Pyramid Imaging Software

User Manual

MediLumine Inc.
+1 844-360-1574, info@medilumine.com
www.medilumine.com

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1. Introduction

Pyramid is an intuitive imaging software designed to help you visualize, explore, and analyze your experimental data with ease. It has been optimized to work with data from the PRISM imaging platform, including bioluminescence, fluorescence and SWIR modalities. **Pyramid** lets you load multiple images, draw and adjust regions of interest (ROIs), and instantly see key statistics.

1.1. Minimum requirements

- Operating System: Windows 10 or 11 (64-bit)
- Processor: Intel Core i5 (8th gen or newer), or AMD Ryzen 5
- RAM: 8 GB
- Graphics: Integrated graphics (Intel UHD / AMD Vega or better) (A dedicated GPU is not required, but OpenGL support improves display performance)
- Storage: at least 500 MB free space for the application + enough space for your image data
- Display: Minimum 1280×720 resolution (1920×1080 or higher recommended for comfortable image and ROI viewing)

1.2. Recommended requirements

- Operating System: Windows 11
- Processor: Intel Core i7 (10th gen or newer), or AMD Ryzen 7
- RAM: 16 GB or more
- Graphics: Dedicated GPU (e.g., NVIDIA GTX 1050 or better)
- Storage: SSD with ≥1 GB free for app and settings + ample space for imaging data
- Display: Full HD (1920×1080) or higher resolution

2. Installation

Pyramid can be installed through its installer: Setup_Pyramid.exe. On installation, a prompt will ask where to install the software, we strongly recommend using the default path and to only keep one installation of **Pyramid** per computer. Multiple installations can generate compatibility issues and break the link with the acquisition software of Prism imaging platform.

On update, first uninstall the old version of **Pyramid** before installing the newest one.

3. Pyramid User Interface

3.1. Main Window

Pyramid main user interface can be divided in five areas:

A. Action buttons (described at section 3.2)

B. Opened acquisitions list (described at section 3.3)

C. Data display (described at section 3.4)

D. Histogram / Contrast adjustments (described at section 3.5)

E. Statistics table (described at section 3.6)

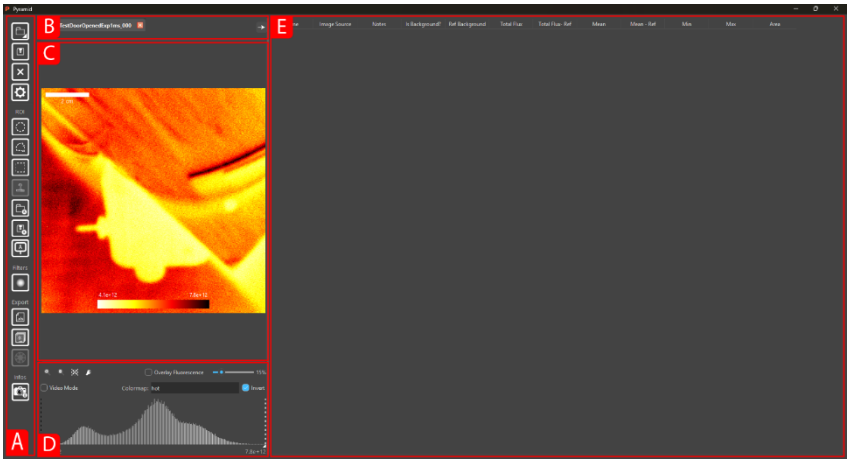

Figure 3-1 Main user interface of Pyramid

Proportion of the C and E panels can be adjusted using the splitting bar (in light gray) that is placed between them. Using this, one can re-distribute the space between the ROI statistics table or the image itself. To adjust the panel proportions, simply left-click on the splitting bar and drag the mouse left or right.

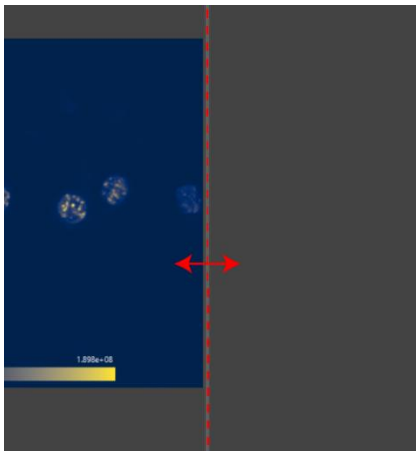

Figure 3-2 Splitting bar to readjust image size

3.2. Action Buttons

Action buttons are used to send the main commands to **Pyramid**: open files, close files, generate ROIs, filter image, export data, etc. They are always situated on the left side of the interface. When an action is not accessible (because no images are loaded for example), the buttons become darker and will not highlight when the mouse hover over it.

File operations:

Load data

This button is used to open data. Three options are possible: open a single acquisition, open one or more folders containing many acquisitions, or open a pre-saved analysis session (see details section 4.1). To access these options, the user must either right-click or press with the mouse's left button for 1 second, then a menu will be shown with the 3 options.

Save current session

Save all the information on the current opened files and ROIs. If the work is not done and the user does not want to lose their work, they can save the current state of the analysis sessions (all opened images + all the ROIs that were drawn) and come back later (by loading the saved session).

Close all

Close all opened acquisition at once.

Preferences

Opens the preferences window, where all the default settings (colormap, position of color bar, scale bar, units, etc.) can be set. For details, read section 3.7

Region of Interest (ROI)

ROI drawing

Used to draw ROI on the current image. User must click on the desired ROI shape (circle, polygon, or square) and then draw the ROI in the image (see section XXX for details)

Stamp tool

Copy the display parameters (see section 3.5 for details) as well as all ROIs of the current acquisition to all other acquisitions.

Open and apply a ROI pattern

Open a pre-saved ROI pattern (ROIs' position, size and name) (*.roi.json file) and apply it on the current displayed acquisition.

Save a ROI pattern

Save the current ROI pattern (ROIs' position, size and name) in a file (*.roi.json) to be reloaded when needed.

Annotation

Add a text note with an arrow to highlight a specific detail on the picture. The color of the annotation can be changed from the Preferences window (section 3.7). The annotation can be adjusted after creation by moving either the circular shape at it's top, or the text label itself. Pressing on the red box with an "x" will delete the annotation.

Filtering

Gaussian Filter

Apply a 2D gaussian filter to the current image. When clicked, a window appears asking for the size of the filter to be applied (default value 2.0 pixel). The larger the value is, the stronger will be the filter effect on the image.

Export data

Export data to .xls or .csv file

Export all the data from the ROIs' statistics table to a .xls (Excel must be installed on the computer, otherwise, it will export only in .csv) or a .csv file.

Export images

Export all the images to the save folder (set in preferences window, see section 4.X). Images can be exported in .png, .pdf or .svg format. All the features (scale bar, color bar, ROIs and annotations) will appear on each image.

Export Video

When in video mode (see section X), this button will export the current acquisition to a video file on disk, with all annotations, colorbar, scale bar and ROI in its display.

Acquisition Parameters

Display acquisition information

Opens a window that list all the saved parameters from the acquisition system (config.txt file)

3.3. List of Opened Acquisitions

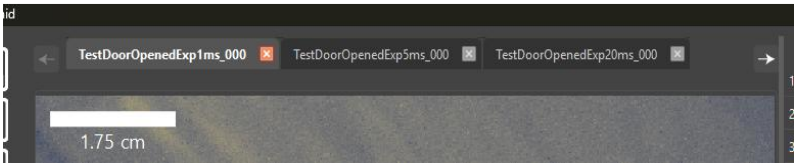

Figure 3-3 Opened acquisitions

For each acquisition that is opened, a new tab is created. User can navigate through all acquisitions by clicking on the tabs. The red *X* button closes the acquisition linked to the tab. The two arrows button move to the next (or previous) acquisition in the list. If the list contains more image tabs that can be displayed, user can scroll using the mouse wheel or the button that will appear at the right of the tabs list.

3.4. Image Display

Image display is in the middle panel, between the action buttons and the statistics table. It consists of the image, in the chosen color map, a scale bar, a color bar and the ROIs. All these elements are interactive and can be moved, resized or removed from the image (either by clicking on them directly or through the preferences window). Also, users can zoom in, zoom out, pan across the image, fit the display back to its normal size (section 3.5).

Scale and color bars

A white scale bar and a color bar can be displayed in any of the corners of the image (on the side for the color bar). For the scale bar, its size and position can be set in the preferences window (see section 3.7). Otherwise, their position can be changed by clicking on it and dragging it to another corner of the image.

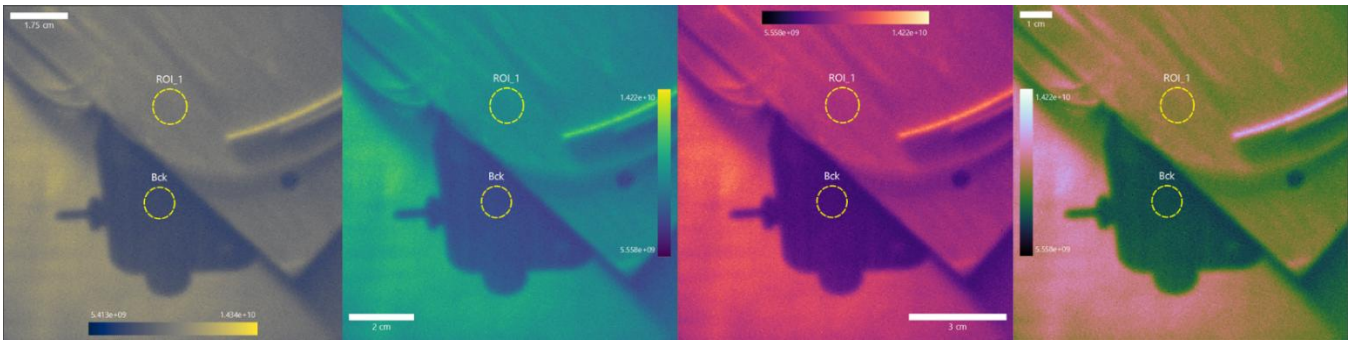

Figure 3-4 Examples of different display setup for the color and scale bars

Region of interest

To create an ROI, the user must first click on the shape button that they want: circle, rectangle or polygon. Then, they can draw the shape over the image, in the region they want to measure. Once drawn, ROI can be interacted with directly on the image display. Users can move, reshape, rename or delete ROI.

All ROIs are represented by a dash yellow line (default color), except for the active one, which is with a cyan dash line.

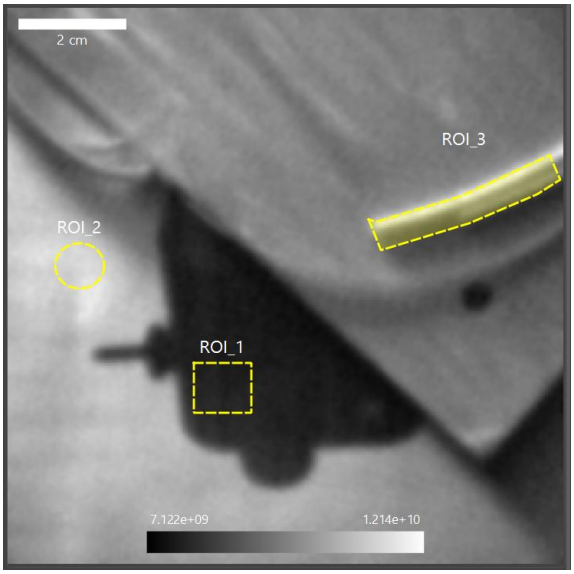

Figure 3-5 The 3 different types of ROI

- Moving the ROI: just press and hold anywhere inside the region and move to where you want to reposition the ROI.
- Reshape the ROI: click on any of the white handles (as shown in figure 3.6) and drag them in the direction you want (to either increase or decrease the size of the shape).
- Rename the ROI: double click on the name of the ROI
- Delete the ROI: click on the red box that appears when mouse is over the ROI (as shown in figure 3.6)

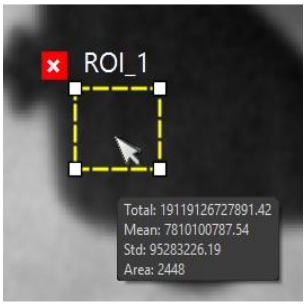

Figure 3-6 Controls for ROI are shown when mouse moves over it

Users can also use keyboard to interact with ROIs:

- **Tab** to cycle through the ROI of the current acquisition
- **Ctrl+R** to rename an ROI
- **Delete** to delete the ROI

Annotations

Annotation markers can be created by clicking on the annotation button in the ROI section. Once clicked, user can choose the starting point in the image by clicking and holding the mouse left-button, and the end point (where the text will be) is set by releasing it. After its creation, the annotation can be modified by moving its pointer (the circle) or the text. Also, the text can be changed by double clicking on it.

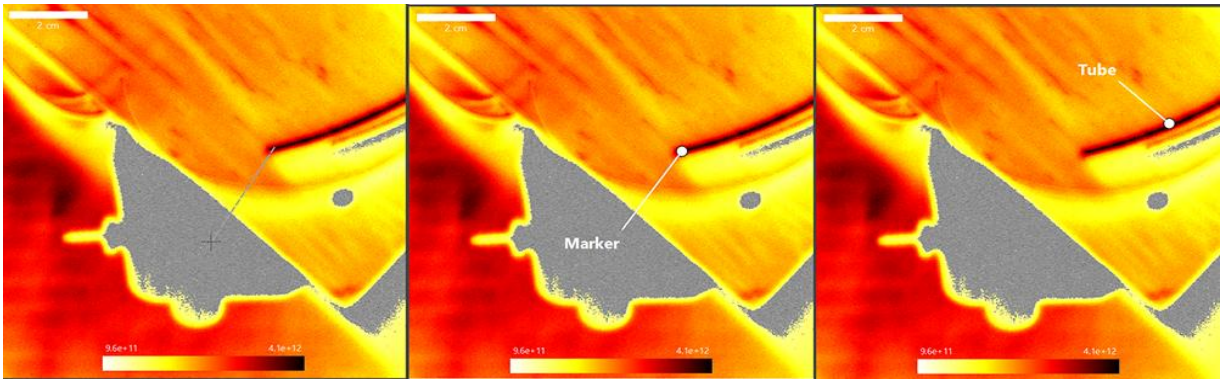

Figure 3-7 Creation, positioning, and edition of an annotation

To remove an annotation, the user can click on the red box with a white X that appears when the mouse hover over the text label.

3.5. Display Interactions

The panel located below the displayed image provides controls to zoom/pan, display mode and to set the image's colormap and contrast. These parameters can be set individually for each acquisition in the list of acquisitions (section 3.3).

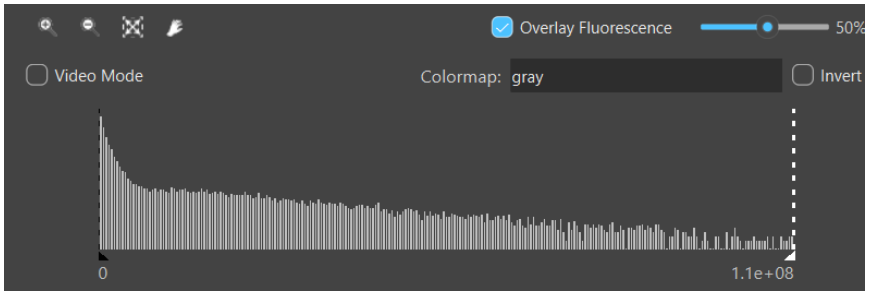

Figure 3-8 Display control panel

Zoom and pan

The zoom and pan buttons are in the top-left corner of the panel:

Zoom in

Zoom out

Fit to view: Fits the image to the data display panel

Pan: Click to activate/deactivate the pan mode. With the pan mode activated (hand cursor), click and drag the image to move the field of view

Video mode

Click the **Video Mode** check box to activate this feature. Video Mode displays a TIFF stack (i.e., an image time series) as a video. Once activated, a **Play** button appears in the center of the image, and the current timestamp is shown in the bottom-left corner.

The timestamp box, like the scale and color bars, can be moved to any corner of the image. Click the Play button to start the video, which will loop continuously until the image is clicked.

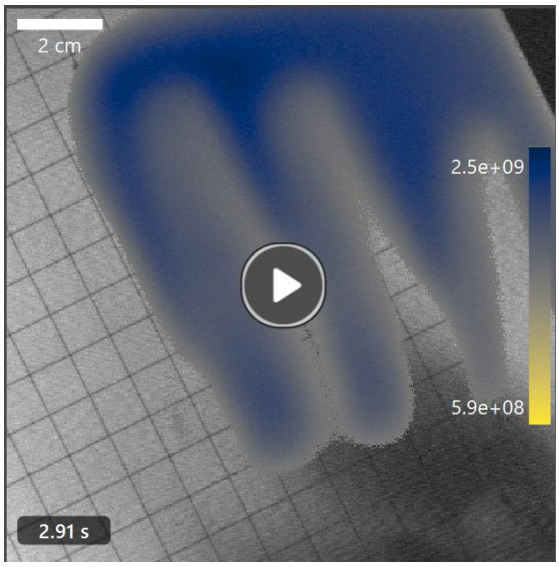

Figure 3-9 Screen shot of the displayed image with the Video Mode activated

Note: The histogram profile updates to match the current frame of the video whenever the playback is stopped.

When **Video Mode** is deactivated, the displayed image is the average of all frames. ROI statistics are calculated based on this averaged image and remain unaffected by Video Mode.

Image display parameters

The user can set the image's colormap, histogram limits and overlay mode.

Overlay fluorescence

Click the **Overlay Fluorescence** button in the top right corner to superimpose the image onto a background image. The background appears in grayscale (see Figure 3-9), while the overlayed image displays pixels with values lower than a specified threshold as transparent. Use the slider to adjust the threshold.

Colormap

Set the image's colormap by clicking on the current colormap name and choosing the desired one in the dropdown menu. Click on the **Invert** check box to invert the colormap.

Histogram

The histogram shows the normalized distribution of pixel values of the image on the Y axis and the color map limits on the X axis. The lower and higher color map limits can be set by clicking and dragging either the black or white dotted vertical bars, respectively. Alternatively, double-click on the lower or higher limits of the X axis (blue arrows in the figure below) to set it to a specific numerical value.

Figure 3-10 Two ways of setting the histogram limits. Set the lower or higher histogram limits by moving the black or white vertical bars (red arrows). Set the lower and higher limit values by double-clicking on the tick labels (blue arrows).

Toggle Y axis scale

The Y axis scale can be set as Linear and Logarithmic. To activate/deactivate the Logarithmic scale, right-click on the histogram and click on the **Log Scale** option. Note that one can set the default Y axis scale in the **Histogram** option of the **Preferences** menu (see section 3.7).

Reset Histogram limits

To restore the limits to the default values, right-click on the histogram and click on **Reset Histogram Limits**. The default limits are set in the **Histogram** option of the **Preferences** menu (see section 3.7).

3.6. Statistics Table

The statistics table displays all descriptive statistics for each ROI created in all acquisitions. For a description of all the available statistics, read section 3.7.

Basic interactions

1) The table's column width can be adjusted by clicking and dragging the division between headers:

Figure 3-11 Column width adjustment

2) Right-click on any of the column headers to select the visible columns:

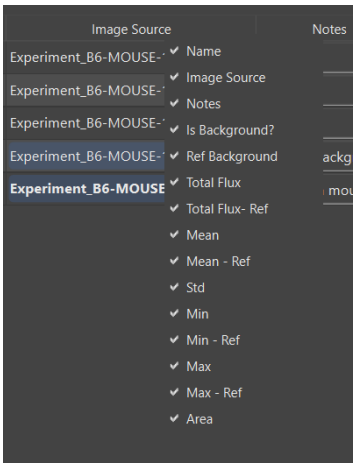

Figure 3-12 Column selection

3) Click on any row to visualize the selected ROI. The selected ROI will be set as active (cyan coloured), and the selected acquisition will be displayed.
4) Hover the cursor over the statistics to show the measurement unit:

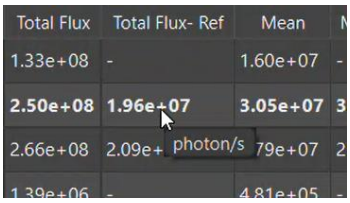

Figure 3-13 Units

Rename ROIs

To rename an ROI in the table, double-click its name in the **Name** column, type the new name and press Enter.

Add notes

The **Notes** column can be used to add comments.

Show differences from Background

You can designate one or more ROIs as **Background** to visualize differences from the background. Follow these steps:

1) Set one or more ROIs as background by checking the **is Background?** column.
2) For the other ROIs in the current acquisition, select the background ROI by clicking the **Ref Background** column.
3) The differences from the background will now be displayed for the other ROIs in the current acquisition (see figure below).
4) Repeat this process for additional acquisitions as needed.

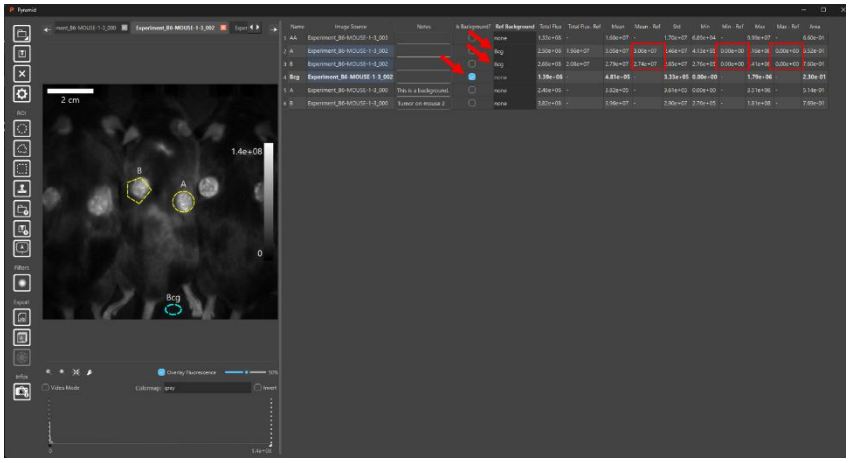

Figure 3-14 How to display differences from background. Set a background ROI and select it as references to the remaining ROIs. The statistics will be updated automatically

Read section 3.7, for details on how the Background value is calculated.

3.7. Preferences

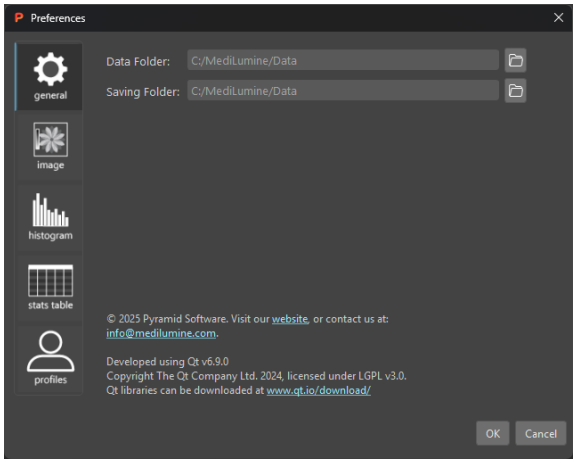

Figure 3-15 View of the Preferences window

The **Preferences** window provides the option to set the app's default parameters. These parameters are applied when the user clicks on the **OK** button. Here is a description of the available settings:

General

- Data Folder: Default folder where the Imaging data are located. This applies for the **Open Image/Acquisition** options.
- Saving Folder: Default folder where all data will be saved including **session files**, **ROI patterns**, **exported tables images** and **movies**.

Image

Here, the user can customize how the images are displayed including the color bar, color map, scale bar and the Font and color of overlayed elements such as annotations and ROI shapes.

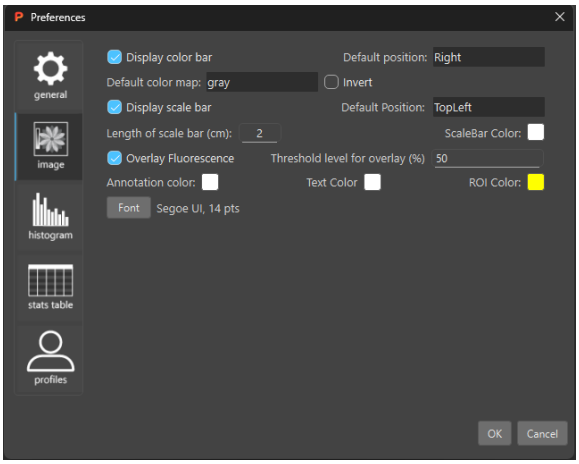

Figure 3-16 Image default parameters

Histogram

Here, the user can set the lower and higher limits of the histogram as well as the Y-scale (linear or logarithmic).

There are four options for lower and higher histogram limits:

a) **Image:** Automatically sets the limits as the minimum and maximum pixel values in the image.
b) **User defined:** Manually set the limit values in the *Default limits* box.
c) **Percentile:** Manually set the limits in the *Default limits* box as percentage of pixel values.
d) **Adaptative:** Automatically sets the limits to optimize the dynamic range of the histogram.

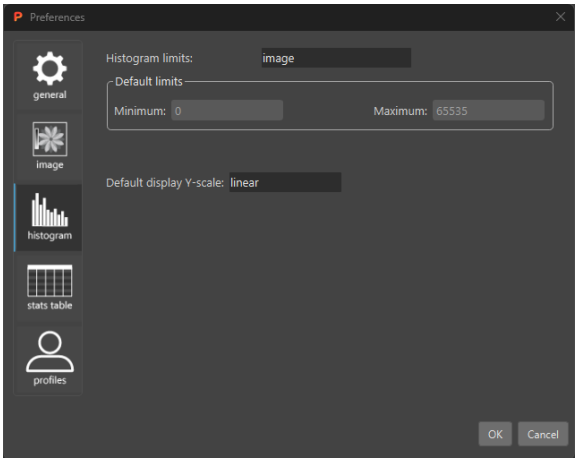

Figure 3-17 Histogram default parameters

Statistics Table

Here, the user can set the **Radiance** to one of the following units:

1) Photons / $cm^2 \cdot s \cdot sr$

2) Photons / $cm^2 \cdot s \cdot sr \cdot (\mu W/cm^2)$

The first unit is mostly used in bioluminescence, while the second unit is useful in epi-fluorescence to normalize by the incident excitation intensity, when available.

The columns to display can also be selected with the descriptive statistics calculated for each ROI.

Figure 3-18 Statistics table parameters

Here is a description of each column:

COLUMN NAME	DESCRIPTION
NAME	Name of the ROI. Double click to edit.
IMAGE SOURCE	Acquisition name
NOTES	Text field to add comments
IS BACKGROUND?	Check box to set the ROI as Reference
REF BACKGROUND	Drop down menu with the names of the ROIs set as background for the acquisition or **none**.
TOTAL FLUX*	Integrated sum of the radiance over the ROI times 4π, in photons/second
MEAN*	Average radiance from the ROI
STD	Standard deviation of the radiance from the ROI
MIN*	Minimum pixel value of the radiance from the ROI
MAX*	Maximum pixel value of the radiance from the ROI

AREA	ROI area in cm^2

* These values can be expressed as a difference from the Reference Background ROI (**Ref Background**). In this case, the value for **Ref** is equal to the mean of the Reference Background ROI times the number of pixels of the target ROI.

Profiles

All default parameters can be saved to a user profile. A **default** profile exists by default, and new user profiles can be created as needed.

To set a new profile:

1) Click the **New** button and enter a name for the profile.
2) Update the default parameters in the previous sections.
3) Click the **Save** button to store the new profile.

To load default parameters from an existing profile:

1) Select the desired profile.
2) Click **Load** — the selected profile will appear in bold.
3) Click **OK** to apply the parameters to the current session.

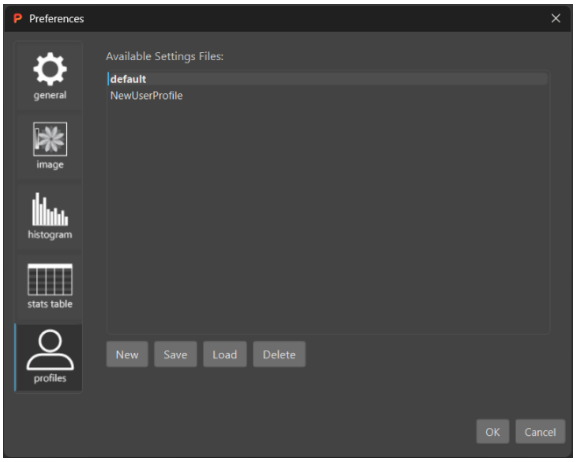

Figure 3-19 User profile section

4. Workflow examples

In this section we describe in more details the basic utilisation example with **Pyramid** application.

First, open the software:

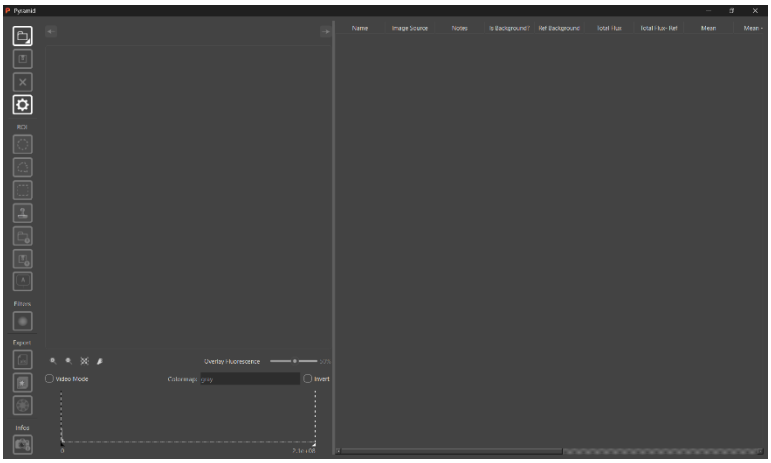

Figure 4-1 Main interface of Pyramid

Only the **Load Data** and **Preferences** buttons are available at this point.

4.1. Opening Acquisitions

There are three option available with the open file button: load a single acquisition, load multiple acquisitions or open a previously saved session file.

Opening a single acquisition

Left-click on the button  and navigate to the acquisition folder and select the file named *Image.tiff*:

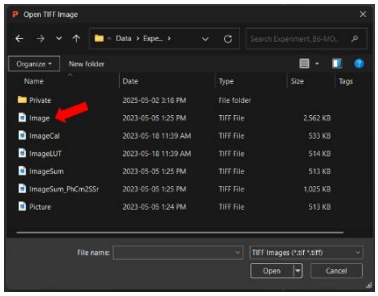

Figure 4-2 Select image.tiff inside the acquisition folder to open a specific acquisition

The acquisition will be loaded in the interface and the action buttons will become available:

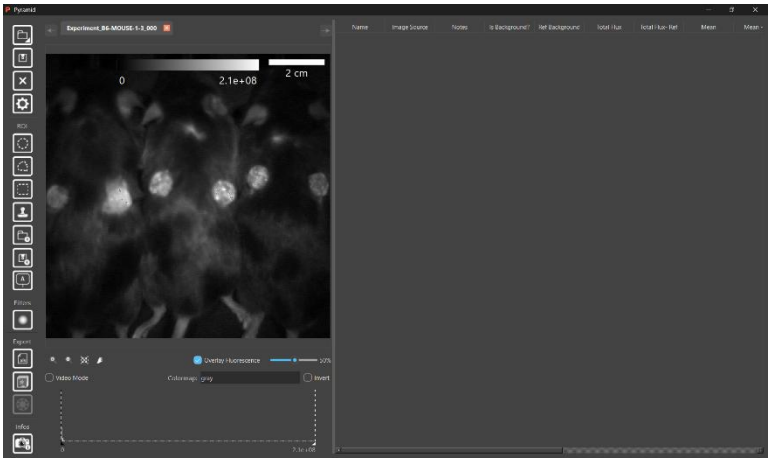

Figure 4-3 Acquisition is loaded and shown in the main window

Opening multiple acquisitions

To open multiple acquisitions all at once, click and hold or right click the Load Data button to access the options menu and click on *Open Folder*:

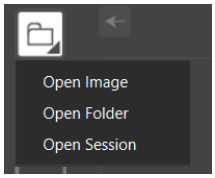

Figure 4-4 Load options submenu

In this case, instead of selecting a TIFF file, select the folder where the individual experiment folders are located. All experiment folders containing the Image.tiff files will be loaded in the interface and their names displayed in the list of opened acquisitions (red rectangle):

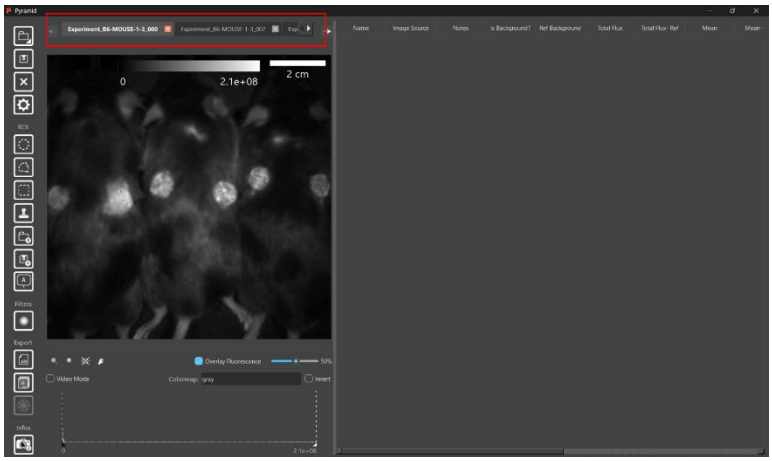

Figure 4-5 Multiple acquisitions opened

4.2. Creating ROIs

Drawing new ROIs

Before drawing the ROIs, make sure that the colormap, histogram Y scale and limits were adjusted to better visualize the boundaries of regions of interest:

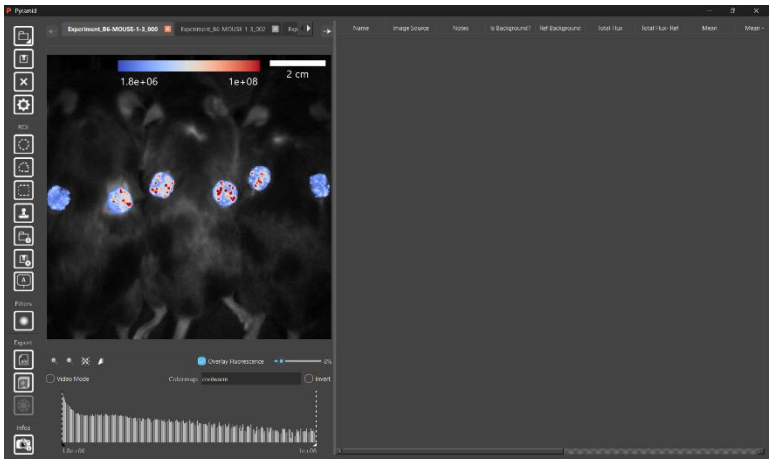

Figure 4-6 Typical image visualisation

To draw an ROI, select one of the three shapes (circle  , polygon  and square ). For the circle and square shapes there are two ways to create an ROI:

1) Click and drag to set the ROI position and final shape:

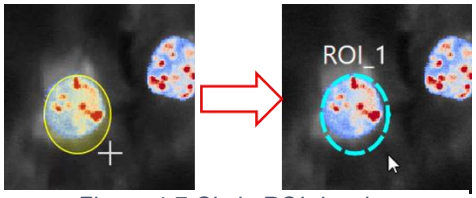

Figure 4-7 Circle ROI drawing

2) Click on the image. A window will appear so the user can type the shape's diameter/side length in centimeters:

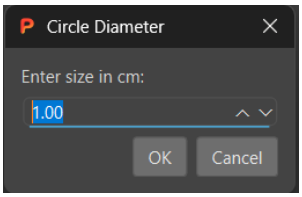

Figure 4-8 Size constrained ROI creation

For the polygon option, draw the shape by clicking the vertices of the polygon. To close the polygon and create the ROI, right-click inside the polygon:

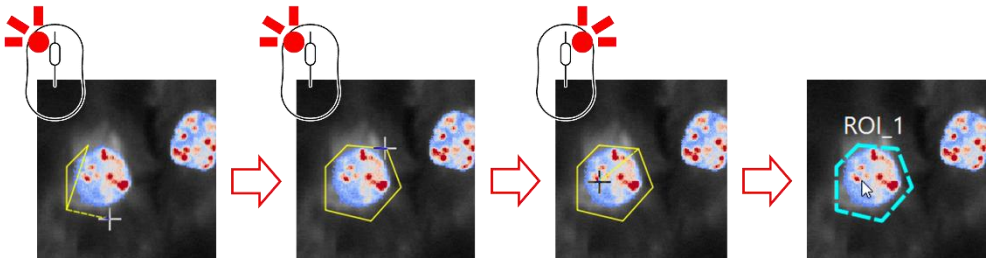

Figure 4-9 Polygon ROI creation sequence

Once each ROIs is created, one can rename them by clicking on the ROI identifier on the image or on the **Name** column of the statistics table. Here is the final ROI pattern created for an acquisition:

Figure 4-10 Multiple ROIs example

At this point, all ROIs' statistics are available in the table. One can also display some of the statistics of a given ROI (*Total Flux, Mean, Std* and *Area*) by hovering the mouse cursor over the ROI:

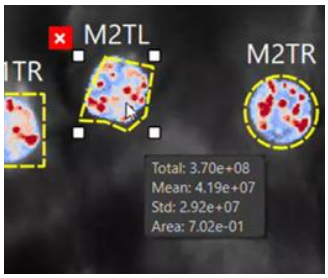

Figure 4-11 Example of ROI tooltip display

Copying ROIs to other Acquisitions

In cases where multiple acquisitions are open, the **Pyramid** software provides three different ways to easily copy ROIs from one acquisition to another.

Copy a single ROI

To copy one ROI to a different acquisition:

1) Click on the ROI to be copied
2) Press **Ctrl+C**
3) Select the target acquisition in the List of opened Acquisitions
4) Click over the image
5) Press **Ctr+V**

To copy all ROIs (ROI pattern) from the current acquisition to a different acquisition:

1) Right-click on the current image
2) Select **Copy ROIs from this acquisition**
3) Select the target acquisition in the List of opened Acquisitions
4) Right-click over the image
5) Select **Paste ROIs to this acquisition**

Using the stamp tool

The **stamp tool**  copies the ROI pattern of the current acquisition to **all** acquisitions on the list. In addition, all parameters of the current image (colormap, histogram limits, etc.) are applied to all other acquisitions. Here is the result of using the stamp tool to apply the ROI pattern to four acquisitions:

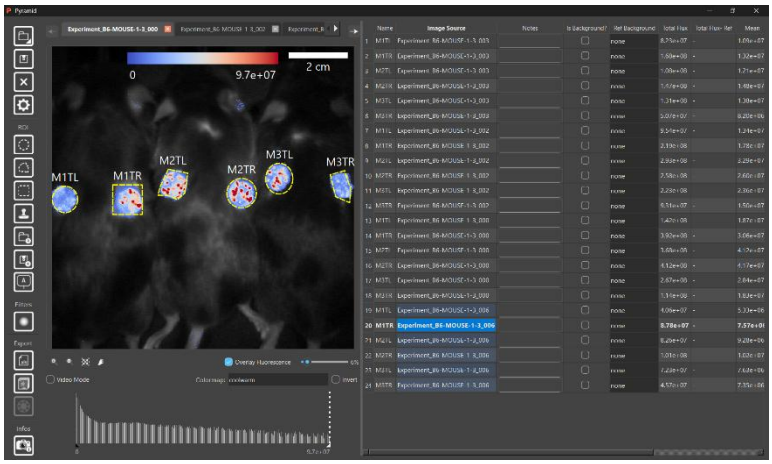

Figure 4-12 Multiple ROIs on different acquisitions

Saving and loading ROI patterns

Pyramid software allows one to save one or more ROI patterns (i.e. the ensemble of ROIs from a given acquisition) to a file for later use.

To save the ROI pattern:

1) Select the acquisition containing the ROIs to be saved
2) Click on the **Save an ROI pattern** button 
3) Select the file name and folder to save the ROI pattern

To load the saved ROI pattern:

1) Select the target acquisition
2) Load the file (*.roi.json) with the ROI pattern by clicking in the **Open and Apply ROI Pattern** button  . The ROIs will be added to the target acquisition.

4.3. Saving and Loading a Session

Pyramid software allows you to save the current session so you can resume work later. This saves the existing ROIs and opened acquisitions.

To save the current session:

- Click Save Current Session , then save the session file (*.session.json) in the Save Folder or elsewhere.

To load a session:

- Click and hold Load Data , then select **Load Session** to load the session file with the stored acquisitions and ROIs.

Note: If the acquisition being loaded is already open, any ROIs from that acquisition will overwrite the existing ROIs with the same name.

4.4. Exporting Data, Images and Videos

At this stage of the workflow, all acquisitions have been processed, meaning all ROIs have been properly created, and the relevant statistics are displayed in the **Statistics Table**:

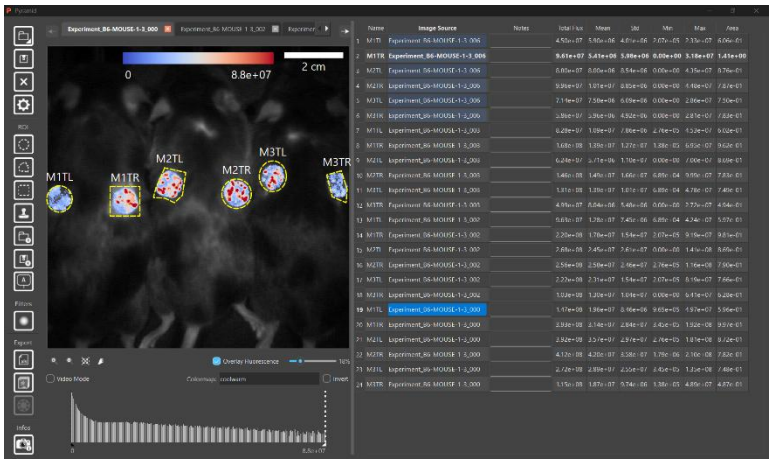

Figure 4-13 Complete statistics table

The final steps involve exporting the statistics table for further analysis and, optionally, exporting images and videos for use in publications, presentations, or other visual materials.

Exporting the Statistics Table

To export the statistics table, click **Export Data**  , then specify the filename and folder to save the table. The table can be saved as a Microsoft Excel file (*.xlsx), if Microsoft Excel is installed, or a comma-separated values file (*.csv).

Note: Only the columns currently displayed in the table will be exported.

Preparing Images for Export

Before exporting, users can enhance their images using the **Annotation** and **Gaussian Filter** tools.

- The Annotation tool  allows users to add markers and supplementary information to the image beyond the ROI itself.
- The Gaussian Filter tool  can be applied to smooth the data for better visualization.

Below is an example where the image has been filtered and the ROIs have been replaced with annotations:

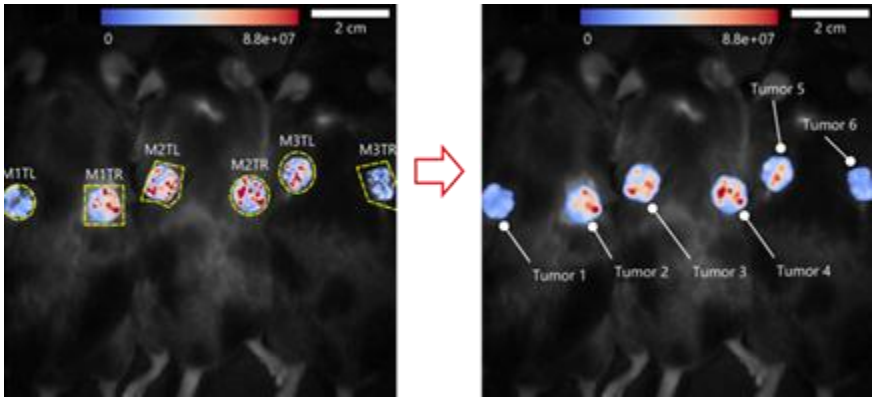

Figure 4-14 Example of Annotation use to marks object in the picture

Exporting images

To export images from the listed acquisitions, click **Export Images**  . The user will be prompted to select a folder where the images will be saved. The images will be exported as they currently appear on the screen with all elements overlayed (see figure above). Next, choose the desired image file format from the available options: PDF, SVG, or PNG. Once the format is selected, all images will be saved as separate files with the acquisition names as file names in the selected save folder.

Exporting videos

If an acquisition contains TIFF stacks (i.e., an image time series), users can export the stack as a movie file (*.mp4). To do so:

1) Activate Video Mode (see Section 3.5) to enable the **Export Video**  option.
2) Click Export Video.
3) Specify the filename and select the save folder.

As for the Images exporting procedure, the video will be saved as displayed, with all overlayed elements.

MediLumine Inc.

For any questions, please contact us:

+1-844-360-1574
info@medilumine.com
www.medilumine.com