iBright™ Analysis Software

Publication Number MAN0017843 Revision H00

iBright™ Analysis Software provides tools for analyzing digital images of electrophoresis gels, western blots, and colony counts. This software performs densitometry, molecular weight determination, relative and absolute quantitation, band purity analysis, and colony counting with the ability to adjust (for example, contrast, rotation, crop) images. The software is available in desktop and cloud versions.

Software installation guidelines

Follow the appropriate software installation instructions based on whether you are using a Windows™ operating system (PC) or Mac operating system (Mac).

Software installation, updates, and system requirements (PC)

System Requirements for PC	
System Configuration	Windows™ 8, 8.1, 10, or 11 with 64-bit[1] operating system
Minimum RAM	8 GB
Internet Connectivity	Required only for software download for installation and updates

[1] The 32-bit configuration is only supported up to version 4.0 of the iBright™ Analysis Software.

Downloading the Installer
Note: The user must log in as an administrator to install the software. **1.** Find the iBright™ Analysis Software installer at **thermofisher.com/ibrightanalysis**. **2.** Fill out the webform. **3.** On the webform thank you page, download the PC-specific installer (iBrightAnalysisSoftware.exe file).

Installation Process
1. Open the PC-specific iBrightAnalysisSoftware.exe installer file. **2.** Accept the terms and conditions (End User Licensing Agreement). **3.** Click **Install**. **4.** Launch the application from the shortcut created on the desktop.

Software Update	
If connected to the internet	When first opening the software, a window will appear indicating a new software version is available. Follow the prompts to install the new version.
If not connected to the internet	You will have to occasionally check to see if software updates are available. Go to **Help menu** ▸ **Check for Updates** and follow the prompts to install the new version.

Uninstallation Process for PC
Note: The user must log in as an administrator to uninstall the software. In the Windows™ control panel: **1.** Click **(Settings)** ▸ **Apps** ▸ **Installed apps**. **2.** From the list of apps/programs, find the **iBright™ Analysis Software** row. **3.** Right-click the **iBright™ Analysis Software** row and click **Uninstall**.

Software installation, updates, and system requirements (Mac)

System Requirements for Mac	
System Configuration	macOS™ 12: Monterey, macOS™ 13: Ventura, macOS™ 14:Sonoma
Minimum RAM	8 GB
Internet Connectivity	Required only for software installation

Downloading the Installer

1. Find the iBright™ Analysis Software installer at **thermofisher.com/ibrightanalysis**.
2. Fill out the webform.
3. On the webform thank you page, click the Mac-specific installer link to download the file.
4. Open the **Download** folder.
5. Double click the iBrightAnalysisSoftware.zip file to extract the contents.

Installation Process

Note: The user must log as an administrator to install the software.

1. Open the download folder and double click iBrightAnalysisSoftware.zip to extract the files.
2. Open the iBrightAnalysisSoftware.pkg file.

   Note: You will see a pop-up window if the Xcode™ software command line tool is not installed on your system. Accept the Xcode™ license and install the software.

   Note: For installation of macOS™ softwares, **right-click** on the iBrightAnalysisSoftware.pkg file and open.
3. Accept the terms and conditions (End User Licensing Agreement).
4. Click **Install**.
5. A prompt will ask for your password to install the application.
6. Launch the application from the **Applications** folder.

Software Update	
If connected to the internet	When first opening the software, a window will appear indicating a new software version is available. Follow the prompts to install the new version.
If not connected to the internet	You will have to occasionally check to see if software updates are available. Go to **Help menu ▸ Check for Updates** and follow the prompts to install the new version.

Uninstallation Process for Mac

Note: The user must log in as an administrator to uninstall the software.

1. Open **Finder**.
2. Select **Applications** from the left panel.
3. Right click **iBright™ Analysis Software** and select **Move to Trash**.

Remove Image Gallery

1. Open **Finder**.
2. Press **Command-Shift-H** to open the **Home** folder.
3. Delete the **iBright™ Analysis Software** folder.

Cloud-based iBright™ Analysis Software

Note: Google Chrome™ browser is recommended for use with the software.

The cloud-based iBright™ Analysis Software is hosted on Thermo Fisher™ Connect Platform (found at **https://apps.thermofisher.com**). The cloud application is not recommended for use with Internet Explorer™ or Edge™ browsers. If the application slows down, return to the gallery and refresh the browser.

Gallery

The gallery contains tools to manage your image files. You can import or export images, select images for editing and analysis, create reports, or delete images.

Import images

The software provides flexibility to import the proprietary .g2i files from the iBright™ Imaging Systems and E-Gel™ Power Snap Plus Electrophoresis System, .vit bitmap files from the E-Gel™ Power Snap Electrophoresis Device and 16-bit unmodified, uncompressed .tiff files from various sources.

To import .g2i or .vit files from the **Gallery** tab:

1. Click **Import ▸ Import .g2i files**.
2. Navigate to and select your image files.

   Note: More than one file can be selected at a time for import. A pop-up window appears showing the upload status of the selected files at the bottom right side of the **Gallery** screen.

Import a single tiff file

- Click **Import ▸ Import single tiff file**. Navigate to and select your tiff image files.

  Note: Only uncompressed, grayscale 16-bit tiff files can be imported.

  Note: Multiple files can be selected for import. A pop-up window appears showing the upload status of the selected files at the bottom right side of the **Gallery** screen.

Import multiple tiff files

1. **Import ▸ Import multiple tiff files**. Drag and drop or browse a folder with individual channel tiff images.

   Note: A maximum of 5 channel images using the same binning can be uploaded at one time. A combination of a folder and individual images is not possible in the software.

2. *(Optional)* Edit the channel name. By default, the file name is used as the channel name.
3. Select the image type from the dropdown menu.

   Note: The background is selected automatically based on the selected image type. Background selection can be changed manually by clicking on  **(White background)** or  **(Black background)**.

4. *(Optional)* Select a false color for the channel.
5. *(Optional)* Click ✕ **(Delete)** to remove a channel image.

6. *(Optional)* Click **Clear All** to remove all uploaded image channels.
7. *(Optional)* Edit the file name by clicking inside the text box with the default file name.

   Note: By default, the date and time of import is used as the file name.

8. Click **Apply** to upload the images and create a g2i file.

   Note: All imported tiff files can be viewed under the tiff import mode when filtering the gallery.

A window will appear showing the status of the imported images at the bottom of the gallery.

Import a g2i image using a designated folder in a network location (compatible with desktop software)

A folder on a local network drive can be set up to export images from an instrument and conveniently import them into the software. The folder needs to be created first, then the location defined in the software as specified below. All files to be imported should be located in this folder. Files within subfolders are not imported.

1. Click **Connect to a network drive**.
   When the software is installed for the first time, or no images are present in the **Gallery**, the **Connect to network drive** option will be displayed on the landing screen.
2. Click **Browse** to define the path in the network drive pop-up menu.
3. Select the desired folder location.
4. Click **Connect**.
   .g2i files present in the network drive folder will be synced with the software and displayed in the **Gallery**. The **Gallery** will be automatically refreshed after every 5 successful file imports. After all the files are synced, the total number of files imported will be displayed. In addition, any files that failed to import will be displayed in the import window in the bottom right corner.
5. Click (**Synchronize**) in the **Gallery** to sync the new files added after the initial network drive set up.
6. *(Optional)* Click **Edit network drive** to change the network drive location synced with the **Gallery**. Click **Change** and select the new network drive location.

   Note: All previously imported images are retained in the **Gallery**.

7. *(Optional)* Click **Disconnect** in the **Edit network drive** window to disconnect the network drive from the software.

   Note: Only one network location can be connected with the software. Upon reconnecting the previously disconnected location, or if the location was edited, then only new files from the last sync date will be synced. Older files will not be synced again.

Import images in the cloud

1. Click **Upload File** from the **Home** or **DataConnect** pages.

   Note: In the analysis application, you can go to the **Home** page or the **DataConnect** page by selecting in the left column.

2. Select your personal folder from the displayed options.

3. Click **Select folder** when needing to upload all of the files from a folder. Click **Select file** to import specific files.

4. Navigate to the folder or files to upload.

   Note: Files for upload cannot be in different folders.

5. Launch the software from the **Home** page to see the uploaded images in the **Gallery** (only .g2i and .vit file types are shown in the **Gallery**).

Manage your image gallery

By default, the image files are displayed in **(Grid view)**, and are sorted chronologically with the newest files on top.

From the **Gallery** tab:

- Click **(Grid view)** to view image thumbnails in the gallery.
  - In the left bottom screen, click on the small, medium, or large circles to change the size of the thumbnail view.
  - To sort images, click the specific option from the drop-down menu to sort images by **Date**, **Month**, **Name**, **Size**, **Mode**, or **Recently used**.
  - Click **(Up or Down arrows)** to reverse the order of the image files. If they are sorted chronologically, you can switch from oldest to newest, or newest to oldest. If they are sorted by name, you can reverse the alphabetical order (A to Z or Z to A).
- Click **(List view)** to view the image files in a table. Use the **(Up or Down arrows)** to reverse the numerical or alphabetical order of the **Date**, **Month**, **Name**, **Mode**, **Recently used**, or **Size** by selecting the option from the drop-down menu.

  Note: Changes to the display settings are retained by the image gallery on subsequent use.

- Click **> (Preview)** to right of the **List View** option to show or hide the **Image Preview**.

  The **Image Preview** shows both an enlarged view of the image, image information, and notes below the preview.

Note: Image information can also be accessed by clicking **(Information)** by the file name within the **Image Analysis** and **Annotation** tabs. The **File Name** and **Notes** sections are editable. Changes are saved upon exiting each section.

When **Image Preview** is on, hovering over a file record or thumbnail causes the corresponding image to display in the image preview pane.

- Right-click on a thumbnail or a row in the list view to access options to export a single image or analysis report for the selected image or delete a single file.

Search the Gallery

The image **Gallery** can be searched by words input into the search field at the top. The **Gallery** is updated in real time as the text is typed. Further search can be refined by using the following filters:

- Search by **Image Details**: Toggle on the section to view search parameters.
  a. Select the **HDR only** checkbox to search all of the HDR images in the **Gallery**. Only the HDR images will be searched for the added text in the search field.

  b. Select the **Notes** checkbox to search the added text in the notes of all the images.

- Search by **Date**:
  a. Select a date range (**From date** and **To date**) in the **Date** section.

  b. b. Click **Apply** to view images in the **Gallery** within that date range. The added text in search field will be searched only for images within the selected date range.

- Search by **Mode**:
  a. a. Deselect one or more modes to restrict search to the selected modes (**Chemi Blots**, **Protein Gels**, **Universal mode**, **Nucleic Acid Gels**, **Fluorescence Blots**, and **Tiff Import**). All modes are selected by default.

  b. Click **Apply**.

Note: The search function cannot use the following special characters:

- **Cloud version:** ` ~ ! @ # $ % ^ & * ( ) - + = [ ] \ { } | ; ' : " , . / < > ?
  The underscore _ character is the only usable character.
- **Desktop:** % and '

Note: All fields are mutually inclusive. At the bottom of the **Search** dropdown, the file names of all resulting images are displayed.

Images for selected modes will be displayed in the **Gallery**. The added text in the search field will be searched only in the selected modes.

Select images to add to tray

Adding images to your tray at the bottom of the window makes them available to Delete, Export, or move to the **Image Analysis** tab for analysis and annotation.

From the **Gallery** tab, in either the **(Grid view)** or **(List view)**:

- Click an image thumbnail or record to add the selection to your image tray at the bottom of the window.

  Note: Hover over an image in the tray, then click the **X** that appears to remove it from the tray. If you have more than 6 images in your tray, use the left and right arrows to scroll through them.

  Alternatively, click on a thumbnail in the gallery at any time to remove it from the tray.

- Click **Delete** to delete images in the tray.
- Click **Next** at the bottom of the screen, or click the **Image Analysis** tab.
- Double-click an image to add it to the image tray and open it in the **Image Analysis** page.

Export an image for publication

Images exported for publication are intended for use in print or digital publications. All image enhancements or adjustments made by the user are retained during export. Publication quality images are not recommended for downstream analysis. If you need to analyze images using third party software, see “Export an image for analysis” on page 10.

1. Export a single image or a batch of images.
   - Right-click over the image and select **Single Image Export**.
   - Select **Export ▸ Batch Image Export** to export all images in the image tray.
2. Select **Publication** to set parameters for publication quality image (24-bit color).
3. Use the menu to select **JPG**, **PNG**, or **TIF** as the file format for the image.
4. Use the menu to select **150**, **300**, **600**, or **Custom** to specify a resolution in DPI (dots per inch) for the image.

   Note: The custom resolution can be set from 72–1200 DPI except for E-Gel™ 96 gels where the resolution for image export is set to 300 DPI.

5. *(Optional)* Enter height or width value for image size. The corresponding value is automatically calculated based on aspect ratio of the image (shown below the **Image size** fields).

   Note: The default image size is set at 10 cm × 8 cm.

6. *(Optional)* Select the **Show Layers** checkbox if layers are desired.

   Note: Layers are only available when the image is analyzed and not added to composite images.

7. *(Optional)* Select **Show Markers** to include markers (where available) on exported images.
8. *(Optional)* Select **Show Annotations** if annotations are present and desired.
9. *(Optional)* Select the **Include Composite Image** checkbox to export composite image along with individual channel images.

   Note: This option is only available for multichannel images.

10. Click **Browse** by Export Directory and define a location for export.
11. Click **Export**.

Your image automatically downloads to your selected location as a folder. The approximate exported file size (or folder size for batch export) is shown in the bottom ribbon. This feature is also available for exporting grayscale mode and inverted images. The channels toggled on in the view port will be exported. The single-channel image downloads directly after single export.

Note: Changes made to the export selections are retained on subsequent use.

Export an image for analysis

1. Export a single image or a batch of images.
   - Right-click over the image and select **Single Image Export**.
   - Select **Export ▸ Batch Image Export** to export all images in the image tray.
2. Select **Analysis**.
3. Use the menu to select **g2i** as the file type if you plan on sharing the file.

   Note: .g2i files can only be analyzed using the iBright™ Analysis Software.

4. Use the menu to select **TIF** as the file format for the image to analyze the image using third-party software.

   Note: The image is exported as a 16-bit grayscale, non-adjusted TIF file.

   Note: When exporting for analysis, all channels in a multichannel image are exported as separate channels.

5. Click **Export**.

Your image automatically downloads to your selected location as a folder. The approximate exported file size (or folder size for batch export) is shown in the bottom ribbon.

Image Analysis

The **Image Analysis** tab contains image editing and analysis tools organized in different accordions.

From the **Image Analysis** tab:

- Select an image from the image tray at the left of the screen to view it in the viewport.
- Select ⓘ by the file name to access image information for image in viewport.
- Change the channel displayed in the viewport for a multichannel greyscale image using **<** or **>** by the channel name on top of the viewport or use controls according to “View and edit channels” on page 13.
- Change the channel selected for editing of multichannel composite images using **<** or **>** by the channel name on top of the viewport or use controls according to “View and edit channels” on page 13.

  Note: Only channels toggled on for display from the **View and Edit Channels** accordion can be changed from the top of viewport in color mode.

- Show or hide all layers using ⊠ on top of the viewport or show/hide layers individually using controls described in “View layers” on page 13.
- Use tools in **Zoom Controls** to adjust the display magnification according to “Use zoom controls” on page 12.
- Use tools in **Adjust Image** to straighten, crop, rotate, flip, or invert an image according to “Straighten, crop, rotate, flip, or invert an image” on page 12.
- Use tools in **Analyze Image** to perform analysis, edit analysis, and view/export data according to “View and edit channels” on page 13.
- Use tools in **View and Edit Channels** to switch the image display between color and greyscale modes, show/hide channels in composite image, select a channel for editing, change/apply false color, and apply image display enhancements according to “View and edit channels” on page 13 and “Adjust image display enhancements” on page 14.
- Use the **Save As** feature to create a copy of the file before editing to retain the original file.

  From the **Image Analysis** or **Annotate** tabs:
  - Right-click on the viewport and select **Save As**.
  - Click ✎ **(Edit)** to edit the copied file name.
  - Click **Apply** to save a copy of the image.

Note: Images adjusted and/or analyzed on the instrument retain those changes and are displayed as they were on instrument. Otherwise, by default:

- The first image in the image tray is selected for viewing and editing if no image was selected in the **Gallery** tray.
- Images from all modes are shown in color mode. No false color is applied to images other than fluorescent images and images captured in **Universal** mode with false color selected.
- All images are auto-contrasted for display. Fluorescent images are also auto-enhanced using background signal correction, but this correction is not applied to grayscale images. Analysis is performed on original images, so these corrections do not impact the raw data.

Use zoom controls

In the **Image Analysis** or **Annotate** tab, use Zoom Controls located on top of the viewport to adjust the display magnification.

- – Hide layers.
- – Pan around an image.
- – Use your mouse to select a portion of the image to zoom in on.
- – Zoom to fit.
- – Zoom out.
- – Zoom in.

Straighten, crop, rotate, flip, or invert an image

In the **Image Analysis** tab, use tools in the **Adjust Image** accordion to straighten, rotate, flip, invert, or crop an image.

- – Click **Straighten**, and crosshairs appear over your image. Use the slider at the bottom of the grid or the text field below the slider to rotate your image to a custom angle, then click again when complete.

WARNING! Straightening can alter pixel intensity values and impact data analysis.

- – Click **Crop**, adjust the region of the image to be cropped by dragging the crop markers, click **Apply**, and confirm your crop to eliminate unwanted area outside of the crop box.

  Manual adjustments performed on the image prior to cropping are preserved in the cropped version, but analysis results are not retained.

  The crop function creates a new image that is automatically added to your gallery and your image tray at the bottom of the screen. The file name of the cropped image is the same as the original file, but with the suffix **cropped** added. The original image remains in the image tray.

- – Click **Rotate**, then click **Rotate Left** or **Rotate Right** under the image to rotate your image by 90° increments.

- – Click **Flip**, then click **Flip Vertical** or **Flip Horizontal** under the image.
- – Click **Invert** to invert the image display. If false color is applied, they will be inverted as well. For a multichannel image, all channels in the image are inverted.

View and edit channels

In the **Image Analysis** tab, in the **View & Edit Channels** accordion:

- Click to turn the display of individual channels on or off.
  If a channel is selected for editing (see below), its display cannot be turned off.
- Click to select a channel to edit. By default, the first channel is selected for editing. The channel selected for editing can also be changed by using < or > on either side of the channel name on top of the viewport.
- Click **Grayscale** to convert and display a fluorescence channel in grayscale.

Note: An overlay of the membrane channel can be created by clicking in the grayscale for Chemi images. This feature is not available in any other mode. Only one channel is displayed in grayscale in Fluorescent and Universal modes. The channel selected for editing will be displayed. Selecting the color scale from the grayscale mode will revert the display back to the composite image, as seen before grayscale selection.

View layers

Note: Layers are analysis objects such as Frames, Lanes, Bands, or regions drawn on an analyzed western blot, protein gels, nucleic acid gels, or wells and colonies.

These functions are available for images that have been analyzed.

In the **Image Analysis** and **Annotation** tabs, click the **Layers** accordion:

- Click **Frames** to view a border around the lanes of your image.
  The frames are numbered from top to bottom and left to right in the viewport.
- Click **Lanes** to superimpose a line vertically down the middle of each band in your image.
  The lanes are numbered from left to right in the analysis frame.
- Click **Bands** to view band boundaries.
  The bands are numbered from top to bottom in each lane.
- Click **Regions** to view regions selected in your image.
  The regions are numbered based on the sequence in which they were created.
- Click **Annotations** to view added annotations on the viewport.
- Click **Markers** to view marker labels on the viewport.

- Click **Wells** to view the border around a group of colonies.
- Click **Colonies** to view colony boundaries.

Adjust image display enhancements

In the **Image Analysis** tab, under the **View & Edit Channels** accordion, in the **Image Display Enhancements** panel:

- Click the **Auto-contrast** checkbox to use the software to determine optimal signal-to-noise contrast.
- Click the **Show saturation** checkbox to highlight saturated pixels. The saturated pixels are displayed using white for fluorescent images in color mode and red in greyscale mode, as well as for other modes.

  Note: **Auto-contrast** and **Show saturation** are channel specific.

- Click the **Auto-enhance** checkbox to view the background-subtracted image display.

  Note: The **Auto-enhance** option is only available for fluorescent images.

Adjust display using the histogram

The histogram is a graphical representation of grayscale intensity, which is the proportion of image pixels with certain grayscale intensity. The vertical axis represents the number of pixels and the horizontal axis represents the pixel intensity.

When using the histogram, any adjustments made will only affect the selected channel, indicated by in the **View & Edit Channels** pane and the channel name shown on top of the viewport. Adjustments will affect the display only, and are not reflected in the data analysis.

In the **Image Analysis** tab, in the **Image Display Enhancements** panel:

1. Use the slider or the text fields below the histogram to designate the black and white signal intensity displayed.
2. Use the gamma slider to adjust the image midtones.
3. *(Optional)* Click **Auto-contrast** to reset default values.

Assign a color to a channel

In the **Image Analysis** tab, in the **View and Edit** accordian panel:

1. Click a color swatch between and the channel name to open the color palette.
2. Select a new color from the color palette below.

The image will update accordingly with the new color.

Analyze Image

The **Analyze Image** accordion contains tools to analyze images of western blots, gels, E-gels (agarose gels), cell-culture/bacterial colony plates, etc. The existing analysis can be deleted and new analysis performed using either sample-specific **Auto-Analysis**, **Templates**, or **Manual** mode.

Note: If analysis was performed on the instrument, the analysis will be retained and shown when the file is imported into the software.

Analyze an image using Auto-Analysis

Auto-analysis is performed on previously unanalyzed images when you move from the **Gallery** to the **Image Analysis** tab. When automatic analysis is performed, the signal in an image is automatically detected using a proprietary frame, lane, and band-finding algorithm measuring band intensities. Manual analysis can be performed on an image already analyzed in the **Automatic** mode.

- “Analyze an image using manual mode” on page 26
- “Edit frames” on page 15
- “Edit lanes and bands” on page 16
- “Perform molecular weight analysis” on page 19
- “View and export analysis data” on page 30
- “Normalize the data” on page 22

Edit frames

- To edit or draw a frame, go to **Image Analysis** ▸ **Analyze Image** ▸ **Western Blot/Edit Layers** ▸ **Frames**.
- If not done previously, click a frame to select it.
- Click — **(Delete)** under **Frames** in the **Edit Layers** panel.
  The frame and corresponding bands and lanes are deleted. You can now replace the frame with a new one.
- Click + **(Add)** under **Frames** in the **tools** panel.
- Use your mouse to draw a frame on the image to cover the area for analysis.
- To resize a frame, click + **(Add)** to deselect the "add frame" selection. Select a frame to resize and use any of the four corner "handles" to resize.
- Click **Apply** to analyze or **Cancel** to remove.

- *(Optional)* Click a frame to select, then click **Ctrl+C** (**Command+C** on Mac) followed by **Ctrl+V** (**Command+V** on Mac) to copy and past the frame for reuse.
  The pasted frames appear below the copied frame with a slight offset. If you are performing repeated pastes and the newly pasted region reaches the image boundary, then the new frame will be pasted over the last pasted frame.
- To move a frame, select the frame and drag it using the mouse or move it using the keyboard arrow keys.
- Select **Apply** to analyze the frame(s) or **Cancel** to remove all edits.
- *(Optional)* Click **(Skew)** and drag one of the handles on the frame to adjust the position.
  Skew handles can be moved up or down to create a curve if needed.
- Select **Apply** to analyze the frame(s) or **Cancel** to remove all edits.

The software automatically identifies and numbers the lanes and bands within the frame.

Edit lanes and bands

You can edit lanes and bands of an image in **Automatic** mode.

In the **Image Analysis** tab, click in the **Analyze Image** accordion, select **Automatic** mode, then click **Edit Layers**.

- Select a lane, then click **— (Delete)** under **Lanes** or click the keyboard **Delete** key to delete a lane.
  To select multiple lanes for simultaneous deletion, use your mouse and click-drag to draw a selection box around the lanes of interest. Alternatively, select individual lanes sequentially using **Shift + mouse right-click**.
- Select **+ (Add)** under **Lanes**, then click at the desired location within the analysis frame to add a new lane.
- Select a lane and click **(Skew)** to skew a lane, pull the handles on the lane to adjust alignment, then click again to deselect **Skew**.
- Select a lane and use the keyboard arrow keys to move the lane.
- Under **Bands**, click **+ (Add)**, then click at the desired location to add a band.
  A band is added. If a lane did not previously exist at the location, a new lane will also be added.
- Select a band, then click **— (Delete)** under **Bands** or click the keyboard **Delete** key to delete a band.
  To select multiple bands for simultaneous deletion, use your mouse and click-drag to draw a selection box around the bands of interest. Alternatively, select individual bands sequentially using **Shift + mouse right-click**.
- Select a band or multiple bands and resize using the corner handles.

- Move band(s) using keyboard arrows.
- Select **Apply** to analyze the adjusted bands and lanes or **Cancel** all edits. Use **Undo** to remove the last edit.

Band addition, deletion, or resizing is specific for each dye channel. Select a channel to make edits.

Use lane profile to adjust bands and perform rolling-ball background correction

Lane profile is the intensity profile plot of the selected lane. It is plotted with Intensity on the x-axis and pixel position on the y-axis. The chart also shows the non-specific background detected by the software in gray. By default, rolling-ball background subtraction is performed using an optimal rolling-ball radius produced by the software to generate a lane profile. This helps to more clearly view the band boundaries and more easily adjust the bands according to profile. Hovering over the profile graph provides values for average pixel intensity and relative front.

In the **Image Analysis** tab, click **Analyze Image** ▸ **Automatic**:

- Click on **View lane profile** at the bottom right of the **Edit Layers**.
- Select the frame and lane to view the profile. By default, frame 1/lane 1 is selected.
- Adjust a band (i.e., add, delete, move, resize). Changes will show in real-time within the lane profile.
- Click **Apply** to save changes.
- To change the default background subtraction, enter the rolling-ball radius. Click ↻ (**Reset**) to reset the rolling ball radius.
  Background from the frame will be subtracted and depicted in the profile. To use this background correction for analysis, click **Apply**. The data table will update to reflect data with the background correction applied.

  Note: By default, the rolling-ball radius changed for one frame will be applied on all frames and all channels. To apply rolling-ball radius change on a single frame and single channel, click **All Frames & Channels** in the tool area and then click **Apply**.

- You can show/hide the tool area in **Lane Profile** using ›. You can adjust the area of the image and lane profile screen by dragging right or left.
- *(Optional)* Toggle off **Background** to view the profile without the background subtraction.
- *(Optional)* Change the channel to view the profiles of lanes in other channels. No lane profile will be displayed for the TPN channel.

Background designation for signal detection

This feature allows the user to change background designation for signal recognition. When **(White Background)** is selected, the algorithm detects black signal on a white background. When **(Black Background)** is selected, the algorithm detects white signal on a black background. If the background of the image is manually changed, the image is reanalyzed accordingly.

Perform relative or absolute quantitation

Determine the relative quantity of signals

Assigning a band or volume region as a relative reference allows comparison of local background corrected volume of the reference area to all other bands and regions on the image. Performing this task normalizes all bands/regions to one band/region of interest. This is a useful tool for comparing experimental samples to a control, where fold-change is interpreted by data normalization.

Relative quantity is the ratio of the region or band volume divided by the relative reference region or band volume. This analysis can be performed in both **Automatic** and **Manual** modes, after you have selected one band or one region to use as a reference. See “Determine the relative quantity of signals” on page 18 for more information.

Relative quantitation is performed at the frame and channel level in **Automatic** mode, and at the channel level in **Manual** mode.

In the **Image Analysis** tab, in the **Analyze Image** accordion, click **Automatic** or **Manual**.

1. Click **Analysis**.
2. Click **Quantitate**.
3. Click **Relative** and select a channel for quantitation.
4. Click to select one of your regions or bands as the relative reference.
5. Select the desired volume type to use for quantitation (see “Select volume type for quantitation” on page 25) and click **Apply**.

Following selection of a relative reference, your data table will reflect relative quantities. The selected **R** has a value of 1.

Determine the absolute quantity of signals

Assigning several bands or volume regions as absolute references with known quantities allows volume (sum pixel intensity) quantity interpolation/extrapolation for all other bands and regions on the image. This is a useful tool for determining amount of a sample when at least two known quantities of sample are on the same image.

Absolute Quantity is the value obtained from the Quantity Curve. The analysis can be performed in **Automatic** or **Manual** modes. See “Determine the relative quantity of signals” on page 18 for more information.

In the **Image Analysis** tab, in the **Analyze Image** accordion, click **Automatic** or **Manual**.

1. Click **Analysis**.
2. Click **Quantitate**.
3. Click **Absolute** and select a channel for quantitation.
4. Click to select one of your bands or regions as the absolute reference.
   A **Standard** text field appears in the **quantitate** pane.

5. Select a unit of measurement for your standard from the drop-down menu by the test box for standard (mg selected by default).

6. Enter the quantity of your standard in the text field provided.

7. *(Optional)* Select one or more additional absolute standards as references, and assign their values as above.

8. Select the desired volume type to use for quantitation (see “Select volume type for quantitation” on page 25) and click **Apply**.

Your data table will reflect calculated quantities based on the standards designated.

Perform molecular weight analysis

Be sure to have the correct channel selected (channel name shown on top or viewport), indicated by , before designating a marker lane.

Note: Only one kind of marker (e.g., iBright™ Prestained Protein Ladder, BenchMark™ His-Tagged Ladder, etc.) can be applied per frame.

In the **Image Analysis** tab, click **Analyze Image** ▸ **Automatic**:

1. Click **Analysis**.

2. Click **Markers**.

3. Click the marker lane in your image to select it.
   The selected marker lane and bands turn yellow (or blue if the image has a light background). The marker list will show the last 3 markers used with a custom marker list and a default marker list.

4. Select a marker from the 2nd dropdown menu.
   See “Add a custom marker to your analysis” on page 20 for information how to create a custom marker.
   The software automatically designates standard sizes to bands, according to the marker selected, in descending order. If the software recognizes more bands than there are standards, it will exclude the smallest size bands. If there are fewer bands than standards, the smallest standards are left off. Band assignment happens in descending order.

5. *(Optional)* Select and deselect extra bands from the list of markers.
   The deselected bands no longer display yellow. In case the software detects more bands than available standards, click on the extra bands in **Image** to deselect them.

6. *(Optional)* Adjust the marker designations or click **X** next to any band incorrectly designated a marker band.

7. Click **Apply**.

You can now view your data as a graph (see “View your data as a graph” on page 21).

Add a custom marker to your analysis

In the **Image Analysis** tab, in the **Analyze image** accordion, under **Automatic**:

1. Click **Analysis**.
2. Click **Markers**.
3. Click **Add/Edit Custom Markers** and then click **Add** to create a new marker.
4. Enter a custom marker name. Use only alphanumeric characters.
5. Select **Protein**, **DNA**, or **RNA** from the **Marker type** drop-down menu.
6. Select the units for the marker, depending on marker type.
7. Select the number of marker values.
8. Enter a marker weight for each band, and click **Save**.
9. Click **X** to exit the custom marker creation/editing workflow.

Your custom marker will be added to your list of custom markers, and can be selected and applied to your image. See “Edit or delete a custom marker” on page 20 for more information.

Edit or delete a custom marker

In the **Image Analysis** tab, in the **Analyze Image** accordion, under **Automatic**:

1. Click **Analysis**.
2. Click **Markers**.
3. Click **Create/Edit Custom Markers**.
4. Click  to select a set of custom markers in the **Custom Markers** list.
   (Optional) You can delete the marker here by clicking **Delete**, then confirming deletion.
5. Edit marker parameters such as **Name**, **Marker type**, **Units for this marker**, or **Number of marker values**.
6. Adjust designated marker weights as necessary.
7. Click **Save**.

View your data as a graph

Once you have assigned marker bands to your image (see “Perform molecular weight analysis” on page 19), you can view your data as a graph.

In the **Image Analysis** tab, in the **Analyze image** accordion, under **Automatic**:

1. Select **Analysis ▸ Marker** from the drop-down menu.
2. Click **Graph**.
   The graph opens. Select a lane on the image to view the corresponding data on the graph. The chosen regression method will be saved as a preference for the next marker analysis.
3. Select a lane or band on the image to view the corresponding data on the graph.
4. *(Optional)* Select **Regression ▸ Linear** or **Linear Semi Log** to change the regression method used. View the **R-squared Value** to determine the "goodness of fit” for the chosen regression method.
   See “Regression methods” on page 21 for more information.
5. Click **Apply** at the bottom of the chart area to apply the new regression method selected.
6. Select another frame in the image to see the graph for that frame. Change the channel using the drop-down menu above the chart.
7. Click **Apply** to save changes or **Cancel** to remove the changes.
8. *(Optional)* Click **Data** to view the data table.

Click **X** at the top of the screen to return to the to return to the **Marker Analysis** screen.

Regression methods

Point To Point – This regression method determines the slope of the line between two markers. Therefore, no single equation and no R^2 term are available for this method. The log of the molecular weight values of the markers is used to generate the slope between each pair of markers.

Linear - This regression method determines molecular weight by a linear equation: y = ax + b, where y is the molecular weight, a is the slope of the line, x is the relative front (R_f) and b is the intercept. This linear equation is calculated with the log of the molecular weight values of the markers. This method minimizes the sum of squared differences between an observed value and the fitted value provided by a model.

Linear Semi Log – The linear equation is y = ax + b, where y is the molecular weight, a is the slope of the line, x is the relative front (R_f) and b is the intercept. This linear equation is calculated with the log of the molecular weight values of the markers.

Note: In some cases the calculated molecular weight varies from visual estimation of the molecular weight based on comparison to the two nearest markers. In such a case, changing to the point-to-point regression method results in calculated molecular weights consistent with visual estimation.

Normalize the data

Normalization is an analytical method wherein data within a set is mathematically corrected for experimental variability, sample loading, and transfer efficiency using an internal control. The internal control can be a housekeeping protein (HKP) such as actin, tubulin, or GAPDH protein that is present in all samples or total lane protein. The total lane protein-based normalization is abbreviated as TPN and uses the sum of the signal intensity in the lane for calculation of normalization factors. Ideally, the internal control should be detected on the same blot as the target protein. Use of HKP for normalization is only recommended under conditions where the expression of HKP does not change with experimental treatment.

Normalization is performed in a multi-step process:

1. Identify the method for normalization (HKP or TPN).
2. Determine normalization factors for each lane.
   Normalization factor for lane equals the **(Observed signal of housekeeping protein or total protein for each lane) divided by the (Highest observed or user-selected, background-corrected volume intensity of housekeeping protein or total lane protein)**.
3. Divide all of band intensities within a lane individually by the normalization factor for their respective lanes.
4. The calculated normalization factors are displayed in the tool area with the data type (volume or background-corrected volume) also indicated.
5. *(Optional)* Change the data used for calculation of the normalization factor by clicking on the **Volume** type used (blue text) and change the volume type from the pop-up screen and apply. Normalization factors are updated to reflect the changes.

Normalize using HKP

In the **Image Analysis** tab, in the **Analyze image** accordion, under **Automatic**:

1. Click **Analysis**.
2. Click **Normalize**.
3. Click **HKP** (HKP is selected by default).
4. Select the channel containing normalization controls (e.g., loading controls).
5. Select a band as a normalization control and select **Apply**.

   Note: A horizontal line appears over the selected band. All the bands falling on this line are automatically designated as normalization controls. If the automatic band selection is inaccurate, select or deselect bands manually.

6. Select a band to act as a reference and select **Apply**.
   A normalization factor is generated, and bands are normalized against this factor.

7. HPK Normalization is allowed across multiple frames. After confirming the reference band, select the frame(s) that need to be normalized using the defined loading controls above. Select **Apply** to confirm normalization. The frames that were normalized and the type of volume data used for normalization are shown in the tool area.

8. Change the volume data used for normalization by clicking on the volume type displayed in the tool area (see details on page 25).

9. *(Optional)* The normalization performed on all frames in that image can be cleared by selecting **Clear**. Normalization on individual frames can be cleared by selecting **X** on the side of the frame in the tool area.

10. *(Optional)* Normalization can again be performed on the frames for which it was cleared (see steps 5–7).

11. To apply the normalization factors to other images in the tray, click on **Select File** in the tool area.

    Note: Only the files that were previously analyzed, and have the same number of frames and lanes can be selected. Images analyzed for colony, E-Gel™ 96, and HKP cannot be selected.

12. Select the file(s) from the pop-up window to apply the normalization factor.

    Note: Images can be added from the gallery by clicking on **Add images from Gallery** in the pop-up window. A confirmation message will appear to exit the current workflow.

13. Click **Apply** to normalize the selected images.
    The software will automatically normalize all of the bands of the selected image(s) using normalization factors from the reference image. The normalization factors and reference image are shown in the tool area of the normalized image. Normalized data is then available in the data table.

14. *(Optional)* To edit normalization factors and reapply to the previously applied images, add the reference image and the applied images to the tray. Edit the reference image as needed and perform HKP across the files again (see steps 11–13).

Normalize using Total Lane Protein (TPN)

In the **Image Analysis** tab, in the **Analyze image** accordion, under **Automatic**:

1. Click **Analysis**.

2. Click **Normalize**.

3. Click **TPN**.

   Note: If a channel was designated as TPN during image capture, software will automatically perform TPN for the frame using that channel as control channel. The lane with the highest background-corrected volume will be used as the reference lane. User can change the reference lane later.

4. If a channel was not designated as TPN during image capture, select the channel containing total lane protein stain (e.g., No-Stain™ Protein Labeling Reagent) and apply.

   Note: Software will automatically perform TPN using the selected channel as a normalization control. The normalization factors will be displayed in the tool area. The normalized data for bands in all other channels is shown in the data table. To apply the generated normalization factors to another image, go to step 10.

5. *(Optional)* Drop lanes that you don't want normalized by selecting + on top of the lane.

6. *(Optional)* Change the reference lane (shown in red) by double clicking on a different lane. The new lane will now be designated as the reference lane.

7. *(Optional)* Increase or decrease the volume of the lane used for analysis by using the handles provided.

8. *(Optional)* The normalization performed on all frames in that image can be cleared by selecting **Clear**. Normalization on individual frames can be cleared by selecting X on the side of the frame in the tool area.

9. *(Optional)* Normalization can again be performed on the frames for which it was cleared by using **Apply TPN Option** under that frame in the TPN workflow.

   Note: Data is only updated after making the changes, then selecting **Apply**.

10. To apply the normalization factors to other images in the tray, click on **Select File** in the tool area.

    Note: Only the files that were previously analyzed and have the same number of frames and lanes can be selected. Files used to generate TPN normalization factors cannot be selected.

11. Select the file(s) from the pop-up window to apply the normalization factor.

    Note: Images can be added from the Gallery by clicking on **Add images from Gallery** in the pop-up window. A confirmation message will appear to exit the current workflow.

12. Click **Apply** to normalize the selected images.
    The software will automatically normalize all of the bands of the selected image(s) using normalization factors from the reference image. The normalization factors used, and the reference image, are shown in the tool area of the normalized image. Normalized data is then available in the data table.

13. Change the volume data used for normalization by clicking on the volume type displayed in the tool area (see details in "Change volume data used for normalization" on page 25).

14. *(Optional)* To edit normalization factors and reapply to the applied images, add the reference image and the previously applied images to the tray. Edit the reference image as needed and perform TPN across the files again (step 10-step 12).

    Note: If the applied image is not present in the tray, it can be added by clicking **Applied Images** in the tool area of the reference image and clicking the image thumbnail in the pop-up window.

Change volume data used for normalization

Volume type selection for Normalization:

In the **Image Analysis** tab, in the **Analyze image** accordion, under **Automatic (Auto analysis/manual frame) ▸ Normalize**:

- Perform HKP/TPN according to “Normalize using HKP” on page 22 and “Normalize using Total Lane Protein (TPN)” on page 23.
- Click on **Volume type** link from the tool area to change the volume type from the local bg. corr. volume.

  Note: Default volume for the TPN channel is rolling ball bg. corr. volume.

- Select any volume or local bg. corr. volume or rolling ball bg. corr. volume.
  - The **Select Volume** pop-up is having all of the frames and channels and volume drop-down for all channels.
  - You can select different volumes for different channels for a single frame.
  - For frames where normalization is not performed, volume selection is not there.

    Note: Message should be displayed under frame number (e.g.,TPN analysis not performed).

- Click **Apply**.
  - The data is recalculated for changed channel/frame based on volume selected and reflected in data table.
  - If the selected background correction is deleted, then by default data gets recalculated using default volume (local bg. corr. volume).
  - If normalization is performed on a membrane channel, then data will be present for the membrane channel in the **Select Volume** pop-up and data table.
  - Volume selection is frame- and channel-dependent.

Quantitation

The **Quantitate** function contains tools for performing relative and absolute quantitation of signal. These functions are enabled when a band or region is selected on the image.

Select volume type for quantitation

The volume type can be selected for Relative and Absolute quantitation.

In the **Image Analysis** tab, in the **Analyze Image** accordion, click **Automatic or Manual ▸ Quantitate**.

1. Click **Relative/Absolute**.
2. Click on **Band** or **Region** to select as a reference or standard.

3. Click on the **Volume type** drop-down menu in the tool area.

   Note: By default, the local bg. corr. volume is selected.
   Volume type options include:
   For **Automatic** mode:
   - Volume
   - Local bg. corr. value
   - Rolling ball bg. corr. volume
   - HKP normalized volume
   - TPN normalized volume

   For **Manual** mode:
   - Volume
   - Local bg. corr. volume
   - Global bg. corr. volume

   The various volume options will be present only when a respective workflow is performed.

4. Select volume and click **Apply**.

Analyze an image using manual mode

When you are interested in analyzing only a portion of your image, **Manual** mode allows you to manually single out the signals you are interested in for analysis.

Note: **Manual** mode and **Automatic** mode can be used simultaneously on the same image.

In the **Image Analysis** tab, in the **Analyze Image** accordion, click **Manual**.

1. In the **Manual** pane, select **Regions**.
2. Select ▭ or ◯ to add a square or round region.
3. Using your mouse, draw borders around the signal to create a region.
   Multiple regions can be drawn at a time.
   The software labels these regions **V1**, **V2**, and so on.
4. *(Optional)* Click a region to select it, then use **Ctrl+C** (**Command+C** for Mac) followed by **Ctrl+V** (**Command+V** for Mac) to copy and paste the region for reuse.
   The pasted regions appear below the copied region with a slight offset. If you are performing repeated pastes and the newly pasted region is out of view, zoom out the image to access them.
5. *(Optional)* To remove a region, click to select it and then press **Delete** or **Backspace** (**+ fn** for Mac) on your keyboard.
6. Using your mouse, move the regions to desired locations.
7. Click **Apply** to confirm the actions on the regions.

You can turn the visibility of regions on or off by clicking **Regions** in the layers pane.

Line Plot

Line Plot is a quick analysis tool that measures the intensity of the signals along a drawn line, box, or segmented line. The line graph is the intensity profile plot of the selected line. It is plotted with Intensity on the y-axis and pixel position on the x-axis. For boxes, the graph uses the average pixel intensity instead of the pixel intensity.

In the Image Analysis tab, click **Analyze Image ▸ Manual**

- Click **(Line Plot)**.
  a. Select **(Line)** to draw a line on the image.
  b. Select **(Segmented line)** to draw a segmented line on the image.
  c. Select **(Box)** to draw a box on the image.
- Click **Apply** to view the respective plots. Label and pixel intensity will display when hovering over a plot line and the line plat graph is automatically saved.
- *(Optional)* Deselect the line label from the tool area on the right side to hide the respective plot.
- *(Optional)* Click **(Edit)** to rename a line, segmented line, or box.
- *(Optional)* Click **(Delete)** to delete a line, segmented line, or box.

Note: All straight lines, segmented lines, or boxes can be deleted by clicking on **(Delete)** at the section level in the tool area or by selecting the line/shape in the viewport and clicking **Delete** on the keyboard.

- *(Optional)* Show legends ( ) or adjust the range of the X- and Y-axis ( ) by using the tools above the line plot.

Note: The **(Show legend)** option is selected by default. Legends are displayed under the line plot.

Note: Adjust the X- and Y-axis ranges by selecting **(Plot range)** and edit the values in the **Plot range** accordion below the plot area. Click **Update**.

Note: Default X-axis values are 0 to a maximum distance (in pixels) of the line/segmented line/box drawn for all enabled channels. The default intensity values are from minimum pixel intensity to highest intensity seen for all the overlayed plots.

- *(Optional)* Click **Reset** to update values to the default values.

Generate a report for a Line Plot

A line analysis report can be exported.

1. In the **Line Plot** workflow, click **Export ▸ Export Line Plot report**.
2. Select or deselect the following features to be included in the report:
   - **Image**
   - **Channel overlay plot**
   - **Single channel plot**
3. *(Optional)* Deselect **Image details** to remove details from your image from the report.
4. Click **Preview**.
5. Click **Print/Download**.

The line plot report is created as a .pdf document and can be saved to any location with the required name. Retaining the .pdf extension is essential for proper opening of the file. Changes made to the settings are retained on subsequent use.

Perform global background correction using manual analysis

The software performs automatic local background subtraction. The raw data and the local background-corrected data are automatically displayed in the Analysis Table. Global background subtraction method is also available using **Manual** mode and rolling ball background correction (see lane profile) using **Automatic** mode.

- Local background subtraction – Local background subtraction is automatically performed by the software. The average intensity of the 5 pixels surrounding the border of each band or region is used to calculate the intensity within the band or region attributed to background. This value is subtracted from each pixel intensity within the band or region, resulting in **Local Background Corrected Volume** and **Density** values in the Analysis Table.
- Global background subtraction – Global background subtraction designates a region as background to be subtracted from volume regions. There are two methods to activate global background subtraction: (1) select a background region and then associate with volume regions or (2) select volume regions or bands to then associate with a background region. Global background subtraction allows designation of multiple background regions for association with a subset of volume regions on an image. For example, when analyzing an image with uneven background, it may be necessary to designate more than one background region for subtraction from certain volume regions of interest.

  Note: For accurate global background correction, designate a background region that is equivalent in size (pixels) to the region of interest.

In the **Image Analysis** tab, in the **Analyze Image** accordion, click **Manual**.

1. Click **Manual ▸ Analysis**.
2. Click **Background Subtraction**.

3. Click **Global**.
4. Select region(s) to be designated as background.
5. Right-click and select the appropriate choice (to designate as background).
6. Select regions that you want to associate with the background region. You can select more than one region by clicking the lasso, then **Shift**, and then selecting the region.
7. Select background region.
8. Right-click and select associated regions from the drop-down options to create a group of the selected regions.
   The selected background region will be used to correct the background of all the associated regions in that group, and data will be available in the data table.
9. *(Optional)* Follow steps 6-8 to create more groups.

Note: Each group can have only one background region.

- Rolling ball subtraction - Rolling ball background subtraction designates a ball of a specific radius that can roll over the selected lane profile and subtract the background. The smaller the rolling ball radius, the more the background subtraction. The background-corrected image can be used for further analysis.
  In the **Analyze** tab of the **Auto-analysis** window:

In the **Image Analysis** tab, in the **Analyze Image** accordion, click **Automatic ▸ Edit Layers**.

1. Click on **View lane profile** at the bottom of **Edit Layers**.
2. Select the frame to apply the rolling ball. Frame 1 is selected by default.
3. Enter the rolling ball radius in the text field below the lane profile or increase the radius using the slider.

Note: An optimal rolling-ball radius generated by the software is applied on all frames of the images by default. Changing rolling ball for one frame and applying changes the value for all frames in all channels. To apply rolling ball on a single frame, single channel, uncheck **All Frames & Channels** in the tool area and then apply. Once selected, the Apply button will be disabled until the rolling-ball radius is changed.
You can reset the radius to the default size at any time using **(Reset)**. After reset, the edited radius size will not be applied for analysis until you click on **Apply**.

View and export analysis data

The data table displays image analysis data, including pixel intensity and density, background subtracted data, retention factor (R_f), molecular weight (MW), relative and absolute quantities, normalization, and band purity. Only available data is displayed. Use the Data Table icon to show or hide the data columns. Changes made to the settings are retained on subsequent use.

In the **Image Analysis** tab, in the **Analyze image** accordion:

1. Click **Data**.
   A table with available data opens and the image appears in the left panel. Use the dropdown menu to select columns. The table displays the following data:
   - Name – Name of the data point or grouping.
   - Vol. (Int.) – Volume – Equals the sum of each pixel grayscale intensity in the region or the band. The units of Volume are intensity. All pixel intensities are displayed as they would be for a 16-bit image.
   - Area – The number of pixels within each region or band.
   - Density – The average intensity per pixel or Volume divided by Area with units of Intensity/Area.
   - Mol. Wt. – Molecular Weight – Weight assigned to bands per the applied marker
   - Rel. Quant. – Relative Quantity – Ratio of the region or band volume divided by the relative reference region or band volume.

     Note: You can use the following volumes for bands: (w/vol), (w/LB corr. vol), (w/HKP Norm. vol), (w/TPN vol), and (w/RB corr. vol). For regions: (w/LB corr. vol), (w/vol), and (w/Global bg corr. vol)
   - Abs. Quant. – Absolute Quantity – Quantity value obtained from the Quantity Curve. The asterisk (*) changes to the mass unit selected from the Units drop-down menu in the Quantity subtab.

     Note: You can use the following volumes for bands: (w/vol.), (w/LB corr. vol.), (w/HKP Norm. vol.), (w/TPN vol.), and (w/RB corr. vol.). For regions: (w/LB corr. vol.), (w/vol.), and (w/Global bg corr. vol.).
   - HKP (Housekeeping Protein) Norm. Factor – HKP Normalization Factor – Background-corrected volume of the control band divided by the background-corrected volume of the reference band.
   - HKP Norm. Vol. – HKP Normalization Volume – Background-corrected band volume divided by the HKP normalization factor.
   - HKP Norm LB Corr. Vol. – HKP Normalization Local Background Corrected Volume – Local background-corrected volume of a band divided by the HKP normalization factor.
   - HKP Norm RB Corr. Vol. – HKP Normalization Rolling-Ball Corrected Volume – Rolling ball-corrected volume of a band divided by the HKP normalization factor.
   - Local Bg. Corr. Vol. – Local Background Correction Volume – Volume minus the local background intensity. This value is automatically calculated for each band or region and cannot be disabled.

- Local Bg. Corr. Den. – Local Background Correction Density – Local Background Corrected Volume divided by the Area.
- Global Bg. Corr. Vol. – Global Background Correction Volume – Volume minus the global background defined area intensity.
- Global Bg. Corr. Den. – Global Background Correction Density – Global Background Corrected Volume divided by the Area.
- Rolling-ball Bg. Corr. Vol. – Rolling-Ball Background Correction Volume – Band intensity minus the rolling-ball background.
- Rolling-ball Bg. Corr. Den. – Rolling-Ball Background Correction Density – Rolling-ball Bg. Corr. Vol. divided by the area.
- TPN Factor (w/RB Corr. Val) – Total Protein Normalization Factor – Background-corrected lane volume divided by Backgound-corrected lane volume of reference lane.
- TPN Factor (w/Vol.) - Total Protein Normalization Factor – Lane volume divided by lane volume of reference lane.
- TPN Volume – Total Protein Normalization Volume – Volume of a band divided by normalization factor for that lane.
- TPN LB Corr. Vol. – Total Lane Protein Local Background Corrected Volume – Local background-corrected volume of a band divided by normalization factor.
- TPN RB Corr. Vol. – Total Lane Protein Normalization Rolling-Ball Corrected Volume – Rolling ball-corrected volume of a band divided by normalization factor.
- % Purity (Band) – A band's local background-corrected volume divided by the local background-corrected volume of all bands in the lane (in that channel) multiplied by 100. If rolling-ball subtraction has been engaged, then the % Purity (Band) is the band's rolling-ball background-corrected volume divided by the rolling-ball background-corrected volume of all bands in the lane (in that channel) multiplied by 100.
- % Purity (Lane) – A band's volume divided by the volume of the entire lane (in that channel) multiplied by 100. If rolling-ball subtraction is engaged, then the % Purity (Lane) is the band's rolling-ball background-corrected volume divided by the rolling-ball background-corrected volume of the entire lane (in that channel).

2. Select **Export** ▸ **Export Data** ▸ XLS to export the table data as an .xls file.

Switch sample type for analysis

Many different sample types can be imaged on the imager, including western blots, protein gels, nucleic acid gels, high-throughput nucleic acid gels, such as E-Gel™ 48- and 96-well agarose gels, and cell culture plates. Samples such as E-Gel™ 96 and cell culture plates use specialized algorithms for analysis, therefore sample definition is required for accurate analysis.

The sample type for analysis can be changed by selecting an accurate analysis option in the tool area. Below are the supported analysis options:

- **Western Blot**: The analysis algorithm is optimized for frame/lane/band identification.
- **Gel**: The analysis algorithm is optimized for frame/lane/band identification of protein gels.
- **Colony**: The analysis algorithm is optimized for identification of wells and colonies.
- **E-Gel 96**: The analysis algorithm is optimized for identification of wells and bands in 96-well E-gels and analysis followed by deconvolution of image and data
- **E-Gel 48**: The analysis algorithm is optimized for identification of wells and bands in 48-well E-gels through default templates.

Analyze high-throughput E-Gel™ gels

High-throughput E-Gel™ gels are available as E-Gel™ 96 gels and E-Gel™ 48 gels.

- E-Gel™ 48 gels contain 52 wells arranged in two rows with 26 wells per row. Default E-Gel™ 48 gel templates are provided for analysis of E-Gel™ 48 gels.
- E-Gel™ 96 gels contain 104 wells arranged to maximize space. The software provides a deconvolution feature to rearrange the wells into 8 x 13, 4 x 26, and 2 x 52 formats for easy visualization and export. E-Gel™ 96 gels can be analyzed manually or through an auto-analysis feature by applying a default E-Gel™ 96 gel template.

Note: The Auto Analysis option draws a default 48-well template on the image, but cannot auto analyze E-Gel™ 48 images.

Note: To use default templates for E-Gel™ images, delete the existing frames, then click **Template** and select the appropriate default template from the **Choose Template** dropdown menu. If the image was captured using an E-Gel™ Power Snap instrument, then select a Power Snap default template. If the image was captured using an iBright™ Imaging System, then select an iBright™ default template.

Auto analyze E-Gel™ 96 gels

1. Select one or more E-Gel™ 96 images in the Gallery to add to the image tray.
2. Navigate to the **Image Analysis** tab and click on the image you want to analyze in the image tray. The image appears in the viewport.
3. Select **E-Gel™ 96** in **Automatic** mode from the **Analyze Image** accordion menu. If you want to reanalyze a previously analyzed gel, then select the frame in the viewport and delete it from the **Edit Layers** tab in the **Analyze E-Gel™ 96** accordion menu.

4. Select **Auto Analysis** to visualize the frame, lanes, and wells on the image.

5. *(Optional)* Select and move the wells, lanes, or frame using the arrow keys to ensure the wells are correctly identified.

6. Click **Apply** to see the bands on the gel. The identified wells and any detected band within the wells are removed.

7. Select the bands and move (using arrow keys), resize, or add and delete from the **Edit Layers** tab to ensure that the bands are identified correctly. Click Apply to save the settings.

8. Click the data to see a non-deconvoluted data table. The data table displays the volume, background-corrected volume, and area of each band detected in the gel. Click **X** to exit the data table.

   Note: The bands in the data table are numbered in sequence from the wells of the top row, disregarding the internal wells. To identify the migration of bands from each well of the gel, generate a deconvoluted image and data table as mentioned in subsequent steps.

9. Deconvolute the E-Gel™ 96 image.
   a. Deconvolute the image by clicking **Deconvolute** in the **Edit** layer. The viewport shows lane boxes on the gel image. You can select and shift the lane boxes for visual clarity. This does not affect calculated data.

   b. To see a preview of the deconvoluted image and data, navigate to **Analysis ▸ Deconvolute**.
      - Select the deconvolution format: **8 x 13**, **4 x 26**, or **2 x 52**.
      - Type an image label.
      - Select the file type from the drop-down menu.

   c. Preview the deconvoluted image and data by clicking **Preview**. By default, the lane boxes and bands are displayed on the deconvoluted image. If desired, you can remove them by unchecking the layers.

   d. Export the deconvoluted image and data table by clicking **Export**. Choose the desired folder and click **Save**.

Templates

The **Template** option is present in unanalyzed images under **Western Blot/Gel/E-Gel 96/Colony** in automatic and manual modes in the **Analyze Image** accordion.

In the **Image Analysis** tab, click **Analyze Image ▸ Automatic or Manual**:

- Click **Template**.
- Select **Existing** or **Custom**.
- Select a saved template from the **Existing** drop-down menu OR create a custom template using **Custom**.

Note: Frame and lane editing options are present in existing and custom panes.

Create an analysis template

An option to **Save as Template** is available in the template workflow and can be accessed by right-clicking in the viewport anywhere in the **Image Analysis** tab.

In the **Image Analysis** tab, click **Analyze image ▸ Automatic/Manual**:

- Click **Save as Template** in the Template (existing/custom) workflow or right-click in the **Image Analysis** viewport.
- Select the required options from the **Save as Template** window:
  - Name of template (editable)
  - Auto analysis/Manual analysis
  - Workflows

  Note: Workflows will be enabled only if they are performed on the image (other workflows will be disabled if not performed).
- Click **Apply** to save the template.

Apply a template

In the **Image Analysis** tab, click **Analyze image ▸ Automatic or Manual**:

- Click **Templates**.

  Note: The Template option is only available when no prior analysis is present.
- Select the template from the **Existing** drop-down menu.
- Select the desired options from the **Save Template** window.

  Note: Frames and lanes will be selected in automatic analysis, and regions will be selected in manual analysis. (Once band is selected in the pop-up window, you can select workflows.)
- *(Optional)* Select the template drawn in the window and press **Ctrl+C** (**Command+C** on Mac) in the viewport. Press **Ctrl+V** (**Command+V** on Mac) to paste a copy of the template in the viewport. The copied template can be dragged and dropped on any other part of the image.
- Click **Apply** on the viewport to apply the template.
  You can view the analyzed data as per workflow selection in the respective workflow panes.

Edit a template

Once the template is drawn on the viewport, the template can be edited before applying.

- Click on **Frame/Lane/Region** to move on the viewport.

  Note: Moving the template will not delete any analysis data.
- *(Optional)* Resize the frames/regions by using the four corner handles.

- *(Optional)* Skew the frame/lane using the toolbar option.

  Note: Skewing and resizing frames deletes bands and respective analysis data. Lanes will be retained.

- Click **Apply**.
  - View the analyzed data as per the workflow selection in the respective workflow panes OR click **Cancel** to remove the template added.

Delete a template

To delete a saved template from the existing template list:

1. In the **Image Analysis** tab, click **Analyze image ▸ Automatic or Manual**. Click **Templates**.
2. Click the delete icon next to a template name from the existing drop-down menu.
   A prompt appears in the viewport asking to confirm the delete template action.
3. Click **Yes** to delete the template from the existing template list.

Use default templates

The software provides default templates for 6- and 12-well plates. To access the templates:

1. Select a colony tab. Images not analyzed will display the **Template** icon ( ). If Automatic analysis is present on the image, then go to the **Automatic** tab and click **Edit Layers ▸ Frames**. Delete the frame and click **Apply** to access the **Template** icon ( ).
2. Click **(Templates)** and choose a 6- or 12-well default template from the **Choose Template** drop-down menu. The template is drawn in the viewport.
3. Resize or move the template in the viewport by clicking and dragging the rectangular boundary around the wells. Individual wells can be moved or resized after selection.
4. Once the template is correctly positioned on the image of the multi-well plate, click **Apply** for colony detection.

Analyze cell culture or agar plates

The cell culture or agar plate analysis uses a specialized algorithm to detect circular spots and is only available when no Automatic analysis is present on the image. Each well within a multi-well cell culture plate is detected separately and the signal within each well is analyzed and grouped under that well. A well behaves similar to a frame in Frames/Lanes/Bands analysis and a colony is similar to a band.

Note:

- Wells will be labeled as W1, W2, W3, etc. starting at the top left side of the plate. Colonies will be labeled as 1, 2, 3, etc. and are displayed with the mouse cursor hovering over the colony. Colony numbering will be per well per channel.
- Wells are common across channels. Identified colonies are channel specific.

If Automatic analysis is present on the image, then go to the **Automatic** tab, then click **Edit Layers ▸ Frames**. Delete the frame and click apply. This will remove all previous automatic analysis associated with that frame. Remove all frames present on the image. In the **Image Analysis** tab, in the **Analyze Image** accordion, click **Automatic**.

1. Select the **Colony** tab.
2. Click **Auto-analysis** to automatically detect well(s) and colonies within the well.
3. Select **+** under Wells to draw well(s) on the image. You can draw circular wells by mouse click and drag in the viewport.
4. *(Optional)* Draw additional wells or select a well by mouse click and copy and paste using keyboard controls.
5. Click **Apply** for colony detection.

Gate colonies

After auto analysis, colony identification can be further refined by applying size, intensity, and circularity gates. Only the colonies lying between the minimum and maximum gate values are displayed and analyzed. To apply the gates on an image with colony analysis:

1. Navigate to the **Edit Layers** panel in **Analyze Image ▸ Colony Accordion**.
2. Select one well in the viewport by clicking anywhere within the well.
   The well number is displayed in the **Edit Layers** panel.
3. Below the well number, sliders for the size, intensity, and circularity gates are displayed.
   a. Size refers to the number of pixels in a colony. Adjust the minimum and maximum size slider to gate out unwanted colonies by size.
   b. Intensity refers to average pixel intensity of a colony. Adjust the minimum and maximum intensity slider to gate out unwanted colonies by intensity.
   c. Circularity refers to the ratio of minor radius and major radius of a colony. Adjust the minimum and maximum circularity slider to gate out unwanted colonies by circularity.
4. *(Optional)* Click ↻ **(Reset)** to set the gates on values obtained by auto analysis.

Note: If you add a new colony to an auto-analyzed well, the gates may readjust to include the attributes of new colonies.

Adjust wells and colonies

In the **Image Analysis** tab, in the **Analyze Image** accordion, click **Automatic** ▸ **Edit layers**.

- To delete wells, select **Wells** and click **Delete**. More than one well can be selected using **Shift + click**. The wells and corresponding colonies are deleted.
- To delete colonies, select **Colonies** by selecting with the mouse or drawing a rectangle around the area and click **Delete**. More than one well can be selected using **Shift + click**. The wells and corresponding colonies are deleted.
- To add colonies, Click **Add**. Click on the desired area within a well to add a colony.
- Click **Apply** to analyze or **Cancel** to remove.
- To resize a well/colony, select a well/colony to resize and use any of the 4 "handles" to resize. On resizing, you can change the shape from circular to elliptical.
- Click **Apply** to analyze or **Cancel** to remove.
- *(Optional)* Click a well/colony to select, then click **Ctrl + C** (**Command + C** on Mac) followed by **Ctrl + V** (**Command + V** on Mac) to copy and paste for reuse. The pasted well/colony appears below the copied one with a slight offset. If you are performing repeated pastes and the newly pasted region reaches the image boundary, then the new well/colony will be pasted over the last pasted well/colony.
- To move a well/colony, select it and drag it using the mouse or keyboard arrow keys.
- Select **Apply** to analyze or **Cancel** to remove all edits.

Note: You cannot add, move, or resize colonies outside of the well.

View and export analysis data

The data table displays the image analysis data.

In the **Image Analysis** tab, in the **Analyze Image** accordion, click **Automatic** ▸ **Data**.

- Click **Data** or **Export Data** from the **Export** drop-down menu.
  The table displays data for **Name**, **Label**, and **No. of colonies**.
  Data Table hierarchy is **Channel** ▸ **Well**. The total number of colonies per well per channel is displayed.

Annotate an image

Annotate allows the user to add text details such as title, lane, and band labels. Furthermore, arrows or boxes can also be added to highlight objects, and images can be labeled from the data table or using annotation tools.

Label an image from data table

Convenient method for labeling analysis objects such as channels, frames, lanes, bands, and regions. The data table is available only when image in the viewport was previously analyzed.

Add text

Note: Click ⇅ to expand the data table for easy viewing and labeling. Only frame and lane labels will be displayed on the image in the viewport. Channel, band, and region labels will only be displayed in the data table. Labels are exported when the data are exported and are also included in the analysis report. Frames and lane labels are displayed in all of the channels of multichannel images.

1. Under the label column in the data table, double-click the cell to label. A text box appears.
2. Type in the text box. Click **Enter** or click outside the text box .
3. Control the display of lane and frame labels in the viewport by clicking on 👁 in the **View labels** column.

Edit text

- Labels can be edited from the table by double clicking on the cell containing the label. User can modify or delete the text.
- Display annotations in the viewport using 👁 to control the display of the channel image.

Filter the data table

Filter the data table to selectively display and export specific portions of the data.

With an image displayed, click the **Data** tab (next to the **Edit Layers** and **Analysis** tabs) within the **Analyze Image** panel.

1. Click ▽ to open a new data table.
2. Add data to the new data table.
   - Click individual bands to add data to the table.
   - Click a lane to add the data for all the bands in the selected lane.
   - Select multiple bands or lanes by clicking while holding down the shift key.
3. (*Optional*) Select **Export** ▸ **Export Data** ▸ XLS to export the table data as an .xls file.

   Note: Only the filtered data is included in the report if the data is filtered.
4. (*Optional*) Click ▽ to return to the original data table.

Label an image using Annotation tools

Annotation tools allow users to label non-analyzed and analyzed images by adding text, arrows, and boxes directly on the image. Annotations added on composite images (color mode) will be added to the channel selected for editing (shown on top of the viewport). The channel selected for editing is also shown by pencil mark and a blue highlight in the **View & Edit Channels** accordion. Annotations added on single-channel images (grayscale mode) will be added to the composite image as well. The labels added using annotation tools are exported with image and are included in report but are not included in data table.

Add text

1. Select text option (**T**) from the annotation tools.
2. *(Optional)* Change the color by clicking on the color box and selecting a new color from the drop-down menu.
3. *(Optional)* Change the text box fill color by clicking on the fill color option and selecting a color from the drop-down menu.
4. Click at the desired location on viewport to add the text annotation
   A text box with a cursor will appear.
5. Type in the desired text.
6. Save annotation by clicking outside the text box.
7. Deselect the text tool after adding all text annotations

Draw a rectangle

1. Select the box (□) option from the annotation tools.
2. *(Optional)* Change the color by clicking on the color box and selecting a new color from the drop-down menu.
3. *(Optional)* Change the fill color by clicking on the fill color option and selecting a color from the drop-down menu.
4. Click at the desired location on the view port and drag the cursor to draw the box.
5. Click outside the box to save.
6. Deselect the tool after use.

Draw an arrow

1. Select the arrow ➡ option from the annotation tools.
2. *(Optional)* Change the color by clicking on the color box and selecting a new color from the drop-down menu.
3. Click at the desired location on the view port and drag the cursor to draw the arrow.
4. Click outside to save.
5. Deselect the tool after use.

Annotate using the line tool

1. Select the line option from the annotation tools.
2. *(Optional)* Change the color by clicking on the color box and selecting a new color drop-down menu.
3. Click at the desired location on the view port and drag the cursor to draw the line.
4. Click outside to save.
5. Deselect the tool after use.

Edit annotations

- Edit labels (move/resize/rotate) by selecting and performing the desired actions.
- Delete labels by selecting and pressing the delete key (fn + del keys for macOS™).

Create and export analysis report

1. Click **Export** ▸ **Export Analysis Report** or right click on the image in the viewport and select **Analysis Report**.
2. *(For SAE secure software version)* Select **Signed** or **Unsigned**. If not fully signed, the signature workflow may be needed.
3. Select or de-select the following features to be included in your report.
   - **Image**
   - **Data table**
   - **Data chart**
4. *(Optional)* De-select **Show layers** to remove the display of lanes, bands, frames, and regions from your image display.

5. *(Optional)* De-select **Image details** to remove the details for your image from the report.
6. Click **Preview**.
   A preview is displayed.
7. Click **Print/Download**.

A pdf of the analysis report is created and can be saved to any location with the required name. Retaining the .pdf extension is essential for proper opening of the file. Changes made to the settings are retained on subsequent use.

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Revision history: MAN0017843 H00 (English)

Revision	Date	Description
H00	26 April 2024	Editing import function for importing g2i and tiff files. Refining topics for accuracy with current software.
G.0	13 December 2022	Refining and correcting text used throughout document. Removing "HT" from "E-gel" usage where applicable.
F.0	28 March 2022	Updating content based on software version 5.0 and combining desktop and cloud versions into one help guide.
E.0	24 July 2019	Updating exisiting help systems to include Annotation section and further updates.