PRISM™ Imaging System

User Manual

ediLumine™

5795 Av. De Gaspé, Suite #228
Montreal, Quebec, Canada
H2S 2X3

TABLE OF CONTENTS

INTRODUCTION

This manual is a reference to use the Small Animal In Vivo Bioluminescence, Epi-Fluorescence & Trans-Fluorescence Imaging System developed by MediLumine. All users must receive a formation before using the system, either by MediLumine or an authorized person.

The system is composed of a cooled EMCCD camera from Nüvü Caméras. This camera has 10 times less noise than any other camera on the market. Excitation and emission filters, depending on the target fluorophores, can be added on request. The system combines fluorescence in reflection and transmission modes. The reflection mode is more flexible and allows imaging samples and ex vivo organs, while the transmission mode is more quantitative and allows performing tomography on the whole body of a mouse.

For the reflection mode, a white light source with a filter wheel excites the target. In transmission mode, one to four lasers in red or near infrared are used. It is not possible to use lasers under 630 nm due to the low penetration of light at these wavelengths. To perform tomography, two motorized mirrors allows the scanning of the laser on the target.

1. HARDWARE

1.1. Starting the System

The imaging device has a switch on the back of the enclosure, on the lower right side. The camera also has a switch on the back. The white light source also has its own switch, but it must stay activated at all time, since it is controlled by the imaging device.

1.2. Anesthetizing the Mice

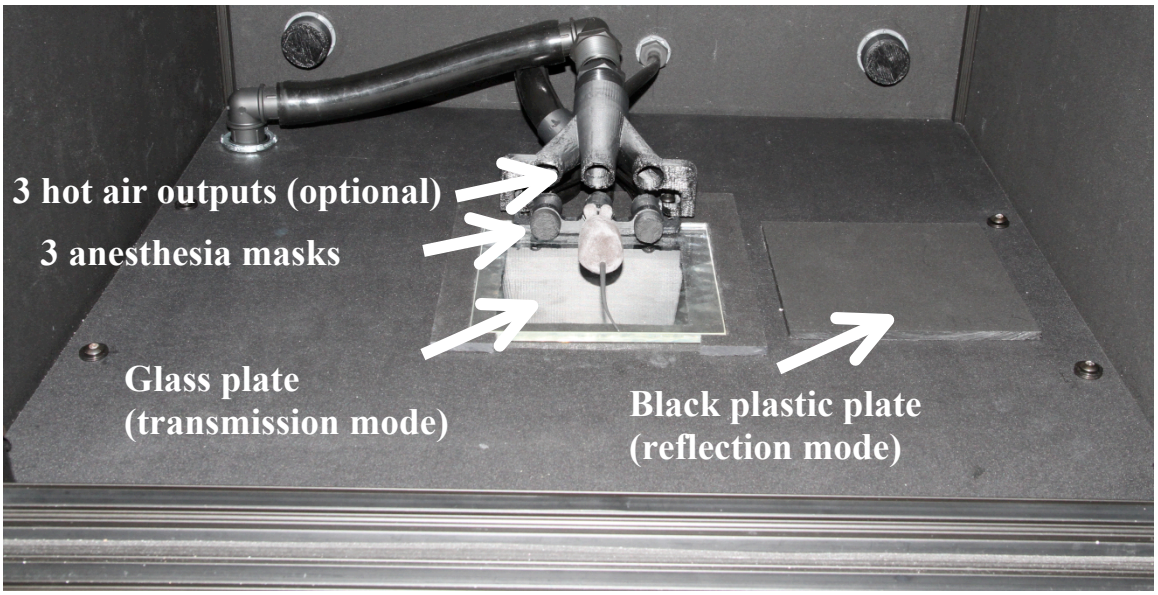
An anesthesia system with an isoflurane vaporizer and an induction chamber may be provided with the system. The imaging system contains the proper tubing to keep the animals anesthetized during the acquisition.

1.3. Installing the Mice in the System

For transmission mode imaging, the mice must be placed on the glass plate to avoid blocking the laser beam. In reflexion mode, it is recommended to use the black plastic plate. The anesthesia masks are placed side by side in the center of the device. Make sure to install the mice correctly in the tube to avoid isoflurane leaks. Unused masks should be blocked with the supplied caps.

The anesthesia masks can be moved from back to forth, allowing an optimal mice positioning.

From the acquisition software, it is possible to activate the white light, allowing the user to better see his manipulations.



1.4. Hot Air Heating (optional)

If equipped, a hot air heating system can push air at 35°C on the bodies of the mice. If using less than 3 mice, do not block the unused air outputs.

1.5. Illumination

In reflection mode, the illumination is a filtered white light. The filter wheel is located inside, at the top of the enclosure and can contain up to 7 filters of 1"/25mm. Multiple filter wheels can be used and they can easily be changed. The filtered illumination is then divided in 2 fibers to expose the mice with a side angle.

In transmission mode, laser diodes are used for the illumination. They are located at the bottom of the enclosure. Motorised mirrors scan the laser beam on the mice during the acquisition.

1.6. Detection

The HNü camera from Nüvü Cameras is used for the detection of fluorescence and bioluminescence. It is fixed on the top of the enclosure. A 50 mm objective with a numerical aperture of 0.95 allows to obtain a field of view of 103 mm X 103 mm. A filter wheel is located after the objective. The wheel can contain up to 4 filters of 50 mm. Multiple filter wheels can be used and they can easily be changed.

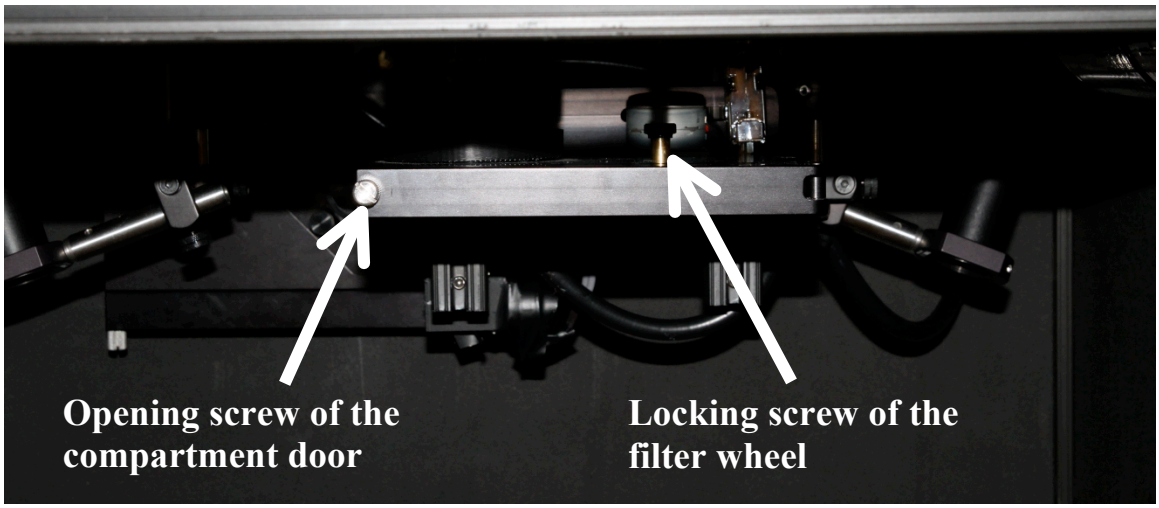
1.7. LEDs

2 LEDs are located on the front of the device. The green LED indicates that the system is powered on. The red LED indicates that an acquisition is running, and that the door should not be opened.

1.8. Changing the Emission Filter Wheel

The emission filter wheel can be changed in just a few seconds and without any tools to accommodate a large quantity of filters. The following steps explains how to change the emission filter wheel.

1. Shut down the imaging system and the acquisition software.
2. Open the compartment door of the filter wheel.
3. Unlock the filter wheel with the screw located on the top.
4. Remove the wheel.
5. Insert a new wheel by making sure the text “IFW 50 mm” is facing down.
6. Lock the filter wheel by tightening the top screw to the end.
7. Close the compartment door.



Changing individual filters inside the filter wheels must be done by MediLumine. Incorrect filter positioning may permanently damage the camera.

Warning: If the software and the imaging system are not shut down during the filter wheel change, the software may not detect the new wheel, which could lead to permanent camera damage.

1.9. Access to the Electronics

For security reason, access to the optical and electronic components under the imaging system is not authorized.

1.10. Moving the System

The system may be carefully moved to a new location. It is recommended to perform a calibration of the laser scanning for trans-fluorescence and 3D profile options.

1.11. Adding Vital Signs Monitoring Devices

The imaging system has 2 holes at the back of the enclosure with a diameter of 2 cm. This allows passing wires to add monitoring devices, such as ECG or rectal temperature.

90° connectors pointing down are used to prevent external light from coming in. Additionally, the holes are blocked with plugs when not in use.

1.12. Connections to the System

Electrical connections

The imaging device is connected to the computer via a single USB cable. The device is powered by a 12V input source. The camera is connected to the computer with an Ethernet cable and to its own power supply. The white light source is connected to the main input power and to the imaging device for remote control.

Optical connections

The white light source is linked to the imaging device with an optical fiber.

Gas connections

An isoflurane vaporiser with an external source of air or oxygen must be connected to the gas inlet of the imaging device (the smaller inlet). The larger connection is for the aspiration. Make sure to properly connect the anesthesia system according to your facility’s regulations.

1.13. 3D Profile

The 3D profile of the animals to image can be obtained with a fringe profilometer (optional). A laser line located at the top of the enclosure is scanning the animal with a motorized mirror. The 3D profile can then be used to perform 3D reconstructions and visualisation of the fluorescence.

2. SOFTWARE

2.1. Warning

The source code of the software is supplied with the system. However, only an experimented and authorised user should modify the code. Note: The source code is set as read-only for the Windows account "User" and can only be modified from the "Admin" account.

2.2. Computer

The computer runs on Windows and it has a Matlab license including the Image Processing Toolbox. The software DAQmx and NuPixel are installed to control the imaging device and the camera.

2 main Windows account are present on the system: Admin and User. The User account should be used. The default password is password. The Admin password is supplied on demand.

2.3. Acquisition Software

Main acquisition window

The acquisition window allows to:

- Obtain the 3D profile of the mice;
- Select the imaging region (transmission mode only);
- Select the acquisition parameters;
- Add comments linked to the acquisition;
- Start an acquisition sequence;
- Display acquisition results;
- Load results from a previous acquisition;
- Open the advanced configuration window;
- Open the saving manager window.

Note:

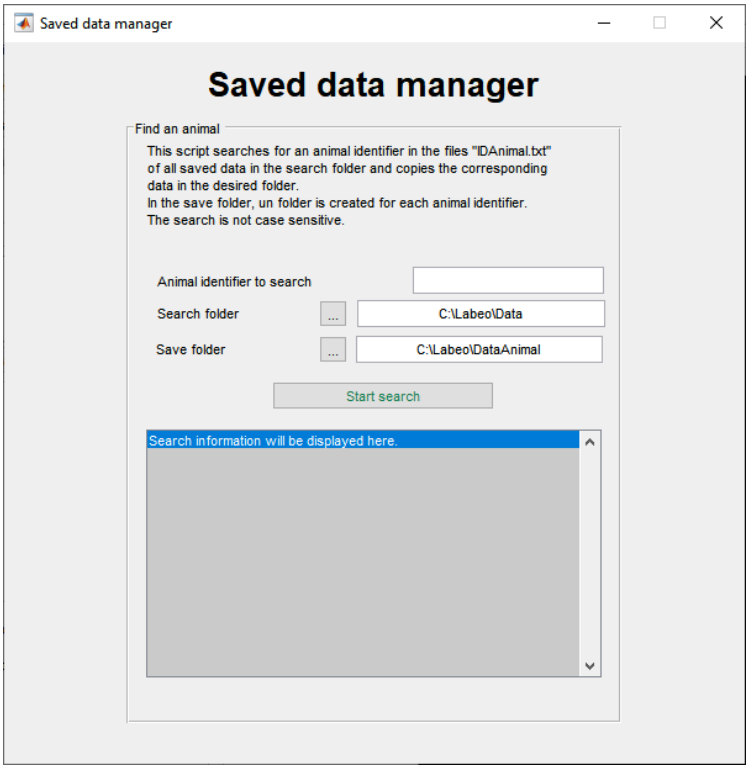
To obtain a detailed description of an object on the software, right click on it.

Advanced configuration window

This window allows controlling different advanced acquisition and display parameters. To open it, select it in the “Parameters” menu of the main window. From this window, it is also possible to realign the motorised mirrors if the system was moved. Moreover, temperature values at different location in the device are displayed.

Saving manager window

This window can be opened from the “Parameters” menu. It consists of a script that allows to search for an animal ID in the file “IDAnimal.txt” of all saved data in the search repository, then copy the corresponding data in the desired repository. In the saving repository, on folder is created with the identifier of the animal. The search is not case sensitive.



Loading or saving default parameters

When opening the software, the default acquisition parameters are loaded from the file “settings/defaultConfig.txt”. In the advanced configuration window, select the “File” menu to load or save custom acquisition parameters. Saving is only allowed with the Admin Windows account.

Saving the results

For each acquisition, a file “config.txt” and “info.mat” containing the acquisition parameters and multiple “.tiff” files containing the acquired images are saved. The image files can be opened with multiple software, such as ImageJ, a free software installed on the computer.

The image files are saved in a repository named with the “output folder name”, the current date, the name of the experiment, an automatically incrementing index and the acquisition mode.

Example: C:\OIS300\2016_08_10\Experiment_001\

If an animal identifier is supplied, it is added to the name of the experiment.

Exemple : C:\OIS300\\2016_08_10\Experiment_ID1234_001\

Acquisition parameters

The first step to perform an acquisition is to configure the acquisition parameters. The main parameters are located on the panel “General Parameters” and are explained below. There descriptions are also available by right clicking on the parameter in the software.

- ***Excitation filter (reflection mode)***: Excitation filter selection depending on the installed filter wheel. The option “No light source” allows to disable the excitation, such as for bioluminescence.
- ***Emission filter (reflection mode)***: Emission filter selection depending on the installed filter wheel.
- ***Fluorophore (transmission mode)***: Fluorophore, associated laser and emission filter selection.
- ***Exposure time (in ms)***: Exposure time of the camera. Accepted values: between 5 and 10 000 ms in reflection mode and between 5 and 1000 ms in transmission mode.
- ***EM gain (1 to 5000)***: EM gain (electron multiplying) of the camera. Warning: first start with an acquisition with no EM gain, then gradually increase the gain to avoid saturating and damaging the camera.
- ***Binning***: Binning of the camera. A binning reduces the size and the resolution of the image, but increases the sensitivity.

- *Number of acquired images (reflection mode)*: Number of images acquired during an acquisition.
- *Minimum time between each image (in ms)*: Minimum time between each image during an acquisition. This setting allows acquiring images at a specific rate for cinetic measurements. This system is capable of acquiring up to 5 images per second with an exposure time of 5 ms. Thus, a value of less than 200 ms will have no effect. Accepted values: Between 0 and 15 000 (4 images per minute).
- *Laser position spacing (transmission mode)*: Spacing between the laser positions in the region to image. The smaller the spacing is, the longer the acquisition will be.
- *Save folder*: Saving repository of the acquired files. A folder with the acquisition date (with the format YYYY_MM_DD) is created inside that repository.
- *Experiment name*: Name of the experiment. A folder with this name is created in the saving repository. Avoid special characters, such as | \ / * > < " '. Spaces and accents are accepted.
- *Animal identifier*: Identification of the animal(s) that is saved with the data in a file named “IDAnimal.txt”. The identification is also added to the name of the saving repository. Separate different identifiers by a space or a comma. Spaces and accents are accepted, but not special characters.
- *Photon counting mode*: Allows to active the photon counting mode during a low light acquisition, such as in bioluminescence.
- *White light*: Allows to activate the white light inside the enclosure when the door is opened, allowing to manipulate the animals more easily.
- *Heating (optional)*: Allows to activate the heating inside the device to keep the animals warm.

The menu “Color Palette” allows to chose the color of the displayed image (excluding the picture). These color palettes are also available for ImageJ.

- *Hot*: Black, red, then yellow.
- *Rainbow*: Black, violet, blue, green, yellow, then red.

Advanced configurations are also available from the “Settings” menu:

- *Target camera temperature (°C)*: Desired temperature for the camera. The optimal temperature is -85°C.
- *Prescan adjustment*: Prescan adjustment in transmission mode. Accepted values: 1 to 10. A smaller number allows a less severe pre-acquisition and a larger EM gain. This parameter should only be modified by an authorized person.
- *Saturation limit*: Limit of saturation in terms of ratio of pixels. The acquisition will stop if the ratio between saturated pixels and the total number of pixels is greater than this limit. Accepted values: 0.01 to 0.3. This parameter should only be modified by an authorized person.
- *Born: Low display limit*: Allows to adjust the Born display by limiting the values that are too low in transmission mode. A value of 0 means there is no limit and a value of 0.4 will cut the lower 40% pixels.
- *Born: High display limit*: Allows to adjust the Born display by limiting the values that are too high in transmission mode. A value of 1 means there is no limit and a value of 0.1 will cut the higher 10% pixels.
- *Exposure time for picture (ms)*: Allows to modify the exposure time for the picture. This parameter could be useful if the picture is saturated or too noisy. If necessary, this value can be saved with the option “Save configuration”.
- *Gain for the 3D profile*: Allows to modify the gain when acquiring the 3D profile. This parameter could be useful if the picture is saturated or too noisy. If necessary, this value can be saved with the option “Save configuration”.
- *Laser power modulation in absorption (%)*: The laser power is multiplied by this factor in absorption mode. Modifying this parameter allows improving the absorption acquisition in transmission mode if there is saturation or if the signal is too weak. This value is automatically adjusted during the acquisition. If necessary, this value can be saved with the option “Save configuration”.
- *Laser power modulation*: Allows to reduce the laser power during the fluorescence acquisition in transmission mode. This parameter should be modified only if the pre-acquisitions failed or if there was saturation.

- *Light power*: Modify this value if the white light source power was manually changed. This has no effect on the acquisition, but it allows to note this change in the saved files.
- *Numerical aperture of the lens*: Modify this value if the numerical aperture of the lens was changed. This has no effect on the acquisition, but it allows to note this change in the saved files.

3D profile acquisition

This acquisition mode allows obtaining the 3D profile of the mice to image. This is mandatory for transmission mode acquisitions, but optional for reflection mode acquisitions. It allows to detect the thickness of tissue to penetrate, which is required for the 3D reconstruction.

To start a 3D profile acquisition, in the acquisition window, press the "3D profile" button and wait for completion.

Select the scan region (transmission mode only)

The scan region selection allows to determine the special position of the mouse and to select the region of interest where the imaging will be performed.

Follow these steps to perform the selection of the scan region:

- Press on the button "Select the scan region" to capture a picture.
- (Optional) Press on the button "Auto-detect" for an automatic detection of the mouse. This option may not work on certain types of mice.
- Manually add/remove regions with the "+" and "-" buttons, then by drawing a rectangular or a free hand shape.
- Press on "Generate grid" to generate a list of points where the laser will be scanned.
  - If needed, press on "Delete" to remove the grid and the scan region.
-
- In the acquisition parameters, the option "Laser position spacing" allows fixing the distance between 2 points in the grid. The smaller the spacing, the longer the acquisition will be.

Reflection mode acquisition

This acquisition mode is the most flexible one. In this mode, the illumination is performed from the top with a filtered white light source.

Follow these steps to perform an acquisition in reflection mode:

- Configure the acquisition parameters in the “General parameters” panel.
- (Optional) Scan the 3D profile.
- Start the acquisition.

A reflection mode acquisition will automatically perform the following steps:

- A picture is taken.
- If the set EM gain is higher than 1, a pre-scan is performed by gradually increasing the EM gain until the desired value is achieved, to prevent damaging the camera. Il there is saturation, the EM gain is automatically reduced.
- The acquisition is started, and the set number of images is acquired.

Transmission mode acquisition

This acquisition mode allows the illumination from the bottom with the scanning of a laser beam. The acquisition is longer, but it allows obtaining quantitative and tomographic images.

Follow these steps to perform a transmission mode acquisition:

- Configure the acquisition parameters in the “General parameters” panel.
- Scan the 3D profile.
- Select the region to image
- Start the acquisition.

A transmission mode acquisition will automatically perform the following steps:

- A picture is taken.
- Pre-scan to determine the laser power at each position of the region to image. Where the laser has less tissue to penetrate, the laser power will be reduced during the acquisition.

- If the set EM gain is higher than 1, a second pre-scan is performed by gradually increasing the EM gain until the desired value is achieved, to prevent damaging the camera. Il there is saturation, the EM gain is automatically reduced.
- Acquisition of the absorption signal with no filter in front of the camera, for each position of the region to image. If the signal obtained is too weak or saturated, another acquisition is performed with the laser power increased or decreased by a factor of 10. This step is repeated until a proper absorption signal is obtained.
- Acquisition of the fluorescence signal for each position in the region to image. If the signal obtained is too weak, a second acquisition is performed with a higher EM gain (only if the set EM gain is higher than 1).

Display options

Multiple display options are available during and after the acquisition:

- *Display the sum of acquired images*: If checked, the sum of the acquired fluorescence images is displayed, instead of only the last one.
- *Picture in background*: If checked, the picture of the mice is displayed in the background of the fluorescence image.
- *Laser points grid in background*: If checked, the grid is displayed in white in the background of the fluorescence image.
- *Signal opacity*: Allows to optimise the display by modifying the opacity (transparency) of the fluorescence, when there is a background picture or grid.
- *Display Born corrected image (transmission mode)*: If checked, the fluorescence signal is corrected by dividing it with the absorption signal to the power of the Born coefficient.
- *Born coefficient (transmission mode)*: Allows to modify the Born coefficient from 0.5 to 1.5 by steps of 0.1, to optimize the Born correction. The absorption signal is first elevated to the power of the Born coefficient before dividing the fluorescence signal.

Display tools are also available in the tool bar at the top of the software. It is possible to zoom on a region of interest with the magnifying glass. The hand allows to move the image when a zoom is applied. The tool “Data Cursor” allows clicking in specific pixels on the image to get the

signal amplitude. The “Colorbar” tool allows displaying a colorbar next to the image, which links a color to a signal value.

Adding comments

Comments can be added at any time using the comments panel. Once the comment is written, press “Save comments” to add the text to the file “Comments.txt”. This file is located in the save repository and it contains all the saved comments.

Displayed image saving

A button in the option panel allows saving the currently displayed image. This option is only available after a complete acquisition.

Opening previous acquisition

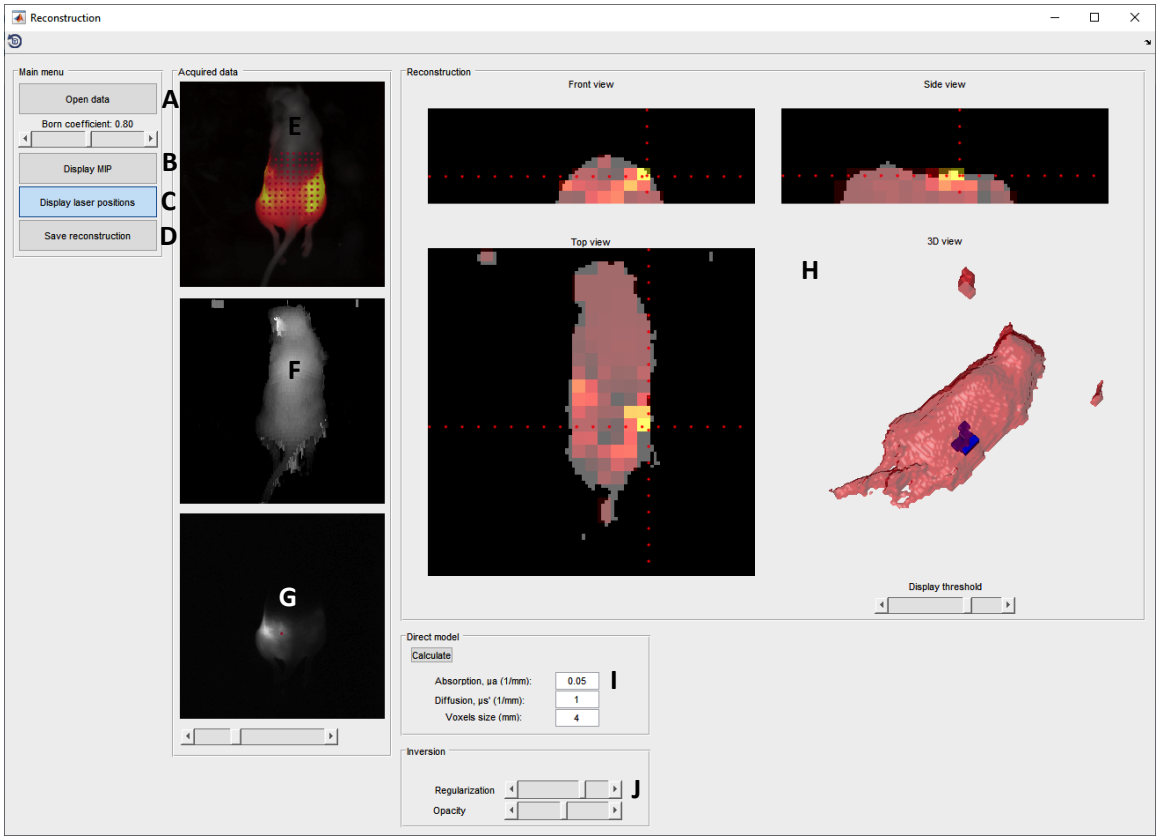
Previous acquisitions can be opened from the “File” menu or with the icon on the tool bar. This is useful to analyse previous data and save the displayed image, with or without the picture in background or the Born normalisation.

2.4. Reconstruction Software

The reconstruction software allows loading data acquired in transmission mode to perform a tomographic reconstruction. This software is entirely opened and can be modified/improved by the user.

The reconstruction in diffuse optical imaging remains a difficult problem that depends on the properties of tissues (distinct for each organ), the illumination configuration and the fluorescent source position. The reconstruction process is first composed of a direct problem: the calculation simulates for each laser position the photon propagation in the target tissue. From this calculation, a “sensitivity” matrix describing the link between the measures and the fluorophores localisation is built. This matrix is at the base of the inverse problem: it is inverted to find the localisation of the fluorescent sources. However, the inverse problem (inverting the matrix) has multiple solutions (there are less measures than possible solutions). It is thus required to discretize the volume in voxels and regulate the problem. The choice of this discretization and regularization is relatively arbitrary and it is let here as an adjustable parameter by the user.

The reconstruction software allows the user to adjust the optical properties of the tissue (absorption, μ_a and diffusion, μ_s) as well as the regularisation parameter of the inverse model. Only the acquisition in transmission mode allows tomographic reconstructions, reflection mode acquisitions don't contain enough information.



Opening and Visualizing the Data

The button "Open Data" (A) is used to select the repository containing a set of data. The user must simply point to a repository saved by the acquisition software. The files are read automatically (transmission images, 3D profile, etc.).

The button "Display MIP" (B) allows opening all the data measured in fluorescence imaging and combining them in a MIP (Maximum Intensity Projection) composite image. This composite image is used to identify the main zones of fluorescence emission and/or potential artefacts at the border of the imaged region.

Over this button, a slider allows adjusting the Born normalisation coefficient, which is the correction of the fluorescence data with the

absorption data. The goal of this adjustment is to obtain the most homogeneous data to model the light propagation in a homogeneous tissue. This adjustment is also useful to favor the reconstruction of a source located close to the top or the bottom of the animal. This enhancement is required, because the light propagation emitted by a fluorophore is affection by the varying absorption and diffusion from different tissues and depending on the wavelength.

The button "Display laser positions" (C), allows showing the acquired laser positions in background.

The MIP image is shown in (E), the 3D profile in (F) and each fluorescence image can be displayed in (G), using the slider below it.

Direct Model

When loading an acquisition, a 3D profile is shown in (H) along 3 view planes. The user can navigate in these views using the left mouse button. A 3D view is also shown, and it can be rotated using the tool "Rotate 3D" in the tool bar. A slider is under this view to select the display threshold of the voxels in the reconstructed volume.

The direct model consists in the calculation of the light propagation. The panel "I" contains all the parameters. To calculate the sensitivity model of the direct model, the user must:

- Choose the absorption coefficient (μ_a): This parameter varies with the imaged organ, the mouse (color of the skin/fur) and the wavelength. An adequate value for near infrared is 0.05/mm. This value should be increased for shorter wavelength or when imaging over the liver or the heart, two very vascularized organs. The absolute μ_a value in the literature is limited and difficult to evaluate, as it is difficult to perform a measurement on a small vascularized organ independently. It is suggested to start with the default value and adjust if required depending on the image quality.
- Choose a diffusion coefficient (μ_s'): Similarly, the diffusion coefficient varies with the imaged region. However, a value of 1/mm remains a good choice for most cases.
- Choose a reconstruction size: The size of the voxels is in mm. For a fast initial calculation, it is recommended to start with a larger voxel size, for example 6 mm, then gradually reduce it. The smaller the

size, the longer the calculation. It is important to note that diffuse optical imaging remains a low resolution technique, using voxel size under 2 mm would not be realistic.

When the parameters are selected, the direct model can be calculated using the button “Calculate”. At this point, the software starts the calculation, which can take up to a few minutes. Once completed, the user can now perform the inverse problem, which is the reconstruction.

Inverse Model

The inverse model aims at estimating the special distribution of the fluorescent sources from the measurements and the direct model. It mainly (and strongly) depends on the regularisation parameter fixed by the user in the panel “J”. For each reconstruction, the user must explore multiple regularisation values: If it is too high, the reconstruction will tend to show an asymmetric solution and non-localised on the borders. If it is too low, then no, or a constant solution will be shown. The adjustment allows choosing the optimal value (which varies with the voxel size and other parameters) and localising the fluorescence sources.

The button “Save reconstruction” (D) allows saving an image in .tiff format of the 3D view displayed, and in .mat format compatible with Matlab.

3. OPTIMISING THE ACQUISITION PARAMETERS

3.1. Fluorescence in Transmission Mode

In this mode, it is recommended to leave the binning at 1x1. The recommended spacing of the laser positions is 2 mm. Only the exposure time and the EM gain will influence the image quality. To find the optimal values, proceed as follow:

- Start with an exposure time of 5 ms.
- Gradually increase the EM gain up to 500.
- If the signal is still weak, increase the exposure time up to 100 ms.

3.2. Fluorescence in Reflection Mode

In this mode, it is recommended to leave the binning at 1x1. Only the exposure time and the EM gain will influence the image quality. To find the optimal values, proceed as follow:

- Start with an exposure time of 5 ms.
- Gradually increase the EM gain up to 500.
- If the signal is still weak, increase the exposure time up to 1000 ms.

The number of images to acquire should be 5. An higher value will allow reducing the noise level, by summing the images, but since the noise is generally low, this should have little effect.

3.3. Bioluminescence in Photon Counting

The photon counting mode automatically applies a threshold on the acquired image of the camera. The displayed result is either 0 or 1, depending if a photon was detected or not. Note that if more than one photon is detected, the value will still be 1. Thus, optimal parameters are required to obtain a reliable result.

Activate the “Photon counting” checkbox, choose a binning of 1x1, 2x2 or 4x4, depending on the desired resolution and a high number of

images, for example 100. Select “No filter” in the “Emission filters” menu.

To find the optimal values, proceed as follow:

- Start with an exposure time of 500 ms, an EM gain of 3000 and 100 images.
- Increase the number of images if the acquisition duration is not an issue.
- Examine individual acquired images. If they contain a low number of active pixels (detected photons), increase the exposure time up to 3000 ms. If they contain a lot of active pixels, photons may be lost (more than 1 photon detected per pixel will still result in a value of 1). In that case, reduce the exposure time down to 10 ms, followed by a reduction of the EM gain.

3.4. Acquisition Over Many Weeks

In some situations, an animal cohort needs to be imaged at different time points to evaluate the evolution of a target, such as a tumor.

The animal should be positioned the same way from one experiment to the other, and the acquisition parameters should be identical. However, it is possible that the EM gain or the exposure time must be modified to keep a good signal or to avoid saturating the camera. In these cases, the images can still be compared, because the signal read from the camera is linearly proportional to the gain, the exposure time and the binning. For example:

- An image acquired at an EM gain of 1000 can be brought back at the same scale as an image with an EM gain of 500, simply by dividing the image by 2.
- An image acquired at an exposure time of 1000 ms can be brought back at the same scale as an image with an exposure time of 500 ms, simply by dividing the image by 2.
- An image acquired at a binning of 4x4 can be brought back at the same scale as an image with a binning of 1x1, simply by dividing the image by 16.

Although the signal is linearly proportional to these parameters, the noise may not be. This is particularly important in bioluminescence, with low and noisy signals.

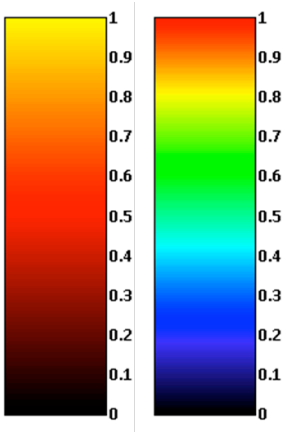
4. IMAGE ANALYSIS IN REFLECTION MODE

The saved files of interest are ReflectionSum.tiff and Picture.tiff. The file ReflectionSum.tiff is the sum of all the acquired images in photon counting mode and the mean of all images in other modes.

4.1. Analysis in the Software

The acquisition software allows saving the picture in transparency under the fluorescence/bioluminescence images, which an opacity adjustment.

Two color palettes are available from the menu “Color palette”: Hot or Rainbow as shown below.



When the checkbox “Display the sum of the acquired images” is checked, the sum of all the images is displayed (or the mean, if not in photon counting mode). Moreover, with this option, the saturated pixel traces from cosmic rays are greatly attenuated.

4.2. Analysis in ImageJ

For advanced image analysis, the ImageJ software can be used. ImageJ is an advanced image analysis and treatment software, which contains all the required functionalities.

The following sections exposes some of the useful functions in ImageJ.

Adjust Brighness/Contrast

The brightness and contrast of the images can be modified in the menu Image → Adjust → Brightness/Contrast. This allows to improvement the picture display, for example.

With these adjustments, it is also possible to fix multiples images at the same scale, using the "Set" button. Thus, it allows a better comparison of multiples images.

Lookup Tables

A color palette can be applied to the image by selecting the propoer lookup table in the menu Images → Lookup Tables.

The color palettes from the software acquisition can easily be imported in ImageJ by copying the ".lut" files provided with the acquisition software in the "luts" folder of the installation repository of ImageJ, for example "C:\Program Files\ImageJ\luts".

The color palette can be displayed on top of the image with the option in the menu Analyse → Tools → Calibration Bar.

Picture Overlay

ImageJ easily allows to superpose the fluorescence signal in transparency with the picture in background. To do so, select the fluorescence image, choose the option in the menu Image → Overlay → Add image and select the picture image with the transparency percentage.

In the case where the color palette is displayed, ImageJ will not allow to overlay another image. It is thus required to superpose the images with the option Image → Color → Merge Channels. The fluorescence signal must be put on the 3 primary colors channels (red, green, blue) and the picture must be put on the gray channel.

Math Operations

It may be required to perform math operations on the images, for example to compare two images acquired with different gains.

Multiple options are available in the Menu Process → Math.

Measurements in a Region of Interest

The main toolbar contains tools to select a square, round or free hand region of interest. When a region of interest is selected, the option in Analyse → Measure displays multiple useful information, such as the area, the mean, minimum and maximum values.

Macros

Wenn multiple operations must be performed on a series of images, it is possible to record a macro by selecting "Record" in the menu Plugins → Macros. Afterwards, perform all the desired operations, press on "Create", and save the ".jim" file. If the macro is frequently used, it can be added in the menu with the option "Install" in the menu Plugins → Macros.

Contrast Enhancement

Note that this option is usually not required, because the acquisition software automatically applies a contrast enhancement of 0.4% to remove saturated pixels.

When the image contains saturated pixels that hide the image because of the modified dynamic range, the contrast of the image can be increased in the menu Process → Enhance Contrast. ImageJ will ask to input a percentage of acceptable saturated pixels. Use 0.4% or 1%.

4.3. Units in Photons per Second

In photon counting mode, the unit of the acquired images is in photons and can easily be converted to photons per second, by dividing by the exposure time in seconds.

The unit of the acquired images, when not in photon counting mode, is the ADU (analog-to-digital unit). This measure can be converted in the number of detected photons by multiplying the value by 17.3 and by dividing it by the EM gain. The number of photons per second is obtained by dividing by the exposure time in seconds.

To obtain the number of photons per cm^2, each pixel must be multiplied by the 2 471. A pixel has an area of 0.0004047 cm^2, since the field of view is 10,3 cm for 512 pixels.

To obtain the number of photons per steradian, each pixel must be multiplied by the 184. Since the camera lens is at 60 cm from the animal, a steradian is equivalent to 3 600 cm^2, and the lens has an area of 19.6 cm^2.

To obtain the number of photons per cm^2 per steradian, each pixel must be multiplied by 453 851.

5. MAINTENANCE

5.1. Cleaning the Glass and Plastic Plates

Do not use cleaning agents containing hydrogen peroxide, such as Peroxigard. The vapors may damage the electronics and corrode metal components. If your facility demands the usage of hydrogen peroxide, make sure to leave the door of the imaging system opened during 1 hour after cleaning.

The glass and plastic plates are used to place the mice during an acquisition. It is recommended to clean them after each use. The plastic plate is in high density polyethylene (HDPE), which resists most cleaning solutions. The glass plate is in borosilicate (Pyrex), also very resistant to chemicals.

5.2. Cleaning the Optical Filters

Dust can accumulate on the filters in the motorised filter wheels, especially when they are frequently swapped. These filters must be cleaned with methanol and dedicated tissue for optical cleaning (Kim-Wipes), when the image quality is affected.

5.3. Cleaning the Mirror Under the Mice

A glass mirror is located under the mice at an angle of 45°. Dust can accumulate on this mirror, it must be cleaned when the image quality is affected. The mirror must be cleaned with methanol and dedicated tissue for optical cleaning (Kim-Wipes), when the image quality is affected. Do not press too hard on the mirror, since it is fixed only on the side and bottom.

5.4. Changing the Carbon Filter (If Present)

The carbon filters have a limited lifetime, which depends on their usage. They can easily be replaced and bought from any anesthesia equipment supplier.

5.5. Changing the Halogen Lamp

When the halogen lamp of the white light source doesn't power on or its intensity is too low, it must be replaced. The halogen lamp has a lifetime of 200 hours. The instructions to replace the lamp are in the white light source manual, supplied with the imaging system. The operation requires no tools and can be done in under two minutes. Replacement lamps can be bought from Edmund Optics (Item #: 39-606) or Thorlabs (Item #: OSL1B).

The intensity of the lamp reduces with usage. An old lamp can have a loss of intensity of 90% and continue to work fine.

To determine the loss of intensity of the lamp from one acquisition to the other, the signal difference of a picture can be used.

The following procedure will determine the loss of intensity of the lamp from one acquisition to the other:

- Put a white sheet a paper in the imaging system.
- Perform an acquisition in reflection mode with the default parameters, in particular a binning of 1x1.
- Open the file "Picture.tiff" located in the saving repository with ImageJ.
- Go to Analyse → Measure and note the value "Max".
- The maximum value should be around 1200 for a new lamp. Note that this value could slightly change from one system to the other. An old lamp could have a value of only 150. It is recommended to change the lamp when the power loss is more than 50%.

○

6. SECURITY

6.1. Optical Danger for the User

The device contains lasers emitting visible and invisible (780 nm) light up to 100 mW. The lasers can only be activated when the door is closed and when the key is at the ON position. For security, the user should avoid looking directly in the center hole when the system is powered on.

The white light source contains a 150W halogen lamp, coupled to an optical fiber. Before using the system, the user must make sure that the optical fiber is properly mounted in the white light source and in the imaging system.

6.2. Optical Danger for the Camera

The camera is very sensitive to light. The lasers, the white light source and even the ambient light are enough to damage the camera sensor. The danger is present when the camera has an EM gain set. For this reason, the user must always perform an acquisition with no EM gain, then slowly increase this parameter to avoid saturating and damaging the camera.

During an acquisition, it is important not to open the door. In case of a problem, stop the acquisition first with the software before opening the door. Only in case of an emergency, shut down the camera first. Note that the camera should not be shut down when the sensor temperature is lower than -65°C.

Moreover, the user should not manually move the adjustment wheel on the white light source during an acquisition. This could cause an incorrect calibration of the EM gain.

Software protections are implemented to avoid directly illuminating the camera with the lasers. The EM gain is carefully activated to avoid

saturating the camera. A software protection cuts the EM gain, the lasers and the acquisition when the door is opened.

The user must make sure that the mice are properly anesthetized before imaging. A mouse that would move during a transmission mode acquisition could cause damage to the camera.

The filters installed in front of the camera allows to protect it by only letting pass certain wavelength of light associated to the fluorescence emission. The user must follow the instructions of this manual before changing a filter wheel. The user must not change or remove any filter from the filter wheels, since software modifications are also required.

6.3. Anesthesia Gases

The system is equipped with tubes for anesthesia gases and aspiration. It is highly recommended to use an active aspiration to avoid spreading anesthesia gases in the imaging device. Moreover, the user must block the unused anesthesia masks with the supplied plugs.

Systems equipped with a hot air heater contain a carbon filter at the top of the imaging system. When the heater is activated, the air aspirated by the device is taken through the carbon filter. It is the responsibility of the user to change this filter regularly.

7. GENERAL INFORMATION

7.1. Warranty Limitations

Unless otherwise noted in the quotation and invoice, MediLumine offers a one (1) year warranty, including parts and labour charges, against defects in material, performance and workmanship, if used according to this manual.

MediLumine will replace or repair a defective system during the warranty period. A Return Merchandise Authorization (RMA) number must be obtained prior to returning the product. Shipment charges must be prepaid by the sender.

This warranty does not cover (a) damage, deterioration or malfunction resulting from accidents, misuse, abuse, natural disasters or failure to follow instructions according to the user manual; (b) systems that have been altered or containing unauthorized replacement parts; (c) normal wear and tear; (d) damage attributable to inadequate electrical power, power lines surge or related electrical abnormalities. This warranty covers only the original owner and is not transferable.

Opening the bottom part of the imaging system without the authorization of MediLumine is not authorized for security reasons.

7.2. Disclaimer

In no event shall MediLumine be liable for any damages of any kind or nature arising from ownership or use of its product including, without limitation, lost profits, lost information and direct, indirect, incidental or consequential damages.

This product is designed for non-human use and is not a medical device. It is thus not authorized for use in human applications.

7.3. Warnings

- The system is not authorized for human use.

- The system may emit visible and invisible light, from the 150 W white light bulb and the 100 mW laser diode.
- Use only three prongs outlets, with an earth ground connection.
- Use the provided power supply only.
- Do not use cleaning agents containing hydrogen peroxide, such as Peroxigard.
- Do not sterilize any parts of the system.
- Avoid spilling liquids in the imaging system.
- Turn the system off when not in use.
- Do not plug or remove connectors when the system is powered.

7.4. Regulations

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

8. TROUBLESHOOTING

The image contails isolated saturated pixels that hides the signal, due to the modified dynamic range.

With an high EM gain (generally over 500), the camera is sensible to cosmic radiation. On average, the camera is hit by an astroparticle every 10 seconds. It is not possible to protect the camera against this radiation, but many solutions are possible to reduce the impact on the image.

When the check box “Display the some of acquired images” is checked, the software automatically reduces saturated pixels, up to an area of 0.4%, which enhances the contrast of the rest of the image.

It is also possible to correct the images in ImageJ by enhancing the contrast, which will eliminate the saturated pixels.

In fluorescence in reflection mode, reduce the EM gain under 500. The acquisition of a larger number of images will allow to attenuate the effects of cosmic radiation.

In bioluminescence, it is strongly suggested to use the photon counting mode. In this mode, a saturated pixel caused by an astroparticle will have the same value as a single detected photon. By acquiring many images (100 for example), the effect of cosmic radiation will be almost inexistent.

The software can’t connect to the imaging system.

It is recommended to power the imaging system 10 seconds before starting the software, to be sure that it is fully booted. Verify that the green LED under the front door is lit and that the camera fun is running.

The acquisition automatically stopped, and the following message is displayed: “Saturation detected on an area of X %”.

First, make sure that the animal is fully anesthetized and that it didn’t move.

In certain rare cases, a cosmic ray may have hit the sensor of the camera in an angle that causes a large region of pixels to be saturated. Since it is not possible to differentiate saturation coming from cosmic rays from optical saturation, the acquisition stopped to avoid damaging the camera. If this was the issue, simply start the acquisition again.

In fluorescence in transmission mode, this error could occur when the pre-acquisition didn't successfully determine the optimal optical power for each laser position. Reduce the EM gain and start the acquisition again.

The EM gain of the acquisition is lower than the initially set EM gain.

A pre-acquisition is performed before each acquisition to make sure that the set ET gain will not saturate the camera. The software will automatically optimize the EM gain to maximize the signal to noise ratio.

In certain rare cases, during a pre-acquisition, a cosmic ray may have hit the sensor of the camera in an angle that causes a large region of pixels to be saturated. Since it is not possible to differentiate saturation coming from cosmic rays from optical saturation, the pre-acquisition blocked the EM gain at a lower value than the set value. In that case, if an higher EM gain is desired, the acquisition must be restarted with the initial EM gain.

The signal is weaker than before in reflection mode.

The intensity of the white light reduces with its usage. An old light bulb can have a loss of power of over 90% and still be functional. Refer to the maintenance section and the white light source user manual to change the lamp.

The 3D profile of the animal is wrong.

Verify the precision of the profilometer by testing a flat rectangular object of a known height between 1 and 2 cm. If the error of the height is over 2 mm, the profilometer must be aligned using the advanced parameters in the software acquisition. Press on the button "Galvo alignment (3D profile)" and follow the instructions.

The laser positions are wrong in transmission mode

During an intensive use in transmission mode, or if the system was moved. It is possible that the motorized mirrors must be realigned using the advanced parameters in the software acquisition. Press on the button “Laser galvo alignment” and follow the instructions to first verify the alignment and, if required, to modify them.

9. CONTACT INFORMATION

MediLumine Inc.
5795 Av. De Gaspé, Suite #228
Montreal, Quebec, Canada
H2S 2X3
Phone: 1-514-360-1574
Email: info@medilumine.com