13, rue Georges Auric
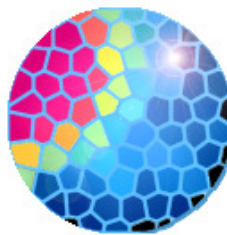**F - 75019 Paris**
www.biospacelab.com
info@biospacelab.com

User Guide

M3 VISION

M3 VISION

Hardware and software requirements

In order to use M3 VISION, your hardware and software should meet the following requirements:

Hardware requirements

- Computer equipped with a Pentium IV or Dual Core type processor.
- 40 Mbytes of free space on the hard disk for full installation.
- 500 Mbytes of free space on the hard disk for standard use.
- 1 Go of RAM
- A graphic board with 16Mbytes of RAM that may display at a minimum resolution of 1280x1024 in 32 bits mode.
- To display 3D data properly, the graphic card should support 3D textures.
- An SVGA or higher resolution monitor. We recommend a monitor size of 19" or larger.

Software requirements

- Windows VISTA/7/8 operating systems either 32 or 64 bits.
- Microsoft .NET Framework 4.5.
- Microsoft Excel for exporting results.

Note: M3Vision will not run properly under Windows XP since "Microsoft .NET Framework 4.5" is not XP-compatible.

TABLE OF CONTENTS

1 QUICK START GUIDE

The purpose of this abbreviated manual is to help users to carry out basic tasts such as Opening acquisitions, optimizing display settings, quantification and export.

1.1 How to install and launch M3 Vision

- any previous version of M3Vision should be uninstalled prior to installing the new version of the M3Vision analysis software. .To Uninstall M3Vision: click on Start in the Windows task bar, Configuration Panel, Uninstall a program, and double-click on M3Vision.

- To work properly, M3Vision requires "Microsoft .NET Framework 4.5", Which may be downloaded from http://www.microsoft.com/en-us/download/details.aspx?id=30653

- Insert the M3Vision installation CD.

- The installation should be launched automatically. If not, explore the CD and double click on Setup.exe.

- Once the setup is launched, follow the instructions, and click Next for each step.

- The using of M3Vision requires the installation of AVI codecs: MPEG-4 AVC codec (for 32 and 64 bits). They are included in the M3Vision's setup

- When launching M3Vision for the first time, a license file needs to be loaded:
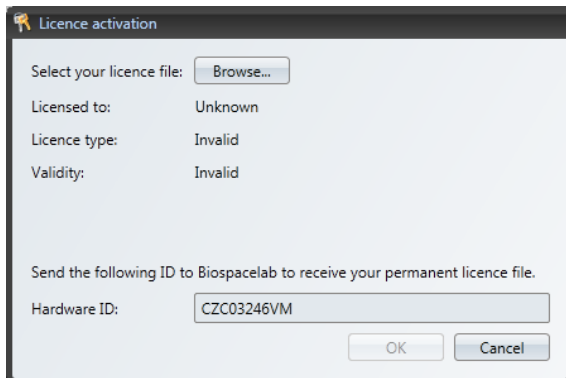
- If you already have a license file, click on **Browse** and select it (**license.lic**)

- If you do not have a license file, you may request one from Biospacelab at support@biospacelab.com

- A temporary demo license or a permanent license can be issued. There are two different types of permanent license: Hardware license or dongle.

- For permanent Hardware licenses, a unique computer **Hardware ID** is required. This ID is automatically retrieved by M3Vision at its first launch and should be emailed to support@biospacelab.com together with the license file request. Each license file is only valid for one computer ID and will not work on another computer.

- Click on the M3 Vision Desktop icon [icon] to launch the software or in the **Windows Start menu**, select **Programs** and then Biospacelab**/M3Vision/M3Vision**.

Once the software is launched and the license loaded, the workspace will appear.

1.2   The M3Vision Workspace

As shown in the figure below, the M3Vision workspace is composed of 6 windows.
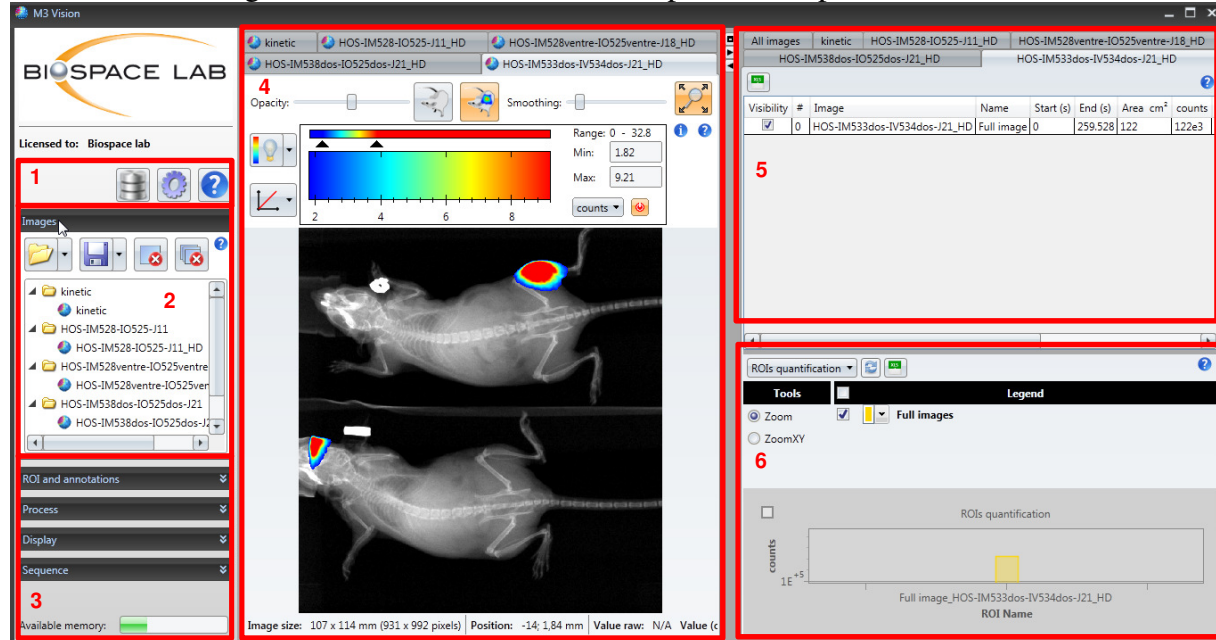
1. The Database /Settings/Help
2. The Workspace Menu
3. Tools
4. Image Viewer
5. Result Viewer
6. Graph window

Database /Settings /Help window

- The Database allows users to access browse and search images with keywords related to metadata information.

- The settings allow users to access advance features such as viewing the software version, and log file, modify the licence, manage units and palettes, and set the maintenance mode.

- The Help buttons gives users access to the interactive user guide.

Workspace Menu window

The workspace menu allows users to open and close acquisitions and save images and workspaces.

Tools window

The tools window is divided in 5 dropdown menus allowing access to various image procession features:

- The **ROI** menu: Access to various ROI drawing tools.
- The **processing** menu: for time extractions and time laps profiling.
- The **Display** menu: to organize the display of multiple images
- The **Sequence** Menu: to create acquisition sequences and export movies.
- The **Maintenance** menu**:** for advanced features and acquisition information.

1.3   **Image Viewer window**
In the image Viewer window each acquisition is sorted as a tab. For each acquisition, image Display features including black and white image overlay, signal offset, threshold and smoothing as well as quantification units can be controlled with the **Image Display panel** (see section 2.2 and 2.3).

1.4   **Result Viewer**
For each acquisition quantification from the drawn ROIs is listed in a table in the **Result Viewer** Window, which can be exported to excel as a report. Again, each acquisition is sorted as a tab but there is also a tab summarizing the information from all open images in one table.

Note: if the Quantification table and the graph window are not visible after starting M3Vision, it can be accessed by clicking on the black arrows on the top left panel of the screen or by dragging the right edge of the Image Viewer window to the right.

Graph window
When created, graphs for time profiles, spatial signal intensity profiles and histograms can be viewed in the graph window. They can also be exported to Excel together with the linked raw data. The quantification values present in the result viewer can also be viewed on a diagram bar.

1.5   **Opening images**

Acquisitions can be opened in two ways:

- Click on the folder button and browse until users find the file they want to open. A double click results in opening the image in M3 Vision. Users can select and open several images at once. Desired file can also be opened by drag-drop.

- Click on the Image Base button and browse the thumbnails or type the keyword in the search space until users find the file they want to open. A double click results in opening the image in M3Vision.

Each opened Image can be viewed in the **Image Viewer** by clicking on the corresponding tab or selecting the name of the image in the **Workspace Menu**. Situated in the upper part of the Image Viewer, the **Image Display panel** allows control of the rendering of the image.

1.6 **Changing the image display**

Various Image Display parameters can be controlled from the **Image Display panel** of the **Image Viewer** window parameters to manage the image display:

The signal contrast can be adjusted by changing the **Min** and **Max** values of the **Look-Up-Table** (LUT) either by sliding the arrows of the LUT bar or by editing the value in the text fields.

- For bioluminescence, fluorescence or autoradiography acquisitions if photographic image available :

  o **The Opacity slide bar** allows adjustment of the overlay between the photographic image and a signal image.

  o **The photographic display** . Allows adjustment of the Brightness and Contrast **Min** and **Max** values of the black & white image either by sliding the arrows of the LUT bar or by editing the value in the text fields.

- For all modalities:
  o **The color coding table**. To change it, select the desired palette, by right clicking on the LUT.
  o **Smoothing option**. Apply some smoothing by moving the bar.

Note: changing the signal contrast does not affect quantification; this action only alters the display of the image.

1.7 **How to quantify region(s) of interest**

- Region of interest (ROI) can be used for quantification of specific areas. It is located on the left panel **ROI and annotations** tab**.**

- By scrolling **ROI and annotations** tab ROI drawing options appear. Select the appropriate drawing tool to create your ROI. You can also draw several ROIs with the same tool by pressing and holding a Space key from the keyboard

- Selection button allows moving the ROIs or other graphics drawn on the image

  o For ellipses and rectangles, Click hold and drag the handle to define the ROI size. Release the mouse button to finalize your selection. The orientation of the ROI can be adjusted clicking on the red handle.

  o For ROIs with multiple handles (polyline, spline), a simple click defines a new handle, and a double-click closes the ROI.

  o For freehand ROIs, click and hold the left mouse button whilst dragging to create the ROI. Release the mouse button to close the ROI. A straight line will automatically be created between the first and last handles.

  o Automated magic wand selection tools draw ROIs according to the Offset and threshold values of the LUT. To use these tools, select the Offset and threshold values defining the ROIs you want to select in the **Image Display Panel** then select your magic wand tool (One flat ROI, one ROI with a hole, Whole image, Whole image with holes), and click on the region where you want to draw your ROI.

- The desired quantification units shown on the image can be selected from the drop down menu in the Image Display Panel.

- The measurement report is synchronized and automatically updated with the state of the different ROIs (except for ROI Grid where the message informs to manually update the data). The results of the measurements are displayed in the **Result Viewer** window on the right hand side of workspace.

- The **Quantification report** can be exported in an Excel format for printing.

  For exports to Excel click on the little green Excel icon in the upper left corner of the **Result Viewer** window or right click anywhere in the **Result Viewer** window and choose export to Excel from the contextual dropdown menu. You can export either the image of interest or choose to export All images which will contain the summary of all images and a separate sheet for each exported image.

1.8 Saving Images

- Images can be exported to different formats including bmp, jpg, png, gif and tif by clicking on

  the save button in the **Workspace Menu** window.

2   General presentation of M3 VISION

2.1   The M3 Vision software for image analysis

M3 Vision is the software for the analysis of the acquisitions performed with Biospace Lab instruments: PhotonIMAGER^{TM} (optical imaging with fluorescence or bioluminescence), BetaIMAGER^{TM} ^{t}RACER and ^{d}FINE (autoradiography), and GammaIMAGER^{TM} (scintigraphy) systems. It provides tools for image post treatment, detection, localization, quantification, and time analysis of signals, and enables exports to Excel for both quantification and illustration.
The software was developed with the aim of providing researchers the best coupling of user friendliness and powerfulness.

2.2   Main window

The Main Window is divided in the three following parts:
1. **Workspace Menu**
2. **Image Viewer**
3. **Result Viewer**
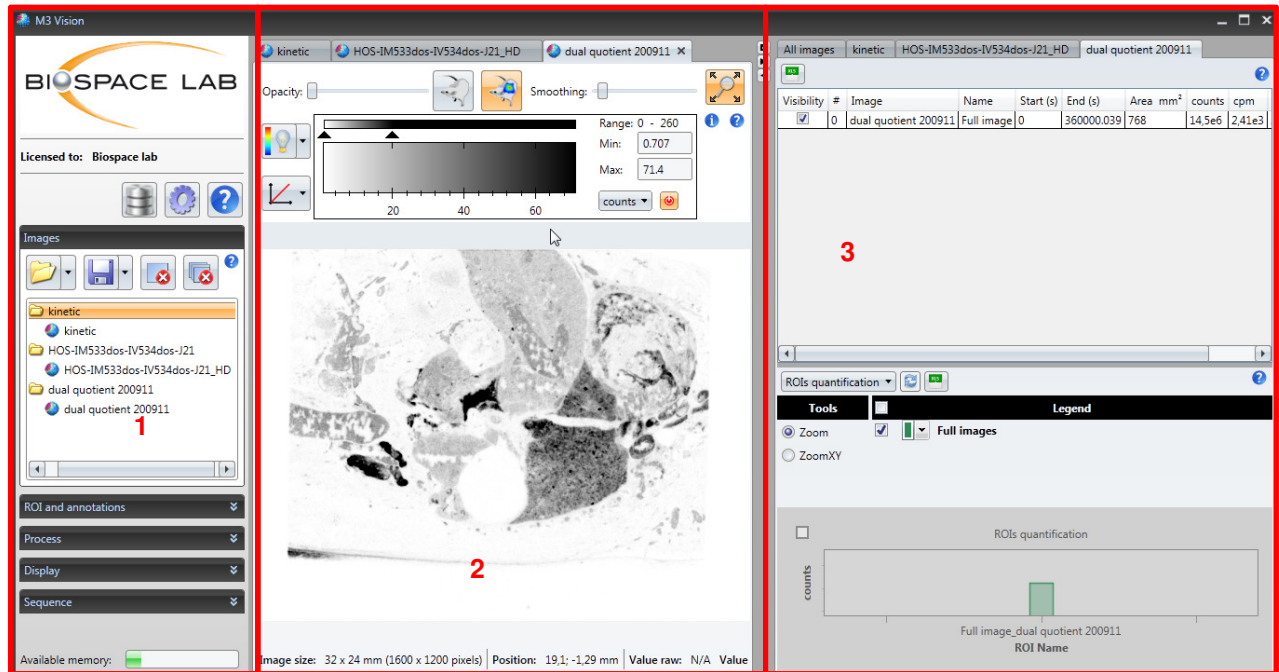
Workspace Menu

The Workspace Menu is located on the left of the M3 Vision main window.
It includes the following buttons: image database, settings, help, open image, save file, close current file, close all files and image management tabs.

On the bottom of the workspace menu the **memory indicator** is located:

This gauge displays the available software memory. The more images uploaded to M3Vision the green gauge will diminish. When it becomes red it means there is not enough memory to work with the best efficiency and M3Vision risks not to work correctly. It is recommended to liberate the space by closing some images or other programs that may be running at the same time.

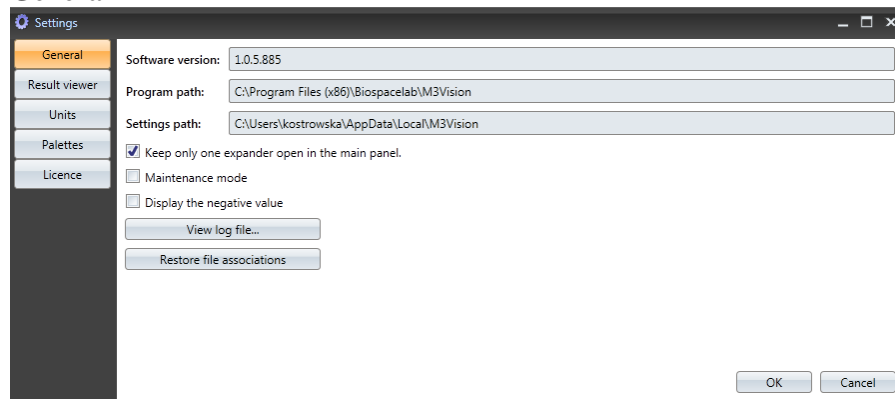On the top of the menu, three buttons are available:

 : Image data base

This button gives an easy and fast access to all images uploaded to M3Vision. Every image opened in the software is automatically added to the Base.

 : Settings

General

Display of the general parameters of the software:

Software version: Current version of M3Vision

Program path: Indicates where the software is installed. (lecture rights only).

Settings path: Indicates where the parameters and user files are stored (personalized pallets, calibrations, etc.). Accessible with lecture and writing rights,

View log file: History file, which may be requested by the support team in case of the problems with the software.

Restore file associations: Allows repair of file associations. When double clicking on a BVR file from Windows Explorer M3Vision should be launched automatically. If not, clicking on 'Restore file associations' should fix the problem.

Keep only one expander in the main panel: when ticked allows keeping open only one of the tabs in the Menu Workspace (ROI and annotations, Display, Process or Sequence). Otherwise one or more tabs can be opened at the same time.
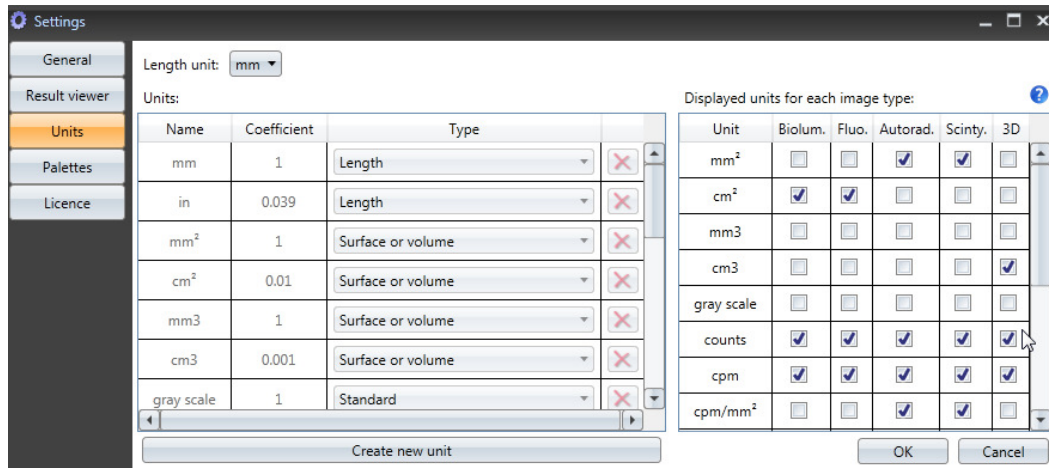
Result Viewer
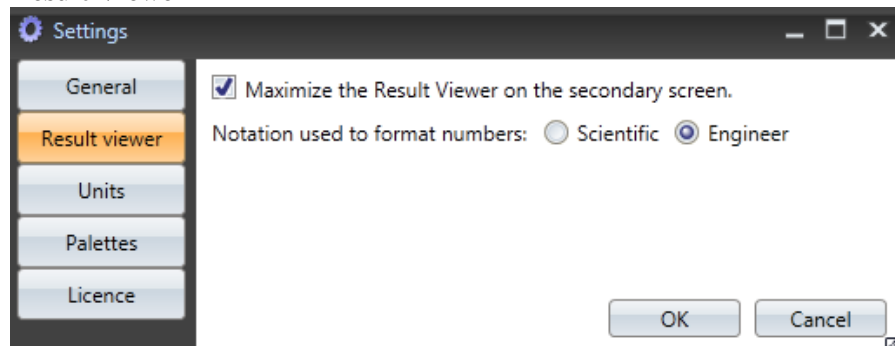
An option **Maximize the result viewer in the secondary is** ticked by default. When using a secondary screen this option makes it possible to open the Result Viewer window on that screen.
One can also choose the way the numbers are displayed. There are two options:
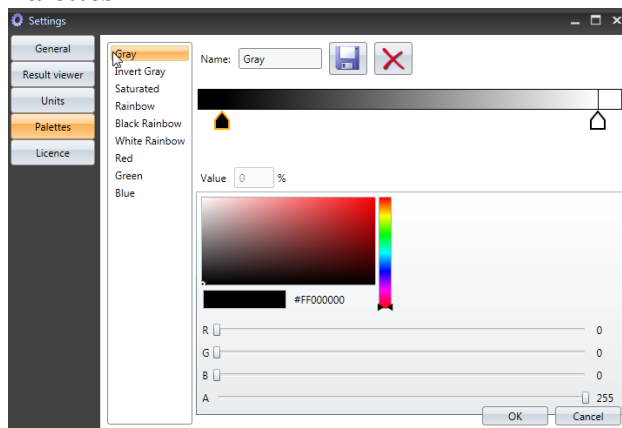Scientific: 33E+3
Engineer: 33e3

Units

This tool allows creating new units (left window) and also shows the displayed units for each image type (right window)

Be careful when you create a new unit, **do not forget to specify for which type of image it will apply**. Coefficient relative to the base unit 'counts'.

Palettes

This tool allows the creation of the customized palettes. To add the control point one must click between two already existing ones. To change the color and the position of the control point one must click below on the color display window.

The color completely on the left of the color window will be used for the values below the minimum threshold of the LUT.

The color completely on the right of the color window will be used for the values above the maximum threshold of the LUT.

The customized palettes can be saved or deleted. The default palettes can't be modified.

License
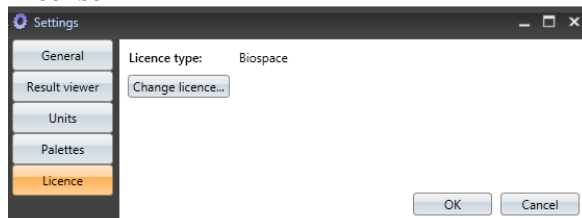
Gives the information about the software license, and the possibility to change it.
Three types are possible:
Demo
Permanent
Temporary

 : Help

This button is found everywhere in the software. Clicking on it allows getting help related to the relevant section.

Under those buttons the **Image Panel** is located.

Image Panel

This panel contains:

 Open file: Opens the 'Load image file' dialog box. The associated arrow gives you the list of last 10 images charged to M3 Vision.

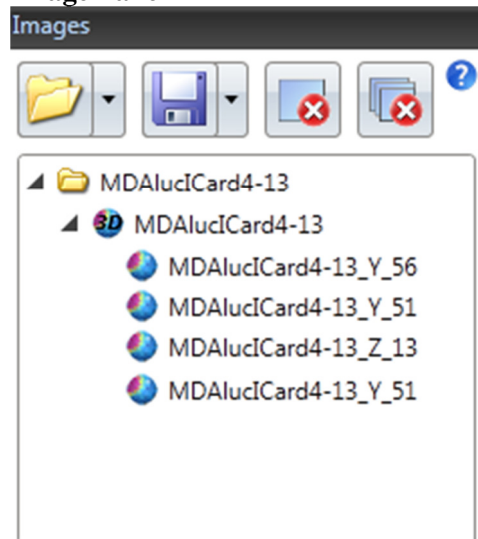When opening an acquisition, M3 Vision opens all the files associated to this acquisition: photographic image, excitation and background images for fluorescence. If the files are not found, a message will be displayed to inform the user.
-   If no photographic image was taken or if the trp or x-ray files cannot be found, it will not be possible to display the photographic image and the fusion images corresponding to the acquisition.

NOTE: This has no other effect on image processing and quantification.

Note: changing the name of files will prevent M3 Vision from correctly linking images. Make sure file names were not modified if images are no longer correctly associated.

 Save file: files can be saved in the following formats:
- Image export for illustration: jpeg (jpg), bitmap (bmp), tiff (tif), graphics interchange format (gif)
- Image export for quantification: tiff (tif)
- RAW: raw data image. The number of rows is saved on a 32-bit integer. The number of columns on a 32-bit integer. Pixels are saved in 32-bit floating mode. This format can be used to load the image into other programs such as Matlab
- AVI for sequence images
- TXT: image in text format. This format can be used to load the image into another program such as Matlab
- Save workspace (wsp). Workspaces files contain the information of the workspace state (images open, ROIs, Display Offset, threshold and smoothing

values) at the moment when the workspace was saved. This feature can be used to work on "projects" when a set of several acquisitions needs to analyzed simultaneously.

NOTE: For acquisitions loaded in M3Vision, the directory of the selected image will be used as the default directory when saving files.

Unload current Image: There are two levels of selection in the **Image window** of the **Workspace menu**. For each acquisition, you can either select an entire folder or an image of that folder. In any case the selection will be highlighted (see image below). When a folder is selected, the **Unload current Image** button will close all images and data opened in that folder (signal, photographic images and images created from the signal image). When only one image of the folder is highlighted, only selected image will be closed.

Unload all Images: this button will close **all** images and data loaded in M3Vision.

NOTE: Make sure your work is saved before closing images. Unsaved data will be lost.
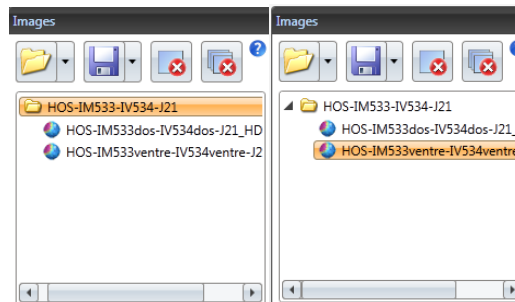
Selected folder   Selected image

The bottom window of the workspace menu, the **Tools window** gives a list of various image management tools classified into 5 tabs:

- The **ROI** menu: Access to various ROI drawing tools
- The P**rocessing** menu: for time extractions and time laps profiling
- The **Display** menu: to organize the display of multiple images
- The **Sequence** Menu: to create acquisition sequences and export movies

Each of these tabs will be described further later on in this user guide (see sections 4, 5 and 6).

Image Viewer

The **Image Viewer** is the central image display window. Visualization and simultaneous work on several acquisitions are possible in the **Image Viewer**. Acquisitions are organized in separate tabs, which can be accessed at the top of the **Image Viewer** or by clicking on the image name in the **Images** panel of the **Workspace Menu**.

Result viewer

Quantification results are displayed in the **Result Viewer** situated on the right hand side of the main window. All created ROIs are listed there together with light intensity and surface measurements as

well as kinetic curves and graphs. When several images are opened at the same time the quantification for each image will be sorted as tab accessible at the top of the **Result Viewer**. An *All images* tab displaying quantifications for all open acquisitions is also available.

Clicking on a data tab will automatically update the **Image Viewer** for the selected acquisition and vice versa. Hence the displayed data should always correspond to the displayed image (Except for the all images tab for which the last selected acquisition will remain in the **Image Viewer**).

The separator between the **Image Viewer** and **Result Viewer** windows can be dragged to adjust their size.

The three buttons on the top of the separator allow the user to toggle between the Maximized **Image Viewer** and **Result Viewer** or to open the **Result Viewer** in the separate window. (by default this window will be open on the secondary screen, when available. This function can be changed by unticking the "*Maximize the result viewer in the secondary screen*" default settings in the **Display Settings** window). **Result Viewer** will automatically return in main workspace when closing the separate window.

Figure 1: main window overview
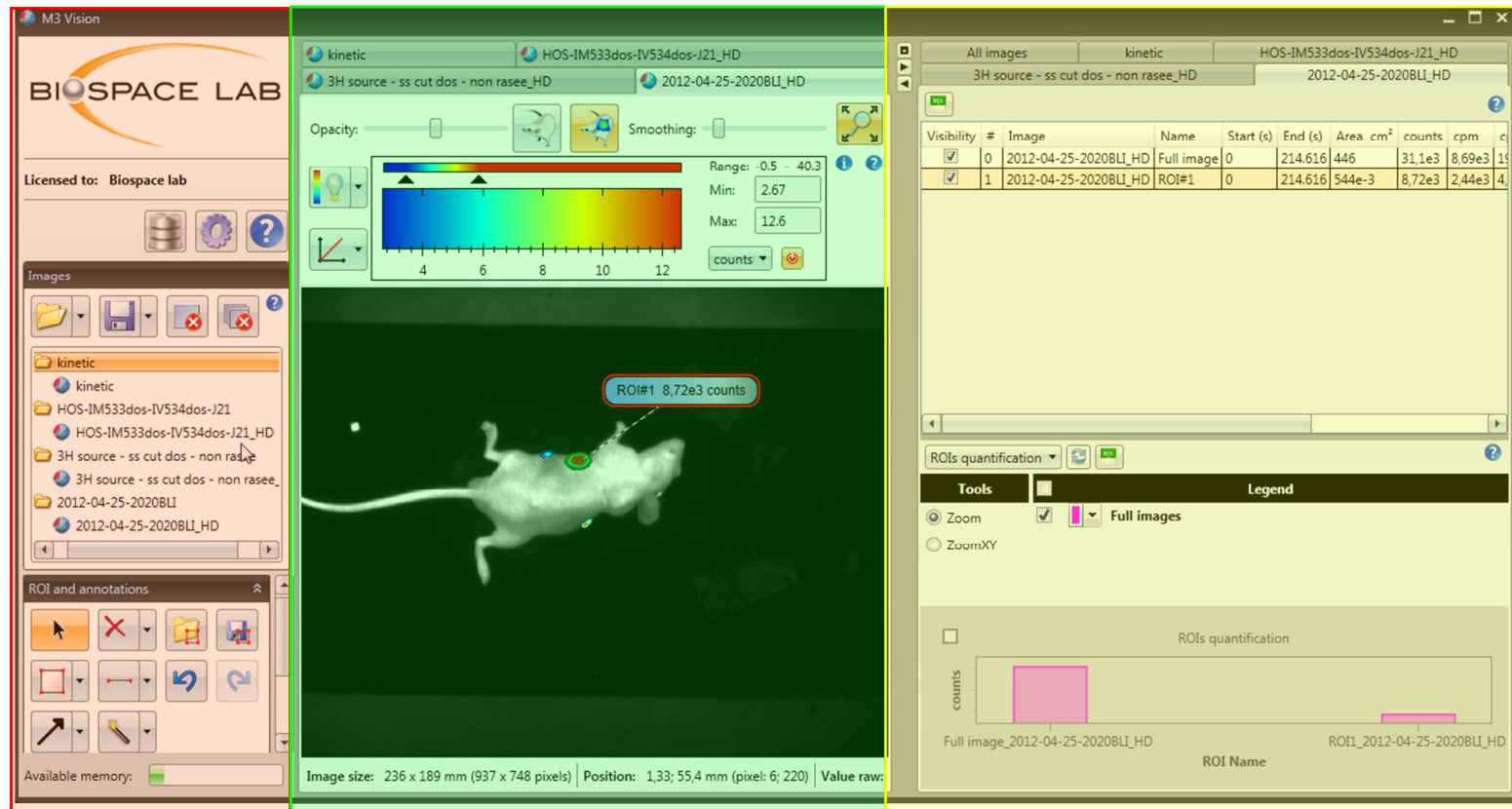
2.3　Workflow

M3 Vision has an ergonomic design with intuitive and user friendly access to all its functionalities. All analysis tools are organized under 5 tabs at the bottom of the **Workspace**. For simple routine analysis, the workflow is optimized with automated processes such as the *magic wand on whole image* and *apply current display to all images* tools. Advanced features such as *Arthur filter* rendering and *spatial data zoom* can easily be found in the **Process** tab of the **Workspace Menu**. Access to the analysis tools is further enhanced by right-clicking in the **Image** and **Result Viewers** to open a contextual menu with shortcuts to routine tools.

3　Getting started

3.1　Introduction

File formats

The following files formats can be opened:

- BVR : signal and background files, bioluminescence, fluorescence, autoradioraphy.
- TRP : Photographic or X-ray image, non quantitative.
- WSP : workspace files, each file contains information and data on all open acquisitions (lists of ROIs, graphic objects, curves etc.) at the moment at it was saved saved. These files may be of particular interest to users working on long analysis projects requiring temporary backup.
- BMP, JPG, PNG, GIF, TIF : non quantitative images, illustration type.
- AVI : sequence of images. Note that the colors are not preserved. M3 Vision uploads AVI files as a sequence of images in gray level.
- RAW : RAW files. A certain number of generic raw data picture files such as RAW files can be opened in M3Vision. Some file format parameters will be requested by M3Vision upon opening this type of files.

For 2D acquisitions created with a Biospace Lab system (PhotonIMAGER, BetaIMAGER DFine, GamaIMAGER), M3Vision will automatically create an overlay of the quantifiable light signal data (BLI, FLI, Autoradiography, Scintigraphy) with the black and white background image (picture or X-Ray image) when available. Should some overlay or temporal data files be missing, the user will be informed by a warning message. Some operations may not be possible in such a case (ex.: fusion image cannot be displayed without the photographic image (trp file); autofluorescence unmixing cannot be done without the background image (fluorescence).

When opening a workspace file a warning message will pop up asking the user to confirm that they want to close all open images.

Note: As the workspace file records the path of the open acquisitions at the moment it is created, it will fail in finding these acquisitions should they be moved or renamed prior to opening the workspace file.

The time information associated with each acquisition is saved in a proprietary format file with the extension '.cri'. CRI files cannot directly be opened in M3Vision. Nonetheless they should remain in the same folder as the BVR and TRP files and should not be renamed or M3Vision will fail to extract any time-related information including time profiles and partial time openings.

Opening acquisitions with M3vision

In M3Vision acquisitions can be opened by clicking on the folder icon (📂) or by browsing the image database (🗄).

Several acquisitions may be opened at the same time. They are displayed in the Image viewer as separate tabs. The names of the opened acquisitions are also listed in the **Images** panel in the **Workspace Menu**. To swap from one image to another simply click on corresponding tab or corresponding name in the Image panel.

The Image Data Base allows easy access to all BVR files uploaded to M3Vision. Every image opened in the software is automatically added to the database.
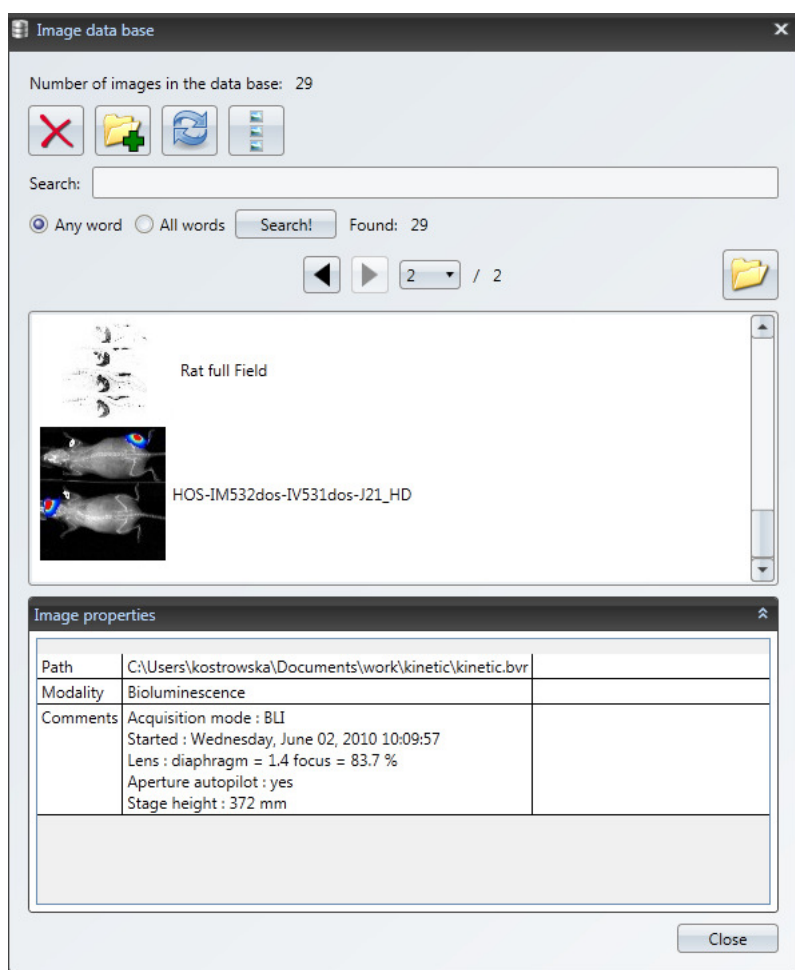
❌ : Erases acquisitions from the database

📂 :Adds acquisitions to the database.

Entire folders from either local (computer hard drive) or remote (server) sources may be added to the database. All the BVRs files present in the selected folders and sub-folders will automatically be

added to the database. This process may take time depending on the amount of the data present in the selected folder.

: Remove all dead images.
This button will remove all the images that are no more present on the local or remote sources

Create thumbnails for every image in the data base.
In case some of the images miss their thumbnails this button can be used to recover them.

The Search button allows to quickly search acquisitions using keywords. One or more keywords can be entered and two options ("any word" or "All words") are given to refined the search. The filtered result will be listed in the browsing window. To display all the images of the database, simply press the **Search** button without typing any words in the search window.

The users will be able to navigate through the retrieved results using the buttons.

On the results list:
-once the image chosen, the properties are displayed. The Browse bases on this information. To search for the image you can type the name of the file, modality, or any word included in the comment.
-to upload chosen image in the M3Vision, double click on it. You can also upload several images by using Ctrl or Shift button ==

3.2   **Display management**

The display tools are available in the **Image display panel** which opens with every opened image and **Display tab** located in the image management tabs in the menu workspace.

3.3   **Image display panel**
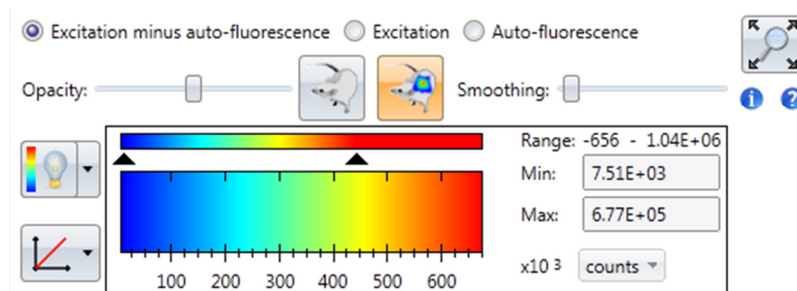
The display control panel is specific to a given image. This control pad is located above the image. It allows adjusting the display properties of each image without changing the quantification and modifying the original data. Display control panels differ a bit depending on the modality.
This panel gives also an access to the acquisition details.

.

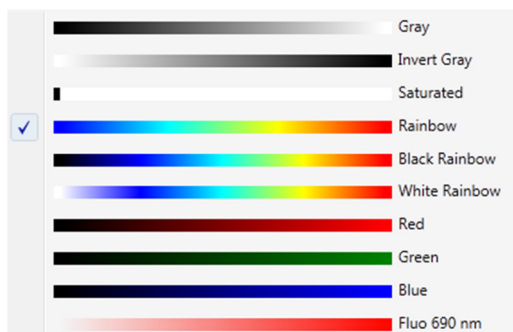| Name	HOS-IO529dos-IV530dos-J21_HD
From file	C:\Users\kostrowska.BIOSPACE\Documents\work\CLIENTS\NANTES\529-530\HO
Size in pixels	940 x 983 pixels
Size in mm	108,1 x 113,045 mm
Pixel size	0,115 x 0,115 mm
Exposure	0:04:03.518
T start	0:00:00
T end	0:04:03.518
Min value	0
Max value	55.30331
Modality	Bioluminescence
Comments	Acquisition mode: BLI (100 ms per frame) Started: Monday, May 06, 2013 3:47:02 PM Lens: 50mm f/1.2; Aperture = 1.2; Focus = 91.0 Filter: No filter Aperture autopilot: yes Distance to lens: 520 Software version: 3.2.6.834 Machine SN: PI3004_DEMO 530- injection missed

Pressing on the 🛈 button opens a window with an acquisition details, such as time of acquisition, its nature and parameters

Opacity (Bioluminescence and Fluorescence only)

The controls of the 'Opacity' enable a precise control of the transparency levels of the signal image and the photographic image. The slider can be moved continuously between the photographic image, and the signal image.

Palette (All modalities)

	Gray
	Invert Gray
	Saturated
✓	Rainbow
	Black Rainbow
	White Rainbow
	Red
	Green
	Blue
	Fluo 690 nm

Palette choice: selects the palette for the display of the active image by right clicking on the LUT.

## Signal setting display or Display range (All modalities)

Max and min of the Look Up Table*
The "max" parameter of the LUT defines the value above which the pixels are displayed with the color of the maximal intensity. The "min" parameter is the display threshold. Any pixel, with a value below the "min", will be displayed as background. If the value chosen for the max exceeds the maximum intensity measured in the image, the figures are displayed in red for rainbow palette or other max color depending on the chosen palette
It is also possible to set the display range automatically.

Min and max automatic settings

Smart autoscale
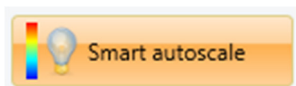
Smart: M3Vision determines the min and max basing on the histogram of the image. The most representative values for the image are chosen.
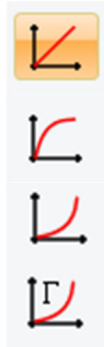**0 to max:** the min is set to 0, the max is set to the max of the signal intensity of the image.

Random: sets the min to 0 and the max to the maximum of a set of pixels chosen randomly in the image

Min to max: the min and the max are set to correspond to the min and max of the signal intensity of the image.

Transform functions

Linear: applies linearly the LUT to the signal range defined by the max and min parameters.

Logarithmic: applies the LUT to the logarithm of the signal intensities.

Exponential: applies the LUT to the exponential of the signal intensities.

Gamma: applies the LUT to a gamma function of the signal intensities. The parameter of the gamma function is defined by a textbox right to the transform functions button.

The Look Up Table
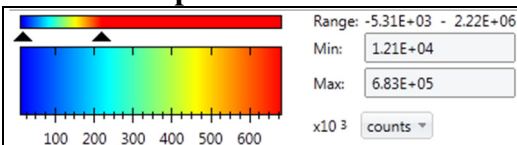
The signal images use pseudo colors to represent the different levels of intensity. The palette defines the list of colors that are used for the different levels; the parameters of the look up table (LUT), the LUT max and min parameters.

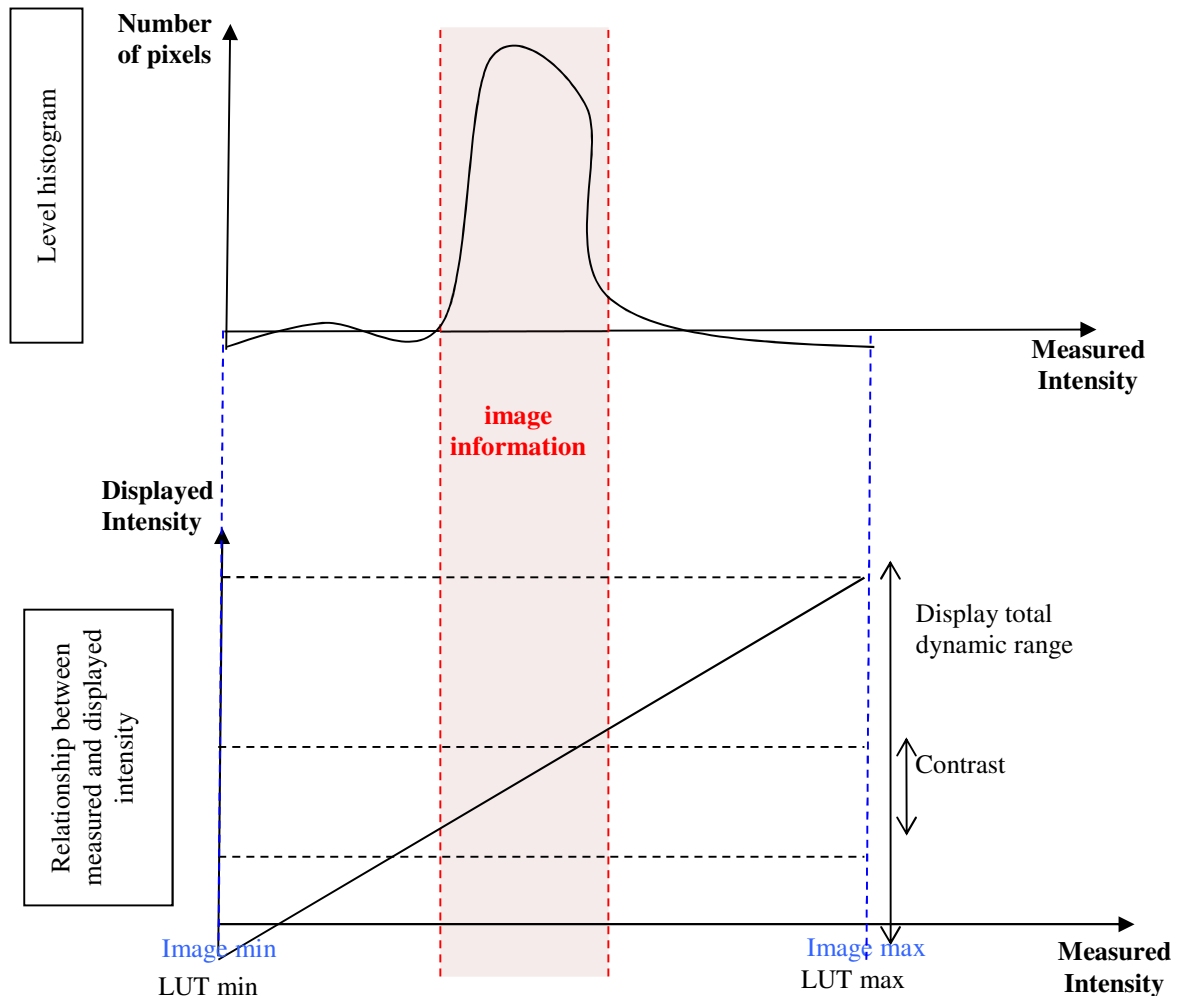The look up table is defined by three parameters:
- The palette: list of colors that encode the different intensity levels
- The max and min parameters: define the dynamic range for which the image offers the best contrast
- The transform function: defines the relationship between the measured intensity and the displayed intensity in the region defined by the LUT min and max parameters

The min/max values can be entered manually in the Min and Max text fields of the Look Up Table, they can be modified by moving the mouse along the main bar or the two triangles below the mini LUT:
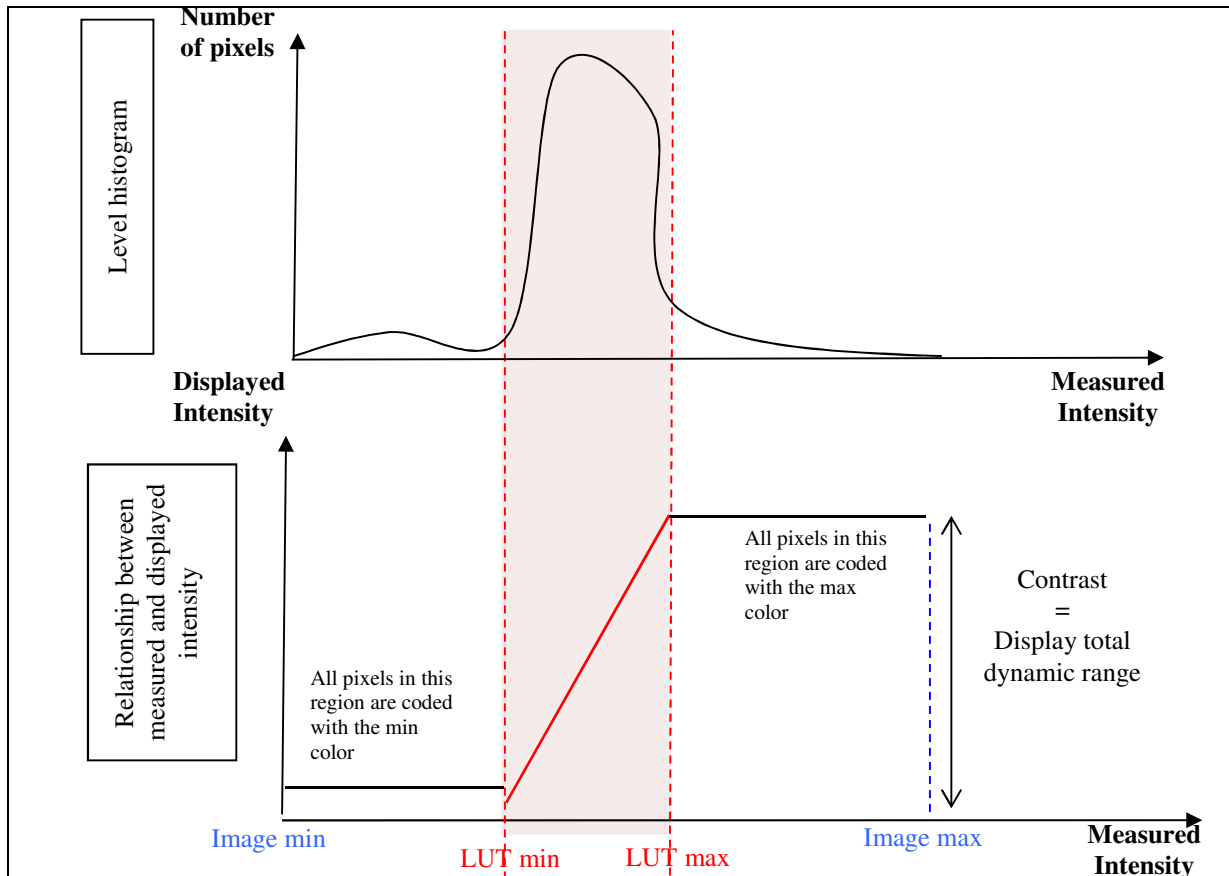- The two triangles represent the min/max position with regards to the real dynamic of the image
- The left triangle represents the min value, moving this triangle changes this value
- The right triangle represents the max value, moving this triangle changes this value
- clicking and holding on the principal LUT bar. The position of chosen this way value is changed while moving the mouse. Example: if the value 200 is chosen by clicking on it, moving the cursor left will result in rising the max value while 200 stays under the cursor.

To change the palette color, right click on the LUT.

The graphics below represent the histogram of an image with a low signal and the different possibilities for the look up table.

Level histogram

Number of pixels

Measured Intensity

image information

Displayed Intensity

Relationship between measured and displayed intensity

Display total dynamic range

Contrast

Image min
LUT min
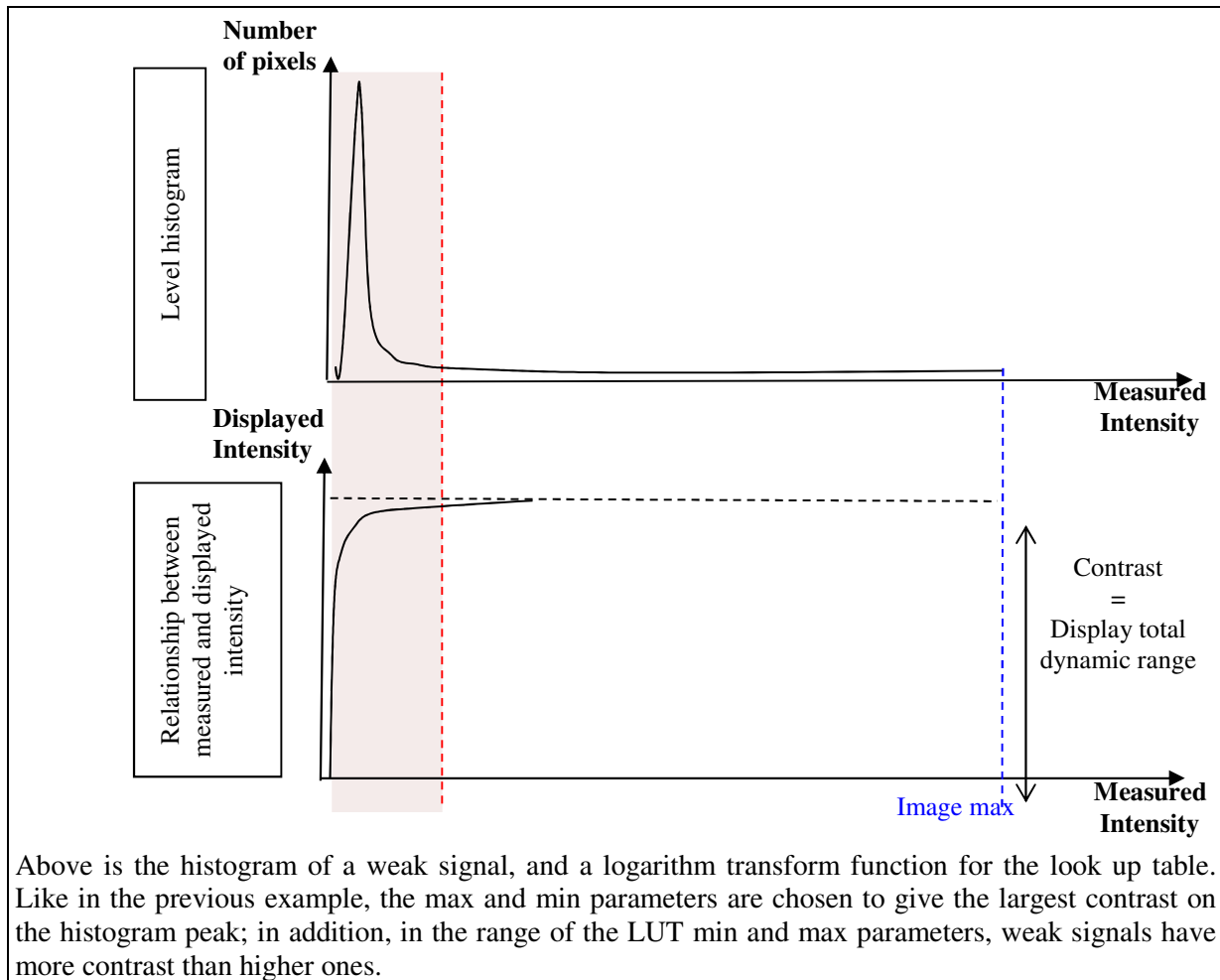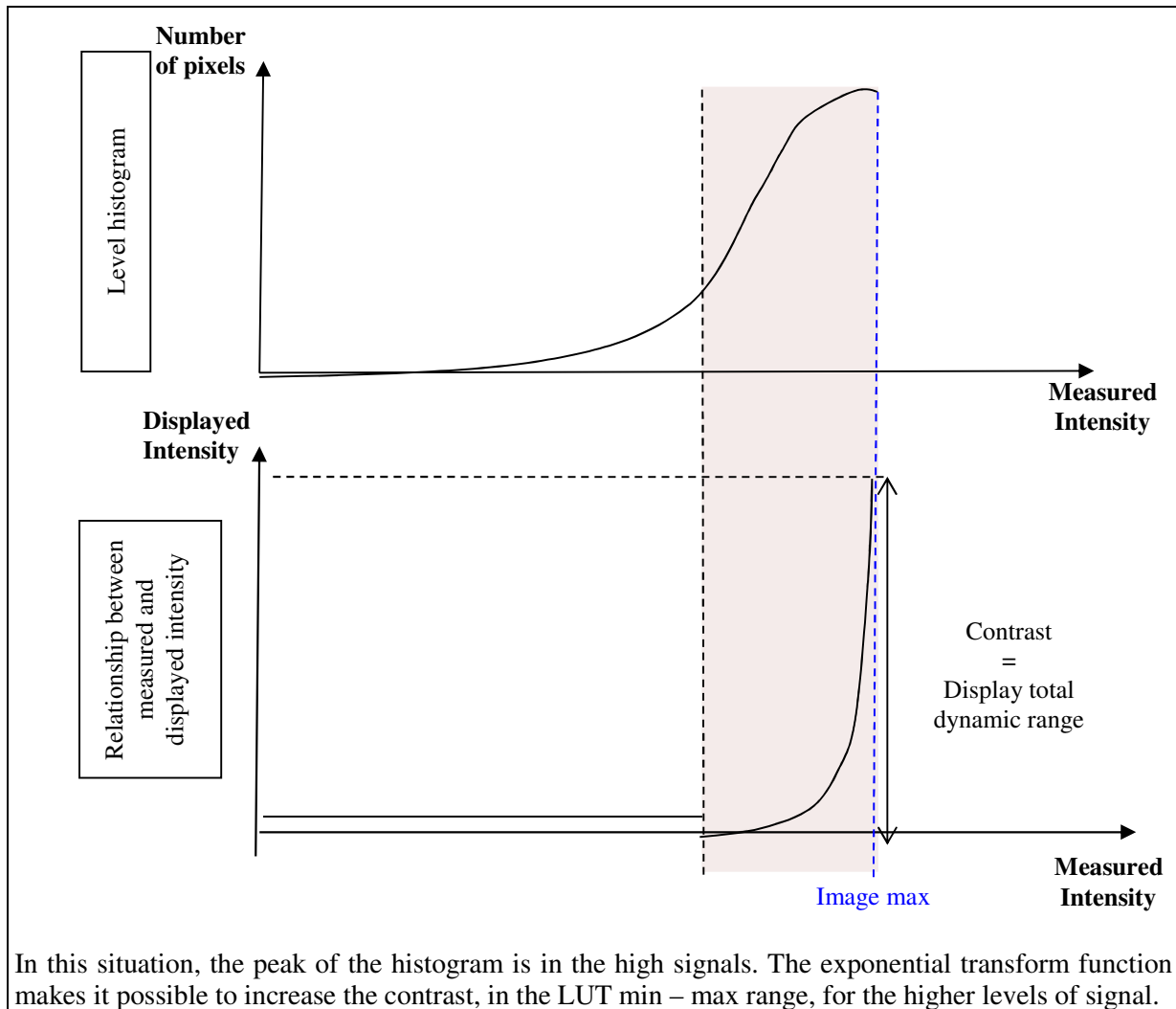
Image max
LUT max

Measured Intensity

The diagram above shows a first possibility, where the LUT max and LUT min are equal to the image max and min intensities. In such a case, the contrast corresponding to the histogram peak, where most of the image information lies, is rather small.

Number of pixels

Level histogram

Measured Intensity

Displayed Intensity

Relationship between measured and displayed intensity

All pixels in this region are coded with the min color

All pixels in this region are coded with the max color

Contrast
=
Display total dynamic range

Image min

LUT min   LUT max

Image max

Measured Intensity

In this second diagram, the LUT min and max parameters are defined based on the histogram peak, in order to maximize the contrast. This contrast is used to display the differences of the intensity levels between the LUT min intensity and the LUT max intensity. All the pixels, the intensity of which are below the LUT min are displayed with the same color (for example, white); all the pixels, the intensity of which are above the LUT max are displayed with the same color (for example, black). As a result, the image, with a display define by such LUT parameters, does not give any contrast information for intensities outside the LUT min – max range.

The two examples above illustrate two possibilities with linear look up tables. Depending on the signal intensities distribution in the image, and the interest for each intensity level, some transformation functions can be of interest.

Above is the histogram of a weak signal, and a logarithm transform function for the look up table. Like in the previous example, the max and min parameters are chosen to give the largest contrast on the histogram peak; in addition, in the range of the LUT min and max parameters, weak signals have more contrast than higher ones.

Number of pixels

Level histogram

Measured Intensity

Displayed Intensity

Relationship between measured and displayed intensity

Contrast
=
Display total dynamic range

Measured Intensity

Image max

In this situation, the peak of the histogram is in the high signals. The exponential transform function makes it possible to increase the contrast, in the LUT min – max range, for the higher levels of signal.

Smoothing or Gaussian filter

The smoothing value defines the width of the Gaussian filter applied to the signal image. It corresponds to the full width half maximum, in mm, of the Gaussian. This filtering is applied on the image used for the display.

Zoom:

Clicking on this button opens the personalized zoom box. Moving the slider up and down changes the zoom in the way that the image takes the maximum of the available space in the Image Viewer (default mode). The zoomed image is represented by the yellow rectangle which changes the size while moving the slider. By moving the rectangle you can change the visualized area in the image.

It is also possible to zoom on one particular pixel : to do that one must move the cursor on the desired pixel, press and hold the control button of the keyboard and use the mouse scroll to reduce or enlarge the zoom factor.

Going back to the original size of the image is possible by clicking again on the zoom button.

: displays all the information on the current image including, among others, the image name and size, the acquisition conditions and eventual comments.

3.4   **Display tab**

Multi-view mode

This mode allows visualization of few images at the same time. They are displayed in the Image Viewer window in the form of a grid. In the multi-view mode an additional panel is displayed in the Image Viewer right above the opened image:

This panel allows definition of the grid size (number of rows, number of columns). Ticking on « Show all pads » allows display of all the panels attached to images (by default only one image is shown). Ticking on « Tracker » allows comparison of the pixel's physical coordinates of the displayed images.

The « counts » scroll allows the choice of the unit to compare. If the unit is not usable for an image type, the "N/A" value replaces the quantification value.

Apply current display settings to all images button

This button allows applying the same display settings adjusted on one image to all images of the same nature (bioluminescence, fluorescence, autoradiography)

Multi label Image display button

Pressing on this option opens the window with all the images opened in M3 Vision.
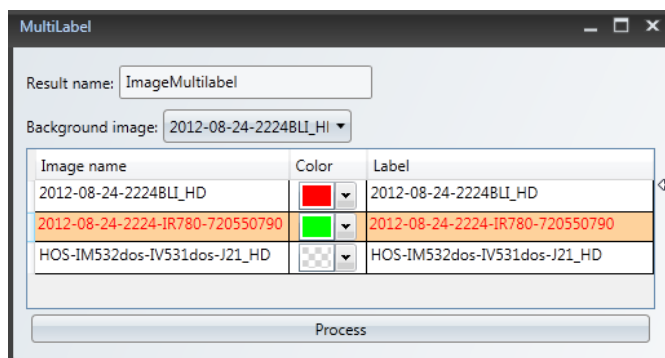
Select two images of the same animal with different marker (ex. luciferase and fluorescent probe, two different fluorescence probes) by choosing a color for each marker. As a background image use the photographic image (trp).
Run tool button validates the choice and opens the merged multilabelled image as shown below.

Note that the two acquisitions done for different markers have to be acquired with the same

positioning of the animal to assure correct superposing of the signal.

## Multilabeling in Bioluminescence and Fluorescence:

Multilabelling image of a mouse with two different markers staining the subcutaneous tumor: fluorescent probe and luciferase.
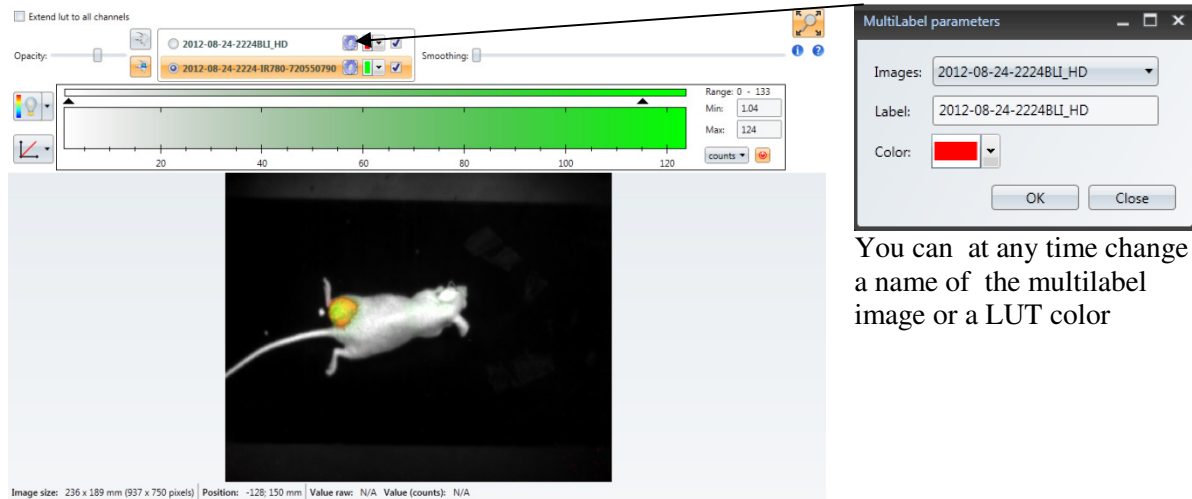
You can at any time change a name of the multilabel image or a LUT color

The Image display panel above allows adjusting the display of the multilabel image. It can be done by selecting the marker displayed. This way the adjustment can be done for each marker separately to obtain the optimal result.

In the active image display window chose the active image to be displayed by ticking the right option on the left (blue box). On the right (yellow box) tick the marker box, to visualize your signal. You can now adjust the settings (LUT, scale). Both markers can be visualized at the same time by ticking the two boxes on the right, but settings can be changed for one marker at a time, depending on the active image ticked.
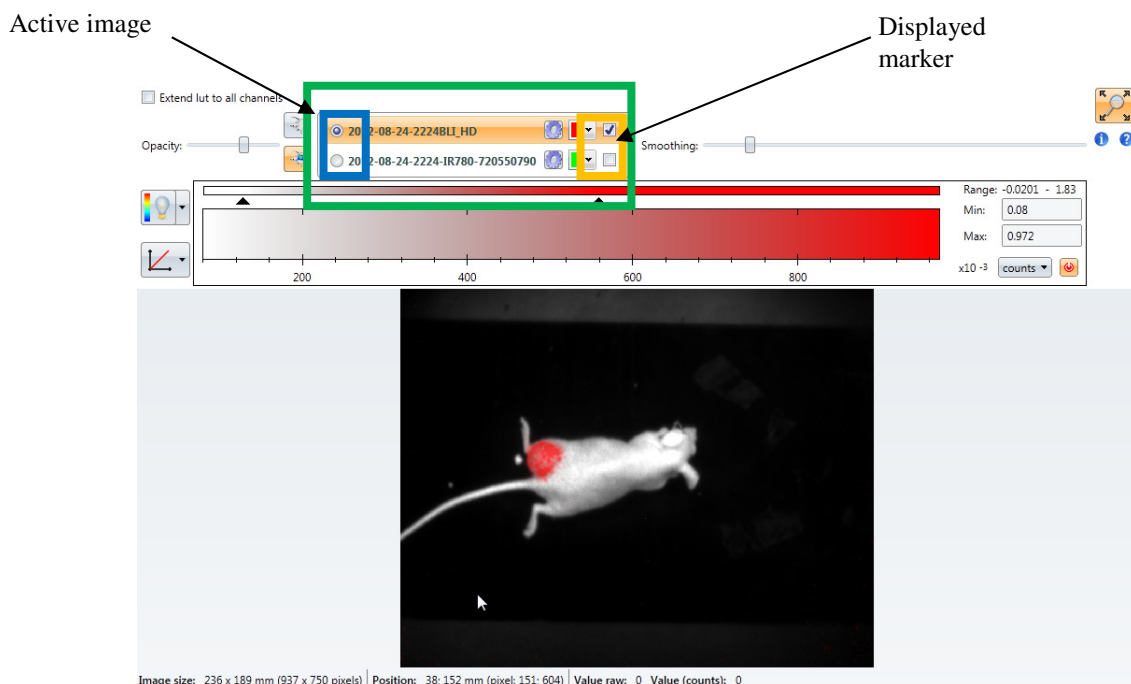
Multilabeling in Autoradiography:
If an autoradiography acquisition was separated using one of multilabel softwares (Decay (DML) or Energy Multilabel Software (EML)) generating a separate files for each isotope used (up to 3 isotopes for EML) it is possible to open those files as a one multilabel image. After the separation the generated files will be automatically registered together with the original acquisition in the same folder. Upon the image opening if there is more than one bvr file in the acquisition folder the software proposes automatically opening in multilabel mode (only for autoradiography acquisitions)
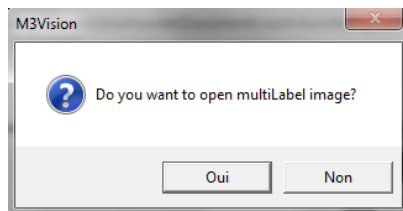
Once accepted the multilabel window pops out like before and the marker color needs to be chosen for each isotope.
Below a tissue sample with two different isotopes 3-H and 14-C
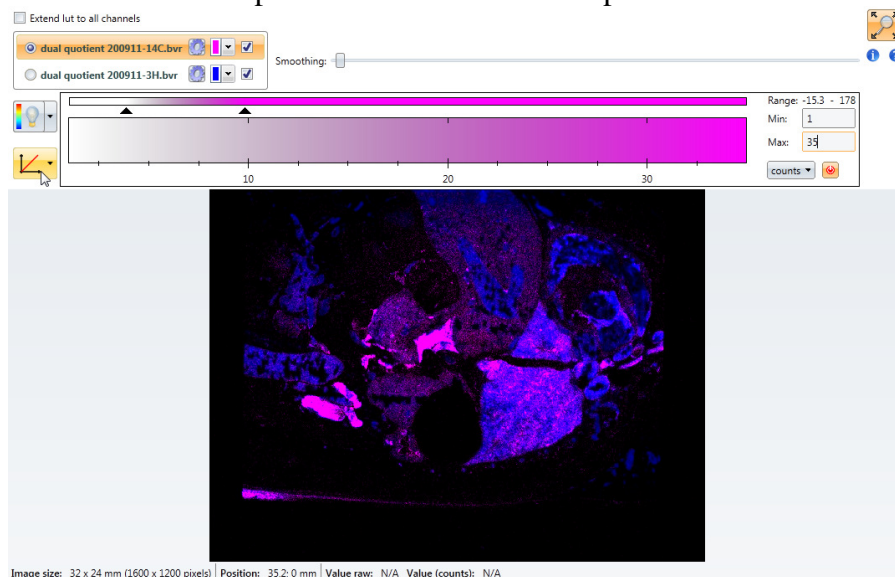
3.5   Image display panels
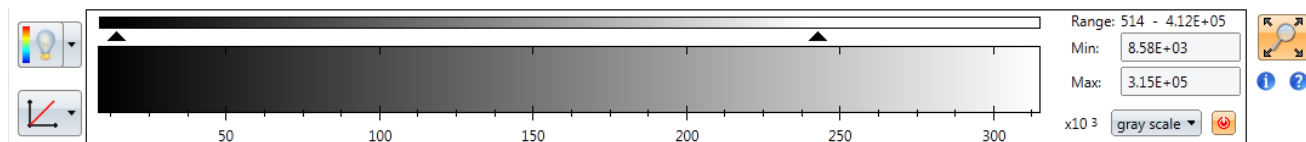
For all modalities including photographic image this Image display panel is always available with the following functions:

- Range : display of the min/max value of the image
- LUT : 30palette of colors used for image display. Each pixel has assigned a color based on the pixel value and the min / max of the LUT
- Min : value associated to the 1st color of the LUT
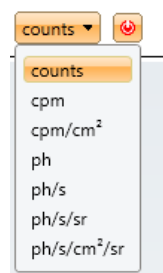- Max : value associated to the last color of the LUT

- • Min and max automatic settings
- • Transform settings
- • Zoom

Image display in bioluminescence
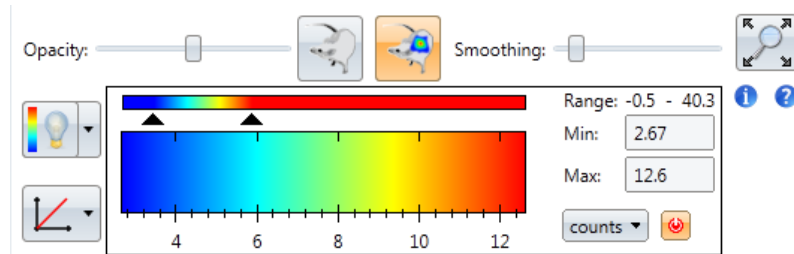
This image display panel in addition to the functions described above contains also:

-Opacity (transparency levels of the signal and the photographic image)
-Smoothing (available for all modalities)

-Units (defines the unit for quantification of the signal)
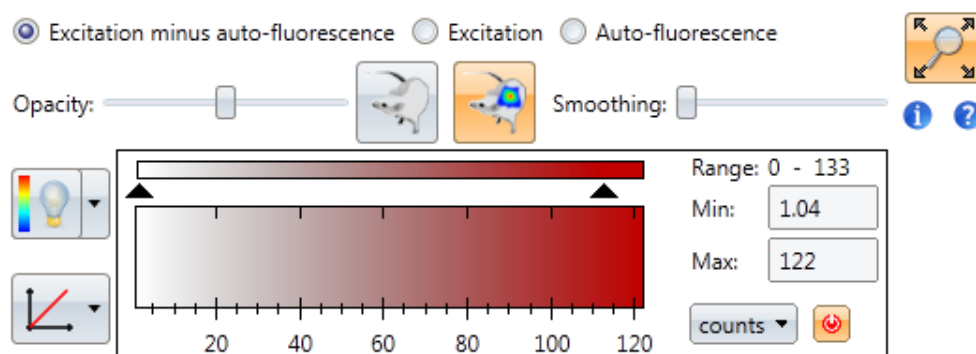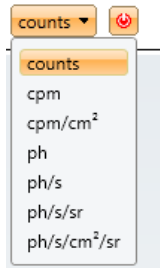-on/off button, displays either the ROI value defined by the unit or a simple label with the ROI number

For bioluminescence image the brightness and the contrast of the photographic image can be adjusted

by choosing the photographic display with the corresponding button

Image display panel in fluorescence

This image display panel in addition to the functions described above contains also:

-Opacity (transparency levels of the signal and the photographic image)
-Smoothing (available for all modalities)

-Signal selection option, just above the Opacity and Smoothing, allows you to navigate between the fluorescence (**Excitation**), background (**Auto-fluorescence**), and unmixed fluorescence(**Excitation minus auto-fluorescence**) images.

By default the unmixed fluorescent image is opened, but it is also possible to open the excitation or background image by ticking the corresponding box above.

-Units (defines the unit for quantification of the signal)
-on/off button, displays either the ROI value defined by the unit or a simple label with the ROI number

For fluorescence image the brightness and the contrast of the photographic image can be adjusted by

choosing the photographic display with the corresponding button

AF unmixing (Fluorescence)

Autofluorescence unmixing toolbox is specific to fluorescence acquisitions.

Autofluorescence and the Photon Imager

Fluorescence imaging is based on photons as a source of information. Fluorescent molecules, after excitation, emit photons, and can this way be localized, and possibly quantified. For this purpose, two types of fluorescence can be used: exogenous fluorescence with dyes like FITC, rhodamines, texas red, indocyanine green; and endogenous fluorescence, with the family of fluorescent proteins: GFP, YFP, dsRed, mCherry, etc.

Unfortunately, living tissue often contain other fluorescent molecules, that produce an additional fluorescence. This phenomenon, commonly referred to as autofluorescence, results in a background that makes more difficult the detection of the fluorescence signal, especially for weak signals. The intensity and the properties of autofluorescence depend on many parameters: the excitation wavelength, the type of tissue, the animal diet, etc.

What can I do about autofluorescence?

Shorter wavelengths (blue green part of the spectrum) usually show a higher level of autofluorescence. With fluorophores in the red part of the spectrum, the autofluorescence may decrease in intensity. Shaving the animal can help you get rid of the autofluorescence from the fur. Yet, if the imaging conditions have to be carefully chosen so to limit autofluorescence as much as possible, it is rarely possible to suppress it totally. This is why the Photon Imager includes a semi automatic image processing feature to help you discriminate fluorescence and autofluorescence.

The model of autofluorescence unmixing with the Photon Imager

The process of autofluorescence unmixing with the Photon Imager is pretty simple. Since it is not possible to get the fluorescent signal without the autofluorescence, we perform an additional acquisition at a shorter wavelength - usually 80 to 100 nm shorter than the wavelength corresponding to the maximum absorption of the fluorophores.

Hypotheses:

- **The autofluorescence has the same spatial distribution for two excitation wavelengths 100 nm apart.**
- **With a 100 nm shorter wavelength, the fluorescent molecules of interest do not contribute to the image, which consists only of autofluorescence**

Even though we consider that the autofluorescence signals have the same distribution at the two wavelengths, they rarely have the same intensity. Therefore, we need to calculate an intensity correction factor to correct for this difference. The unmixing process includes therefore a step where the user has to select a region of the image with autofluorescence only, with no fluorescent contribution. The software compares the relative intensities of autofluorescence in the two images, and infers the intensity correction factor.
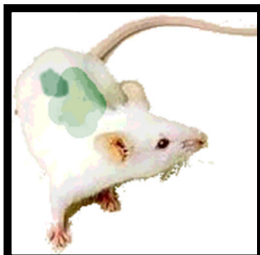
Fluorescence and autofluorescence (excitation image)
Excitation wavelength: λ_f

Autofluorescence only (background image)
Excitation wavelength: $\lambda_{af} = \lambda_f - 100$ nm

Fluorescence only (unmixed image)
Image resulting from the subtraction
Excitation image $- \alpha$ Background image

How should I choose the 'autofluorescence only' area?

Several possibilities, which depend on your protocol, can be used:
- If you have a control mouse with no fluorescence, it provides you with an ideal zone for comparing the two autofluorescence signals
- If you know approximately where to expect the fluorescence signal, choose any other region which showed some background signal. Similarly, you can select any region where you know there cannot be any fluorescent signal. Typical selections are: fur, legs, ear…

It is important, however, that the regions you select have the same optical properties as the 'region of interest'. It would not be the case, for instance, with a living animal and an ex vivo sample, where the autofluorescence would unlikely show the same properties on both regions.

Validation of the unmixing process

After the unmixing process, make sure the result is relevant. If it is not, you may consider changing your protocol or the unmixing process. You may consider the following questions:
- Is the fluorescent signal really present and strong enough to be detected?

- Is it possible to adapt the protocol to reduce autofluorescence:
  - With red shifted fluorescence
  - With shaved animals
  - With black fur animals
  - With a change in animal diet
- Do I have additional information about the properties of the fluorescence and autofluorescence signals? (ex.: spectral properties of my tissue autofluorescence)
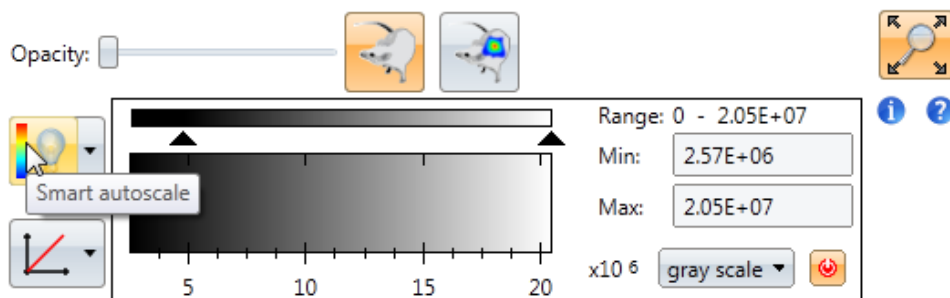
Image display panel in autoradiography
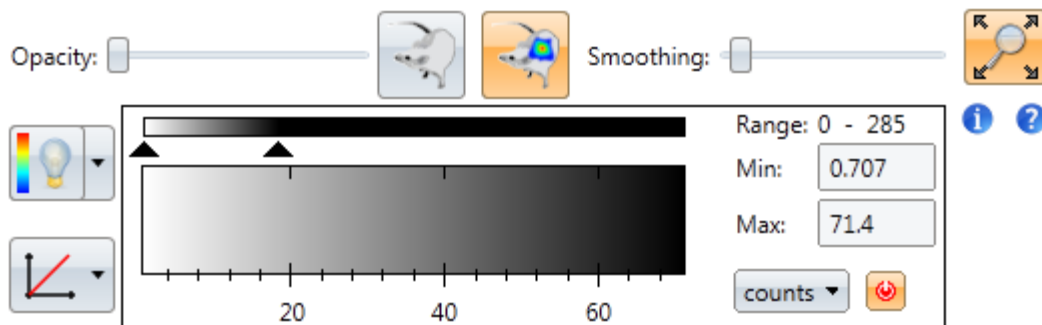
This image display panel in addition to the functions described above contains also:

In autoradiography mode the photographic image is only available for the acquisitions done on Beta Imager ^{D}Fine.

Image display panel in picture mode

This image display panel allows playing on the contrast and brightness of the photographic image by changing the **Min and Max values of the Look-Up-Table** (LUT) by moving the arrows of the LUT bar or by editing the value in the text fields.

Note: the brightness and contrast controls have no effect on images other than the photographic image of fusion images.

4 Measurement management (All modalities)

4.1 Data table and graph zone

Visibility	#	Image	Name	Start (s)	End (s)	Area cm²	counts	cpm	cpm/cm²	ph	ph/s	ph/s/sr	ph/s/cm²/sr	
☑	0	kinetic	Full image	0	1917.273	330	425e3	13,3e3	40,3	22e9	11,5e6	912e3	2,76e3	
☑	1	kinetic	ROI#1	0	1917.273	2	23e3	718	360	1,19e9	619e3	49,3e3	24,7e3	
☑	2	kinetic	ROI#2	0	1917.273	2,85	101e3	3,17e3	1,11e3	5,25e9	2,74e6	218e3	76,4e3	
☑	3	kinetic	ROI#3	0	1917.273	651e-3	10,4e3	325	500	538e6	281e3	22,3e3	34,3e3	
☑	4	kinetic	ROI#4	0	1917.273	1,29	33,6e3	1,05e3	817	1,74e9	908e3	72,2e3	56,1e3	
☑	5	kinetic	ROI#5	0	1917.273	2,61	36,3e3	1,13e3	435	1,88e9	978e3	77,8e3	29,9e3	
☑	6	kinetic	ROI#6	0	1917.273	20,5	21,3e3	666	32,4	1,1e9	574e3	45,7e3	2,22e3	

For every opened image the quantification report of the total signal is displayed in the Result Viewer window by default.
Measurements are done through the creation of ROI, the value of signal intensity of which is then displayed in the same quantification report. This report is specific for each image and appears as a separate tab in the Result Viewer window. One can navigate between images on changing the corresponding tabs. There is also a tab 'All images' which displays the summary report for all opened images and drawn ROIs.

Below the data table there is a graph zone
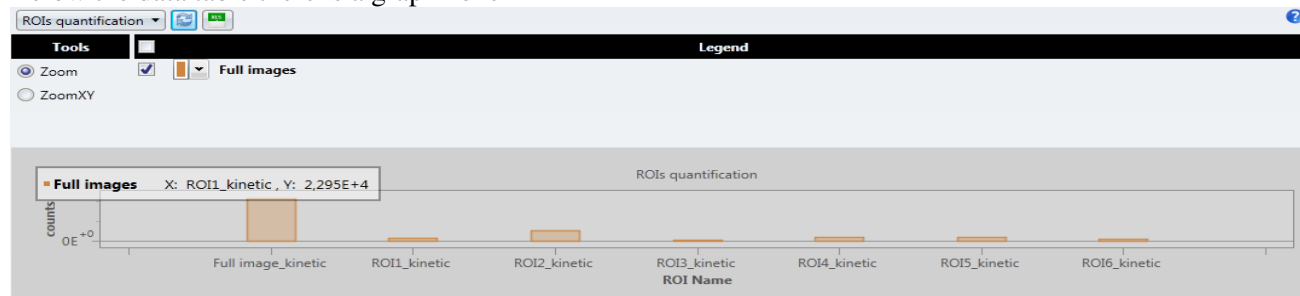
All drawn ROIs including the full image ROI are displayed as the bar graphs. By default all of them are represented that way unless the Visibility tick box for the chosen ROI (1st left column of the data table) is unticked. If the position of ROI changed the value follows automatically (except of ROI Grid where it has to be done manually) in the data table, but graph zone remains unchanged untilthe refresh button clicked ( )

4.2 Region Of Interest drawing

ROI tools are available in the **Menu workspace** where the **ROI and annotations** tab is localised. Ten possibilities are available to create regions of interest (ROI)
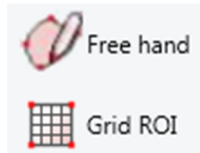
Standard tools
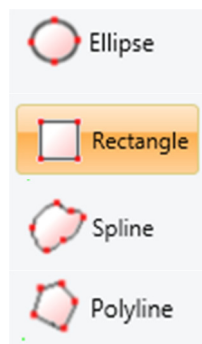
Ellipse/circle: creates an ellipse. To create a circle, press SHIFT while drawing.

Rectangle/square: creates a rectangle. To create a square, press SHIFT while drawing.

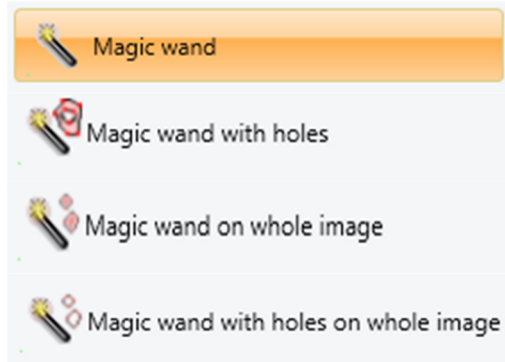**Splines:** creates a ROI defined by splines.

Polylines: creates a polygon**.**

Freehand: creates a ROI from 'manual' drawing.

Grid ROI: creates a grid of ROIs rectangles or ellipses

Automated tools

Classical Magic wand: creates a single ROI.

**Magic wand with holes**: creates a set of ROI from a min and a max threshold.

Magic wand whole image: creates a set of ROI in the entire image.

Magic wand with holes on whole image: creates a set of ROI from a min and a max threshold in the entire image

Once ROI drawn its value is displayed in the text field above. The unit can be changed with the use of the **counts drop down list** in the Image display panel. This list allows you to choose the type of information displayed in the labels of the ROI: signal intensity. To hide the ROI value and display just a simple label id with the number of ROI click on the red on/off button. The surface information can be viewed by a tooltip, displayed when the mouse is maintained over the ROI.
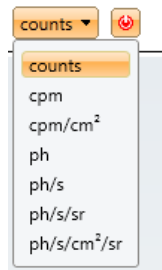
Units: defines the unit for a quantification of the signal
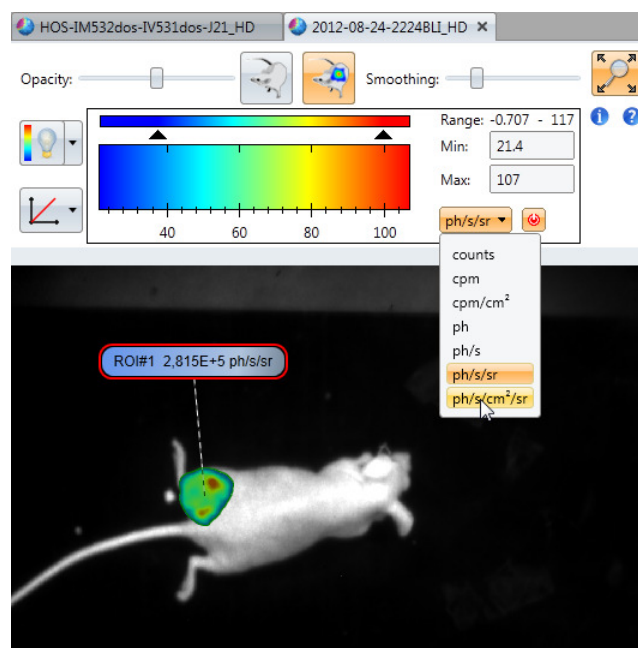
ROI creation with Magic Wand

Three different magic wands are available. Such tools create regions of interest defined by splines.

Magic wand   **Classical Magic wand**

The classical magic wand creates a single region of interest in the image from a given pixel and a given tolerance. It selects a region in the vicinity of the selected pixel, with all contiguous pixels that have an intensity greater than the selected pixel minus the tolerance.

To select the specificity (size) of the ROI, the tolerance parameter can also be seen as a threshold parameter. The relation between the two values is simple:

Threshold = Selected pixel intensity – Tolerance

To get a larger value, the tolerance should be increased, or the threshold decreased. This tool can create regions of interest as small as 20 pixels.
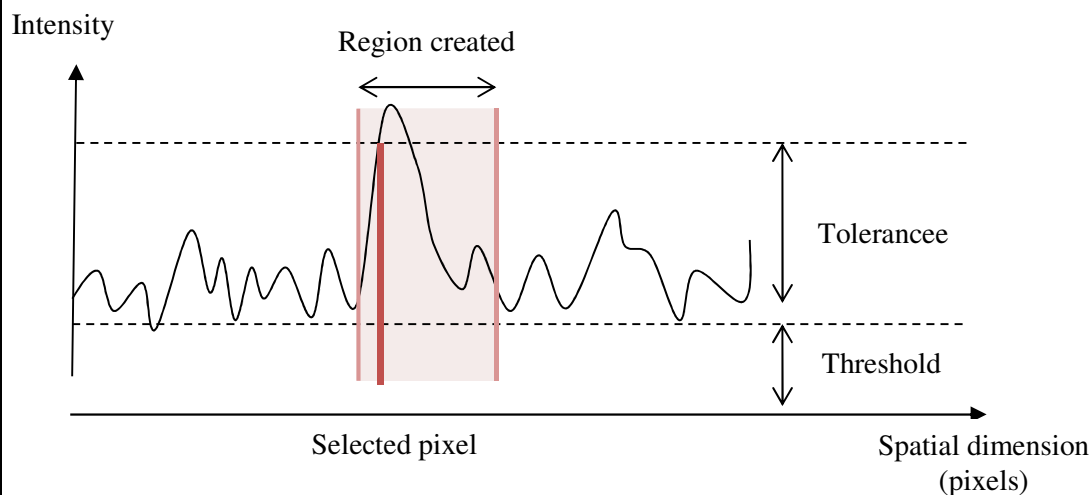
Figure 1: Creation of a ROI with the classical magic wand displayed on a spatial profile. The ROI includes the contiguous pixels around the selected pixel with a greater intensity than the threshold
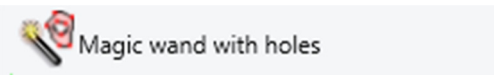
Magic wand with holes   **Magic wand with holes**

The magic hand with holes can generate more complex set of regions of interest, with a threshold min and a threshold max. This tool creates a first outer region of interest, based on a threshold min principle, similarly to the classical magic wand. It also creates, when relevant, inner regions of interest, the pixels of which are either above the threshold max or below the threshold min.

The threshold min, threshold max, tolerance and selected pixel intensity have the following relationships:

Threshold min = Selected pixel intensity – tolerance
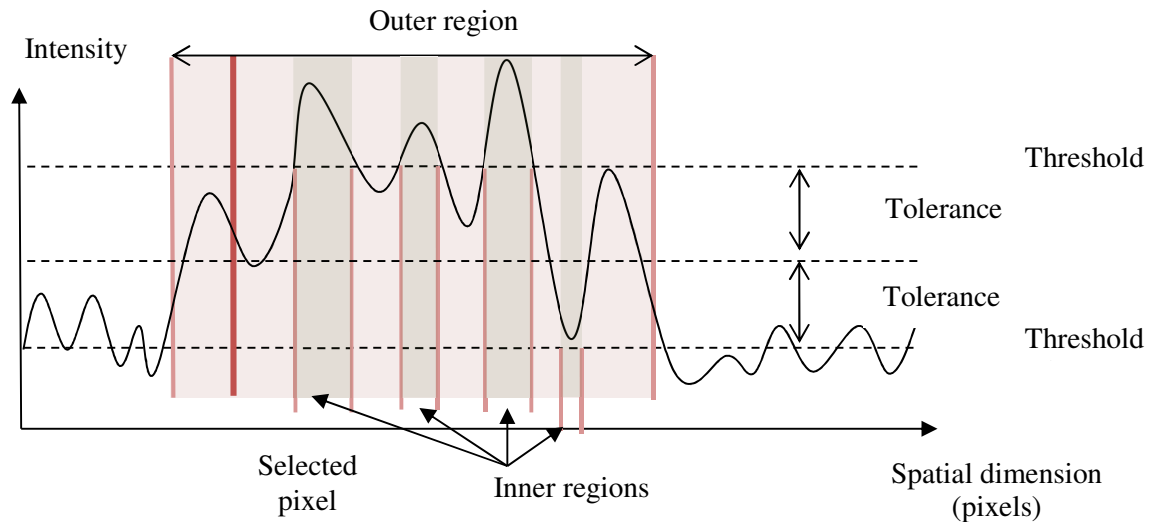Threshold max = Selected pixel intensity + tolerance

Figure 2: Creation of a set of ROI with the magic wand with holes displayed on a spatial profile. The outer ROI is similar to the one created with the classical magic wand, and the inner ROI include the contiguous pixels above or below the thresholds.

Magic wand whole image: creates a set of ROI in the entire image.

The magic wand whole image creates several regions of interest with all the groups of pixels that have an intensity greater than the selected threshold.
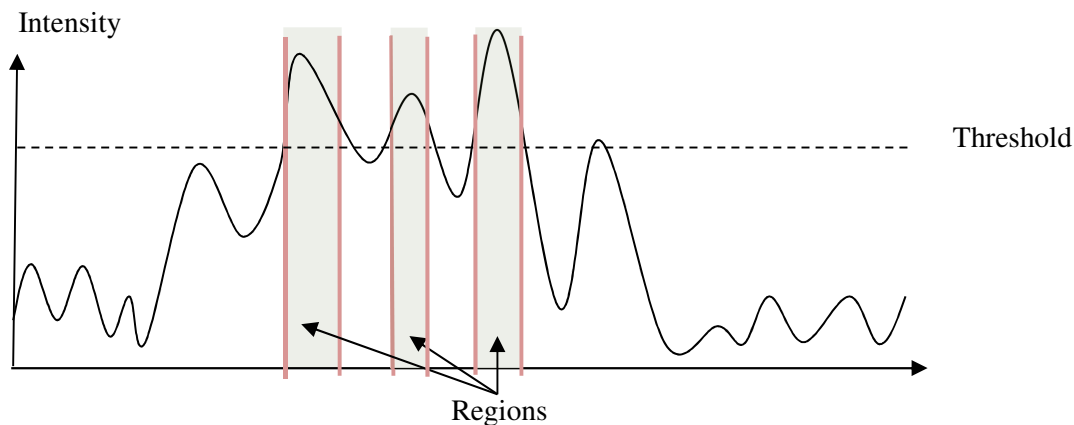

Figure 3: Creation of a set of ROI with the magic wand whole image displayed on a spatial profile. Each contiguous group of pixels above the threshold becomes a ROI.

4.3   **Region of Interest management**
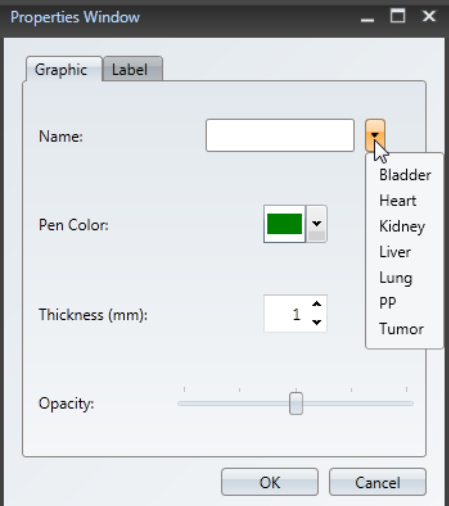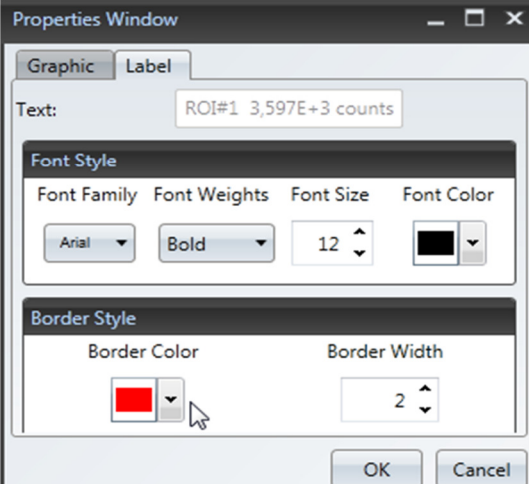
The ROI management is done through the use of selection tool from the **ROI and annotations** tab and the context menu which can be opened by right clicking on the previously drawn ROI. To draw few ROIs with the same tool keep the space key pressed

Selection button

Selection: makes ROI selection with the mouse available. With the use of this button one can also move the ROI over the image by pressing and holding the left mouse button and dropping it once the desired position chosen. Quantification of the region will be changed in order to fit the newly chosen position. To follow the changes on the graph zone, the refresh button needs to be pressed. Double clicking on the ROI opens a Properties window. This window has two tabs:
  o   Graphic-graphic properties of the drawn ROI
  o   Label-graphic properties of the label/text

Properties Window — Graphic tab: Name, Pen Color (green), Thickness (mm) 1, Opacity. Name dropdown list: Bladder, Heart, Kidney, Liver, Lung, PP, Tumor.	Graphic: **Name:** give the name to your ROI or choose one from the predefined list. The name is given in addition to an index assigned automatically to the ROI **Pen color**: change the color of your ROI, green by default **Thickness**: change the thickness of the border line of your ROI **Opacity:** change the transparency level of your ROI
Properties Window — Label tab: Text ROI#1 3,597E+3 counts. Font Style: Font Family Arial, Font Weights Bold, Font Size 12, Font Color black. Border Style: Border Color red, Border Width 2.	Label: The text style and color can be chosen and the border color and border width as above.

Context menu

Right clicking on the image viewer opens a context menu.
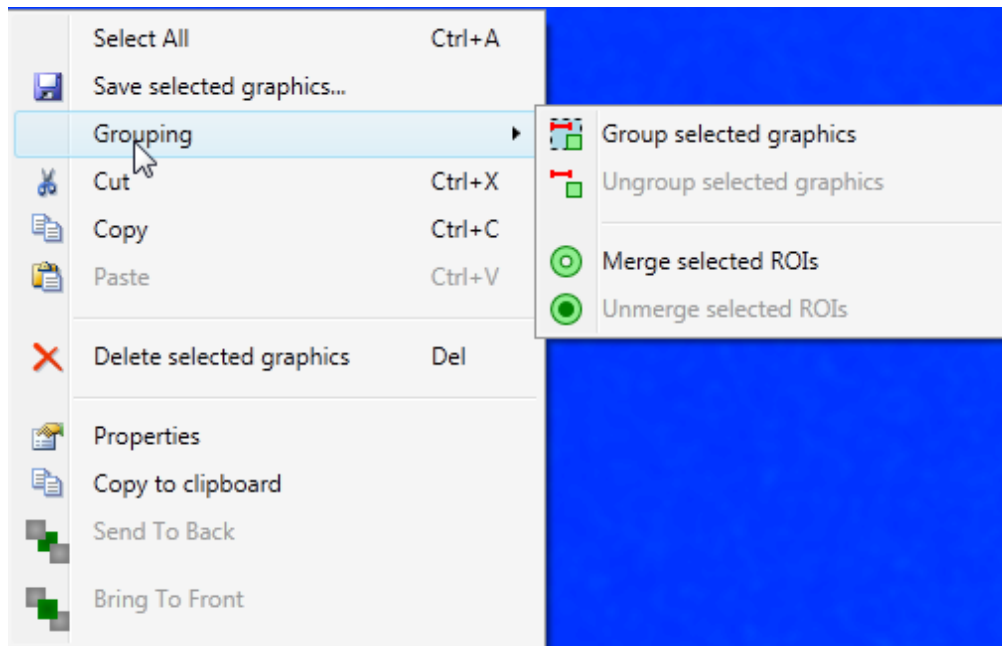
This menu gives an access to additional options. It is possible to:

- o Save selected graphics to open them later on the same or another image
- o Duplicate existing ROI to paste elsewhere on the same or other opened image
- o Delete selected graphics: ROIs or others available in ROI and annotation tab
- o Group and ungroup selected graphics
- o Merge/unmerge ROIs
- o Bring ROI to the front or send it to the back for overlaid ROIs
- o Open ROI properties window

ROI duplication can also be obtained with copy and paste with the keyboard **Ctrl + C** (copy) and **Ctrl + V** (paste)**.** Selection of all graphics can be done with the use of **Ctrl + A** buttons.

Region Of Interest load/save

Regions of interest (ROI) can be saved in graphic objects files, with the extension **grx**.
Load ROI file: loads the ROI of the selected graphic file (grx or gr6).

Save ROI: saves ROI in a graphic file (grx).

Quantification report

- • The **Quantification report** can be exported in an Excel format for printing. For export to Excel click on the Excel icon in the left upper corner of the Result Viewer .

In the following screenshot three ROIs have been detected by the tool **Magic wand whole image.** Their respective total counts are displayed on the image, and a report shows their measures in the different units. With the contextual menu which appears with right clicking on the displayed data one can change the columns visibility. By default all the available units are shown.
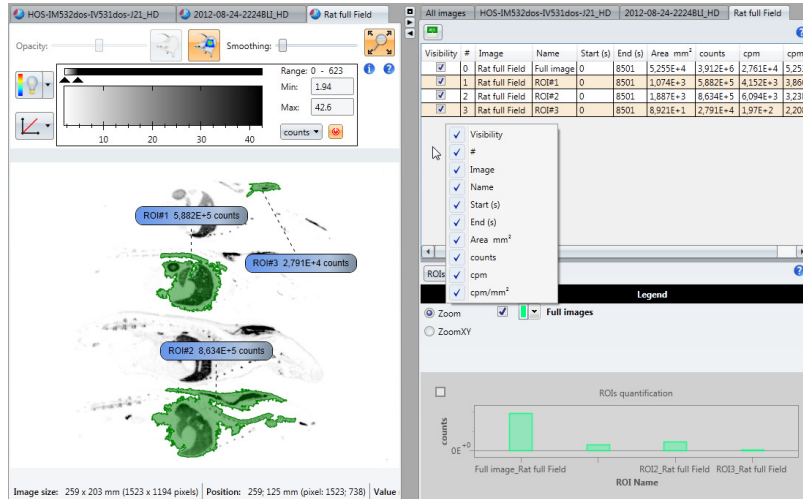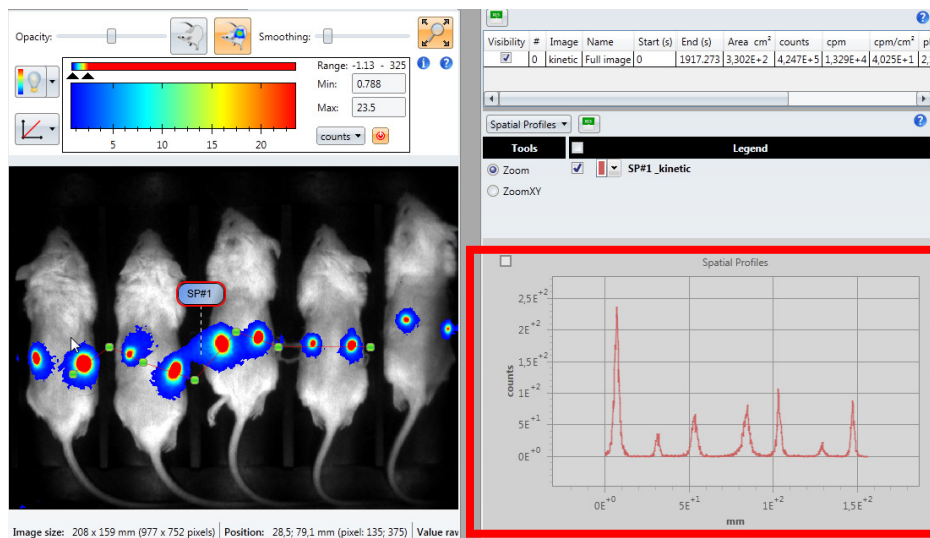

4.4   **ROI and annotations:**

Apart of the ROI management tools this tab contains also:

Spatial profile

spatial profile button creates a line or polyline and displays its spatial profile in the Result viewer window, like on the screenshot below:

   undo and redo buttons

Other graphic tools

Other graphic tools

Arrow: creates arrows in the image

Text: adds text zones to the image

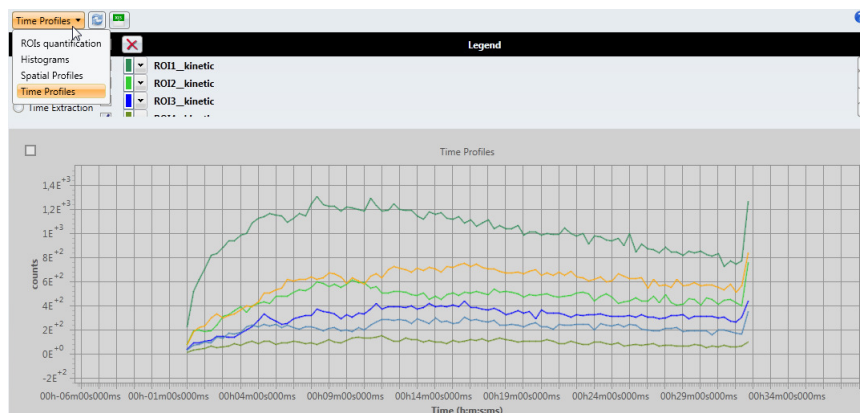**Length:** measures the distance between two points

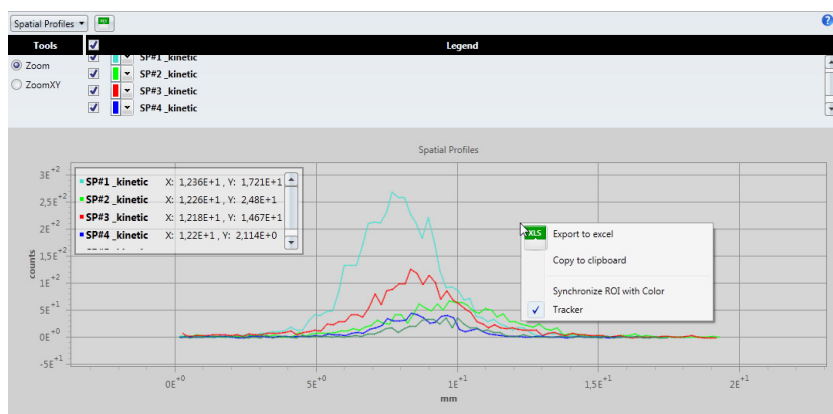**Angle:** measures the angle, in degrees, between two lines

Graphic zone

In the Result Viewer window in the zone where the graphs are displayed one can navigate between curves created for:
- ROI quantifications
- Histogram
- Spatial profile
- Time Profile

Additional options are available for the graphic zone:

- Legend above the graphic zone which allows displaying or hiding the created curves and change the color
- Traker (for spatial profiles) shows on the profile where are we on the graph (Contextual Menu)
- Zoom : selection of the zone on the graph to be displayed. Double click on the graph zone to zoom out.
- Synchronization of the color of the curve with the corresponding graphic objects (ROIs for histograms, spatial profiles for the graphs of the spatial profiles) (Contextual Menu)

Concerning time profiles, partial time profiles can be applied by selecting on the graph the zone of interest. This option is disabled in the "All images" tab.The curves of Histogram and Time Profile can be hidden or deleted independently from the graph zone.
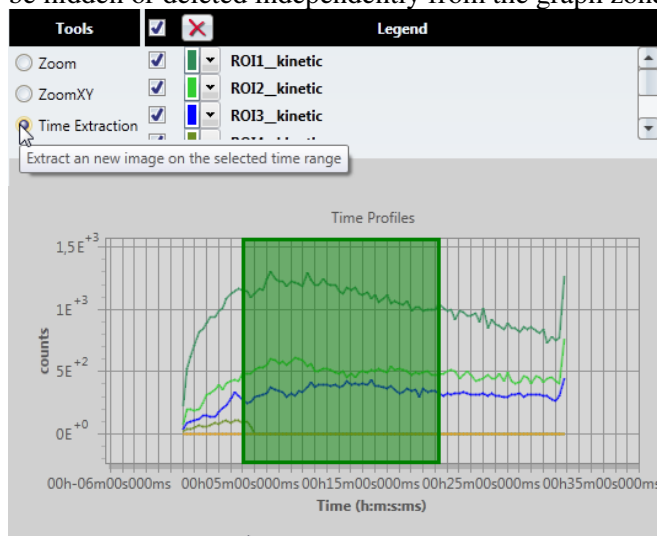
Navigation in the graph zone:
- Zoom : either by zone selection or by using the scroll (double-click to zoom out)
- Change the scale (move the cursor on the scale and use the double arrow)

5   PROCESS tools

Another tab in the Workspace menu. This tab contains Time analysis and Partial time opening options and also some advanced calibration tools and resampled image creation possibilities.

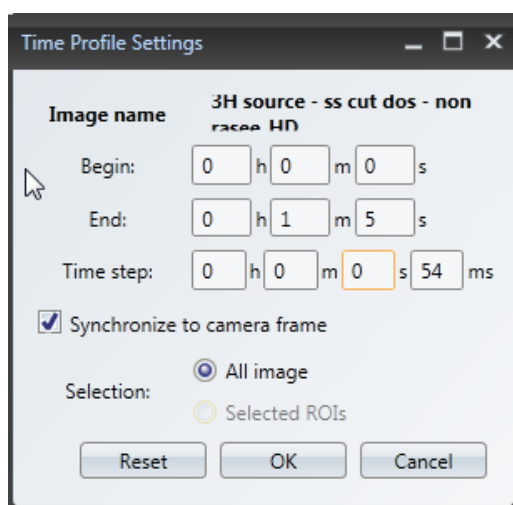## 5.1 **Time analysis (Bioluminescence)**

The time analysis toolbox is specific to bioluminescence acquisitions in order to show the signal kinetics but might also be used in autoradiography to show the isotope decay curve for isotopes of a short half-life. This tool is also available from the Image display panel for bioluminescence and autoradiography modes. To use this tool make sure the CRI file of the chosen acquisition is present otherwise Time analysis cannot be performed.

Time analysis

Use this control group to create the curves showing signal kinetics of ROIs or the whole image.

Pressing on the time analysis button opens a Time profile settings:

Time step: defines the time step for the kinetics curve, in second. By default, the mean sampling (camera rate) is used as a time step.
Sync. To camera rate: checked by default, allows the software to match the time step chosen by the user with the camera rate. The time step for the sampling of the curve is in this case the multiple of the camera time step the closest to the time step chosen by the user. If the box is unchecked, the curve is created with the exact value chosen by the user.
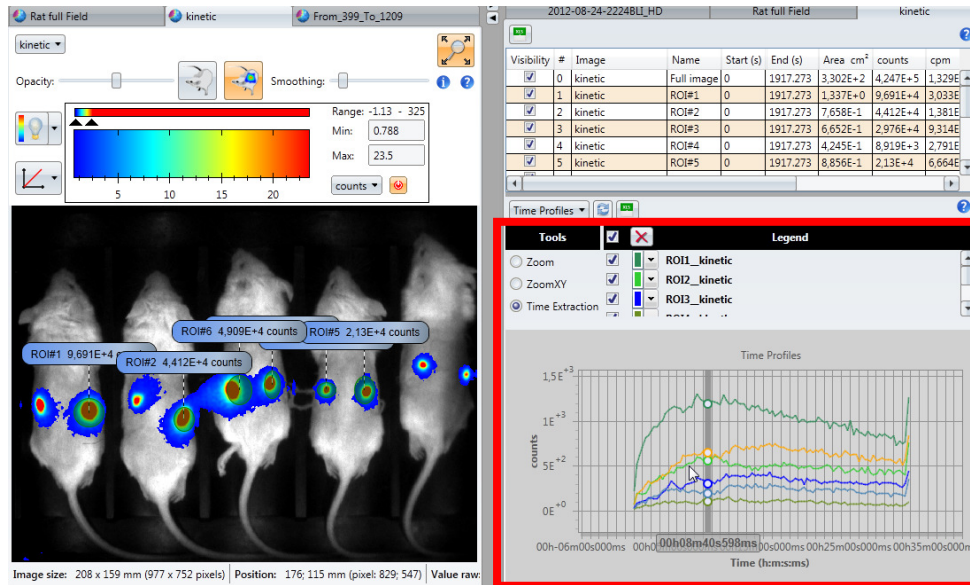**Begin (s):** defines the starting point of the time selection, in seconds, relatively to the beginning of the acquisition.
End (s): defines the ending point of the time selection, in seconds, relatively to the beginning of the acquisition.

OK button validates the chosen settings and creates and displays the curve of the signal kinetics of ROIs or of the whole image in the Result Viewer.
Reset button resets the **Begin, End** and **Time step** fields. **Begin** is reset to 0, **End** to the end of the acquisition and Time step to the camera rate.
On the screen shot below, the kinetic curves for 3 selected ROIs drawn with an ellipse ROI tool are displayed. The legend on the right allows the display of chosen kinetic curves only.

5.2 Partial time opening

Use this control group to create an image with the signal of a given time period only.

Pressing on the time analysis button opens a Partial time opening settings:

Begin (s): defines the starting point of the time selection, in seconds, relatively to the beginning of the acquisition.
End (s): defines the ending point of the time selection, in seconds, relatively to the beginning of the acquisition.

OK button validates chosen settings and creates the image with the signal of the selected time period. This image appears in the Image Panel under the original image with the chosen time opening.
Reset button resets the **Begin** and **End** fields. **Begin** is reset to 0 and **End** to the end of the acquisition.

5.3 Histogram

Histogram: creates a graphic showing the repartition of the whole image intensity levels or of the selected ROI. This representation is composed of one hundred slots based by default on the displaying scale. Adjust the extremes and the step to change the histogram representation.

5.4 Calibration

Calibration: redefines the image dimensions (spatial and intensity). This tool changes the correspondence between detected signal and quantification values.
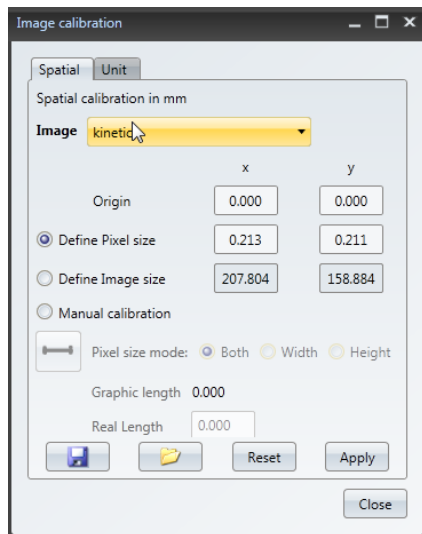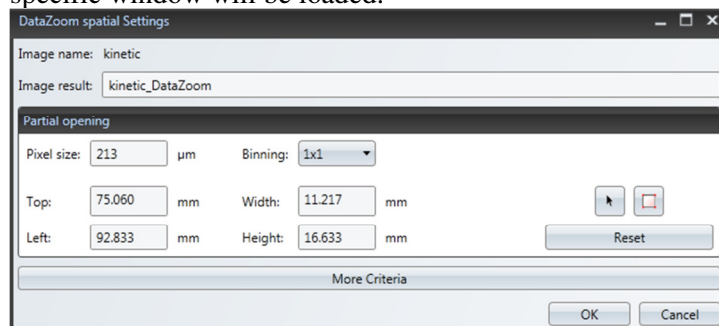
2 Calibrations are possible:
- Spatial calibration: redefining the size in mm of the image and / or its origin
- Unit calibration

5.5   Spatial data zoom

Spatial Data zoom

Use this control group to create resampled images. Only the events belonging to the specific window will be loaded.

Pixel Size (µm): specifies the size of a pixel in micrometers.
Binning: groups a neighborhood of pixels and set them to the same value.
Coordinates (mm): defines the position of the zoom windows for the extract image. This window is defined by three points, the **Top-Left corner Width** and **Height**. Their coordinates are in millimeters.

The selection can be done with the text fields or with the rectangular selection tool.

OK button creates the image corresponding to the different spatial parameters **Reset:** resets the **Pixel Size** and **the coordinates** fields. **Pixel Size** is reset to the original value stored in the image properties.

More criteria

Opens a new window with some advanced features of spatial data zoom.
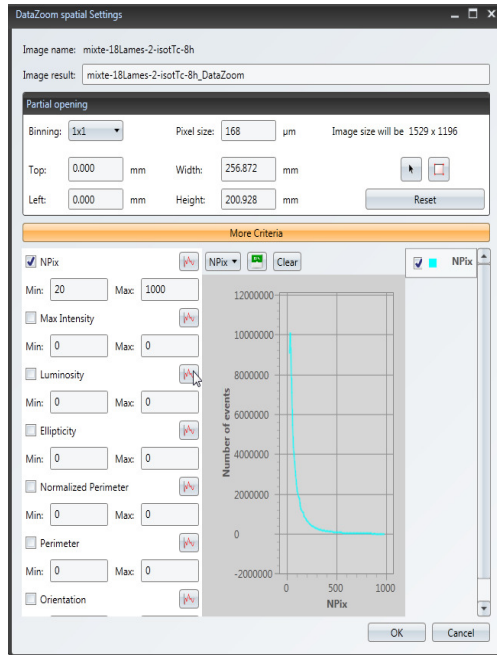
nPix: if checked, sets the range of the events size to filter.
For example : *Min:1 Max:5*
Only the events with a size between 1 and 5 pixels will be loaded.

Max intensity: if checked, sets the range of the events maximum intensity to filter.
For example : *Min:20 Max:22*
Only the events with a pixel at a value between 20 and 22 (grey level) will be loaded.

Luminosity: if checked, sets the range of the events intensity (sum of each pixel intensity composing the event) to filter.
For example : *Min:100 Max:150*
Only the events with an intensity value between 100 and 150 (grey level) will be loaded.

Ellipticity: if checked, sets the range of the events ellipticity to filter.

$$\text{ellipticity} = \frac{4\pi \times Area_{Event}}{Perimeter_{Event}{}^2}$$

For example : *Min:0.5 Max:1*
Only the events with an ellipticity value between 0.5 and 1 will be loaded.

Normalized perimeter: if checked, sets the range of the events normalized perimeter to filter.

$$\text{Normalized perimeter} = \frac{Perimeter_{Event}}{nPix_{Event}}$$

For example : *Min:0.5 Max:1*
Only the events with a normalized perimeter value between 0.5 and 1 will be loaded.

Perimeter: if checked, sets the length of the border of the detected spot.

Orientation: angle of the detected spot. 0 means the spot hit the detector horizontally. pi/2 or –pi/2 means the spot hit the detector vertically

5.6 **Apply some processes**
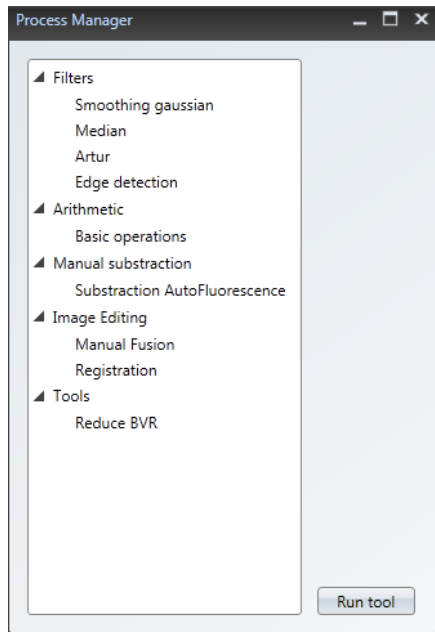
Apply some processes button gives an access to more advanced tools. Pressing on it opens a **Process Manager** window

Filters

Filters can be used for smoothing, median filtering, pass band filtering, Artur algorithm application and edge detection.

- **Smoothing**

Smoothing (mm): defines the width of the Gaussian filter applied to the signal image. It corresponds to the full width half maximum, in mm, of the Gaussian.

- **Median** filter

Window size (mm): defines the size, in mm, of the window to be used for the filtering.

- **Artur**

Regularization coefficient (mm): defines the regularization coefficient for the application of the ARTUR relaxation algorithm. Combines a smoothing and a sharpening filter.
Enhances the display of the image by reducing the noise and keeping the bounds of the objects.

Arithmetic

Opens window which allows some arithmetic operations like adding the images or multiplying pixels. Each function has a short tooltip explaining its use. To see the tooltip simply move the cursor on the chosen function.
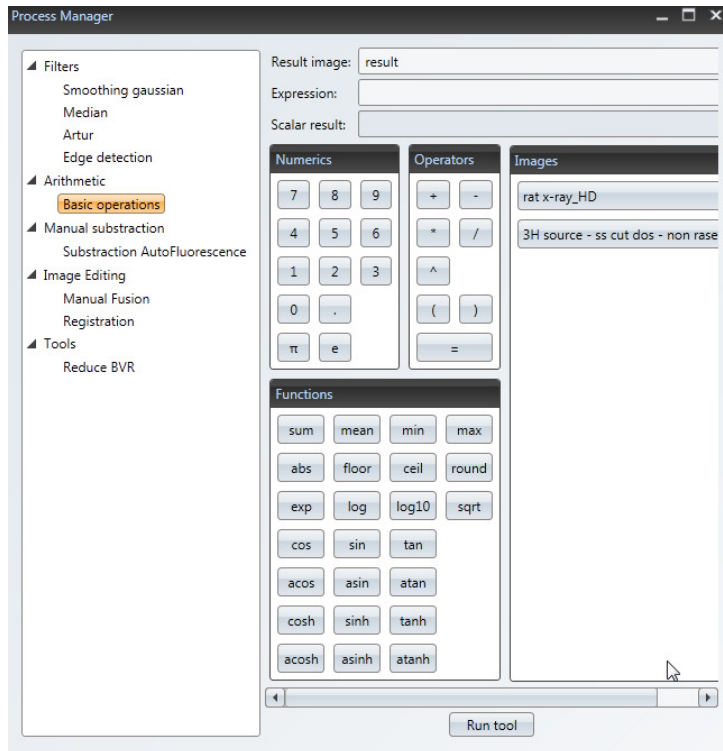
Manual Subtraction (fluorescence)
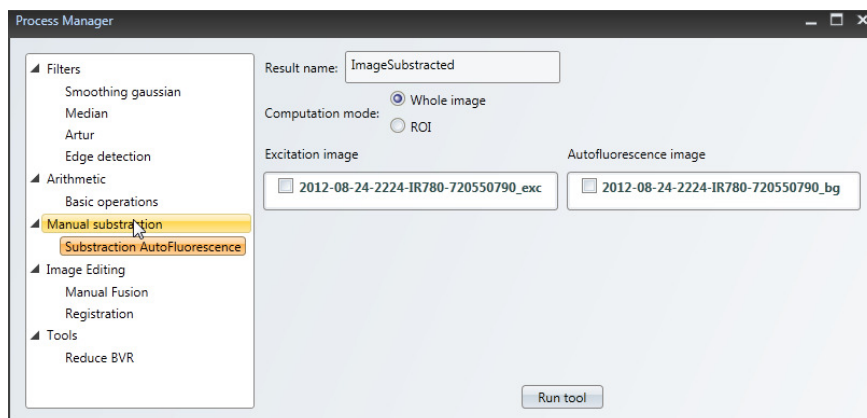
This tool allows manual subtraction of the autofluorescence from the real signal, if the automatic unmixing doesn't bring expected results. The correlation coefficient of manual subtraction can be calculated on the whole image or only on a ROI.

Image editing

This option allows the overlay of the images. Refer to section 8 Standard Operating Procedures.

5.7   Hot spot remover

Hot spots are regions with very high signal values because of artifacts. The hot spots button launches a detection of such spots. The signal will be set to 0 in such regions after the userconfirmation. Be careful, this operation will change your quantification

To localize hot spots one has to adjust the threshold by moving the slider
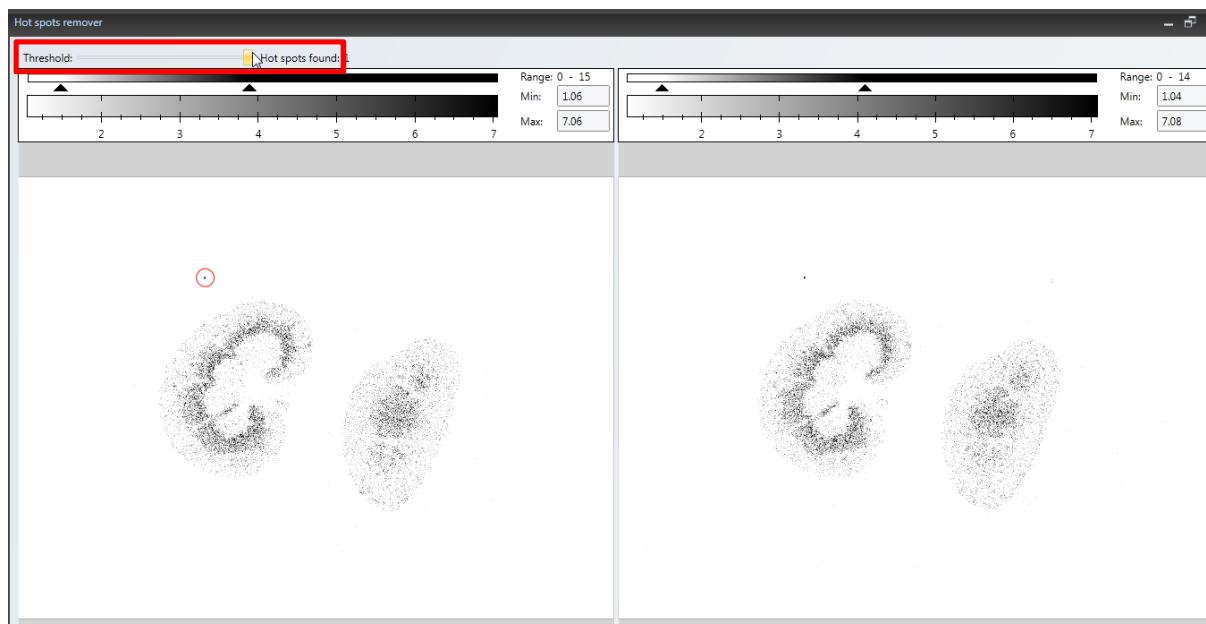
5.8 Tools

Reduce bvr tool for HD files in bioluminescence only
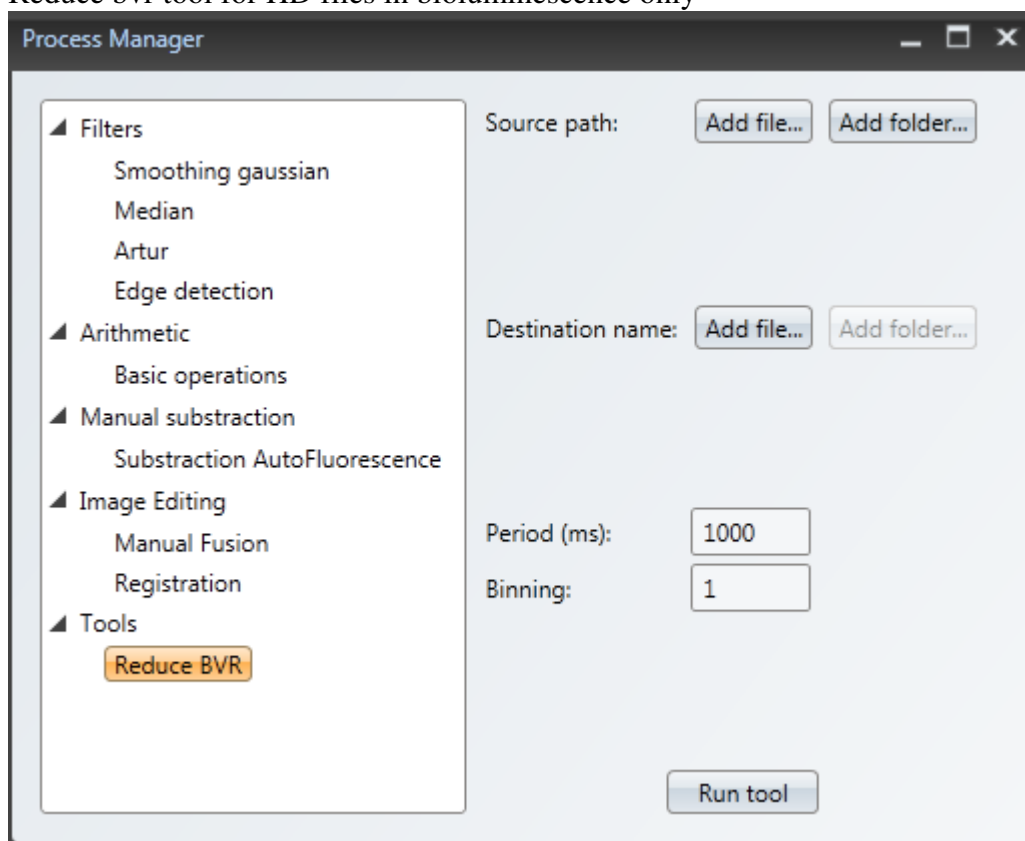
This tool allows changing the size of your bvr file if too big to analyze. By default frame rate corresponds to the one of the camera which may results in the very big acquisition files if the signal of the sample is very strong and time of acquisition long. This tool allows considerably reduce their size.

In Source path choose the file to be reduced or add a folder containing more files. Then choose the name of the future reduced file or a destination folder for more files. Choose the time frame period; you can also add some binning. Run tool to validate.

This process won't cause the signal loss or affect the quantification.

6 SEQUENCE tab

4th and the last tab in the Workspace menu. The sequence toolbox is specific to all modalities.

6.1 Sequence creation

Create sequence button opens the window of sequence creation, where the user can define the different parameters of the sequence (frame duration, sequence duration, number of frames).
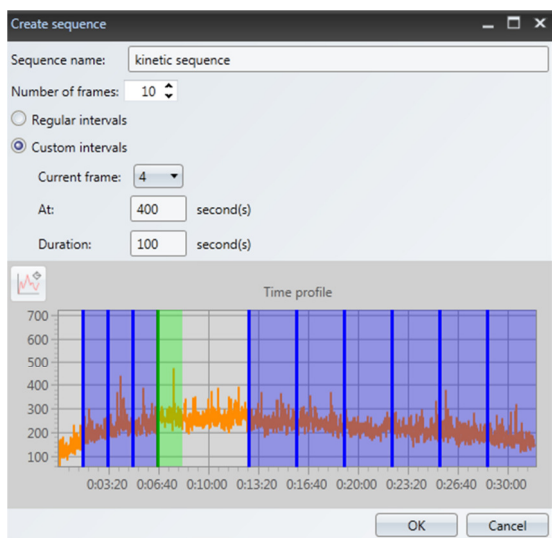
It is also possible to display Time profile by pressing on Time profile button (bioluminescence, autoradiography)

User can use either regular intervals for every frame, or custom intervals (time interval chosen for each frame).

On the screen shot on the left the custom interval settings for the 4th frame are shown, the frame duration 100s, frame acquired at 400s, color green on the time profile graph.

OK button validates chosen sequence settings and results in opening the panel which controls the navigation through the different frames of the sequence.

This panel appears in the Image display panel just above the analysed image.

Displays the first frame of the sequence.

Steps back to the previous frame.

Loops through the sequence at the reverse side.

Loops through the sequence.

Steps forward to the next frame

Displays the last frame of the sequence.

Stops the loop

Extracts the active frame to create an independent image.

Frame: 6 / 10   Indicates or sets the displayed frame.
Indicates the total number of frames.

When you create a ROI, the Result Viewer displays the quantification for all the frames.

6.2 **Sequence measurement**

Sequence measurement button, measures the signal for each frame (over chosen ROI or all image) and displays the results in the Result Viewer window.

7 Standard operating procedures

6.3 **Overlay of images from Beta Acquisition**
with the images from other modalities saved in the known format (tiff, PNG, jpeg) in M3 Vision.

1. Open a Beta Acquisition image which will serve as your reference image
2. Go to **Process tab => Data zoom** and choose a part of the image which will be overlaid.
   The newly created reference image will appear in the Images window under the name xxx_DataZoom
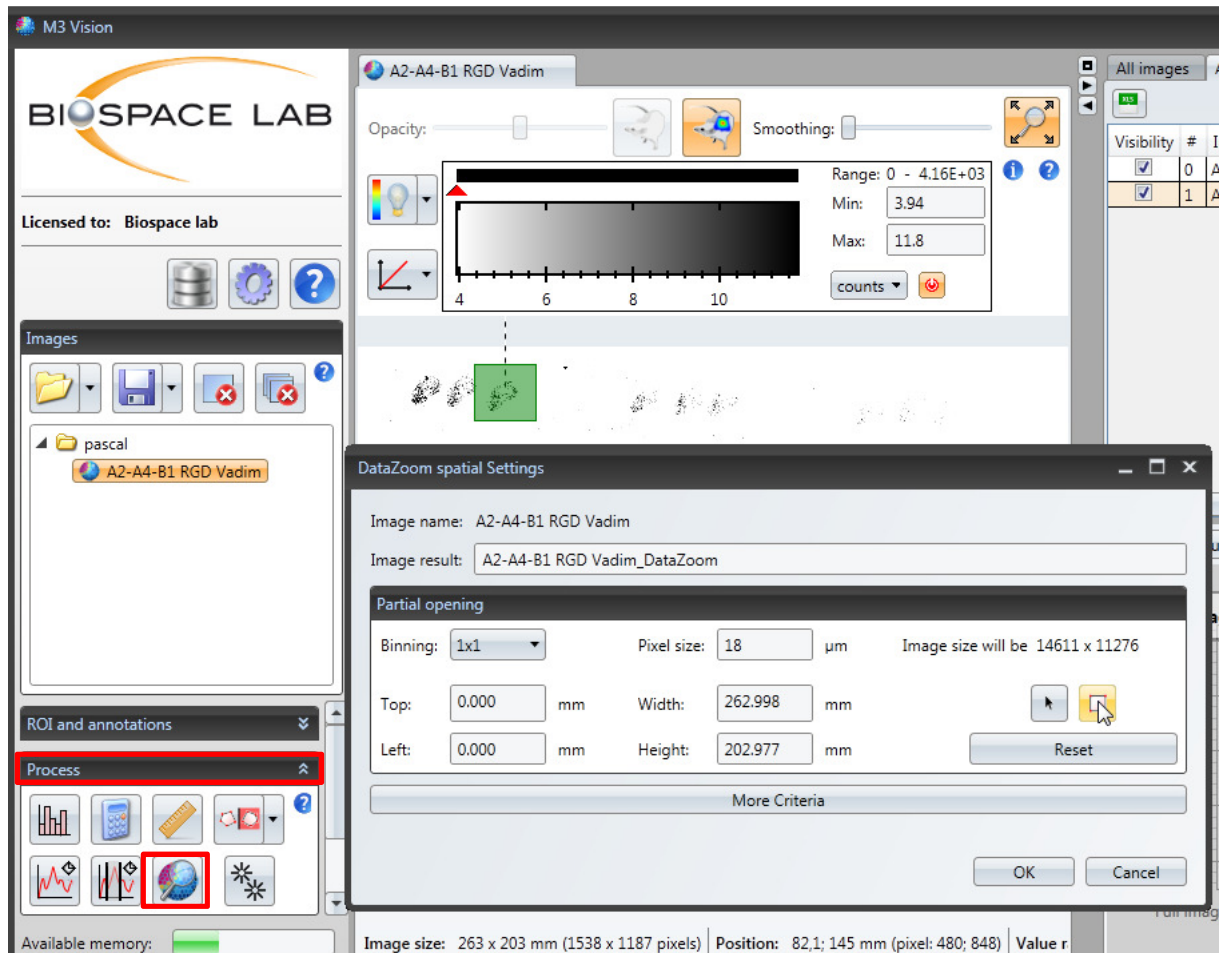
3. Go to the **Apply some processes** in **the Process tab** and choose **Image Editing=> Manual fusion**
4. Choose your reference image and Browse for the background image to be fused.
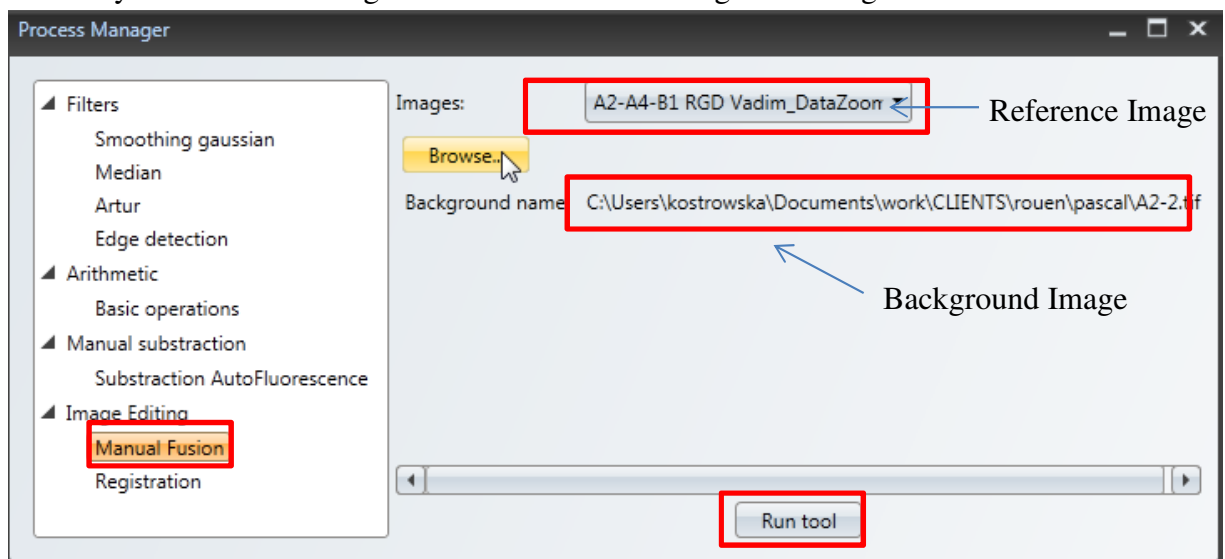
5. Press on Run tool button, the chosen image will be automatically added to your reference image and appear under the same name: xxx_DataZoom

6. Choose the **Registration button**
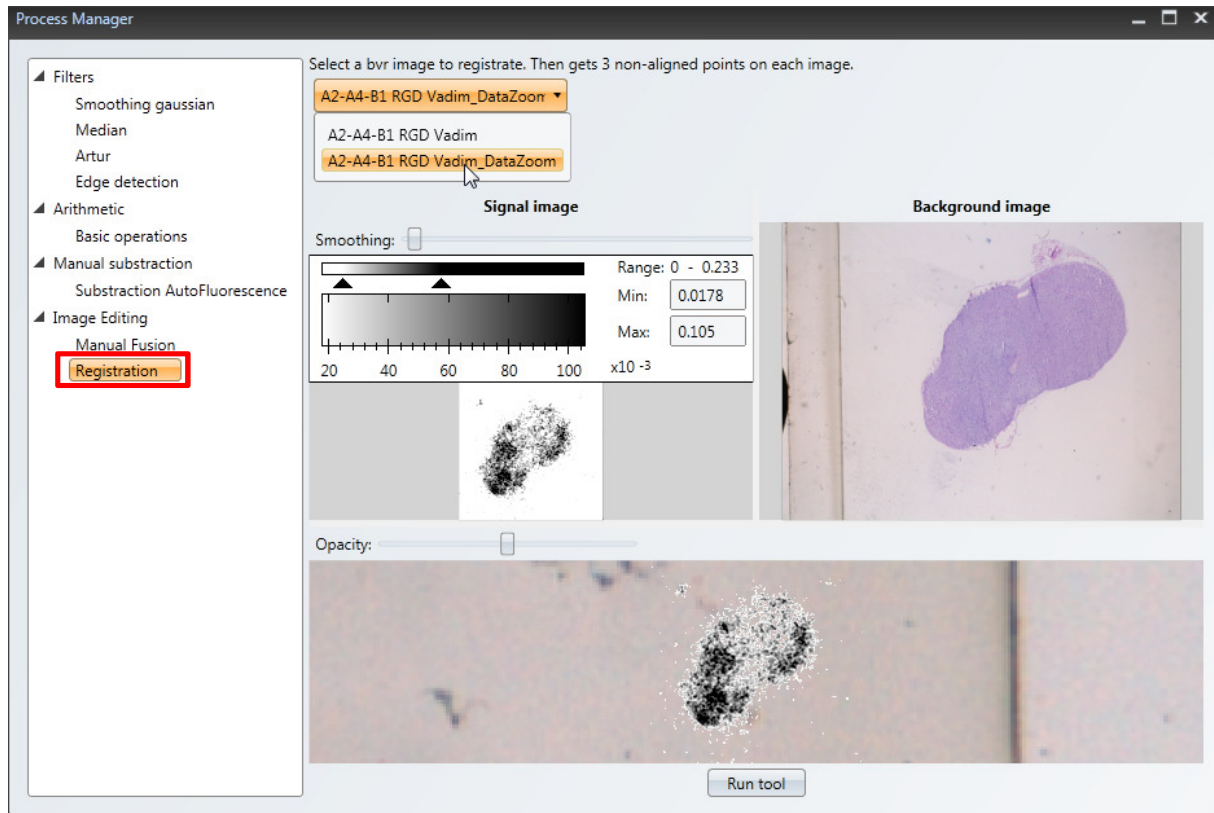
7. Select a fused image for registration. Once fused image charged you can adjust the display of the reference image in the left window and change opacity of the fused image in the bottom window. You can also change the size of each window to adjust the size of displayed image. The background Image will appear in the right window

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8. Select **Create marker** to draw the reference points (at least 3) on the reference image (bvr) on the left
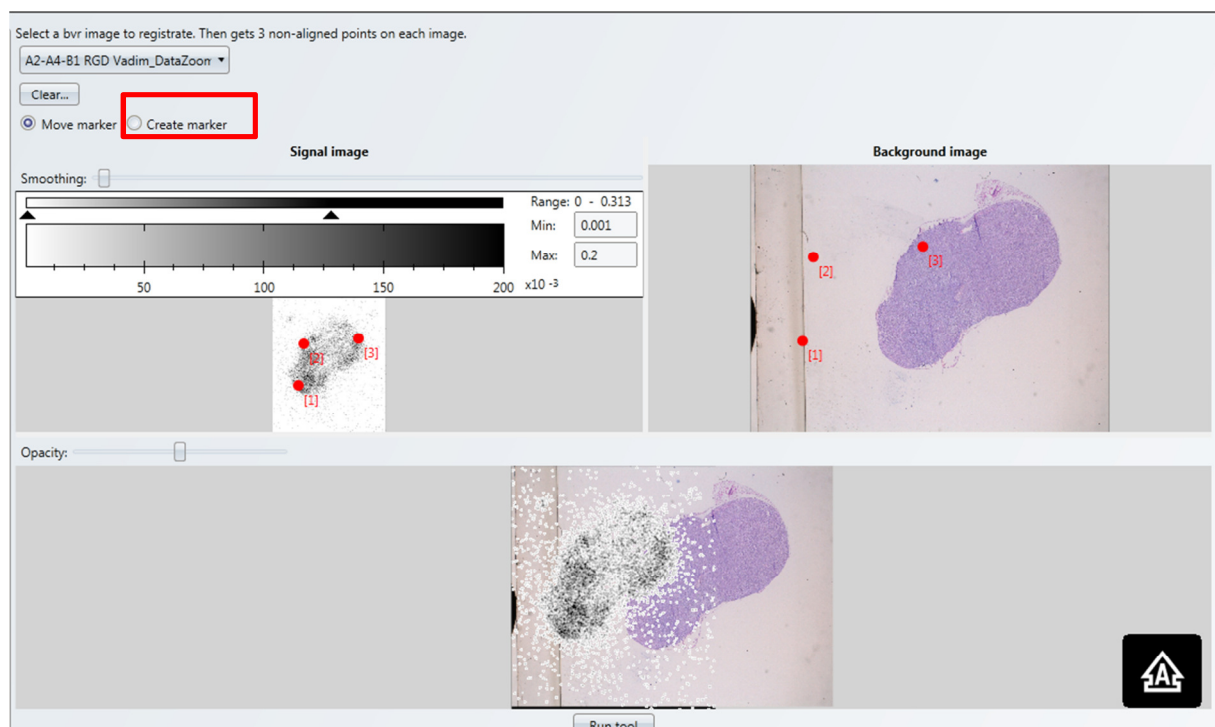
9. The reference points will appear automatically on the background Image. Chose **Move marker** button to move the reference points of the Background Image in order to adjust them with the reference Image. While moving the points over the Background Image you will see the creation of the fusion image on the bottom window. Once the

fusion result is correct press on Run tool. The fused image will appear in the Result window. You can continue your quantification analysis.
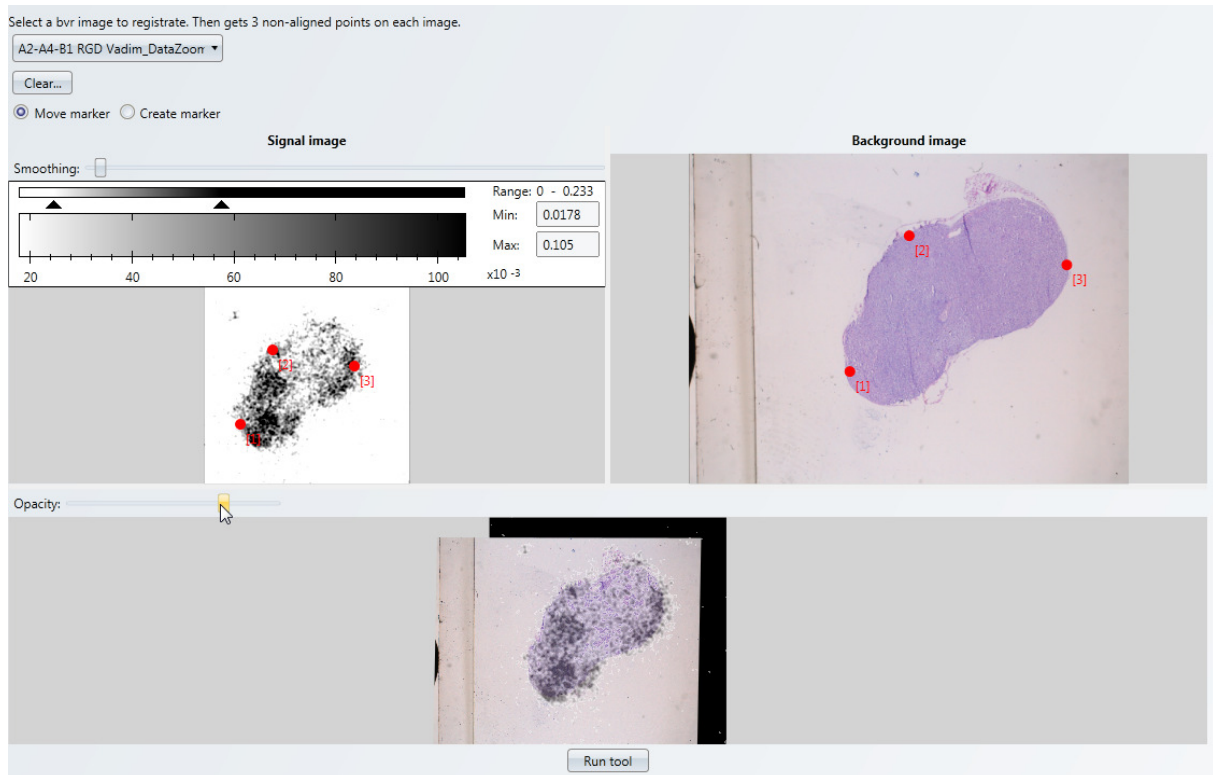
## 6.4   Overlay of X-ray image with the signal image

1. Open an image of interest in M3Vision
2. Go to **Process tab => Apply some processes=> Image Editing=> Registration**

If more then on image is opened in M3Vision, choose your image of interest to be registered
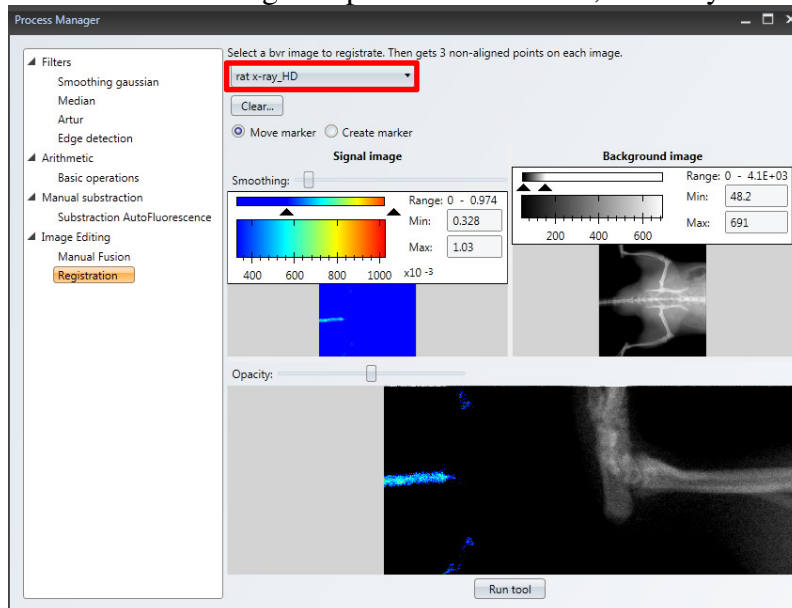
The signal Image will appear in the left window, the X-ray image will appear as a background image in the right window, and the fused image will appear at the bottom under those two images.

3. Once the right image charged you can adjust the display of the signal image in the left window and change opacity of the fused image in the bottom window. You can also adjust the size of each window to adjust the size of displayed image with the double arrow.
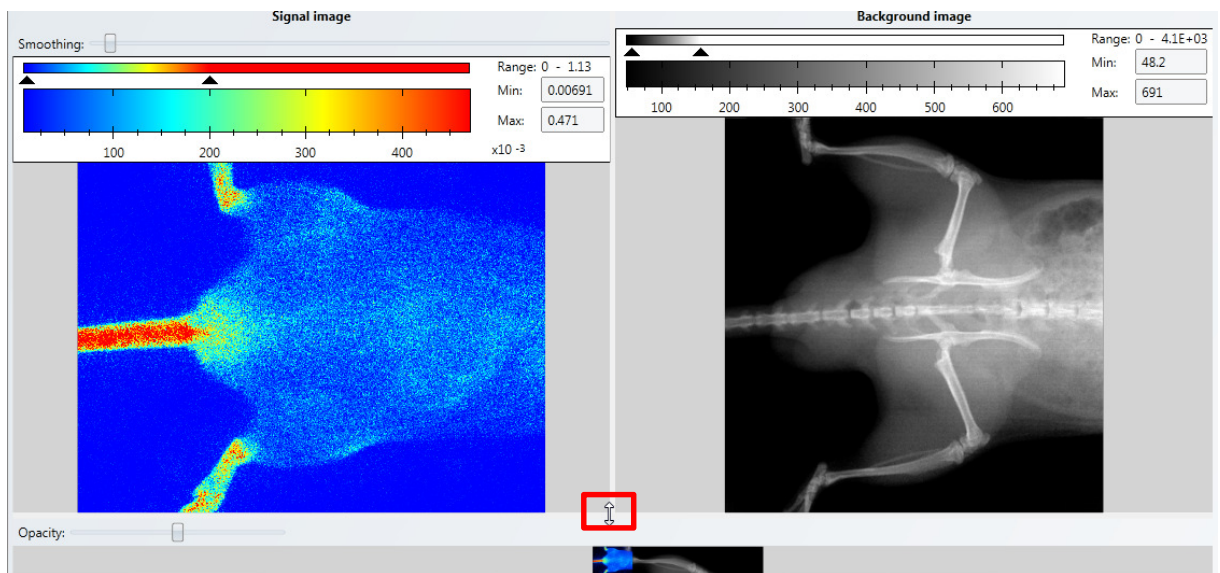

4. Select **Create marker** to draw the reference points on the reference signal image on the left
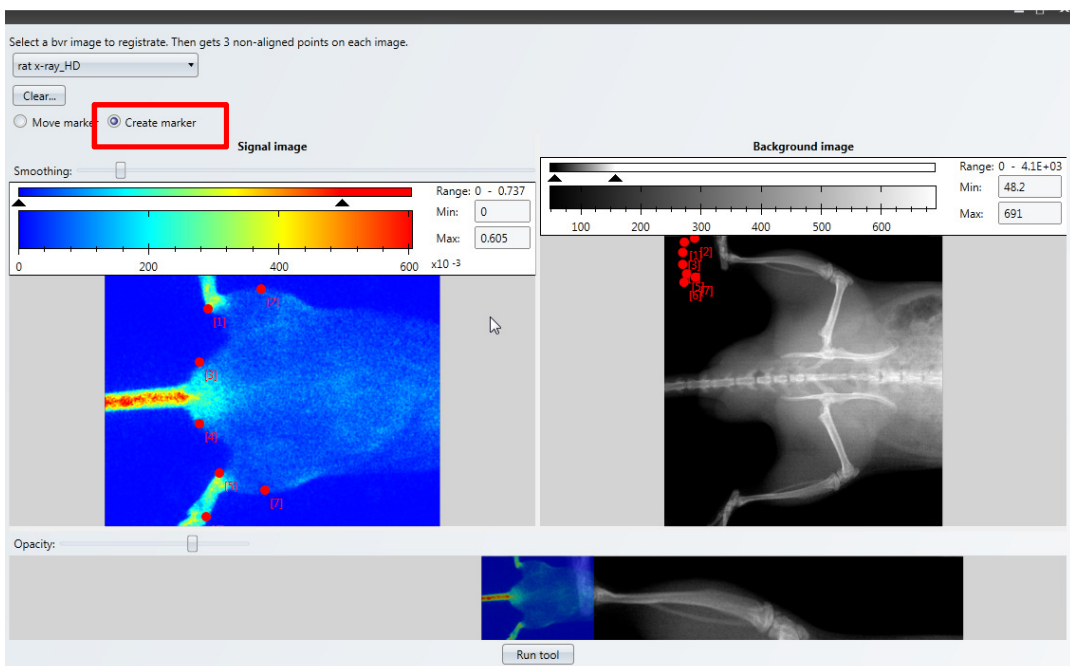
5. The reference points will appear automatically on the background Image. Chose **Move marker** button to move the reference points of the Background Image in order to

adjust them with the reference Image. While moving the points over the Background Image you will see the creation of the fusion image in the bottom window. Once the fusion result is correct press on **Run tool**. The fused image will appear in the Result window. You can continue your quantification analysis.
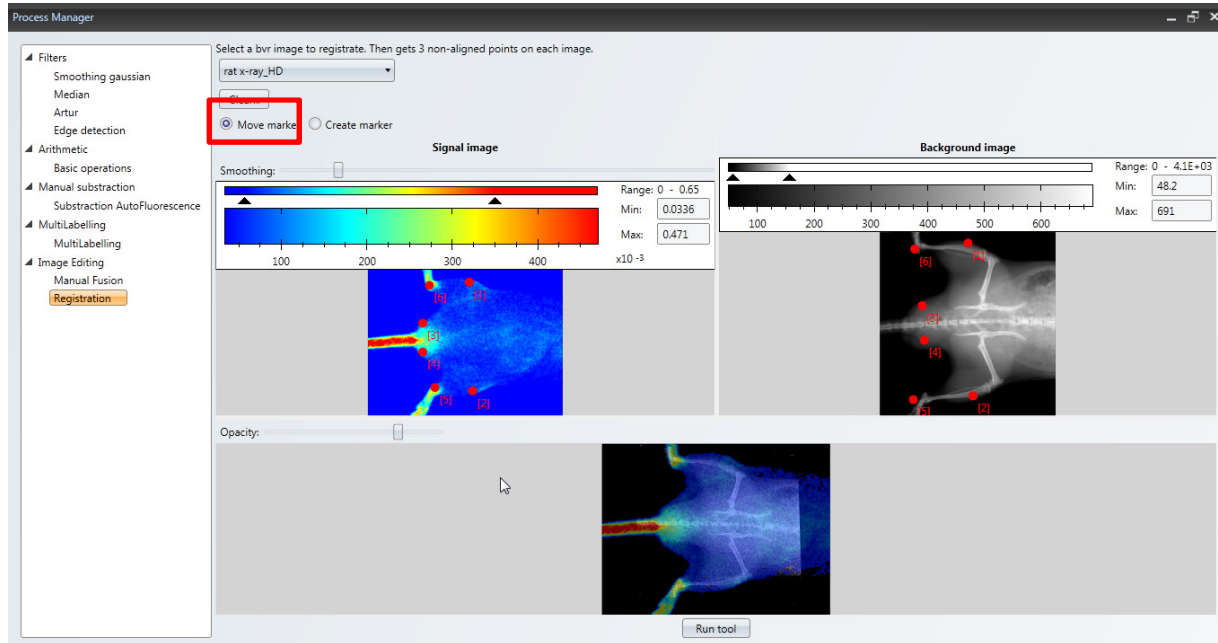
# 8	Customer Support

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