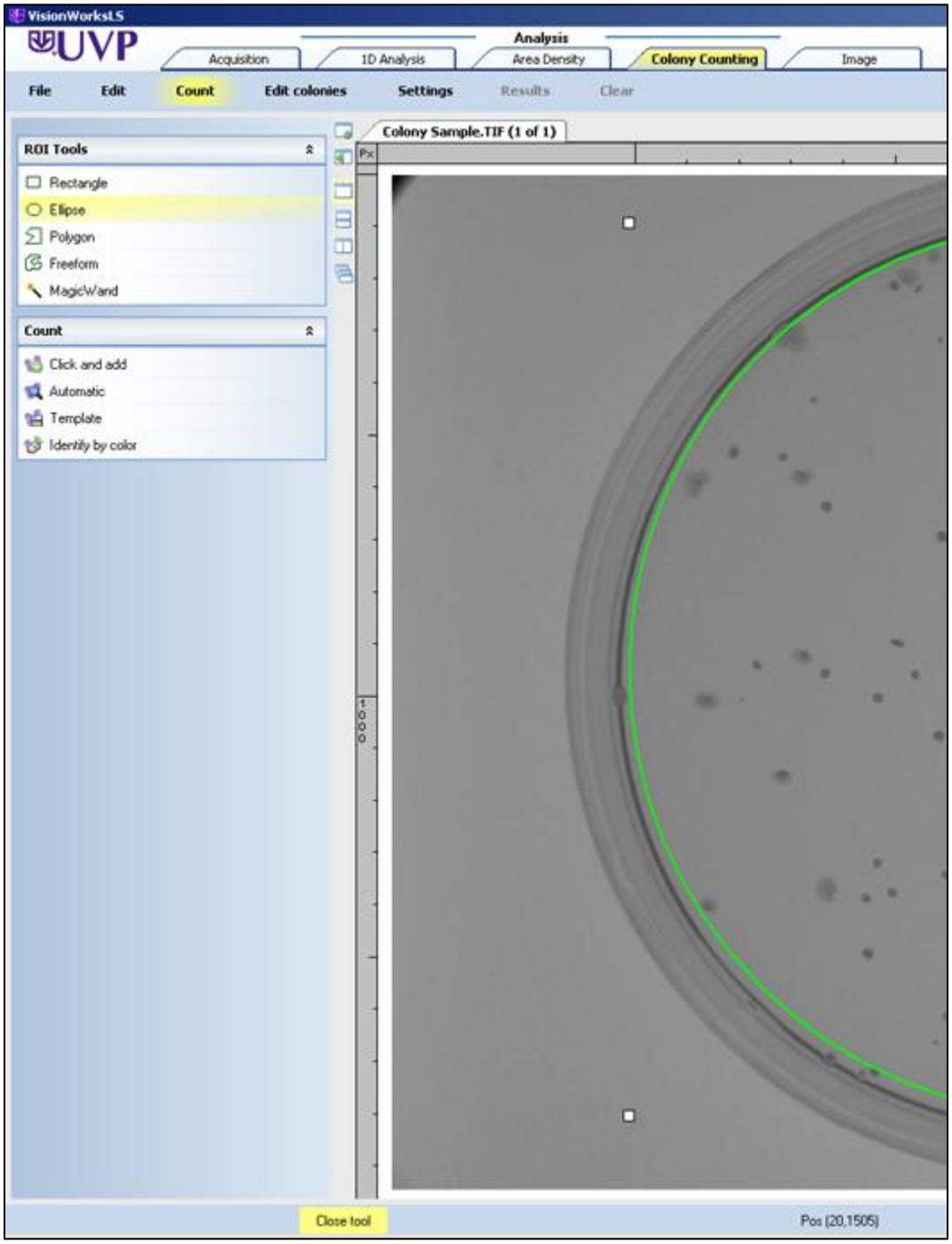
Export the Results

- From the **Area Density Results** window **> Data** menu select from multiple options to export analysis data:
    - Copy to Clipboard
    - Export to CVS
    - Export to Excel

Perform Colony Counting

Colony Count Action Tab

The **Colony Counting** Action Tab contains information that allows users to perform analysis on the captured image.



Functions within the Colony Counting Action tab are:

Count

- Define Region (ROI Tools)
    - Rectangle
    - Ellipse
    - Polygon
    - Freeform
    - MagicWand
- Count
    - Click and Add
    - Automatic
    - Template
    - Identify by Color

Edit Colonies

- Edit Colonies
    - Add Colonies
    - Delete Colonies
    - Manually Split Colonies
    - Automatically Split Colonies
    - Merge Colonies

Settings

- Settings
    - Analysis Display Settings

Results

- Spiral Plate
    - Spiral Plate Analysis
    - Align Spiral Plate Overlay
    - Clear Spiral Plate Analysis
- Estimate Area
    - Define Image Scale

Clear

Count Colonies

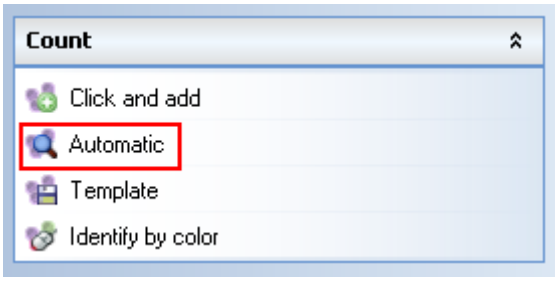
The software offers several functions for counting colonies.

- Automatic Counting (described immediately below)
- Template Counting
- Identify by Color Counting
- Click and Add Counting
- Zone Analysis
- Spiral Counting

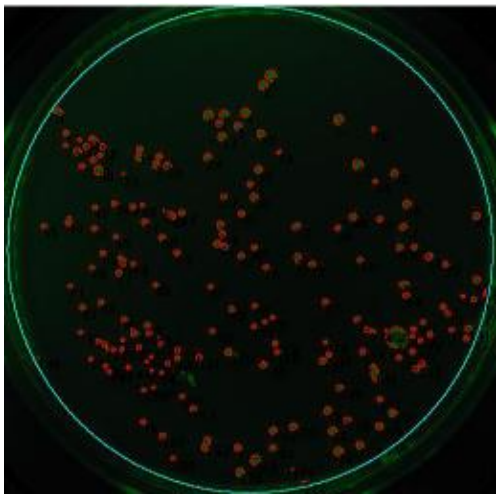
Automatic Counting

Automatic Counting allows users to generate a colony count for a Petri dish or plate using the Automatic function.

- To begin automatic counting click onto the **Colony Counting** Action Tab and then onto the **Count** menu button.
- Define the region of interest to count using the tools provided in the module (Rectangle, Ellipse, Polygon etc.)
- Select **Automatic.**



- The automated count will be displayed in the Results window.
- Colonies can be filled in with red or outlined. Colonies can also be numbered or annotated using multiple colors. Go to the Colony Counting Settings section for more details concerning changing the appearance of the image.

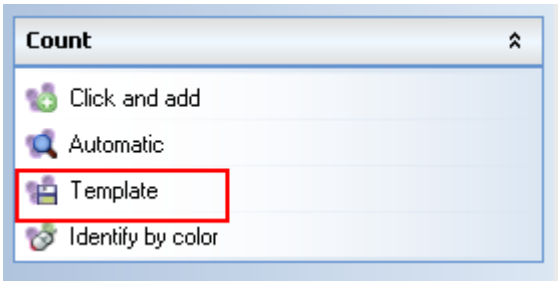


- If desired, colonies can be added, deleted, split, or merged. (See Editing Colonies)

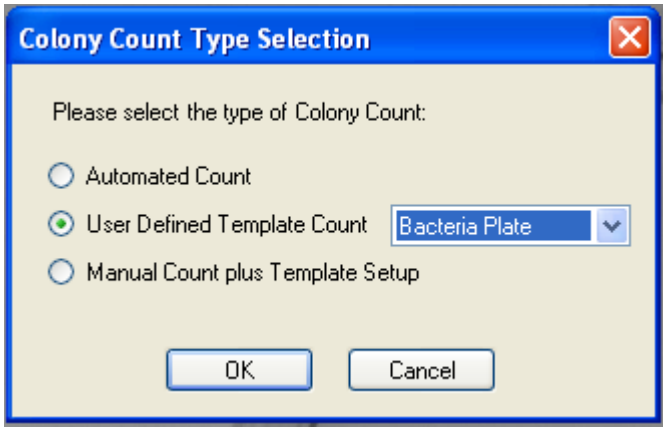
User Defined Template Counting

Before proceeding through the next steps, a template must have been saved using the **Manual Count Wizard**. If there are no templates, create a template.

- Select the **Colony Counting** Action Tab, click on the **Count** menu button.
- Define the region of interest to count using the tools provided in the module (Rectangle, Ellipse, Polygon etc.)
- Click on the **Template** function.



- A new window appears. Select the template to use from the dropdown menu.



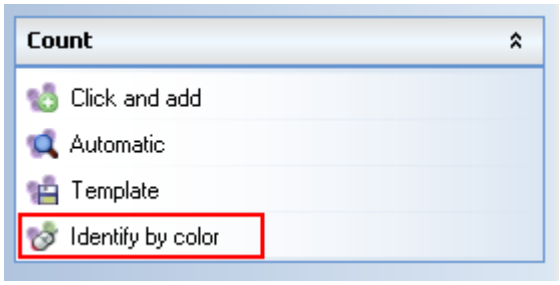
- Click **OK**.
- The count total will be displayed in the **Results** window.

Identify by Color Counting

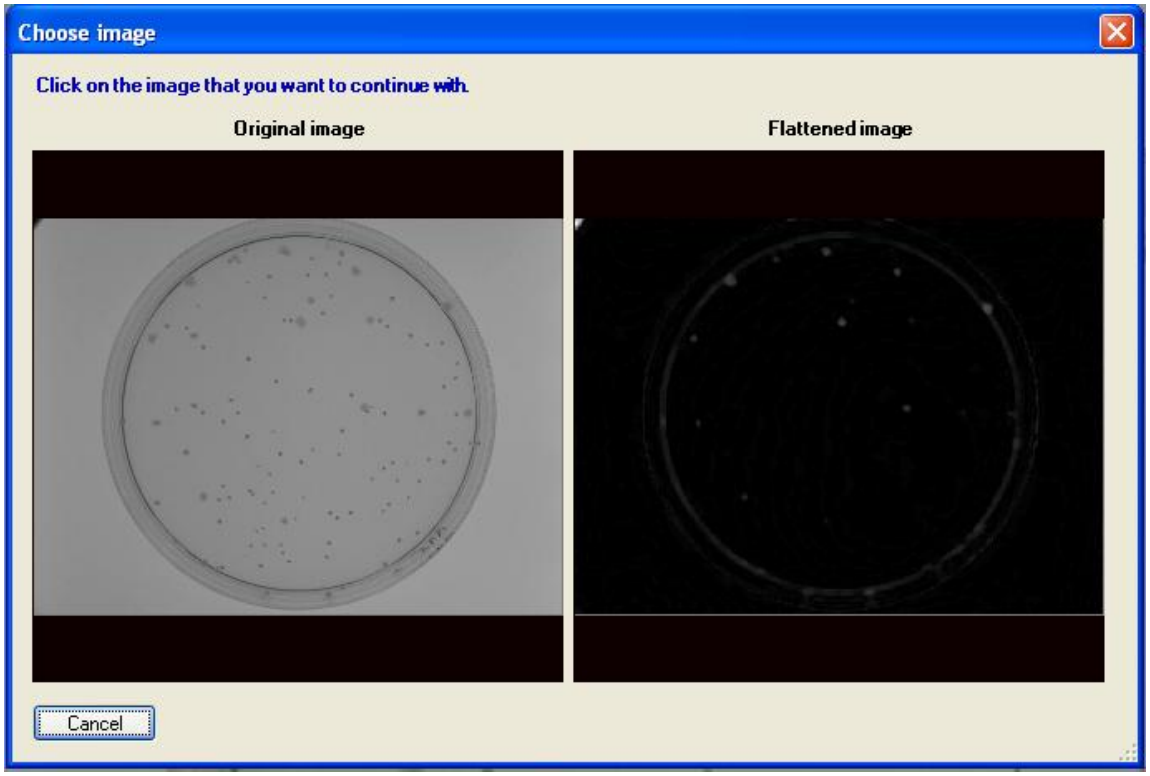
Identify by Color Counting

Frequently colonies are identified by their color. The software allows users to capitalize on this inherent distinction of colonies and generate a total count based on the color properties of individual colonies.

- Open the **Colony Counting** Action Tab and then select **Count.**
- Define the region of interest to count using the tools provided in the module (Rectangle, Ellipse, Polygon etc.)
- Select **Identify by Color**.



- Click on the **Original Image** or **Flattened Image** to continue. (The flattened image will sometimes produce improved counting results)



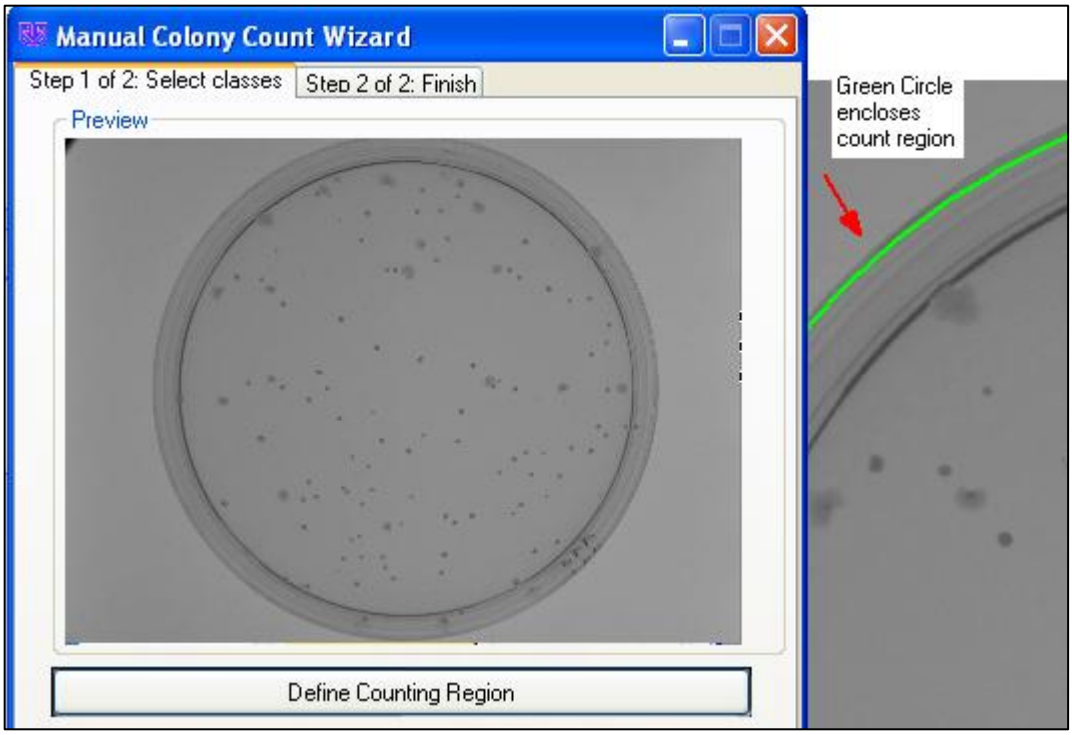
- The **Manual Count Colony Wizard** window will open.
- Proceed to the Identify by Color Counting **Step 1: Select Classes** and **Step 2: Finish.**

Identify by Color Counting Step 1: Select Classes

Please refer to the Identify by Color Count page before proceeding. When counting colonies by color, the window that appears after choosing to use the **Original Image** or the **Flattened Image** is the **Manual Colony Count Wizard**. It contains two tabs. The first tab is **Step 1 of 2: Select Classes** and the second tab is **Step 2 of 2: Finish**.

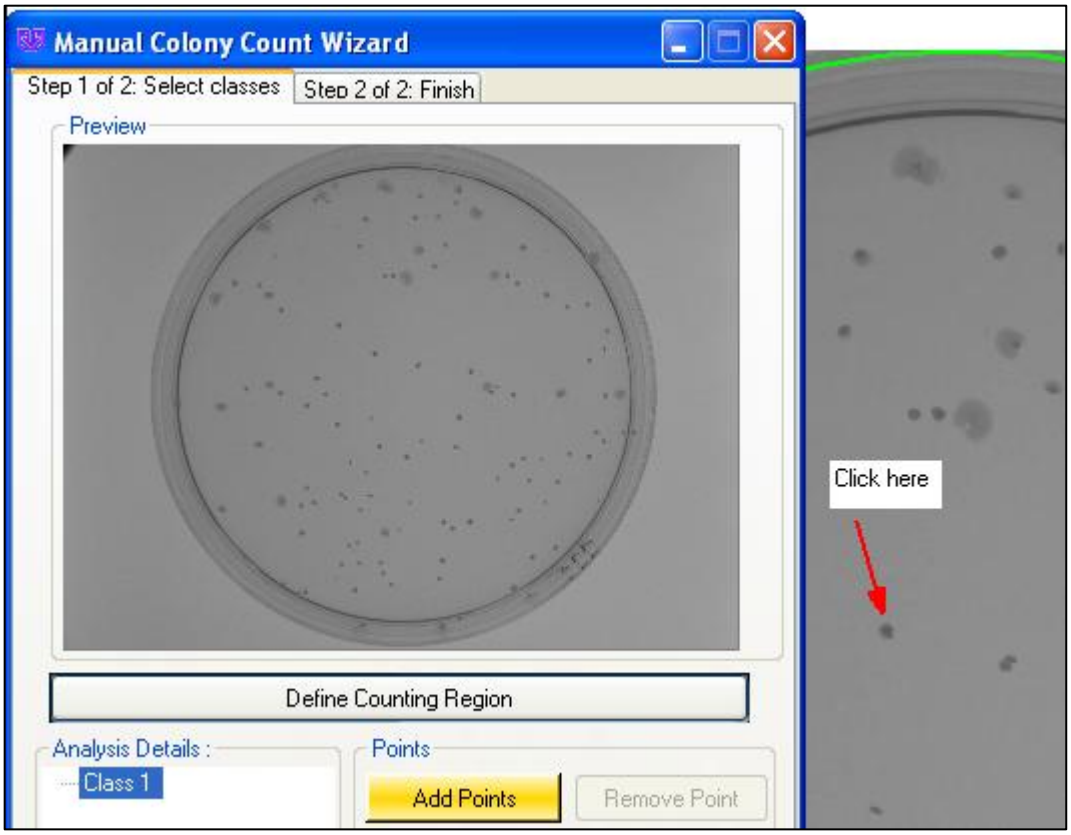
Define the Counting Region

- In Step 1 of 2: Select Classes, users may 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, the user can increase or decrease the circle size.
- If a smaller or larger region of interest is desired, click the **Define Counting Region** button and hold and drag the pointer over one of the corners of the circle to change the size.

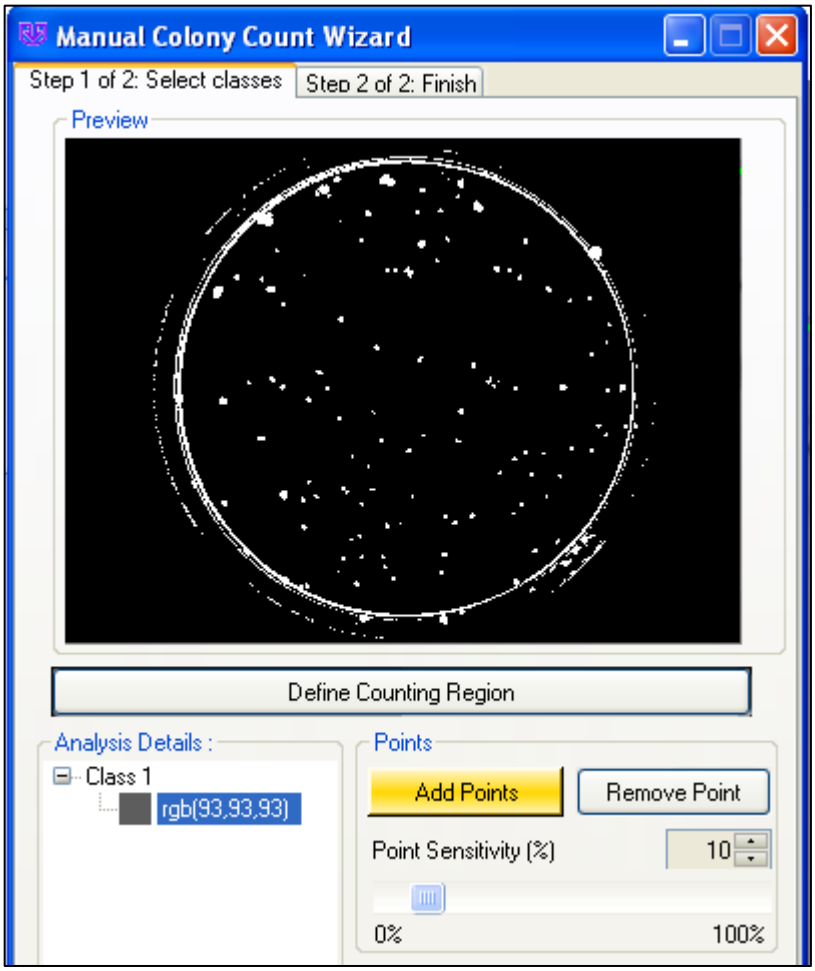


Select Classes

- Next the user should 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 Points** 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 1 of 2: Select classes window.)
- Classes can be 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.



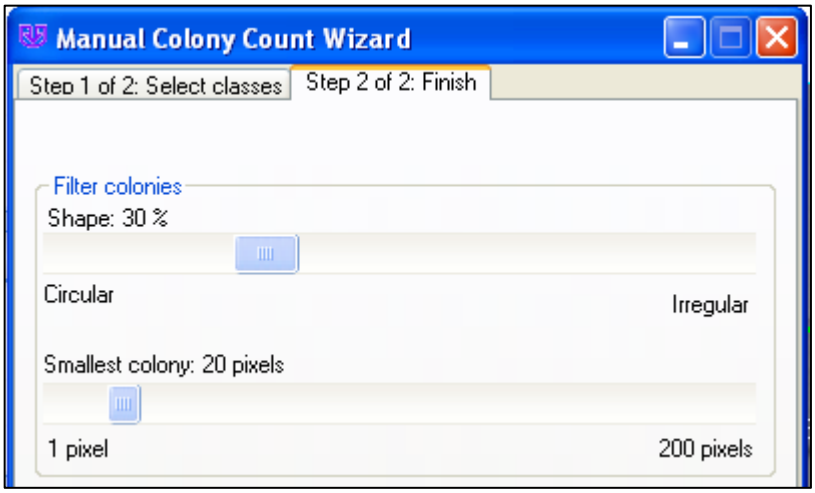
- The color of the point selected will also appear in the **Analysis Details** box (for example: rgb (93, 93, 93).
- Continue adding points and classes as necessary until the black and white image shows all the colonies of interest in white.
- To remove a class or a point, highlight the class or point in the **Analysis Details** box and click the **Remove Point** button.
- Click the **Count** button to go to **Step 2 of 2: Finish.**

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.

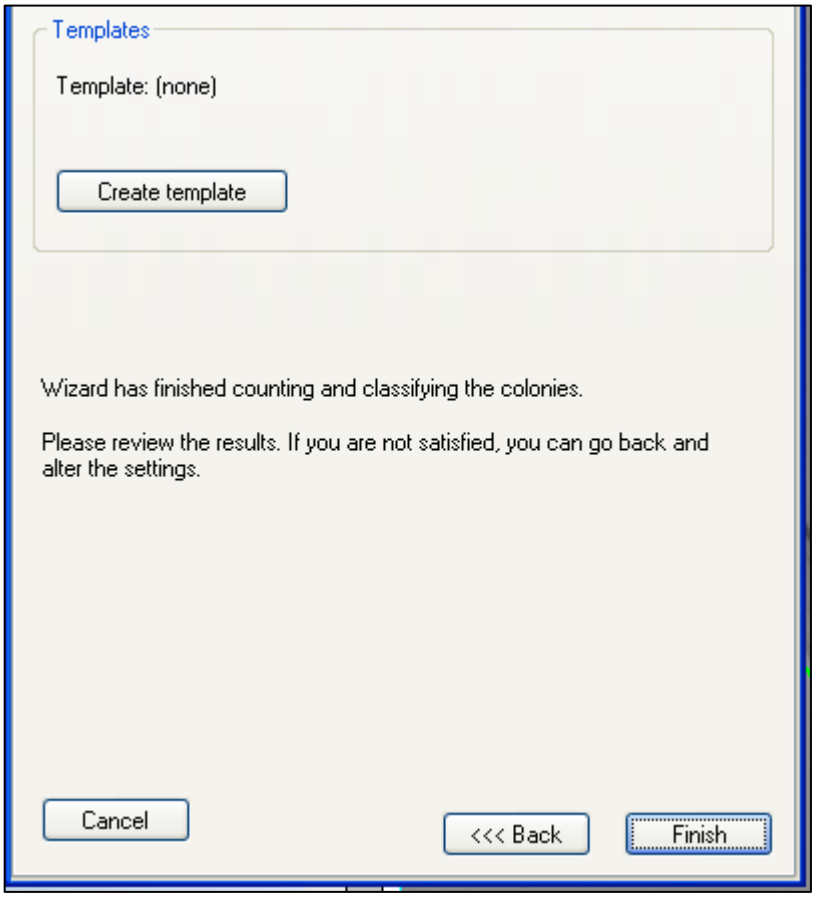
Identify by Color Counting Step 2: Finish

After completing Identify by Color Count Step 1: Select Classes the software brings up the Identify by Color Step 2 of 2: Finish tab. This tab allows users to filter the colonies (or zones), adjust the **shape** or **colony size** (or add and subtract zones).

- 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. To save a template, click onto **Create Template** and assign a name to the template to be used in a future counting session.
- Click **Finish** to exit the Identify by Color count mode.

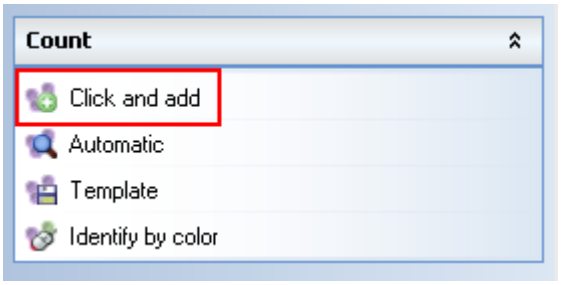


The software will redirect to the initial screen. To view the analysis data for the zones click onto **Results** menu button. A window appears that provides information relating to the classes, zones (listed as colonies), statistics, and distribution of the zones. In the report, users may view the area and perimeter of the zone.

Click and Add Counting

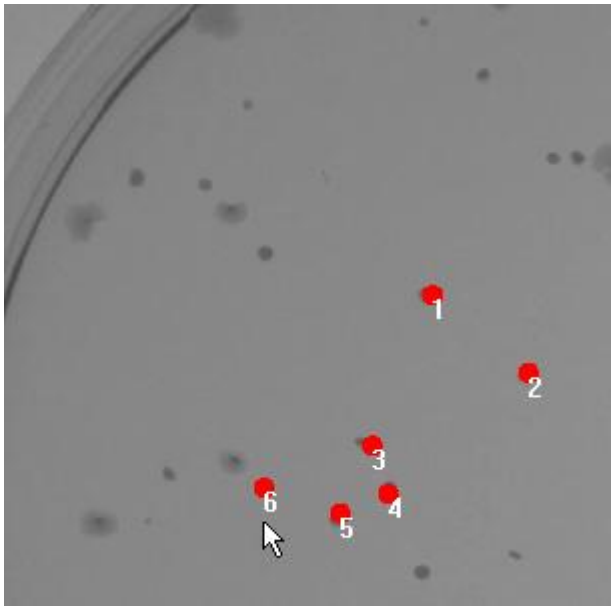
The **Click and Add** counting method allows users to count colonies by clicking onto each colony in the captured image.

- To begin Click and Add counting click onto the **Colony Counting** Action Tab
- Click onto the **Count** menu button.
- Select the **Click and Add** counting method then use cursor to click onto each colony to be counted in the captured image.



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The resulting count total will appear once the **Results** menu button is depressed in the **Colony Counting** Action Tab.



Spiral Counting

- Perform Spiral Count
- Adjust Grid Size and Volume
- View, Save and Print Spiral Plate Results
- User Tips

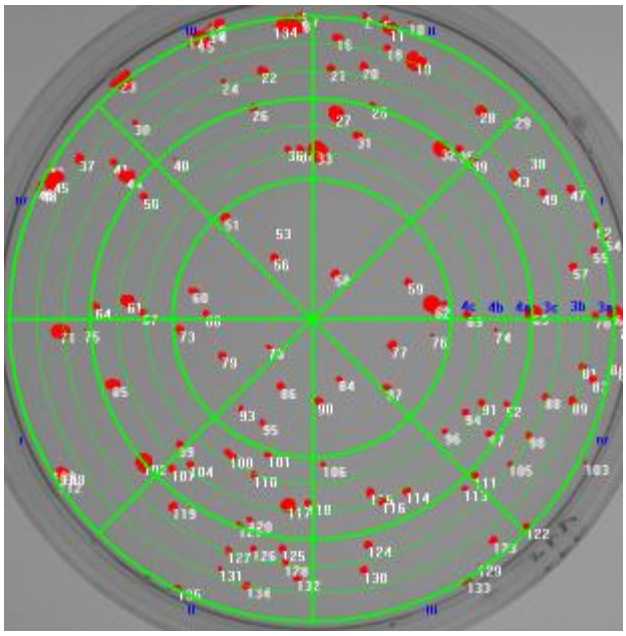
Perform Spiral Count

To perform a spiral plate count:

- First, use the Automated, Identify by Color, or Template counting function to generate an initial count of the spiral plate.

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 Wizard** functions (in Identify by Color count) to store template settings.

- Click on the **Results** menu button then click onto the **Spiral Plate Analysis** function. A green grid will appear over the counted image.



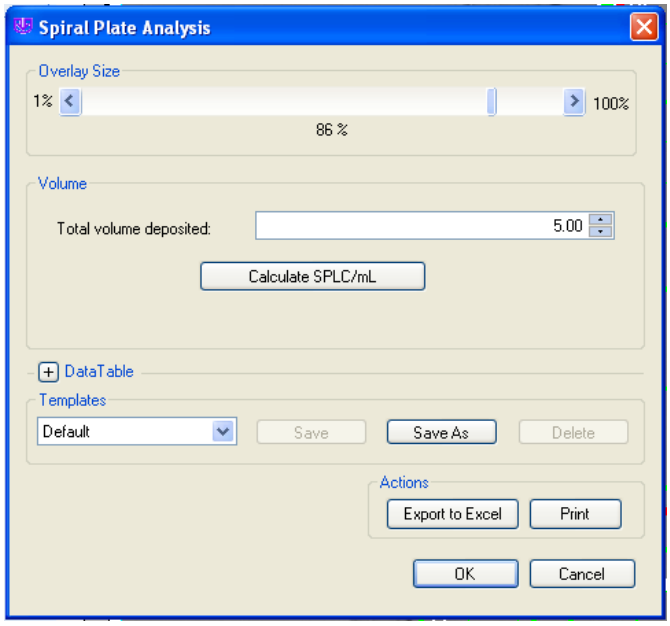
Adjust Grid Size and Volume

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:

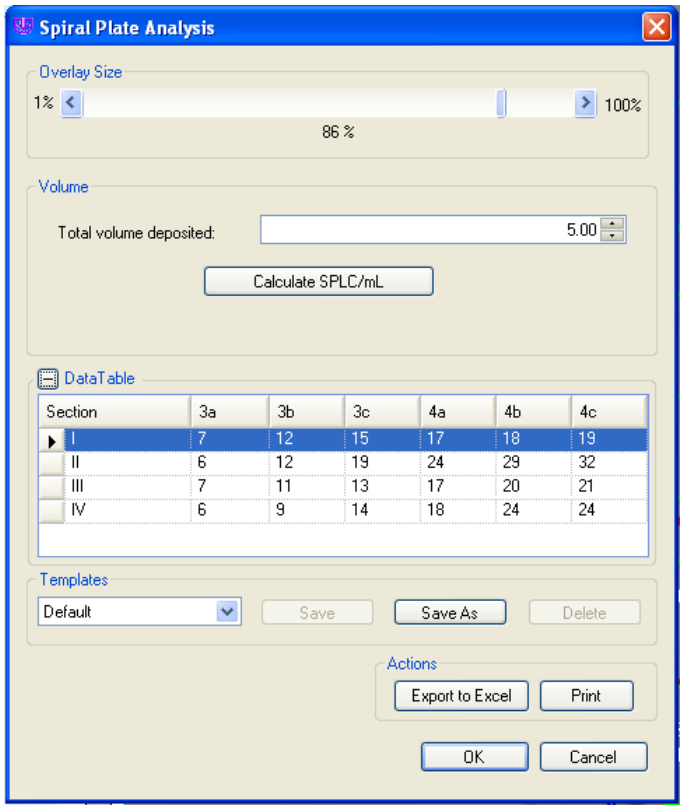
- Type in or use the up or down arrows in the **Total volume deposited** box provided. The calculated amount will appear immediately below the **Calculate SPLC/mL** button.



View, Save, and Print Spiral Plate Results

To view the resulting **Data Table** analysis for the spiral plate count:

- Click onto the + sign (expanded report will be displayed 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** and then 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.

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 **Colony Counting** Action Tab then click onto the **Count** menu button.
- Select the **Identify by Color** Counting method.

NOTE: While performing the Identify by Color counting method, select a point inside the inhibition zone to measure.

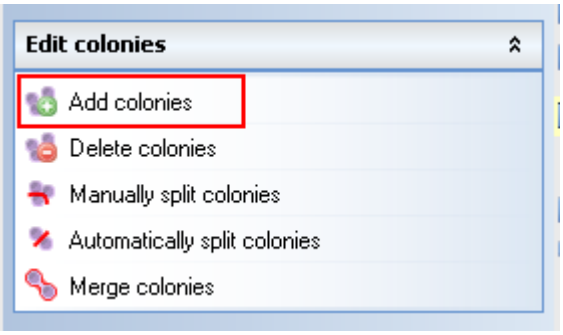
- Go to the **Results** page to get the readout of the zone measurement.

Edit Colonies

Add Colonies

Users may add colonies by performing an initial count (Automated Count, Identify by Color Count, or User Defined Template Count), and adding colonies manually.

- To add colonies manually, go to the **Edit colonies** menu button under the Colony Counting Action Tab and click on **Add Colonies.**

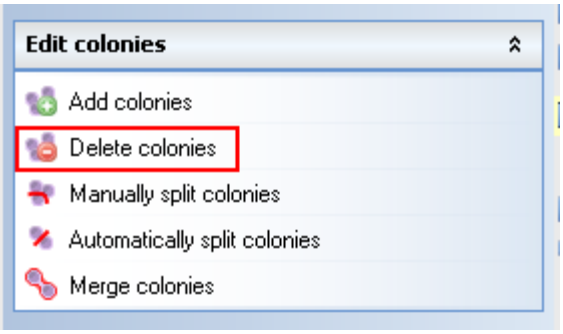


- Click on a colony in the image to add.
- The colony selected will now have a circle (filled in or outlined) around the point selected.
- The total colony count will change to include the colonies added.
- If the colony is hard to see, zoom in on the colony by using the zoom/pan functions at the bottom of the screen. Click on the plus (+) sign to increase the zoom.
- To use a larger circle to highlight the colony, go to the Settings menu button and choose to use a **Circle Radius** of 5, 10, 15, or 20.

Delete Colonies

Users may delete colonies by performing an initial count (Automated Count, Identify by Color Count, or User Defined Template Count), and deleting colonies manually.

- To delete colonies, go to the **Edit colonies** menu button under the Colony Counting Action Tab and click on **Delete Colonies**.
- Click on a colony in the image to delete.



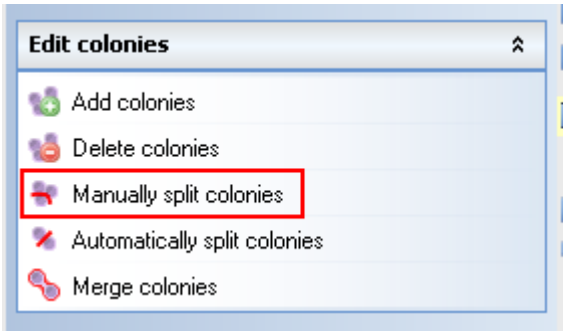
- The colony circle (filled in or outlined) will now disappear. The total colony count will change to remove the colonies indicated.
- If the colony is hard to see, zoom in on the colony by using the zoom/pan functions at the bottom of the screen. Click on the plus (+) sign to increase the zoom.

Split Colonies

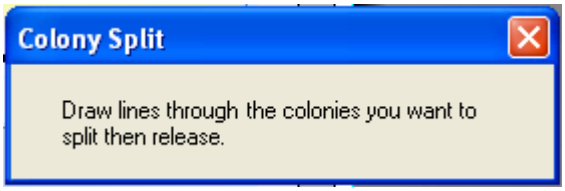
Manual Split Colonies

Users may split colonies by performing an initial count (Automated Count, Identify by Color Count, or User Defined Template Count), and splitting colonies manually. Colonies should be split when two or more colonies are very close together and are counted as one colony by the software.

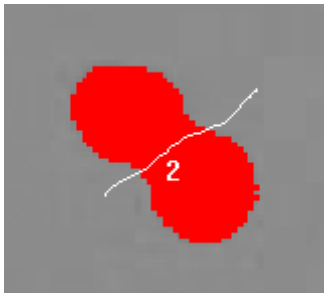
- To split colonies manually, go to the **Edit colonies** menu button under the Colony Counting Action Tab and click on **Manually Split Colonies**.



- 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.

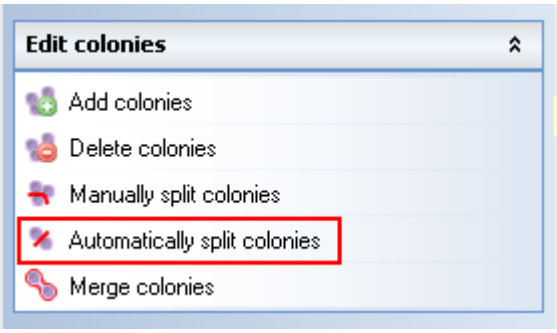


- The total colony count will change to add new colonies identified by the split.
- If the colony is hard to see, zoom in on the colony by using the zoom/pan functions at the bottom of the screen. Click on the plus (+) sign to increase the zoom.

Auto Split Colonies

Users may split colonies by performing an initial count (Automated Count, Identify by Color Count, or User Defined Template Count), and splitting colonies by clicking on the colony to split. 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 automatically, go to the **Edit colonies** menu button under the Colony Counting Action Tab and click on **Automatically Split Colonies.**

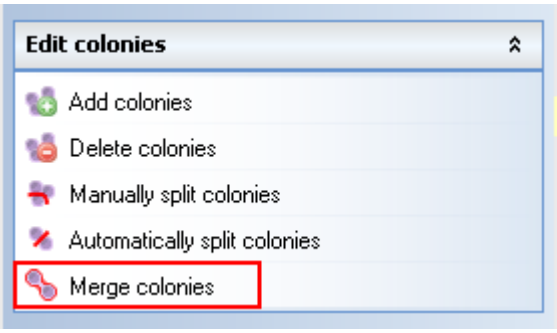


- Click onto the colony in the image to split.
- If the colony is hard to see, zoom in on the colony by using the zoom/pan functions at the bottom of the screen. Click on the plus (+) sign to increase the zoom.

Merging Colonies

Users may merge colonies by performing an initial countt (Automated Count, Identify by Color Count, or User Defined Template Count), and merging colonies manually. 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, go to the **Edit colonies** menu button under the Colony Counting Action Tab and click on **Merge Colonies**.

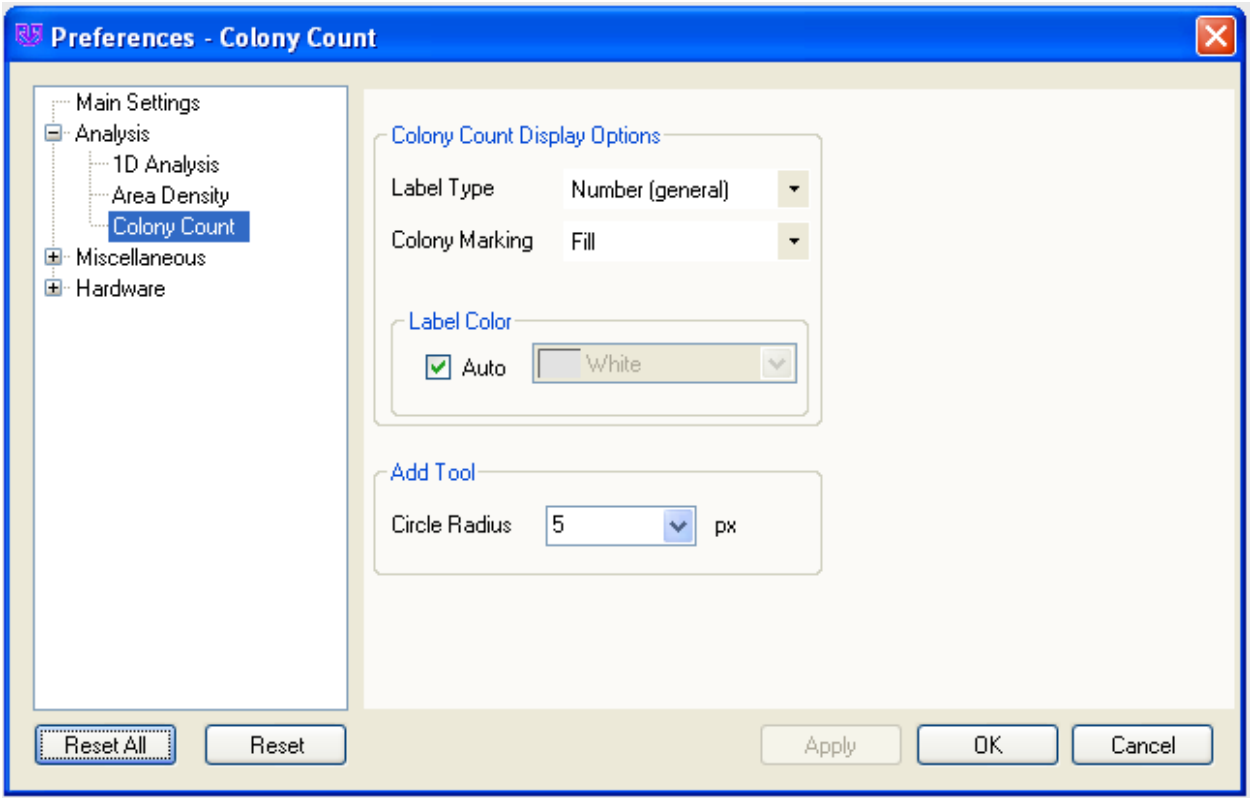


- A new window will ask the user to "**Select the desired colonies and then press the Merge button to join them**."
- Click onto the colonies to be merged.
- The total colony count will change to reflect the changes due to using the merge function.
- If the colony is hard to see, zoom in on the colony by using the zoom/pan functions at the bottom of the screen. Click on the plus (+) sign to increase the zoom.

Colony Counting Settings

Open the Preferences window from the **Colony Counting Active Tab** and select the **Settings** menu button. This window allows users to set new defaults for the colony counting display.

- Label Type
- Colony Marking
- Label Color
- Circle Radius



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

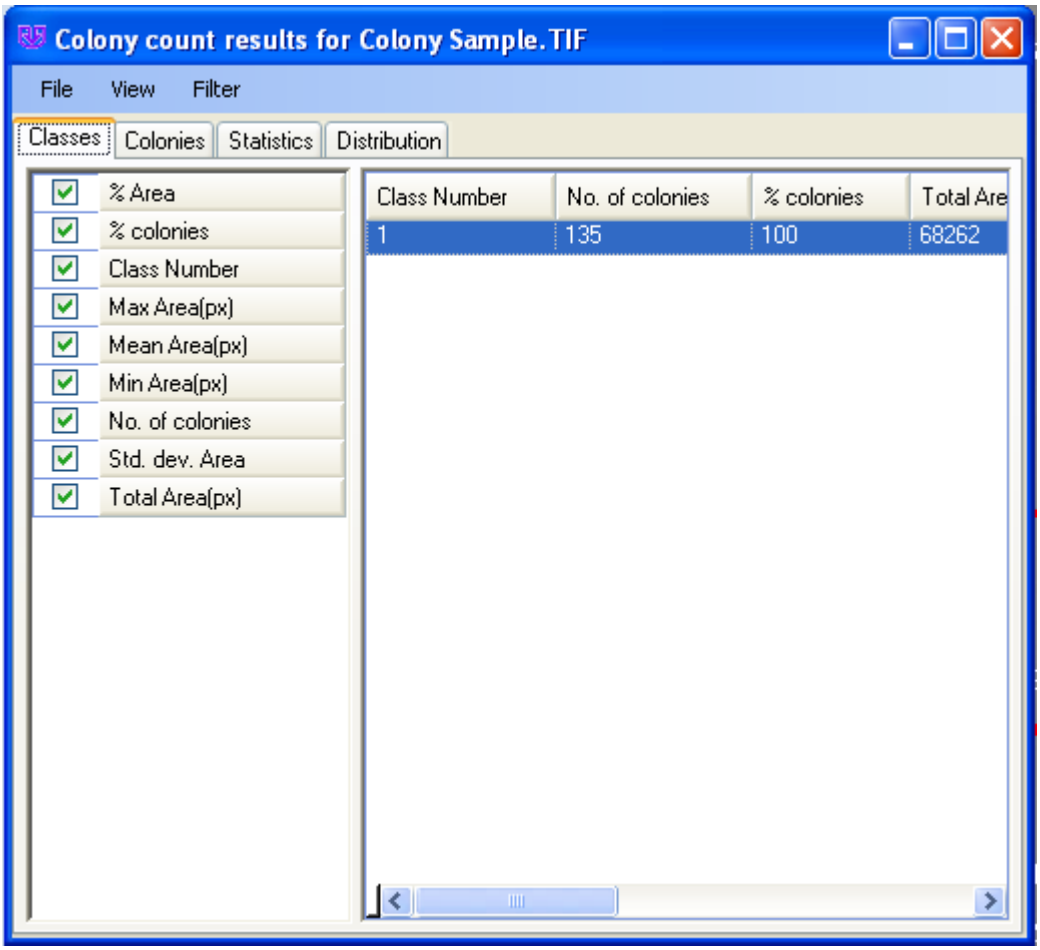
- To change the **circle radius**, click the drop down arrow and select from the numbers to change circle pixel radius.

Colony Counting Results

Colony Counting Reports

The results of the colony count can be displayed in the results window. To show the results:

- In the **Colony Counting Action Tab** click the **Results menu button** 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
    - Colonies
    - Statistics
    - Distribution

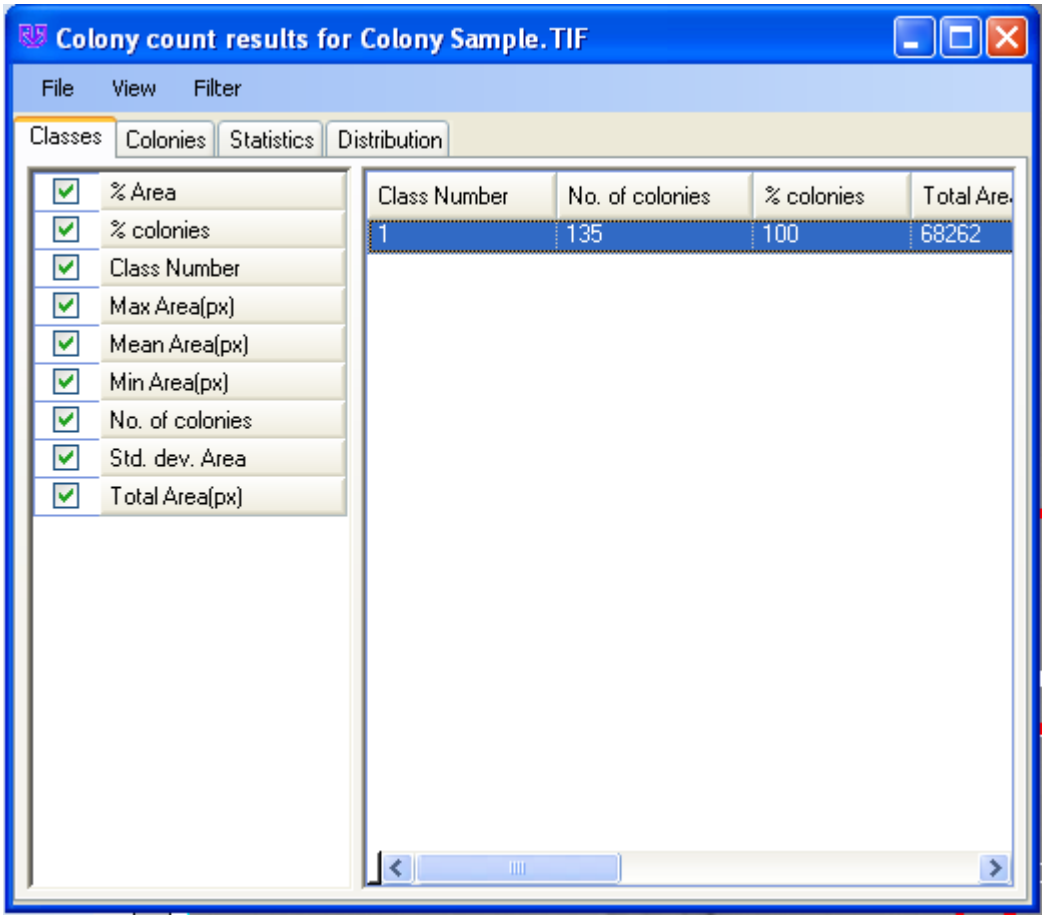


Reporting Colony Class Information

Colony class types can be displayed in the Colony Count Results window. To access the window click onto the **Results** menu button from the **Colony Counting** Action Tab. A **Colony count results** window will appear. Then click onto the **Classes** tab after the new window appears. The Classes tab displays:

- 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)

All dimensional values are reported in pixels unless the calibration process is performed on the image.

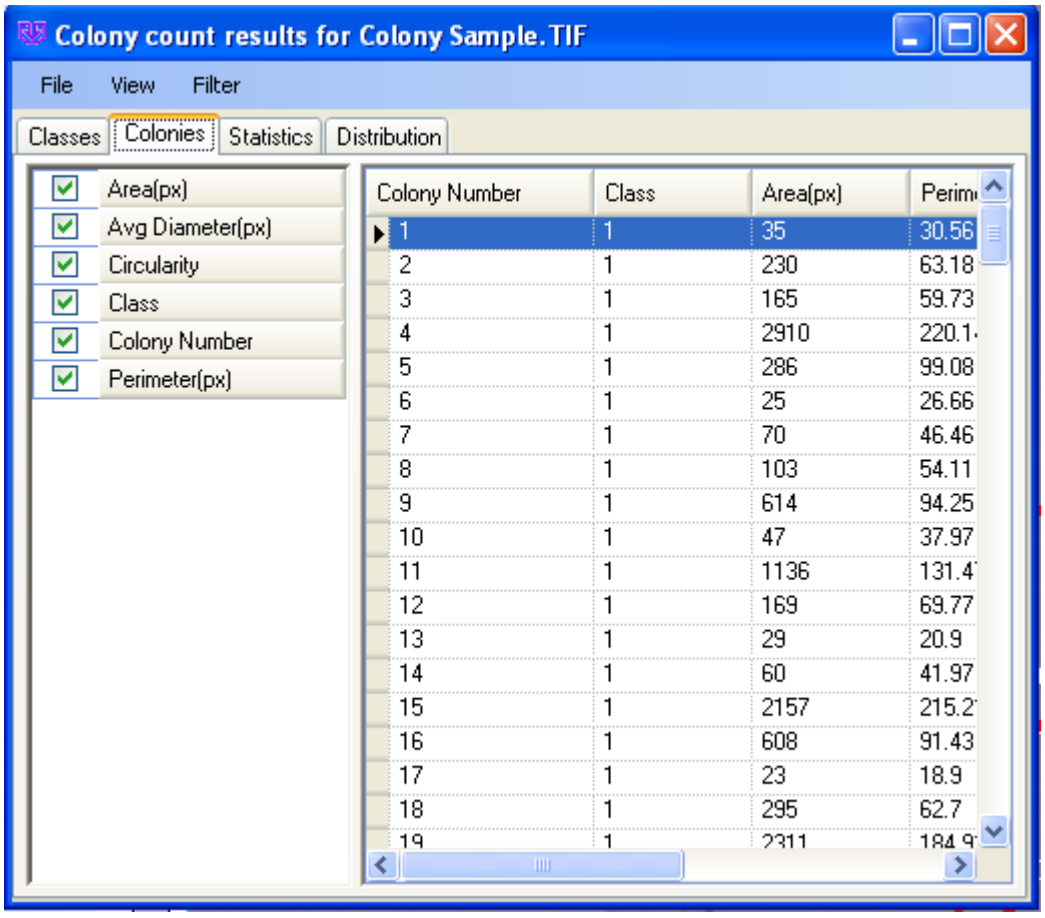


Reporting General Colony Information

Colony information can be displayed in the Colony Count Results window. To access the window click onto the **Results** menu button from the **Colony Counting** Action Tab. A **Colony count results** window will appear. Then click the **Colonies** tab after the new window appears. The Colonies tab displays:

- Class number
- Total area of that colony
- Perimeter of the colony
- Average diameter of the colony
- Circularity of the colony (numerically depicts roundness of the colony)

All dimensional values are reported in pixels unless the calibration process is performed on the image.



Reporting Colony Statistical Information

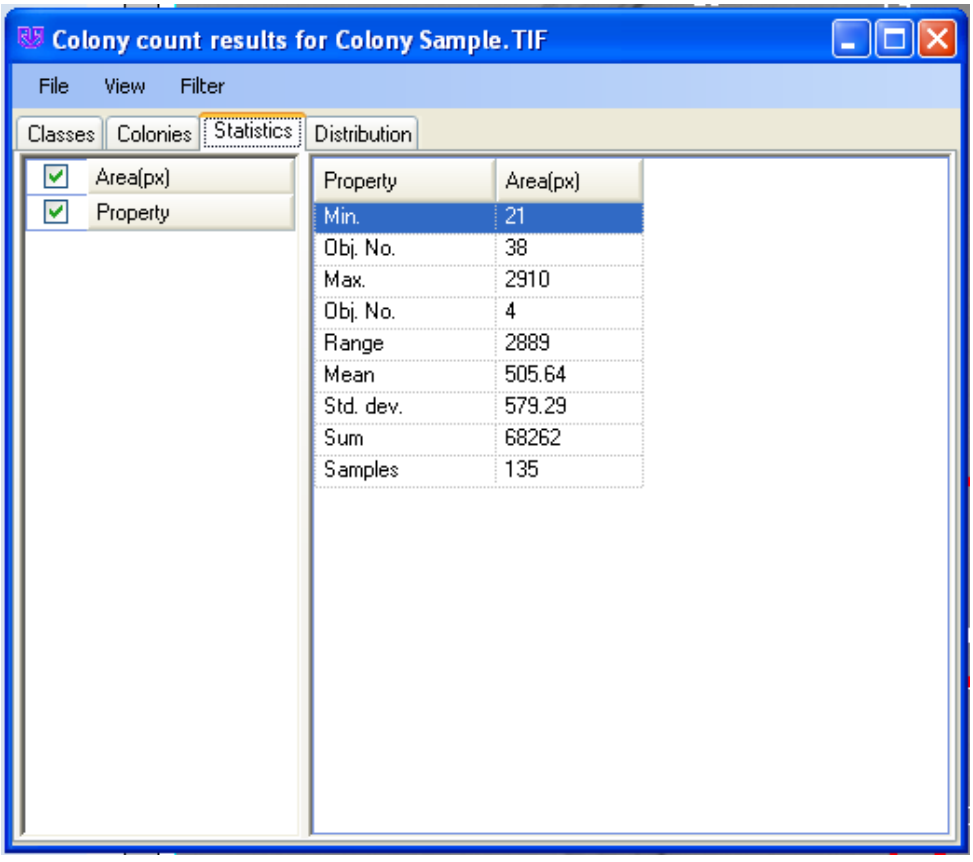
Statistical colony information can be displayed in the Colony Count Results window. To access the window click onto the **Results** menu button from the **Colony Counting** Action Tab. A **Colony count results** window will appear. Then click onto the **Statistics** tab after the new window appears. The Statistical tab displays:

- 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.

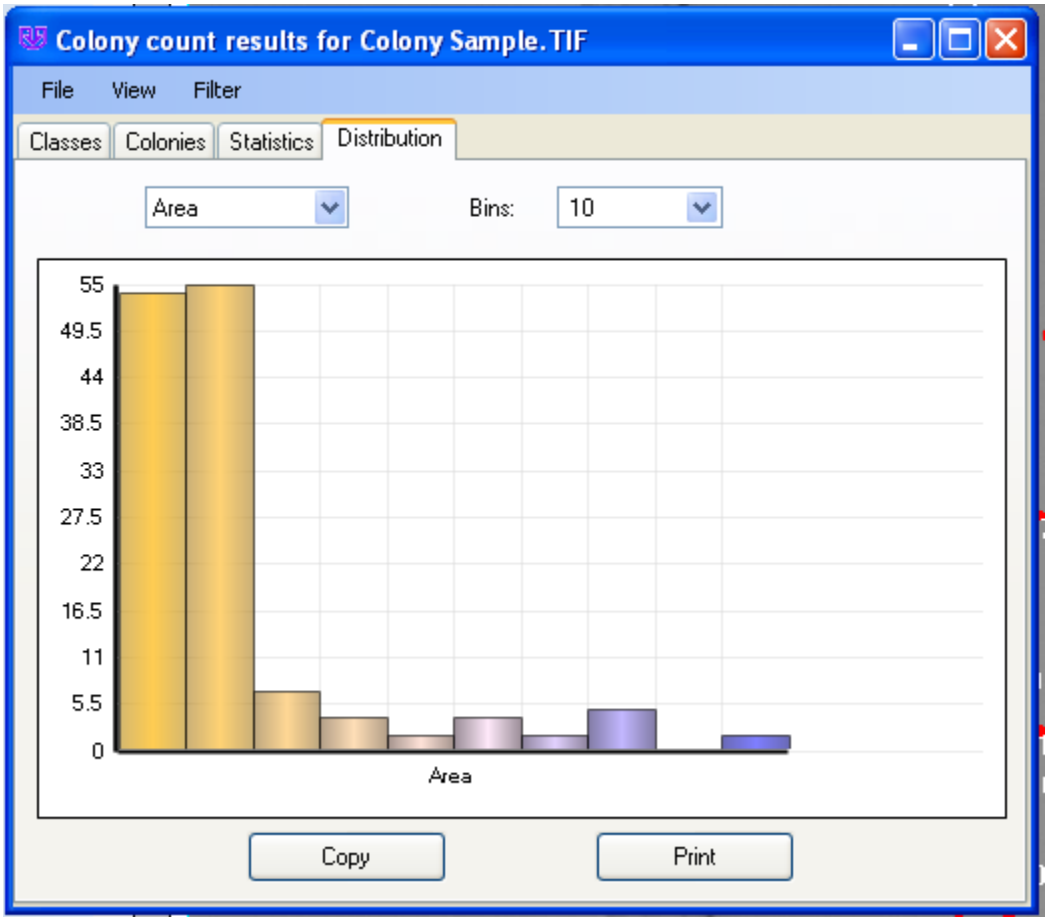


Reporting Colony Distribution Information

Distribution information can be displayed in the Colony Count Results window. To access the window click onto the **Results** menu button from the **Colony Counting** Action Tab. A **Colony count results** window will appear. Then click onto the **Distribution** tab after the new window appears. The Distribution tab displays graphical information.

- 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.

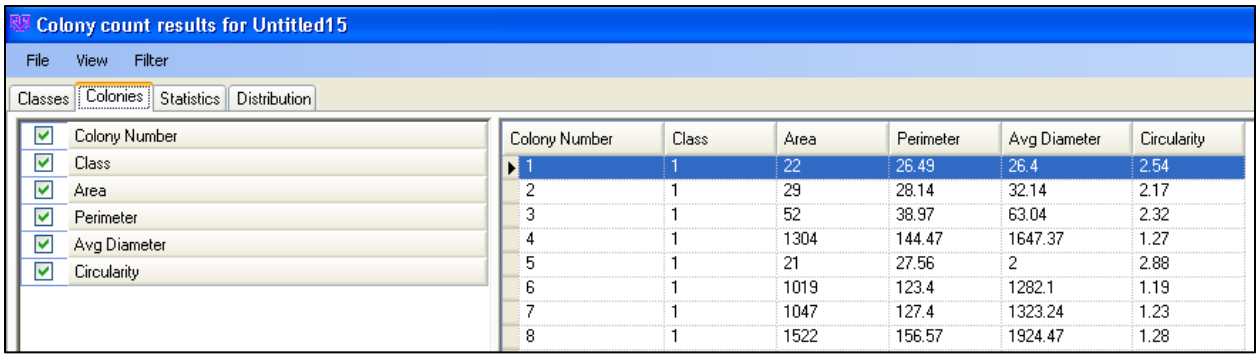
All dimensional values are reported in pixels unless the calibration process is performed on the image.



Export and Print Colony Results

Exporting to Excel

- To export data, click on the **Results** menu button from the Colony Counting Action Tab. A **Colony count results** window will appear. 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.** The fourth tab shows **Distribution.**



Colony Number	Class	Area	Perimeter	Avg Diameter	Circularity
1	1	22	26.49	26.4	2.54
2	1	29	28.14	32.14	2.17
3	1	52	38.97	63.04	2.32
4	1	1304	144.47	1647.37	1.27
5	1	21	27.56	2	2.88
6	1	1019	123.4	1282.1	1.19
7	1	1047	127.4	1323.24	1.23
8	1	1522	156.57	1924.47	1.28

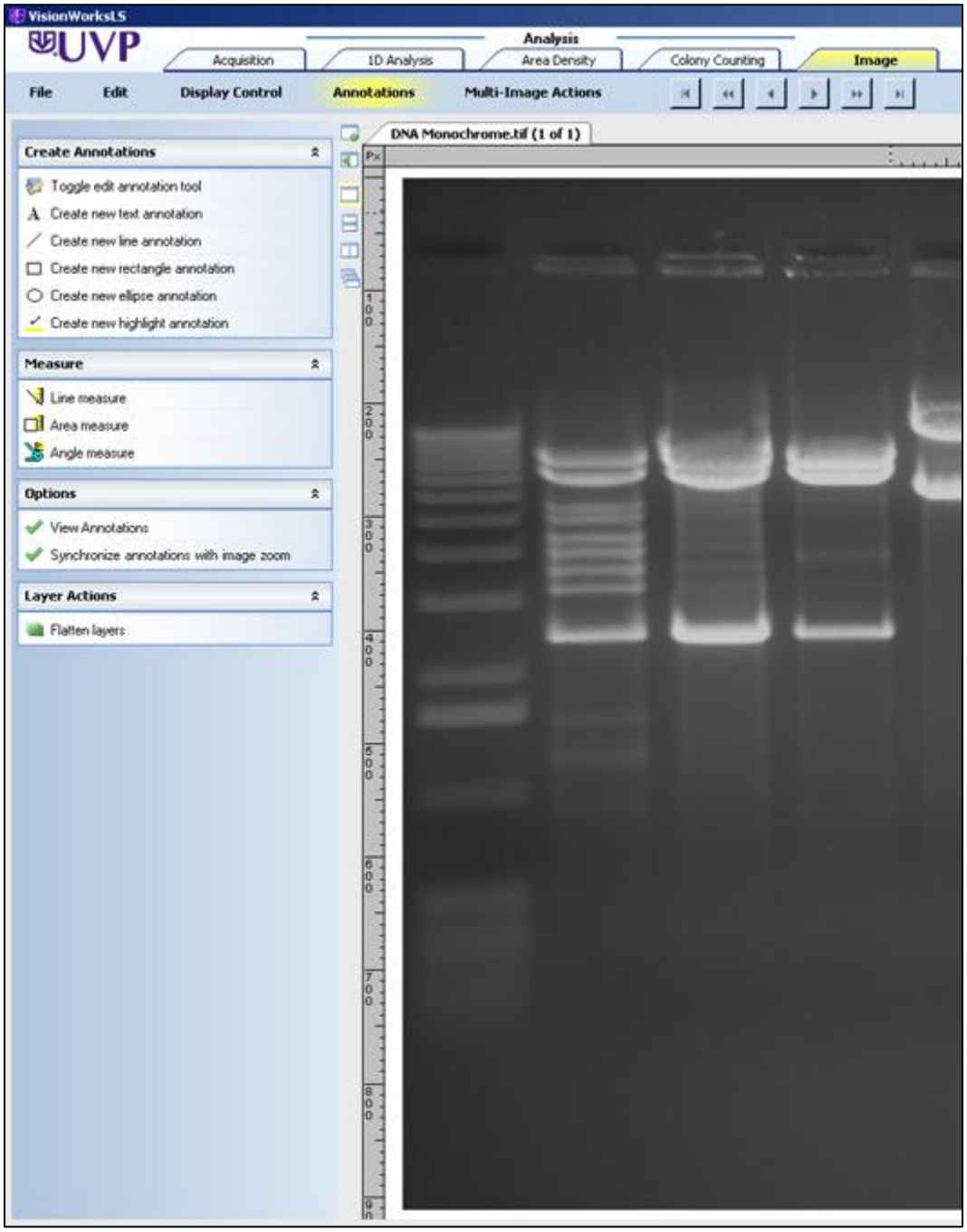
Printing Colony Results

Use Excel to open and print these reports.

Modify Images

Image Action Tab

The Image Action Tab provides the means to modify captured images with the UVP system. The window below shows the **Image Action Tab**.



Contained in the Image Action Tab are the following functions:

Display Control

- Corrections
    - Brightness
    - Contrast
    - Gamma
- Histogram
- Pseudocolor
- 3D Plot

Annotations

- Create Annotations
    - Toggle Edit Annotation Tool
    - Create New Text Annotation
    - Create New Line Annotation
    - Create New Rectangle Annotation
    - Create New Ellipse Annotation
    - Create New Highlight Annotation
- Measure
    - Line Measure
    - Area Measure
    - Angle Measure
- Options
    - View Annotations
    - Synchronize Annotations with Image Zoom
- Layer Actions
    - Flatten Layers

Multi-Image Actions

- Background
    - Background Correction
    - Background Subtraction
- Compositing
    - Multiple Color Channels
    - Composite

- Sequences
    - Extract
    - Merge
    - Enable Tile View
    - Play Options

Display Control

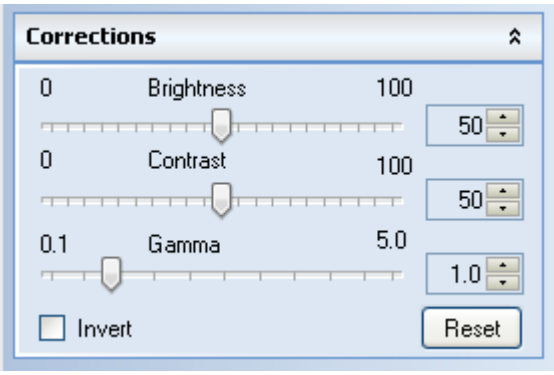
Image Corrections

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

To access the Corrections functions, click on the **Image Action Tab** and then select the **Display Control** menu button.

Corrections

- Brightness
- Contrast
- Gamma
- Invert



The 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.

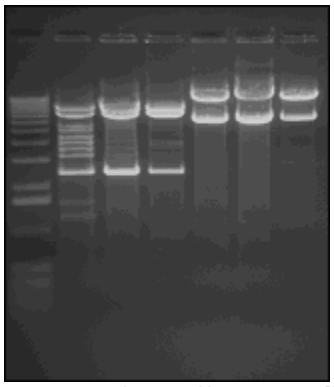
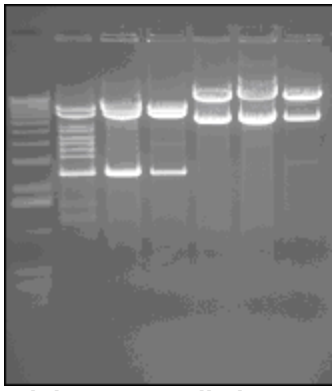


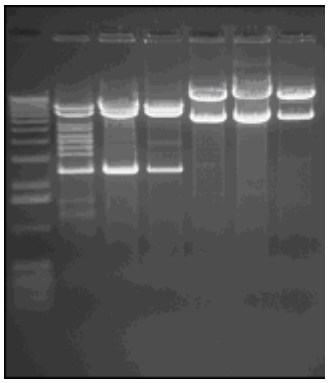
Image Before Effects Applied



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.



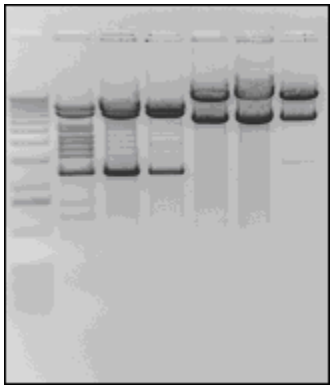
Contrast Enhanced

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.

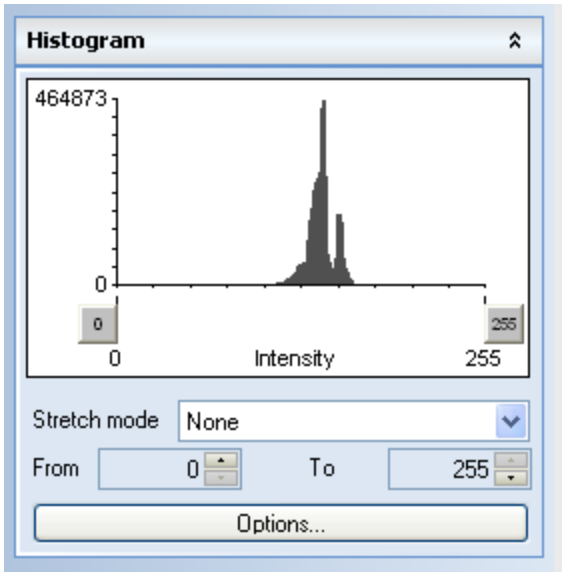


Inverted Image

Histogram Controls

The Histogram Controls offers options for viewing tonal and color information about an image. By default, the histogram displays the tonal range of the entire image. To display histogram data for a portion of the image, first select that portion.

To access the Histogram functions, click on the **Image Action Tab** and then select the **Display Control** menu button.



Apply a Histogram

- From the **stretch mode** drop-down list, select **None**, **Automatic** or **Manual**.

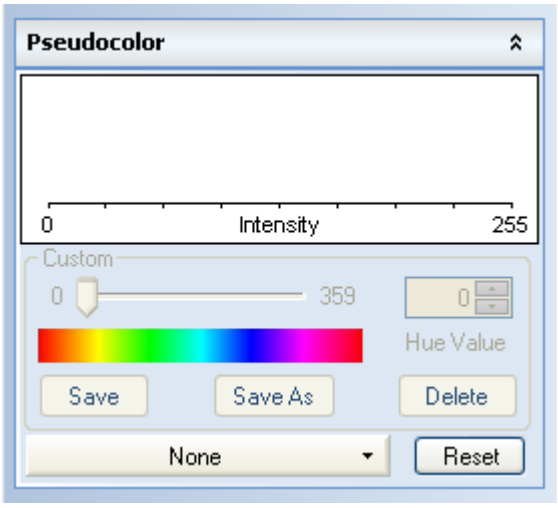
- In the **Options** button select: Y-axis, rest zoom or copy graph.

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.

To access the Pseudocolor functions, click on the **Image Action Tab** and then select the **Display Control** menu button.



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

- 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**.

3D Plots

- Using the 3D Plot Function
- Viewpoint Tab
- Output Tab

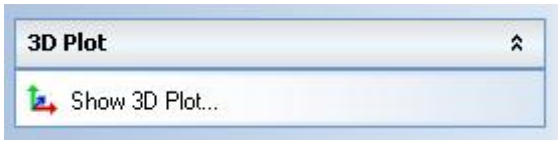
The 3D Plots plug-in allows the user to see a three dimensional view of the sample. For example, if two bands look equally bright in an image, or with naked eye, a 3D plot can actually show if there is a quantitative difference in intensity. A 3D plot can also be used as a great presentation tool.

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

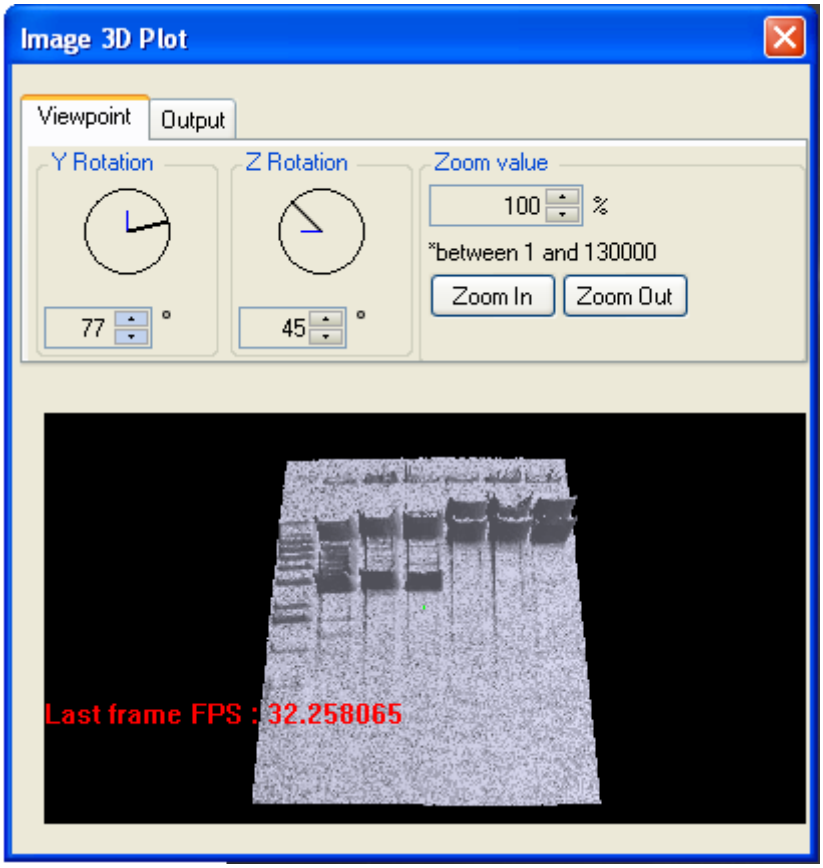
Using the 3D Plot Function

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.)
- To access the 3D Plot function, click on the **Image Action Tab** and then select the **Display Control** menu button.



- This will bring up a separate window that shows the 3D plot.



Viewpoint Tab

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

Rotation Controls

One can rotate the image in Y as well as Z axes, using the ’r;Y’ rotation and ’r;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

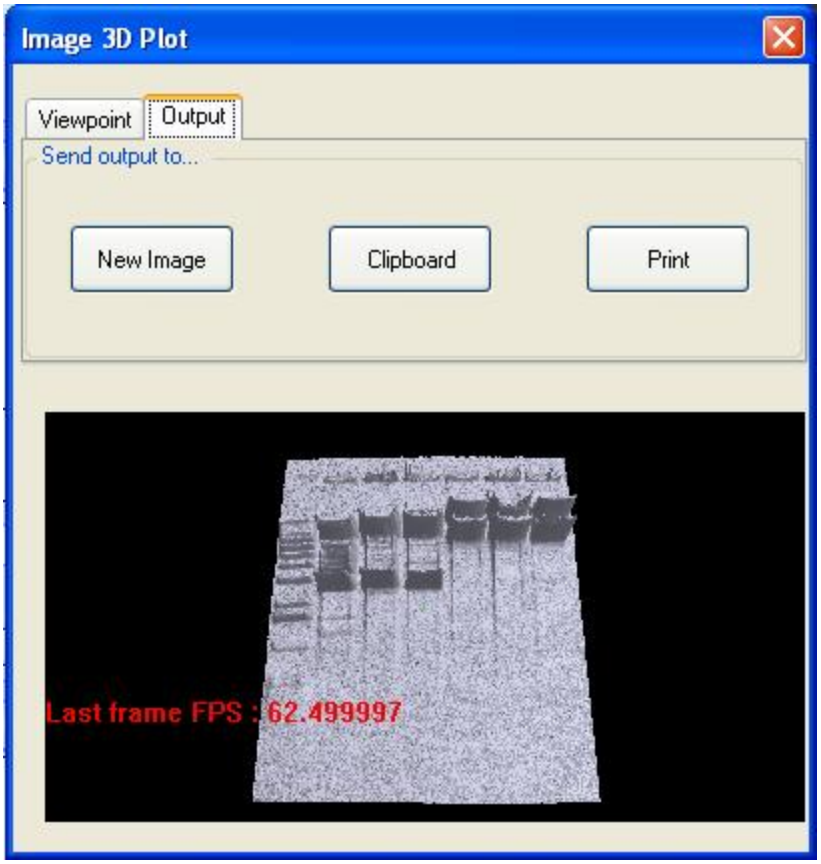
Zooming in and out of the plot is possible in two ways:

- Using the buttons labeled ’r;Zoom In’ and ’r;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.)
- **Print**: Pressing this button prints the 3D plot on a desired printer. Pressing it brings up the list of available printers.



Annotations

- Overview
- Creating Annotations
- Viewing and Hiding Annotations
- Modify Annotations
- Synchronize Annotation with Image Zoom
- Measurement Tools
- Layer Actions

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 viewed or hidden to see the image with or without annotations.

Users may add and edit annotations through the **Image Action Tab** > **Annotation** menu button.

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 Annotations** with image zoom.

- ***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.

Create Annotations

Create a Text Annotation

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.

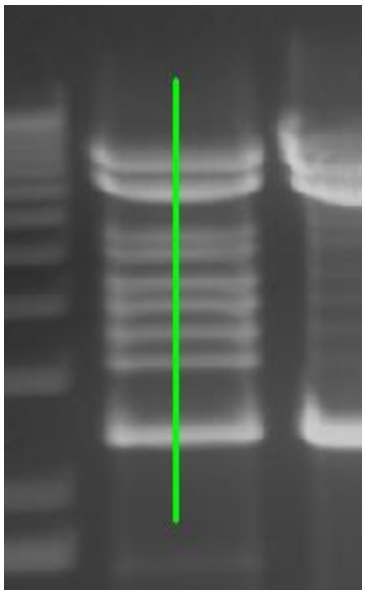
The New Annotation window saves the last text entered, making it the default for the next text annotation to be created. To enter new text, simply start typing and the existing text will be replaced.

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.

- Select the **Image Action Tab > Annotations menu button > Create new text annotation**.
- 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.

Create Line Annotations

- Select **Image Action Tab > Annotations > Create new line annotation**.
- 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.

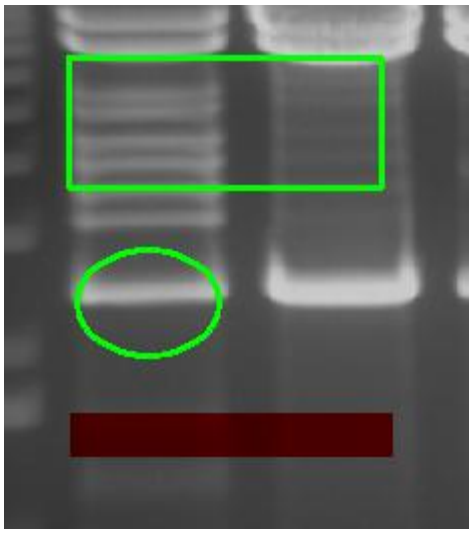


Create Shape and Highlight Annotations

Create a Rectangle, Ellipse, Highlighter Annotation

- Select **Image Action Tab > Annotations** 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.

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

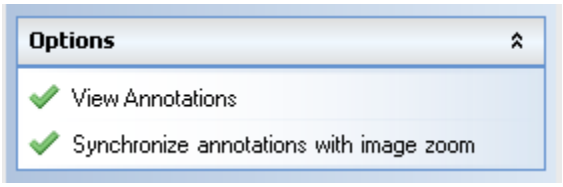


View Annotations

Although annotations are useful for drawing attention to important features, they may cover-up important features. LS software allows annotations to be viewed or 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.

- Click the **Image Action Tab > View Annotations**. If this menu option is checked but no annotations appear, no annotations exist for this image. Image below shows a check next to **View Annotations**.



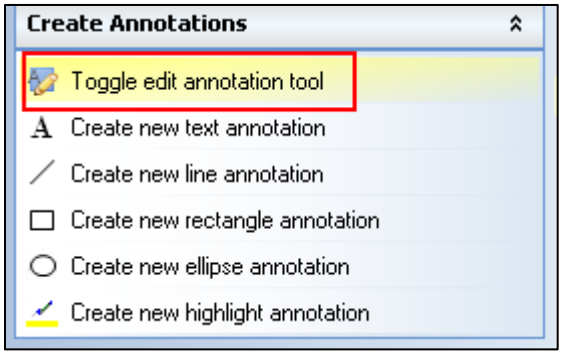
Note: To **edit an annotation**, ensure that the **Toggle edit annotation tool** is turned on. Select the annotation in the image, right mouse click on the annotation, then select from the menu options to change the annotation. Also refer to the **Modify Annotations** section of this chapter for more information.

Modify Annotations

- Select Annotations
- Edit Text Annotations
- Move/Resize Annotations
- Rotate Text Annotations
- Format Annotations
- Delete Annotations

Select 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 Toggle Edit Annotation Tool -- to select and edit annotations. Every time an annotation is added, the tool switches to the Toggle Edit Annotation Tool automatically so that the new annotation can be edited immediately.



- Choose **Image Action Tab > Toggle Edit Annotation Tool**.
- Click on any part of the annotation to edit. 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.

Edit Text Annotation

- Double-click the text annotation.
- In the **New Annotation** window, make any changes to text.
- Click **OK**.

Move an Annotation

- To move an annotation, select **Toggle Edit Annotation Tool** if not already selected; 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.

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.

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.

Rotate Text Annotations

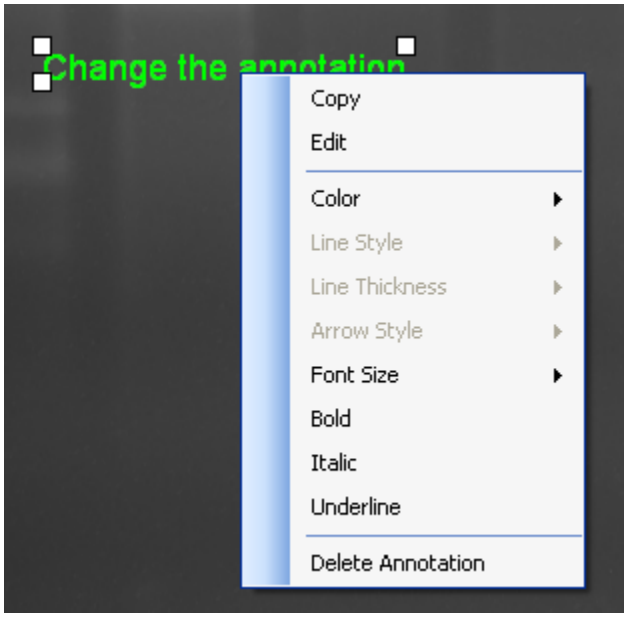
- Select the annotation as described above.
- Drag any control point around the center of the annotation until the desired location of rotation is reached.

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

Format Annotations

The annotation formatting contains options to format a selected annotation.

Change the formatting of an annotation by selecting the **Toggle Edit Annotation Tool** and then **right click** on the desired annotation.



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.

Delete Annotations

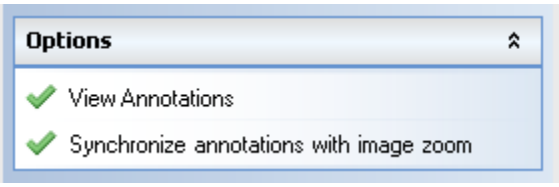
Deleting an annotation removes it permanently from the image.

- Delete an annotation by selecting the **Toggle Edit Annotation Tool** 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.

Synchronize Annotations

The default value for LS is **Synchronize annotations 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, uncheck the **Synchronize annotations with image zoom**. The image below shows the **Synchronize annotations with image zoom** function checked.



- To change this setting go to **Image Action Tab** > **Synchronize annotations with image zoom**.
- Click onto the **Synchronize annotations with image zoom** to uncheck (or check) the option**.**

Measurement Tools

- Line Measure
- Area Measure
- Angle Measure

Line Measure

- Choose **Image** Action Tab **> Annotation > Line Measure** or choose the **Area Density** Action Tab **> Calibration > Line Measure.**
- 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

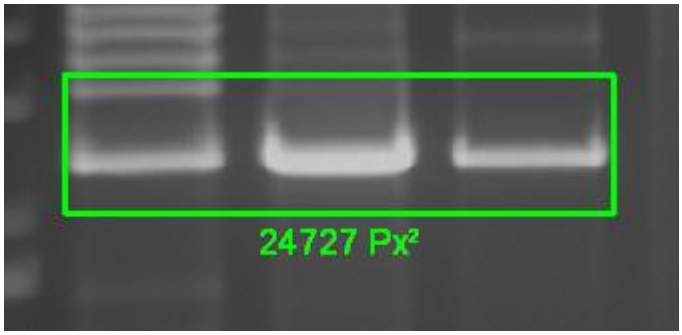
value.

- 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.



Area Measure

- Choose **Image** Action Tab **> Annotation > Area Measure** or choose the **Area Density** Action Tab **> Calibration > Area Measure.**
- 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.

Angle Measure

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

- Choose **Image** Action Tab **> Annotation > Angle Measure** or choose the **Area Density Action Tab > Calibration > Angle Measure.**
- 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.

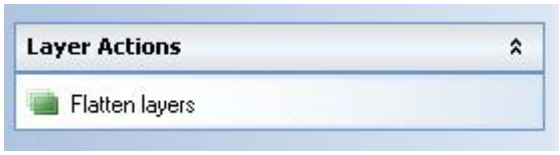
- Click to freeze the angle in the desired position. The new angle measurement annotation now appears.
- Right click on the new measurement annotation displayed to change the appearance of the annotation. If desired, change the line thickness, line style, font size, or general formatting of the line and value displayed.



Layer Actions

The Flatten Layer function embeds the annotations and analysis markings in the image. This feature is useful when users want to open and see the analysis and annotations in a program other than LS. If the layers are not "flattened" markings may seem to disappear after opening the image in another program.

To access the Flatten Layers function go to the Image Action Tab and click on the **Flatten Layers** button.



Multi Image Actions

- Background Filters (described immediately below)
- Image Composite
- Player

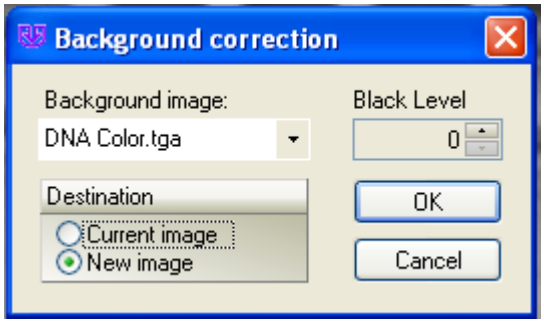
Background Filters

Background Correction

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

To access this function go to the **Image Action Tab** and click on **Background Correction**.

Select a **Background image** and the **Black Level.**



Background Subtraction

This command allows users to subtract one image from another.

To access this function go to the **Image Action Tab** and click on **Background Subtraction**.

Select a **Background image.**

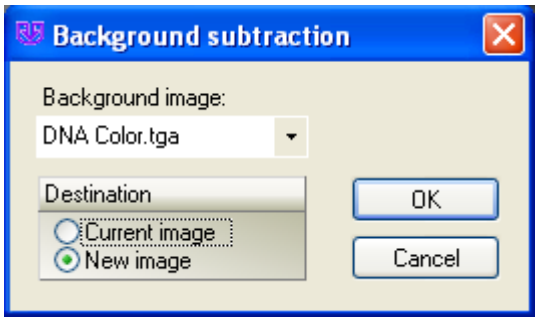
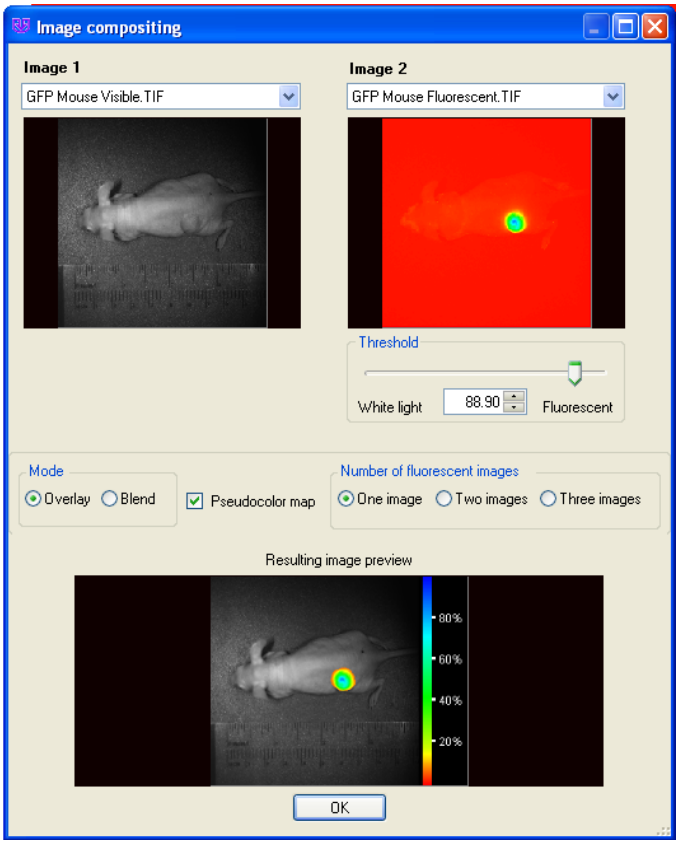


Image Composite

The Composite function allows the user to take two or more images and create a composite of the two images. For example, the user may take a white light image of a mouse and a fluorescent image of a mouse. These two images can be used to combine into one composite image that outlines the location of the fluorescence inside the mouse body.

Create a Composite Image

- Open the images that will be used in the composite image. Use the Pseudocolor function to colorize the fluorescent image, if desired and as shown below.
- Go to the **Image Action Tab** > **Multi-Image Actions** button to select the image **Composite** feature.
- Load the **White light image** and the **Fluorescent image(s)** from the workspace window by using the dropdown menu. (LS allows for more than one florescent image to be used in the composite.)
- Change the **Threshold**, if necessary, to show an image with a stronger **White light** image or **Fluorescent** image by adjusting the slider bar.
- Select to view the composite image in an **Overlay** or **Blend Mode**. The overlay mode creates a sharp contrast in color transition. The blend mode is a blended, or smoothed, transition between colors.
- When only one fluorescent image is loaded, click onto the **Pseudocolor map** checkbox to show the color mapping to the % of maximal fluorescence intensity in the resulting image. The resulting image preview is displayed in the Image compositing window.
- When finished, select **OK.**



Player (Image Sequences)

- Purpose of the Player
- Using the Player
- Merge Functions
- Player Features
- Player Options
- Extract or Delete Individual Image Files (Frames)
- Saving_.AVI_Files

Purpose of the Player

The player 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.

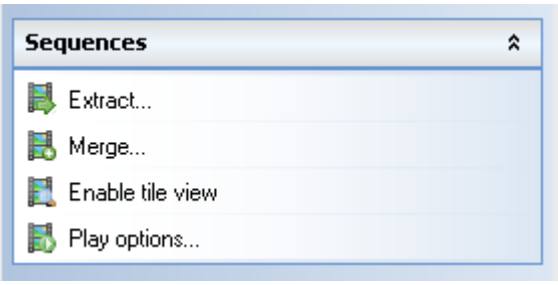
If a sample is to be observed over a period of time, (e.g. Chemiluminiscence blots), it is required to snap multiple pictures at regular and definite time intervals, maybe with progressively higher integration times.

Depending on the camera used, LS software provides two features - **Sequential Integration** and **Dynamic Integration**. Sequential and Dynamic integration functions produce a series of images as output. LS software serializes these images into a single file in the workspace, with extension .sqv that Image Sequences player can utilize.

Using the Player

To access the Image sequences player function:

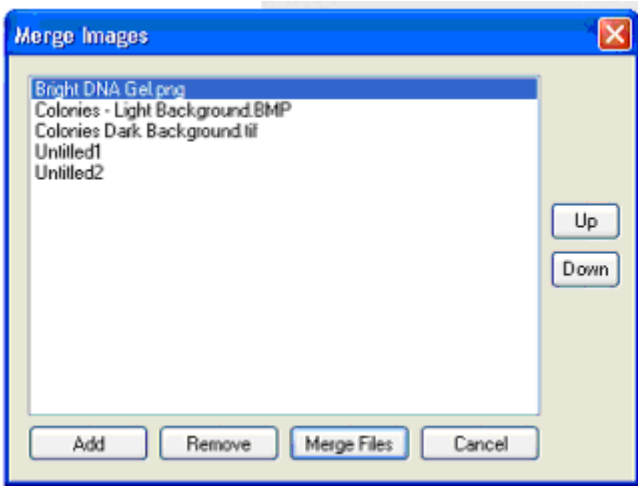
- Go to Image Action Tab and click the **Multi-Image Action** button. The following **Sequences** menu will display.



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

To play a sequence of images:

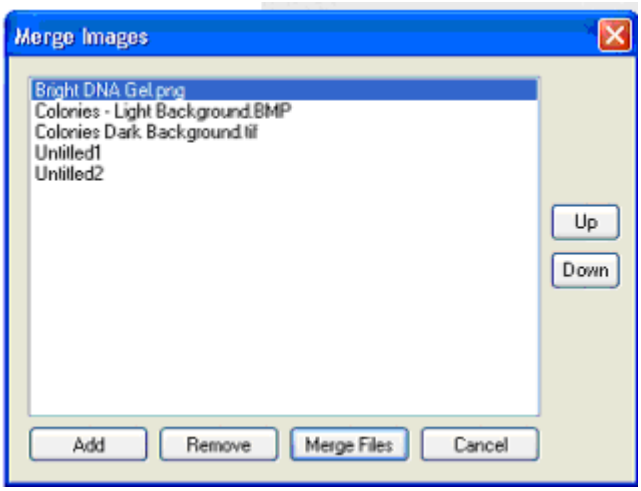
- Open images.
- Click **Merge** from **Images** action tab > **Multi-Image Action** > **Sequences** menu.
- Click **Merge Files** from the Merge Images window.



- A new file is generated which includes all open images (file name is untitled).
- Save the file (.sqv). Ensure that file is selected.
- Click **Play** button  or select other **Play Options** from the **Sequences** menu.

Merge Functions

These functions can be used to modify the merged images.



- **Add**: Click the **Add** button to find files from the hard-disk to merge.
- **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.

Player Features



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

BUTTON FUNCTIONALITY



Views the next image.



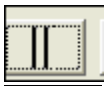
Views the previous image.



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.



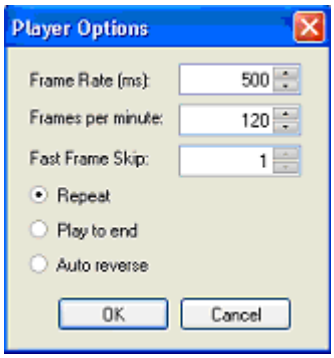
Plays the sequence with a number of images to **skip**. The number of images to skip can be set in the Options dialog.



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.

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:



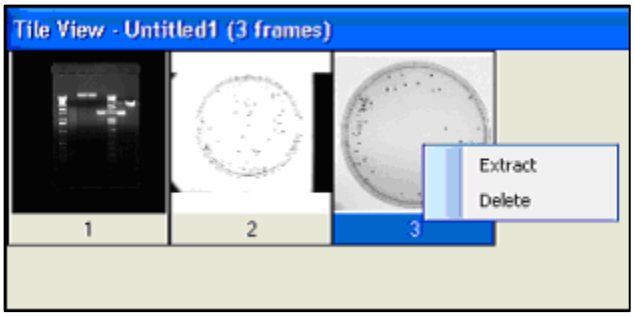
Frame Rate	This is the interval (in milliseconds) between two consecutive images that are displayed. For example, a value of 500 means that a new image will be displayed a half second after the previous image. 'r;Frames per minute' is automatically calculated based on this value.
Frames per minute	This 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  or  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.

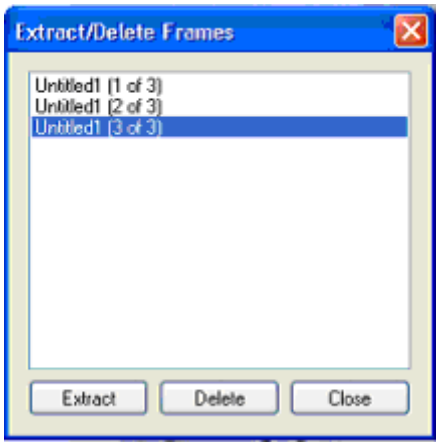
Extract or Delete individual Image files (frames)

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 **Enable Tile View** from the Multi-Image Actions under the Image Action Tab, which brings up the following window. Just right click on any image to extract or delete.



The following window opens 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.

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

Create 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 Camera Templates (described immediately below)
- Set-up Canon Capture Templates
- Set-up Darkroom/Lens Templates
- Create an Auto BioLite Template
- Creating Colony Templates

Set-Up Camera Templates

A template is simply a group of all configurable camera settings grouped under a single name. After the ideal combination of settings for a sample has been documented for the first time, settings can be ***saved*** as a Template and easily ***applied*** next time a similar sample is imaged.

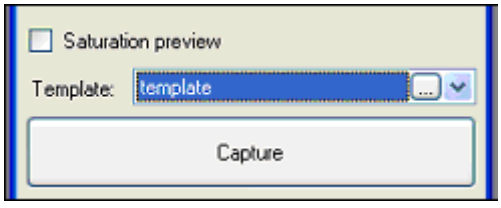
Explanation of main buttons discussed here:

- **Edit Name:** Change the existing name of the template.
- **New:** Create a new Template.
- **Save:** Save this template and continue working.
- **Delete:** Delete this template and create a new one.
- **Sync:** Synchronize the settings from capture panel. This could be a very useful button if a Template changes. Press it once after the settings have been changed. In one click, all settings get copied (or synchronized) from the Capture Panel to the Template window.

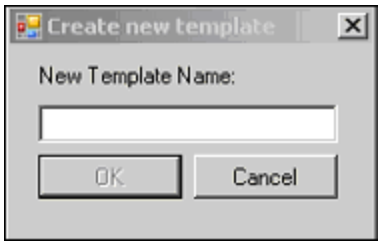
After the values of the template are entered, click **Sync, Save** and **OK** to save the template.

Create a New Template

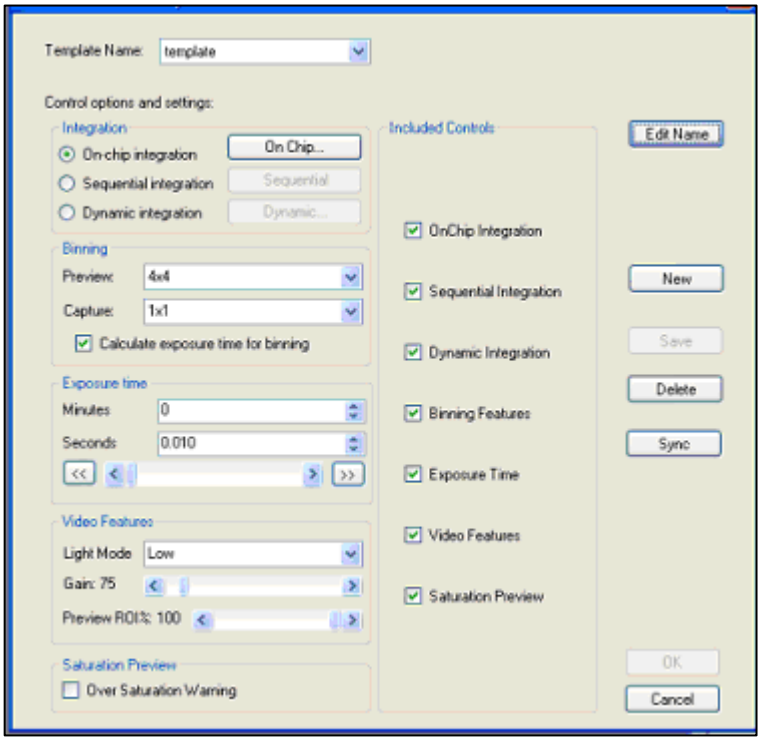
- The user starts by clicking onto the three dots in the camera module to create the template. A sample screen shot is shown below; each camera plug-in may look different.



- A new window appears that asks the user to provide a new template name.



- A new template window appears that allows the user to select various integration, binning, video, and saturation preview settings. **Note**: Camera templates vary from camera to camera.



- On the left side of this window, specific values for all the available options can be selected. For example, a specific exposure time can be set that provides a sharp image.
- 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.

Set-Up Canon Capture Templates

- Create a New Template
- Edit a Template
- Delete a Template

Capture Templates are groups of preset camera settings. They are used on the Camera module 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

selected. If a setting (or group of settings) is not excluded, it can be overridden on the camera module before each capture.



Create a New Template

- On the Camera module 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.

Edit a Template

- On the Camera Module click on the **Templates** drop down menu. The Capture Templates window will appear.
- Select the template to edit from the drop-down list. The default values and included controls check boxes will be set to the values used for that template.
- To edit the name, click **Edit Name** to the right of the template drop-down list. Type a new name and then click **OK**.
- If changes were made while in the Camera module to the same template, the user may make the template take the changed settings by clicking **Sync**. This allows experimentation with changes to settings and then save those changes.
- To change any setting default, select the new value for that setting.

- To include or to show a setting or a group of settings, select or clear the related **Included Controls** check box.
- Click **Save** (or **OK**) to save your changes.

Delete a Template

- On the Camera module click on the**Templates** drop down menu. The Advanced Capture Templates window will appear.
- 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 one time by using **Save** instead of **OK**. **OK** will close the Advanced Capture Templates window, saving the latest changes.

Set-Up 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.

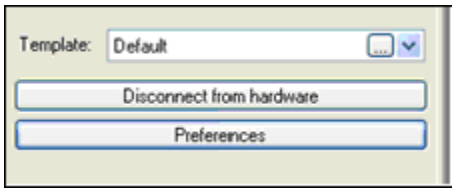
To set a template for any Petri dish, select the Identify by Color Count method from the **Count** menu button in the **Colony Counting** Action Tab. A template can be saved during operation **Step 1 of 2: Select classes** or **Step 2 of 2: Finish**.

- 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.

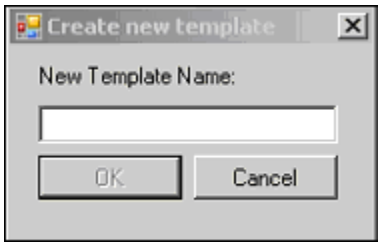
Set-Up Darkroom/Lens Templates

A template is simply a group of all configurable darkroom and lens settings grouped under a single name. After an ideal combination of settings for a sample has been documented for the first time, settings can be ***saved*** as a Template and easily ***applied*** the next time a similar sample is imaged.

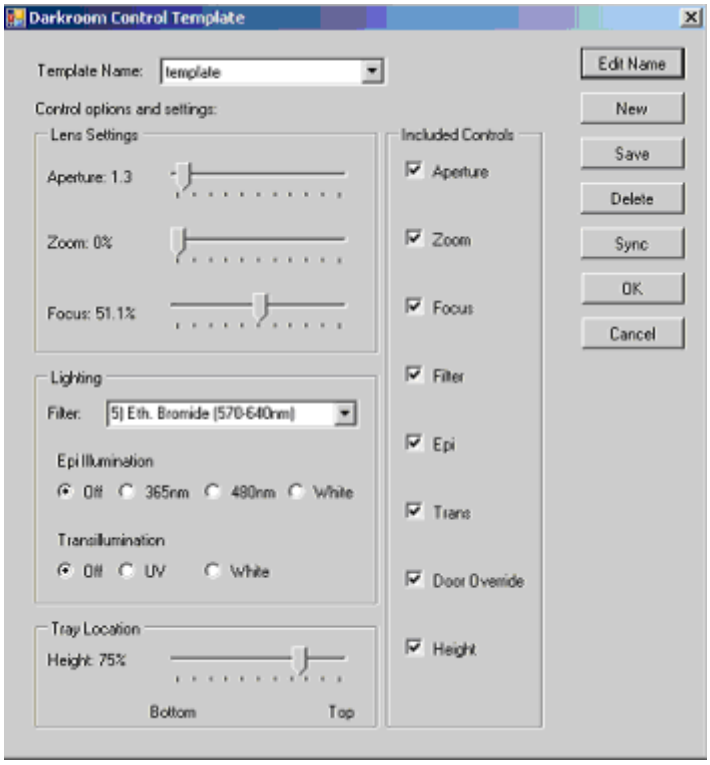
- The user starts by clicking onto the three dots in the Darkroom and Lens control window next to the Template drop down box.



- A new window appears that asks the user to provide a new template name.



- Once the template is assigned a name, a new template window appears that allows the user to select various lens, lighting, and tray height settings.



On the left side of this window, specific values for all the available options can be selected. For example, a specific light source can be set that provides a sharp image.

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.

Explanation of main buttons:

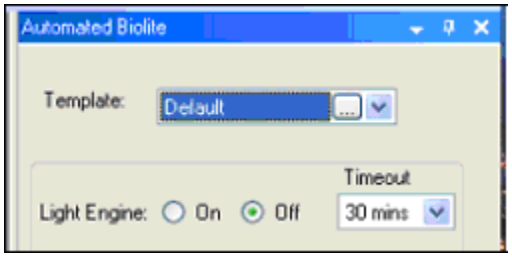
- **Edit Name:** Change the existing name of the template.
- **New:** Create a new Template.
- **Save:** Save this template and continue working.

- **Delete:** Delete this template and create a new one.
- **Sync:** Synchronize the settings from capture panel. This could be a very useful button if a Template changes. Press it once after the settings have been changed. In one click, all settings get copied (or synchronized) from the Capture Panel to the Template window.
- After the values of the template are entered, click **Sync, Save** and **OK** to save the template.

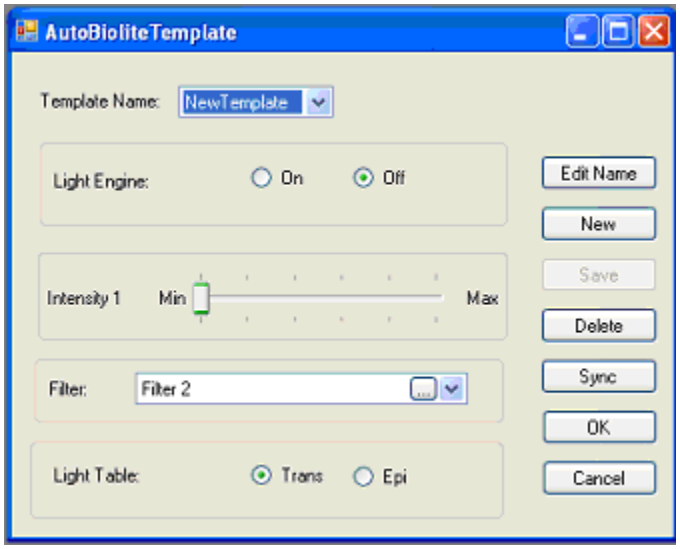
Set-Up an Auto BioLite Template

A template is simply a group of all configurable darkroom and lens settings grouped under a single name. After an ideal combination of settings for a sample has been documented for the first time, settings can be ***saved*** as a Template and easily ***applied*** the next time a similar sample is imaged.

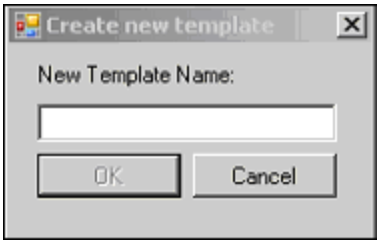
- In the **Automated Biolite Plugin**, click the three dots next to the drop-down **Template** menu.



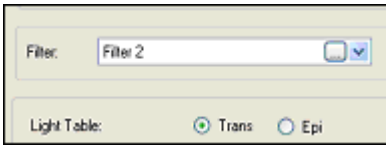
- An AutoBiolite Template will appear.



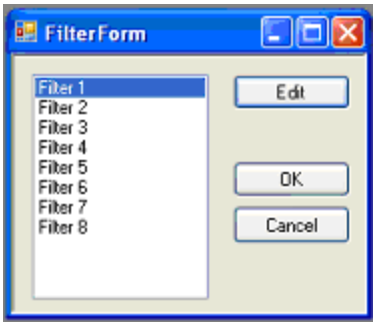
- Click onto New in the **AutoBiolite Template** window. A **Create new template** window will appear.
- Enter a New Template Name.



- Set the appropriate **Light Engine**, **Intensity**, **Filter** and **Light Table** settings.
- To change the filter names in the template, click onto the three dots next to the drop-down **Filter** menu.



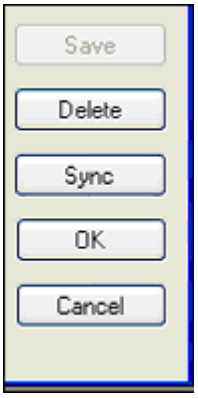
- A **FilterForm** window appears. Click onto the **Edit** button.



- A **FilterEditForm** window appears. Type the new **Filtername** in the box provided. Select **OK.**
- Continue to enter new filter names as necessary. When finished entering new filter names click **OK** in the **FilterForm** window.



- When finished selecting the appropriate settings for the Automated BioLite, select **Sync, Save, OK** in the **AutoBiolite Template** window.



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.

Edit and Delete Templates

Edit a Template

- While in the module 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 module 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**.

Delete Colony Counting Templates

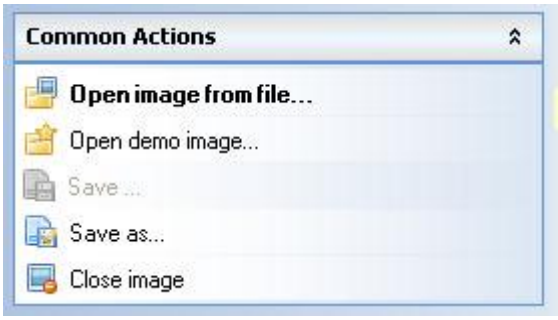
To delete a **Template**, from the **User Defined Template** list:

- Click Identify by Color Count.
- On the **Step 2** tab, under the **Template** section, click the drop down menu.
- Select the Template name to delete.
- Click **Delete**.

Open and Save Images

Open and Save Images

- Open Images
- Save Images
- Image File Types



Open 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 the **Image Files** topic below.

Open a Previously Saved Image

- From the **File** menu button, choose **Open image from file**.
- 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.

Open a Demo Image

- Go to **File** menu button and click on **Open Demo Image**.
- Select the desired demo image file from the list of available files.
- Click **Open**. An Image Window containing the desired image will appear.

Save Images

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

- Click on the **File** menu button choose and select **Save** or **Save As**. The Save window will appear.
- Select the file type 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**.

Save Changes to an Image

- From the **File** menu button choose **Save**.
- Changes will be saved to the existing file, overwriting the previous image data.

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**.

Image File Types

When analysis is performed, images are saved as two separate files. While the two files have the same name, they have different extensions. The two files are Image File and the Analysis File (Extended Attributes - TIF/TIFF).

NOTE: Images saved in TIF/TIFF format stores both image and analysis data in a single file. The file must be opened with VisionWorks version 7 or later.

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 TIF/TIFF by default. The file extension will reflect the file format (e.g. ".TIF" or ".TIFF").

NOTE: If the image is not saved as a TIF file, the analysis will be saved in the archive folder.

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.

Extended Attributes File

The image will also have a second file that ends with the extension ".TIF." 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.TIF," the matching extension file would be "MyImageJPG.TIF" To copy an image, move an image or send an image to another LS software user, copy, move, or send both files.

Support 21 CFR Part 11 Compliance

Supporting 21 CFR Part-11 Compliance

- Purpose
- Features
- Usage

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:

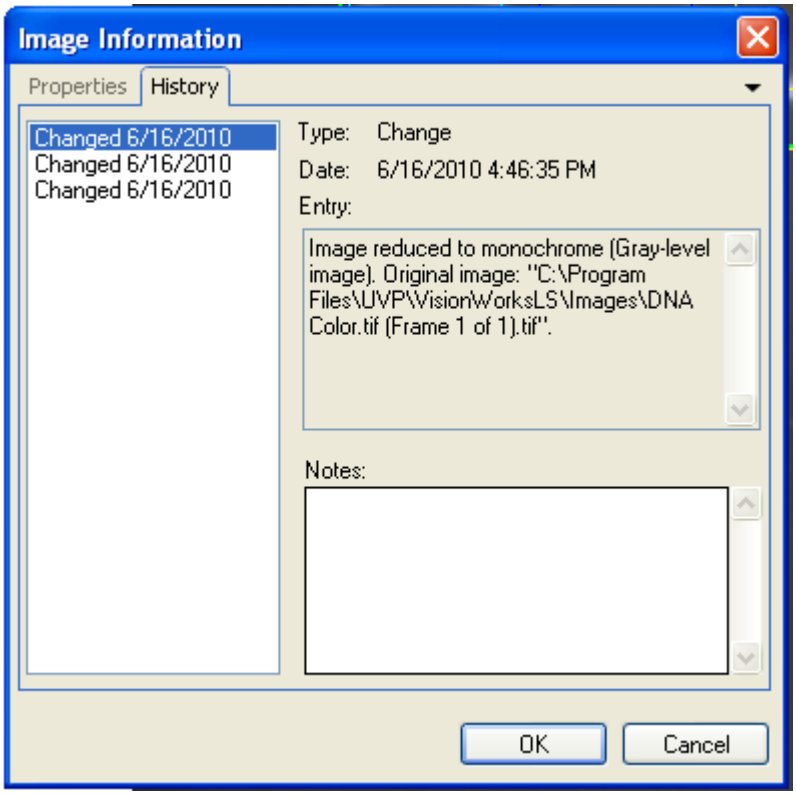
- Section 11.10 (e) – For electronic records, this section requires the use of computer- generated, time-stamped audit-trails to track changes.
- The software keeps track of all changes that affect image-data. Any action in the software that modifies the original data of an image open in the LS workspace, is logged. The log of such changes is individually maintained for each image and is referred to as ’r;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

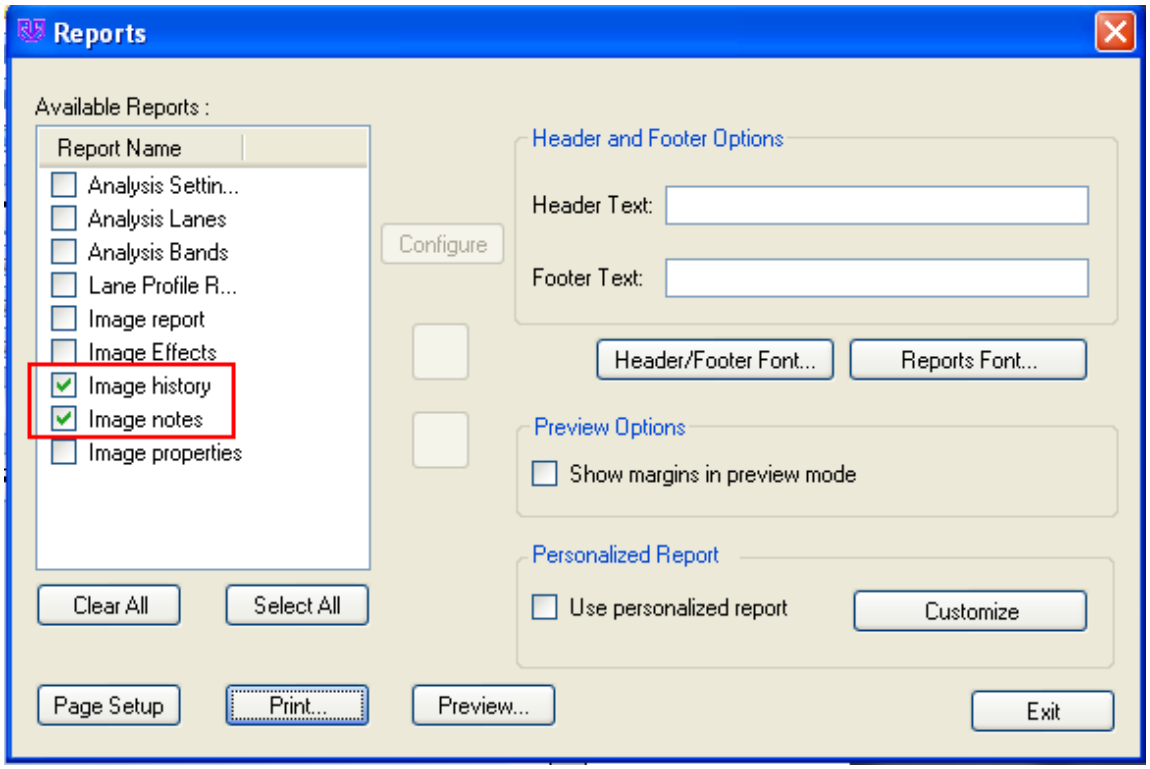
View an Audit Trail (History)

- 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.



Print an Audit Trail (History)

- Open the image for which to print the Audit Trail.
- Go to **File > Print a Report**. (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.



For information on user accounts and passwords, go to: User Administration

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**.

View 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.

Add 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 Supporting 21 CFR Part-11 Compliance.

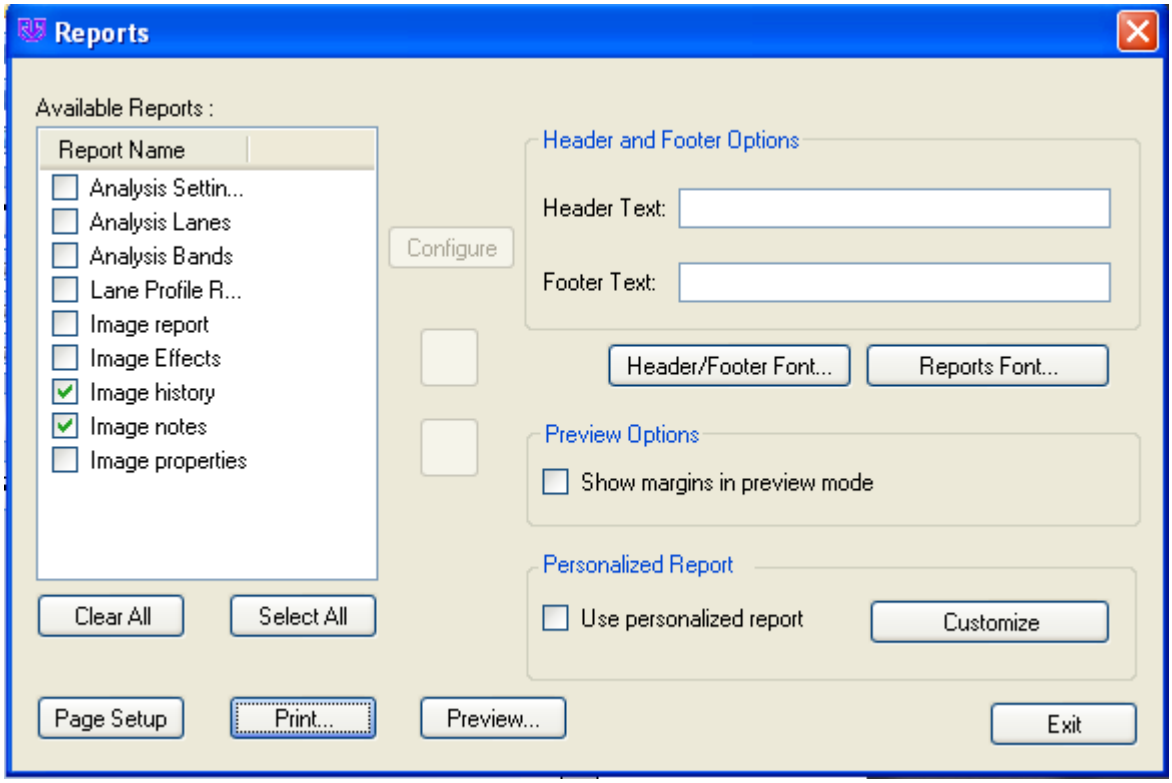
Print Image History

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.



View and Print a Report

- Choose **File > Print a Report** 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.

- Open an Image.
- Choose **File > Print**.
- If necessary, choose the target printer, paper size, paper source (tray) and page layout.
- Click **OK**.

Note: The **Print** command uses exactly the same format as the Image Report in Reports.

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.

Glossary

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.

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