NEWTON© SOFTWARE & HARDWARE

USER & SERVICE

MANUAL – V18.0X

VILBER
Smart Imaging

NEWTON© SOFTWARE
USER GUIDE – V18-0X

Thank you

Dear Customer,

On behalf of Vilber, we would like to thank you for choosing the Newton imaging system.

In order to learn the capabilities of your Newton imaging system, we kindly ask you to read this manual. This manual details how to install and to operate the hardware and the software components.

Vilber is dedicated to your satisfaction and we will be pleased to answer any question you may have. We are also very receptive to your suggestions. Many of the new features and enhancements in this system are a direct result of conversations with our customers. Please do not hesitate to contact us to let us know what you would like to see in the next version of this system.

You can contact us at the following addresses:

⇨ Worldwide: Vilber Lourmat SAS
                      BP31 - ZAC de Lamirault- Collegien
                      F-77601 Marne-la-Vallée Cedex 3
                      France
                      T.: 33 (0) 1 60 06 07 71 / Email: info@vilber.com

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                      Wielandstrasse 2
                      D-88436 Eberhardzell
                      Deutschland
                      T.: 49 (0) 7355 931 380 / Email: info@vilber.de

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                      北京市丰台区玉泉营 111 号北京 111 文化产业园 A 座 127 室
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Do not hesitate to visit our website at www.vilber.com

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Introduction

Using this manual

This manual contains software and hardware instructions for the following imaging systems:
• Newton FT500
• Newton FT100
• Newton BT500
• Newton BT100
Please refer to the specific pages of your instrument.

This manual is part of the product. Please read this manual in full before using your imaging system
• Store this manual at an easily accessible location
• The device may only be transferred with the operating manual
• If the operation manual is lost, replace it immediately. Please contact us for further details.

Newton FT100 / Newton BT100

Newton FT500 / Newton BT500

Prerequisites

The User Guide provide you with the instructions needed to operate, and maintain the Newton imaging system in a safe way.

In order to operate the Newton imaging system in the appropriate way, the following prerequisites must be fulfilled:
• You have read and understood the safety instructions in this User Guide, particularly in regards of powerful light danger.
• You should be qualified to use general laboratory equipment and to handle biological materials.
• You should use the instrument only for the intended purpose of gel and blot image acquisition in research laboratories.
• You accept the terms and conditions of the end user license agreement
• The instrument is connected to a grounded power source and to a circuit breaker.
• The instrument is installed by a Vilber Lourmat representatives.
• This instrument is suitable for research use only and shall not be used in clinical procedures, or for diagnostic purposes.
• Some techniques or reagents for generating and / or detecting light in biological samples are patented and may require licenses from third parties. Users should independently determine for themselves whether their activities infringe any valid patent before operating the Newton imaging system.

Disclaimer: Vilber's Newton 7.0 Imager may be used in a wide range of imaging applications for research use only, including in vivo, ex-vivo and in-vitro imaging in animals. No license under any third-party patent is conveyed with the purchase or transfer of this product.

No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation, reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. Therefore, users of the Newton should seek legal advice to determine whether they require a license under one or more of the exiting patents in their country.

About the Newton imaging system

The Newton imaging system is a scientific instrument designed to capture bioluminescence or fluorescence gel or blots images. The Newton system uses the Evolution-Capt software to control image capture and optimization for selected applications

A cooled scientific CCD camera is used to capture high resolution digital images of your sample. The instrument can capture images of chemiluminescent, fluorescent, and colorimetric samples, depending on the system configuration. The instrument can only be used for research purposes in the academia and life sciences industry. The instrument cannot be used for diagnostic purpose.

Newton offers exquisite precision and resolution, which mean reliable results for both quantification and documentation. The advanced imaging electronics has been developed by our experts especially for your scientific applications. This association of our exclusive electronic, high-quality optics and advanced software delivers outstanding performance. With Newton, you simply reach the lowest limits of detection on all of your samples.

Warranty

The Newton imaging system is warranted against faulty construction or defective material for a period of two years from the Vilber Lourmat invoicing date. If any defect occurs in the instrument during this warranty period, Vilber Lourmat will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

• Defects caused by improper operation, incorrect use or bad maintenance
• Repair or modification done by anyone other than Vilber Lourmat or the company's authorized agent
• Use of spare parts supplied by anyone other than Vilber Lourmat.
• Damage caused by accident, misuse or disaster
• Corrosion caused by improper solvents or samples

This instrument should not be modified or altered in any way. Modification or alteration of this instrument will:
1. Void the manufacturer's warranty.
2. Void the conformity certifications.
3. Create a potential safety hazard.

The light sources (LED panel, …), the filters, the power supplies, the batteries, and the consumables are not covered by our warranty. The use of consumable products or non-original spare parts not recommended by our service department is at the user's own risk and therefore automatically invalidates the warranty.

We reserve the right to decide where the faulty goods will be repaired (in our workshop or elsewhere), and whether or not the faulty part is to be replaced; all other freight charges incurred being at the cost of the purchaser. Returned goods will not be accepted for repair unless previous written authorization is obtained from our service department. A request for authorization must be accompanied by an itemized list of products, model numbers and the corresponding invoice numbers under which they were originally shipped. All returned goods should have a certificate of decontamination. The Buyer must bear all costs and risks incurred during the transportation of the goods from their collection at Vilber Lourmat factory. In the case Vilber Lourmat incorporates some devices or equipment from another supplier in the manufacture of its products, the extent and the duration of the warranty will be those conceded by the suppliers or sellers.

Vilber Lourmat cannot be held responsible for any loss, bodily injury or material accident incurred by any failure of this supply, whatever the origin of this failure may be. Vilber Lourmat is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Vilber Lourmat. The responsibility of Manufacturer is strictly limited to its staff and to its own supplies. In the case of dispute, only the commercial court of Meaux (FRANCE) shall be competent, even in third party claims proceedings or when there are several co-defendants.

Safety instruction

Introduction

PLEASE READ CARFULLY THESE INSTRUCTIONS BEFORE OPERATING THE INSTRUMENT.

The Newton imaging system is powered by mains voltage, and is used to capture images of samples that may be hazardous. Before installing, operating or maintaining the instrument, you must be aware of the hazards described in the user documentation. Follow the instructions provided to avoid personal injury or damage to the instrument.

Safety notices

This user documentation contains safety notices (WARNING) concerning the safe use of the product. See definitions below.

WARNING
Whenever you find this pictograph, be sure to refer to this Manual.

WARNING indicates an imminently hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

NOTICE
NOTICE indicates instructions that must be followed to avoid damage to the instrument or other equipment or to ensure the instrument is properly working.

General precaution

WARNING
Do not operate the Newton imaging system in any other way than described in the User Manual.

WARNING
Use of this instrument in other ways than those specified in the user documentation may result in physical damage because of exposure to irradiated light or electric shock or injury from touching an operating part.

WARNING
Do not use the Newton imaging system in dangerous atmosphere or with dangerous materials for which the Newton imaging system has not been designed for.

WARNING
Do not use the instrument if smoke, atypical noises or odors can be perceived, or if the instrument becomes unusually hot as this may result in fire or electric shock. Stop using the instrument immediately, turn off the power switch, and unplug the instrument from the power outlet. Contact Vilber Lourmat representative to request repair.

WARNING
To prevent fire or shock hazard, do no expose the unit to rain or moisture.

Do not pour liquids directly on or inside the instrument.

Switch off all the lights immediately after use.

Clean the transilluminator platen after use.

WARNING
Ensure that all the ventilation-opening systems are not obstructed. The obstruction of the air admission grids may affect the performance of the system and cause operational failure. To ensure adequate cooling ensure there is at least 40 cm of free space in front of the instrument and at least 10 cm on all other sides to walls or other equipment.

WARNING
The use of accessories not supplied by Vilber Lourmat can damage the system or create safety hazard.

WARNING
When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the system.

WARNING
Do not use the instrument within or near a sink, or in humid (above 70% RH) or dusty environments. This can result in fire or electric shock.

WARNING
As the equipment is heavy, it cannot be carried by one person. Two service persons are necessary when moving or relocating safely the main body of the equipment. If the equipment drops, it may be broken, or you may get seriously injured if it drops on your foot.

WARNING
Do not touch the light source in the instrument. The light source may be hot.

WARNING

• Do not place objects on top of the instrument.

• Use the handle to open or close the instrument door.

 • Handle sample trays with care to avoid dropping them.

• Do not leave samples in the instrument after exposure. If left, these may degrade and cause damage to the instrument.

• Do not disable the interlock of the equipment as light can be emitted if the door is opened.

• Do not open the instrument door during exposure. This will stop the exposure and image data will be corrupted.

• Do not use abrasive cleaning materials for cleaning. This may cause damage to the instrument.

• Do not use excessive amounts of liquids for cleaning

Emergency procedure

WARNING

In case of emergency:
• Turn off the power by pushing the Power switch to the O position on the top rear side of the instrument.
• Disconnect the power cord from the AC voltage outlet.

Access to power switch and power cord with plug:
For your security, do not block the access to the power switch and to the power cord. The power switch must always be easy to access and the power cord must always be easy to disconnect.

Important electrical power safety information

WARNING

Electrical requirements are located on the imager label on the rear panel of the imager. Please ensure these electrical requirements are compatible with your AC voltage outlet.

• Connect the imaging system to an appropriate AC voltage outlet that is properly grounded and protected by a circuit breaker.
• Connect the imager power cord to the rear left side panel of the system and plug into a grounded power outlet. Connecting to ground constitutes an obligatory protection.

WARNING

• Power down the system and disconnect the AC main from the unit before performing any disassembly or repair the instrument.
• The system must be unplugged from the AC voltage outlet if it is not intended to use it before a long time.

WARNING

Before plugging or unplugging the camera cables, ensure the system is off and disconnect the Newton imaging system from the AC voltage outlet.

WARNING

• Do not damage the power supply cord by bending, twisting, heating or allowing them to become pinned under the equipment. Using damaged power cords could result in fire or electric shock. If the power supply cords are damaged, contact us for replacements.
• Never pull the cable itself. Disconnect the cable only by grasping the plug.

WARNING

Do not block access to the power switch and power cord. The power switch must always be easy to access. The power cord with plug must always be easy to disconnect.

WARNING

The Newton imaging system has serviceable fuses which are located on the rear panel and are a part of the power entry module. See the Maintenance chapter of this guide for information about replacing the fuses

> **WARNING**
> All equipments connected to this instrument shall be certified according to IEC 60950 standard.

> **WARNING**
> In case of thunder, do not touch the power supply plug, as this can result in electric shock.

> **WARNING**
> Turn off the power switch before cleaning the inside of the instrument.

> **WARNING**
> Do not turn off power during image acquisition as this can cause loss of data and damage the instrument. Only turn off power in an emergency situation.

> **WARNING -** Electrical shock hazard.
> All instrument repairs or modifications should be performed by service personnel authorized by Vilber Lourmat. Do not open any covers or replace parts unless specifically stated in the user documentation.

Instrument serial number and manufacturing information

The instrument serial number is found on the label located on the back of the instrument. The two first digit refer to the manufacturing year. For instance, the serial number 18-123456 has been manufactured in 2018.

The instrument manufacturer name is found on the label located on the back of the instrument.
Manufacturer name: Vilber Lourmat SAS
　　　　　　　　　　 BP31 - ZAC de Lamirault- Collegien
　　　　　　　　　　 F-77601 Marne-la-Vallée Cedex 3
　　　　　　　　　　 France
　　　　　　　　　　 www.vilber.com

Vilber Lourmat is proudly ISO-9001 certified since 1994.

CE conformity

This system complies with the requirements of the EC Directive 2004/108/EEC, 2006/95/EEC and EN 61010-1, relating to Electro-magnetic compatibility and low voltage.

The Electro-magnetic susceptibility has been chosen at a level that gains proper operation in residential areas, on business and light industrial premises and on small-scale enterprises, inside as well as outside of the buildings. All places of operation are characterized by their connection to the public low voltage power supply system.

$$\mathsf{C}\,\mathsf{E}$$

The CE marking and the corresponding EC Declaration of Conformity is only valid for the instrument when it is:

• used as a stand-alone unit, or

• connected to other products recommended or described in the user documentation, and

• used in the same state as it was delivered from Vilber Lourmat, except for alterations described in this user documentation.

International standards

This product fulfills the requirements of the following standards:

IEC 61010 -Safety requirements for electrical equipment for measurement, control and laboratory use
EN 61326-1 - Electrical equipment for measurement, control, and laboratory use - EMC requirements

Recycling information

This product fulfills the requirements of the following standards:
2011/65/EU Annex II – Restriction of Hazardous Substances (RoHS) Directive
2012/19/EU – Waste Electrical and Electronic Equipment (WEEE) Directive

Decontamination

The equipment and the accessories must be clean from contaminants before decommissioning and all local regulations must be followed with regard to waste disposal. Samples are to be disposed of according to local regulations.

Please always enclose the Decontamination Declaration form for product return. We require the fully completed decontamination declaration for accepting and processing the return. If it is not enclosed with the return, we will return the product at your expense.

Recycling of lamps

The LED lamps must be recycled or disposed of in a manner compliant with national and local environmental regulations.

France only:
The buyer ensures and finances the decontamination, the collection and the disposal of waste electrical and electronic equipment (WEEE) under the conditions provided in the Articles 21 and 22 of the Decree No. 2005-829 dated of 20 July 2005. In France, for tubes recycling, contact the Recylum, www.recylum.com Improper disposal may be harmful to the environment and human health.

Disposal of electrical components

Waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately.
When taking the instrument out of service, the different materials must be separated and recycled according to national and local environmental regulations.

Considerations when setting up an imaging laboratory with the Newton system

Considerations for outfitting a Newton system in your lab will depend on the anticipated workflow of studies that will be made. Additionally, some aspects of laboratory set-up are necessary to meet safety and regulatory requirements. We recommend to considered your institutional, local, and national agency guidelines at first before to install the Newton system in your lab environment.

The general need for solutions for animal transport, anesthesia delivery, animal preparation and monitoring, and data management is first to be considered.

Animal transport

Access and transport of animals in to an imaging facility is almost always a consideration for any imaging laboratory. For facilities where imaging equipment will be shared by multiple researchers this can be a particularly important consideration. Complications can arise when animals are to be transported form a dedicated animal housing facility that employs barrier and isolation policies. Many imaging facilities designed for institute-wide use are established within the facility's animal housing area. To some extent this can overcome some of the barrier and transport logistical issues for imaging. Solutions for isolating animals during transportation and imaging, even when animals are housed within an animal facility, may be required.

Hepa filtered animal chamber are ideal for such animal handling requirements and can be supplied. Supporting infrastructure, including clean benches and sterile biosafety cabinets are mandatory to handle and prepare the animal(s) under sterile conditions and to transport them to the imaging system.

Animal care and Anesthesia

Optimum configurations for delivery of gas anesthesia for animal preparation and imaging, as well as end-user safety, should also be determined. Scavenging and ventilation solutions, storage and access of anesthetics supplies that conform to regulatory and legal requirements and proper mounting solutions for carrier gas (e.g., oxygen or medical air) cylinders should be included in the anesthesia setup design.

Vilber could supply several solutions to facilitate functional and safe anesthesia delivery as well as waste gas exhaust with up to 99.9 % scavenging of isoflurane.

Laboratory space to perform animal procedures including injections and necropsies should be allotted. Compartments for waste (e.g. syringes and decontaminated materials) and other laboratory consumables should be included. Autoclaves, sterile hoods, interim animal cages including sterile ventilation and exhaust as well as sample preparation materials and space should be considered as well.

IT system

Support for data reconstruction solutions and data management/backup solutions should also be considered. Implementations of data storage solutions should provide reliable data retrieval.

Imaging analysis may be extensive for some applications. Depending on the anticipated level of use for equipment, it may be advisable to configure separate workstations that are dedicated for analysis only. Workstations should be configured with sufficient processers to support image analyses which can be computationally demanding.

NEWTON system installation

Unpacking the system

⚠	**WARNING** The NEWTON imaging system is powered by mains voltage, may be equipped with powerful light sources, and is used to capture images of samples that may be hazardous. Before installing, operating or maintaining the instrument, you must be aware of the hazards described in the user documentation. Follow the instructions provided to avoid personal injury or damage to the instrument.

Please, open the NEWTON box carefully and verify the contents:

NEWTON system	1
Power cable	1
USB cable	1
Instruction manual	1
Installation CD-Rom inside the instruction manual	1

⇨ Remove carefully each component from the box.
⇨ To remove the NEWTON from the box, please use the belts which surround the system and designed for this purpose
⇨ Do not handle the system by the camera or the camera support.
⇨ Remove their protective plastic cover.
⇨ Place the darkroom at its permanent location, the door facing forward. The cabinet has to be placed near the computer used for image acquisition.

Protect the environment!

Dispose of packaging properly, according to existing and applicable waste management rules and regulations.

WARNING

It is recommended that the NEWTON darkroom be carried by at least two people – one on each side- holding the instrument from the bottom side. Be sure that the door is properly closed when carrying the instrument.

The imager weighs 40kg. Using two people, place the imager onto the benchtop given proper space, electrical and environmental requirements. Remove the outer packaging materials.

WARNING

Do not connect the power cable to a power source until all connections are made. The power source has to be grounded and protected by a circuit breaker.

WARNING

The NEWTON system is designed to fit a specific voltage. Please, check the voltage to ensure it corresponds to the NEWTON specifications.

WARNING

Please keep an open area of at least 20 cm at the rear of the cabinet to ensure a proper air circulation for the system. The system should be located in an area free of excessive dust or moisture, strong magnetic fields or ionising radiation. It is also recommended that the ambient temperature be stable and within the range of 15°C to 25°C (20°C recommended) and that the relative humidity not exceed 70%, non-condensing.

WARNING

Ensure that all of the systems ventilation openings are free of interference. Excessive heat build-up in the instrument may affect performance or cause operational failure.

The NEWTON system should be located away from water, solvents, or a corrosive material, on a bench top that is dry and stable. The system should be placed away from interfering electrical signals and magnetic fields. A dedicated electrical outlet should be used to eliminate electrical interference from other instrumentation in your laboratory

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WARNING

Do not defeat any instrument interlocks; they are designed to prevent user injury.

⚠️ It is compulsory to power down the system and disconnect the AC mains from the unit before performing any disassembly or repair to the system.

Installing the hardware

Environmental conditions

Placement	Place the NEWTON on a level-working surface. The imager requires a stable laboratory bench or table capable of supporting 110kg. Provide a minimum clearance of 8cm at the rear of the imager to allow adequate ventilation for camera cooling and access to the power cord and switch. In addition, allow sufficient space to open the front-facing imager door to place sample within the imager. The NEWTON is intended for indoor use only.
Free space	Computer LCD panel operation free space required: 50 cm Right, left, rear and top minimum free space required: 20 cm
Other conditions	• Do not install the equipment near a window and avoid direct sunlight. We recommend attached blinds to nearby windows • Do not install the instrument where it may get wet or flooded • Do not install the instrument in a dusty environment • Do not install the instrument in a place constantly or excessively exposed to vibration or impacts (next to a centrifuge or to a compressor for instance) • Remove all hot or heat source near the Newton, specifically from the air intake. • Do not place objects near the power outlet to ensure easy access to the power cord for disconnection in case of emergency.
Environmental conditions	Temperature: 18° C to 25° C Humidity: 20% to 70% RH (no dew condensation)

Minimum computer configuration

Main board: Desktop computer format

Processor: Intel CoreI7 and upper recommended.
AMD processor and chipset are not supported

Operating system: Windows 10

Memory: 8 GB ram and above

Storage: 250 GB internal hard drive. 50GB should remain available for the Newton imaging system.

CD-Rom: CD player necessary to install the software

Monitor/display: minimum 1280x1024/ 16 million color mode (24-bit). Upper resolutions supported.

USB port: At least two USB ports available in the computer rear side.

USB-3 is required for the Newton system

Please make sure to enable the "XHCI" BIOS option to fully activate the USB-3 ports capabilities

NOTICE
Windows 10 offer advanced control over your computer power options. No BIOS or Windows energy savings should be active when using the instrument.
Please refer to the Trouble shooting section of this User Guide for more information on how to access advanced Windows power options.

Windows 10 must be installed in the computer intended to be used to control the Newton imaging system.

Please run completely the Windows Update before installing the software.

Software installation

Prerequisite

⚠️	**WARNING** The imaging system should be **switched off** before the software installation. Do not switch on the system before the installation is fully completed.

🔍	**NOTICE** The CD disk provided with your Newton system contains the Newton software and driver. You must have Windows™ administrator permission to install the Newton software

Evolution-Capt software installation

🔍	**NOTICE** Please refers to the CD-Rom software name to ensure you have the appropriate software for your Newton system

Please ensure the Newton system is switched-off before the software installation. Do not switch on the system before the installation is fully completed.

Step 1
⇨ Insert the CD-ROM in the CD-ROM drive
⇨ Ensure that all other application programs are closed. Windows 10 users should also ensure that they are logged on with administrator privileges.
⇨ If the Autorun option is set on your computer, the installation automatically starts. If not, double click on the Autorun.exe file to start the installation of the software.
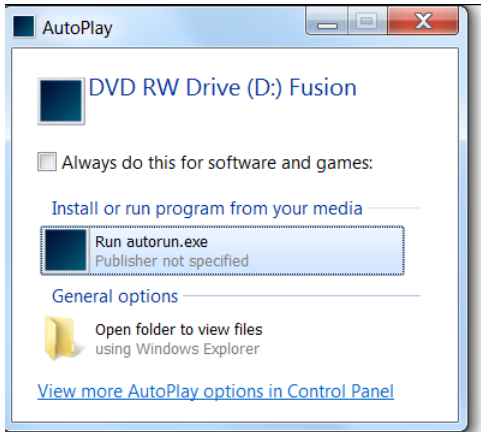
⇨ Please click on the run autorun.exe icon. The Newton Setup window will appear, welcoming you to the Install Wizard for Evolution-Capt Setup.
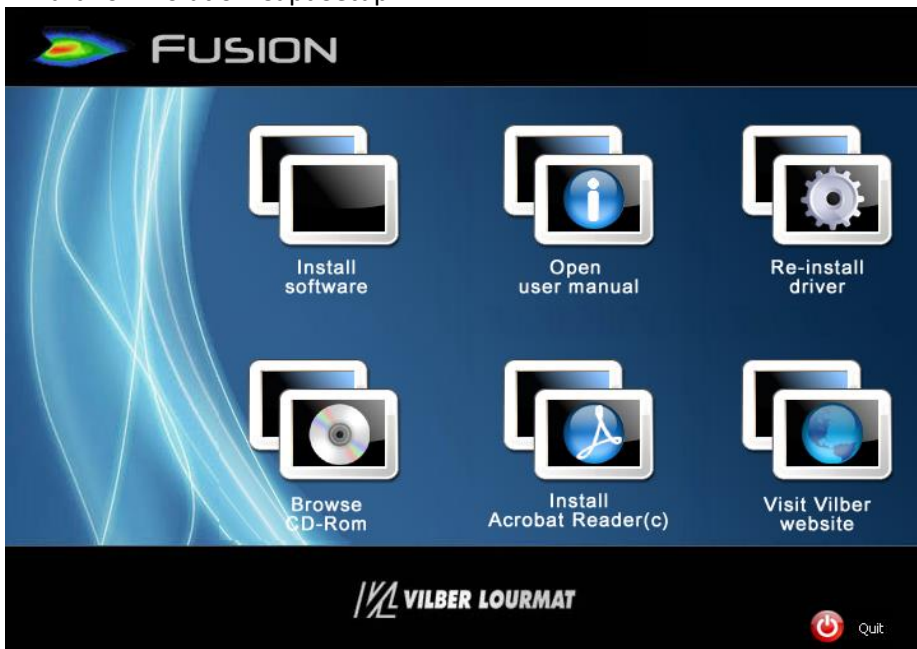
⇨ Please click on the Install software icon.
The software installation process will start:
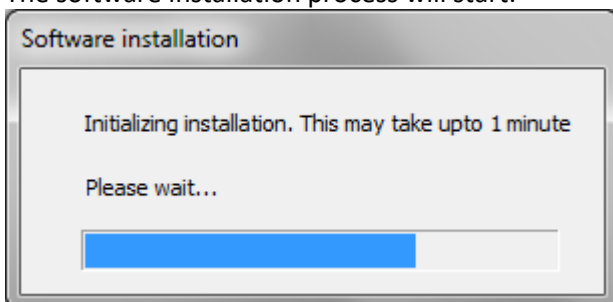
A pop-up window displays the following Windows installation authorization message:
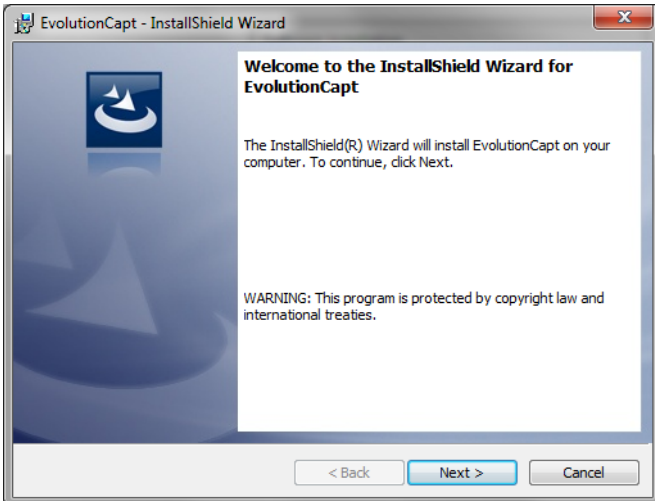
Click on NEXT to continue.
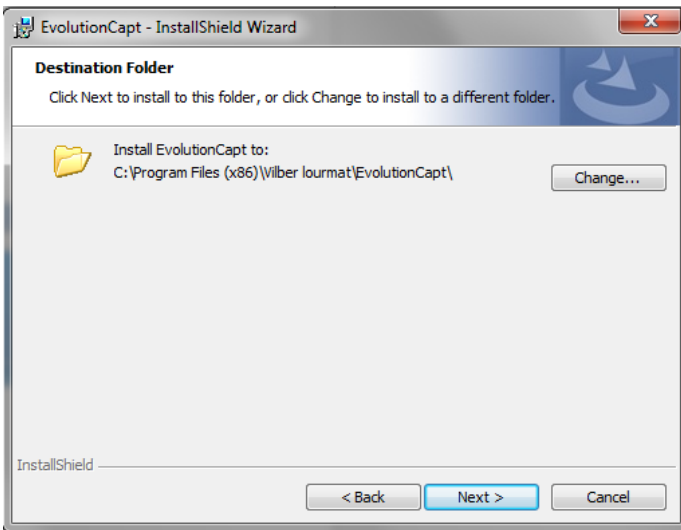
⇨ Select the Destination directory ("c:\Program files\Vilber Lourmat\EvolutionCapt" by default).
⇨ Select on Next to continue
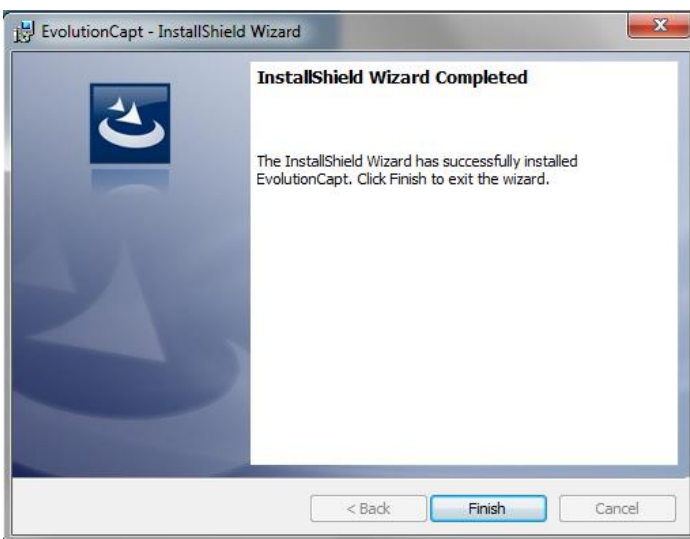
⇨ After the software installation, click on Finish to start the driver installation
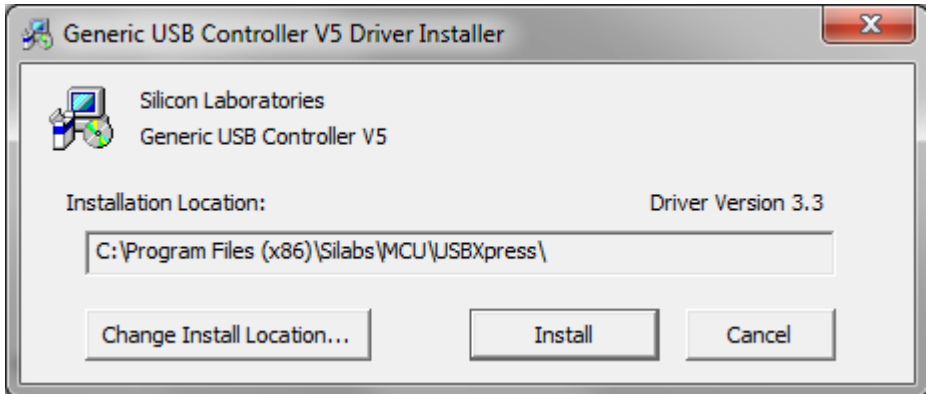
The setup wizard will then install the USB controller driver.
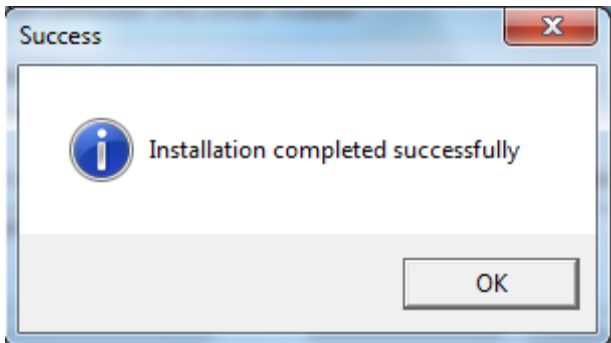
When finished, click on OK.
A pop-up window displays the following camera driver installation message:
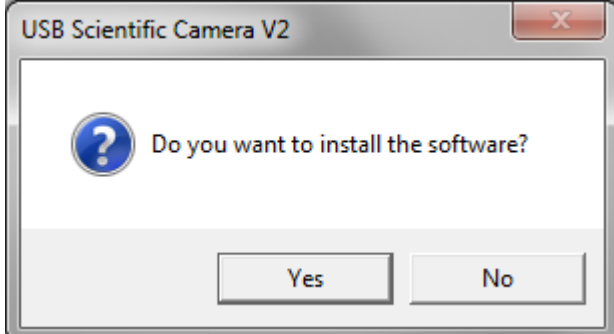
⇨ Select on Yes to continue
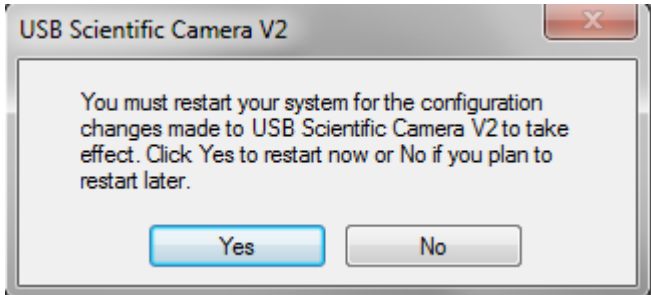
When finished, click on Yes to restart the computer.
After the restart, switch on the Newton system. Keep the Newton-Capt CD-Rom in the CD-Rom drive of your computer.

The darkroom driver is then installed by Windows:

Installing device driver software

Click here for status.

Generic USB Controller (V4)

Device driver software installed successfully.
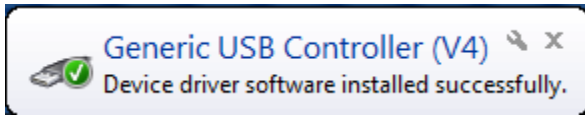
This driver is related to the Newton darkroom management.

Wait for 10 seconds and double click on the Newton icon: .

The camera driver is then installed by Windows:
You can now properly use the Newton software and hardware.

Trouble shooting – Windows device manager

In case of installation issue, we recommend to check the driver status using the Windows Device Manager.
To do so, from the Windows start menu, select "Control Panel".
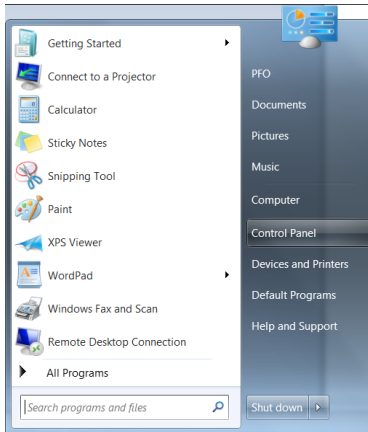
⇨ The software opens the Windows setting options; Select System and security, then system, then Device Manager:
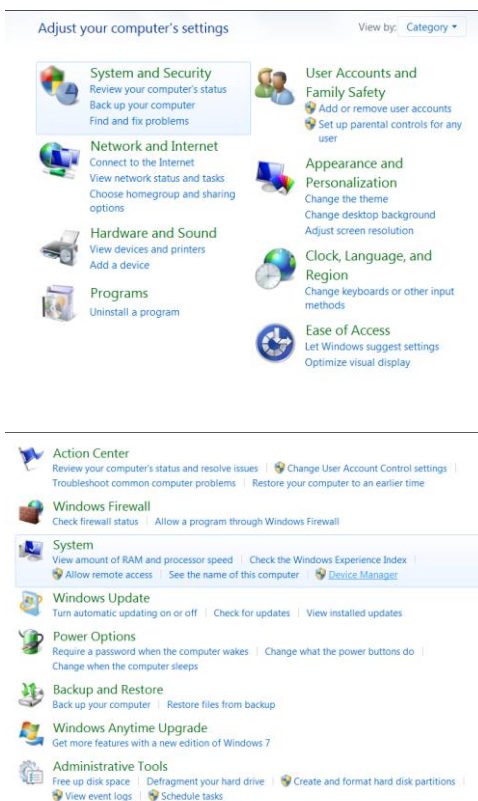
The Device Manager should appear as below for the Newton FX7, Pulse 7, SL7 and Solo 7:
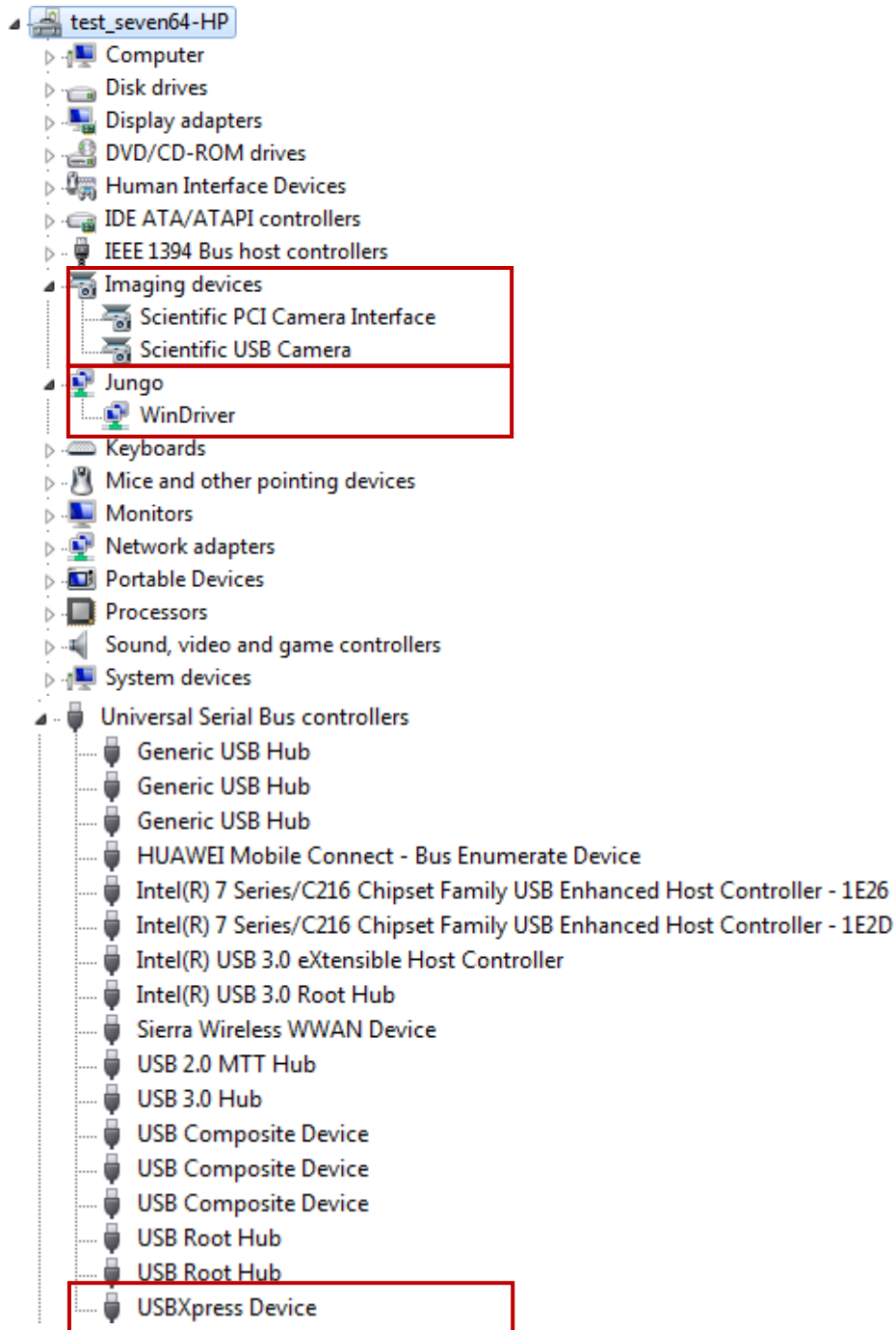
The Device Manager should appear as below for the Newton FX6, Pulse 6, SL6 and Solo 6:
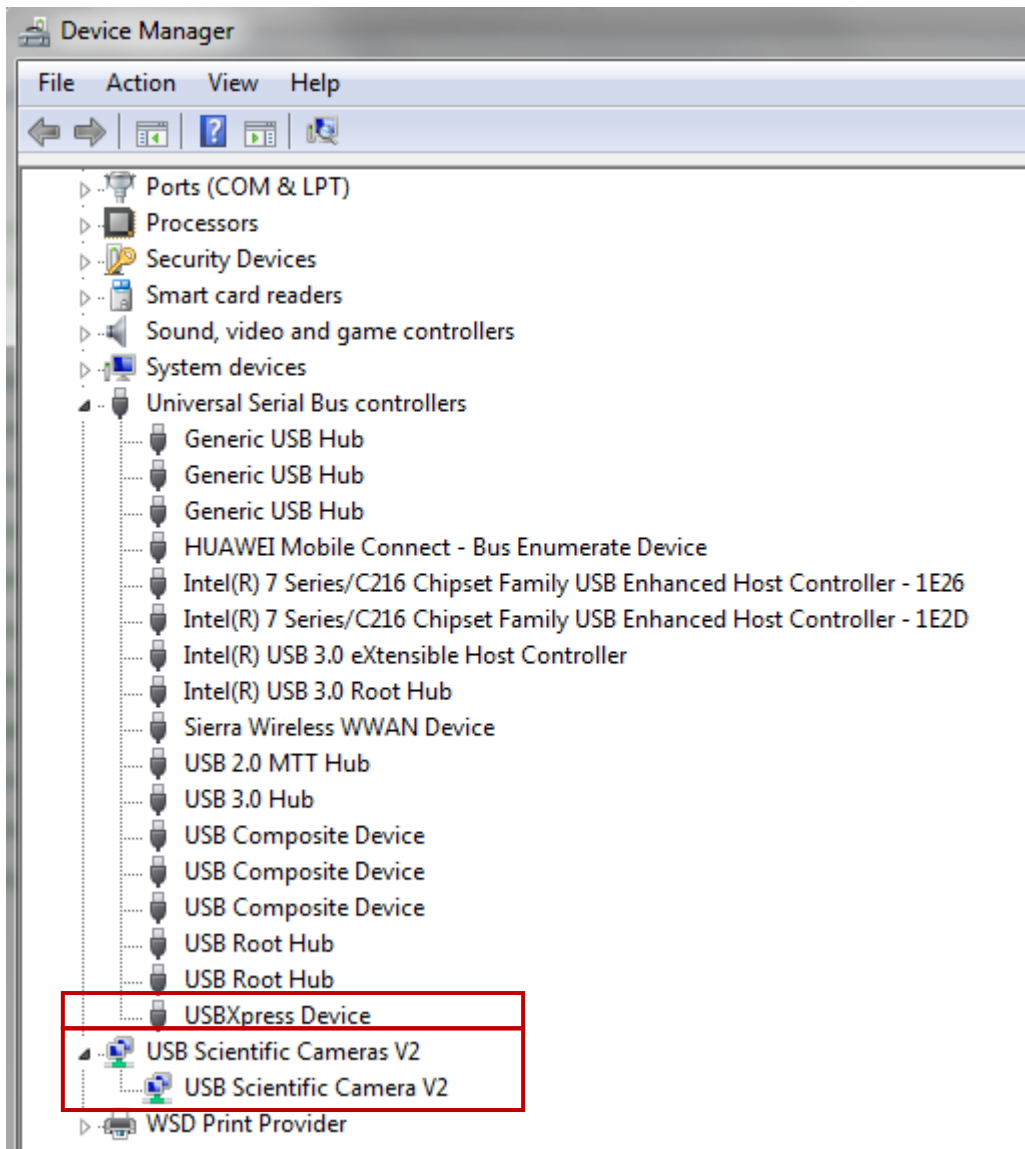
Configure your PC to improve the system performance

This technote outlines known good practices which are likely to improve camera performance.
Our imaging system is a real-time streaming device which compete with other computer devices for system resources. The computer power efficiency management makes the CPU constantly changing its speed and performance. This leads to image artifacts, camera timeouts, and other unwanted imaging errors. In order to overcome these performance issues, certain settings must be adjusted to ensure the system performance is not hindered.
The computer Bios and Windows offer advanced control over your computer power options. You can ensure maximum real-time image streaming performance occurs throughout your image acquisitions, by using the recommended power settings below.

Bios settings – Disable the CPU EIST function

On Intel Core i3/5/7 computers, the mother board BIOS has enabled CPU speed throttling and power saving by default (called CPU EIST – Enhanced Intel Speed Test – or "SpeedStep" or "C State"). Depending on CPU loading, Intel EIST technology can dynamically and effectively lower the CPU voltage and core frequency to decrease average power consumption and heat production. These settings do not allow for stable image acquisition because the speed of the CPU is constantly changing.

Follow the suggested settings below to ensure maximum performance. Note: please consult with your computer/motherboard operating manual or 'boot screen' for instructions about how to access settings in the BIOS.

• Disable the Intel CPU EIST (or Intel SpeedStep or CPU C State).
This feature could generally be found in the following Bios menu: Advanced / CPU configuration / CPU Power management configuration.
Some BIOS menus have grouped these settings into more generally-named options, set these sorts of power savings options to 'maximum power'/'least savings' if there is any doubt.
In some Bios menu, you need to select the "Best performance" option in the advanced CPU configuration.
Please refer to your computer Bios manual for more detailed information.
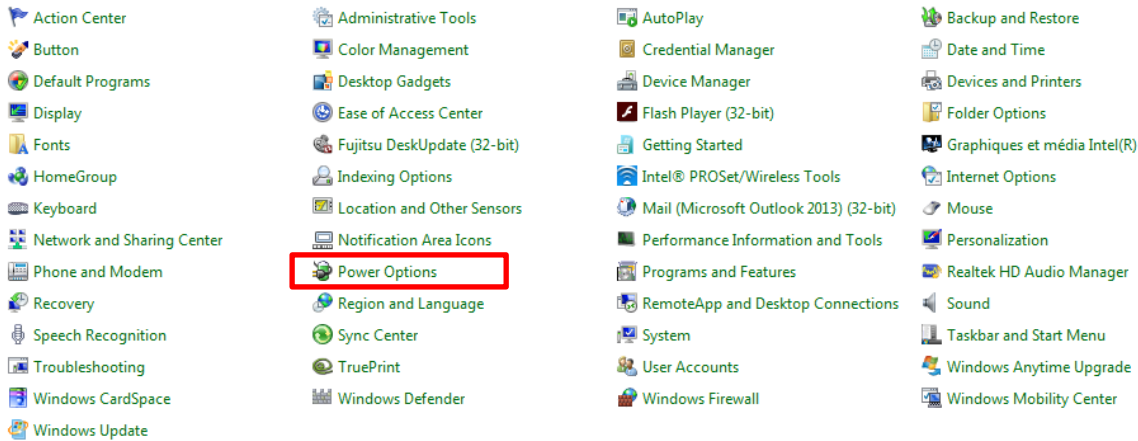
USB Advanced Power options

Please ensure the camera and the system are connected to the USB rear port of your computer. The front port are usually not designed to support device powered by high voltage such as the Newton darkroom and camera.

Windows tries to determine which USB devices are not actively in use and temporarily suspends them. This can cause performance degradation for USB cameras and USB storage devices (which a user may use for storing images).
In the advanced power options, find and set:
• USB settings - USB selective suspend setting Setting: Disabled

To access the power option, open the Windows control panel and select the Power Options

Action Center	Administrative Tools	AutoPlay	Backup and Restore
Button	Color Management	Credential Manager	Date and Time
Default Programs	Desktop Gadgets	Device Manager	Devices and Printers
Display	Ease of Access Center	Flash Player (32-bit)	Folder Options
Fonts	Fujitsu DeskUpdate (32-bit)	Getting Started	Graphiques et média Intel(R)
HomeGroup	Indexing Options	Intel® PROSet/Wireless Tools	Internet Options
Keyboard	Location and Other Sensors	Mail (Microsoft Outlook 2013) (32-bit)	Mouse
Network and Sharing Center	Notification Area Icons	Performance Information and Tools	Personalization
Phone and Modem	Power Options	Programs and Features	Realtek HD Audio Manager
Recovery	Region and Language	RemoteApp and Desktop Connections	Sound
Speech Recognition	Sync Center	System	Taskbar and Start Menu
Troubleshooting	TruePrint	User Accounts	Windows Anytime Upgrade
Windows CardSpace	Windows Defender	Windows Firewall	Windows Mobility Center
Windows Update			

Then select High performance and Change plan settings:

Select a power plan

Power plans can help you maximize your computer's performance or conserve energy. Make a plan active by selecting it, or choose a plan and customize it by changing its power settings. Tell me more about power plans

Plans shown on the battery meter

○ **Balanced (recommended)**   Change plan settings
Automatically balances performance with energy consumption on capable hardware.

◉ **High performance**   Change plan settings
Favors performance, but may use more energy.

Find and set • USB settings - USB selective suspend setting Setting: Disabled:

Change advanced power settings

Restore default settings for this plan

Power Options

Advanced settings   Utilitaire Économiseur d'énergie

Select the power plan that you want to customize, and then choose settings that reflect how you want your computer to manage power.

High performance [Active]

USB settings
  USB selective suspend setting
    On battery: Enabled
    Plugged in: Disabled
Intel(R) Graphics Settings
  Intel(R) Graphics Power Plan
Power buttons and lid
PCI Express
Processor power management
Display
Multimedia settings

Restore plan defaults

OK   Cancel   Apply

USB Hub Advanced Power options

In the Windows Device Manager, each USB hub need to be set not to turn off.
To access the Windows Device Manager, open the Windows control panel and select the Power Options
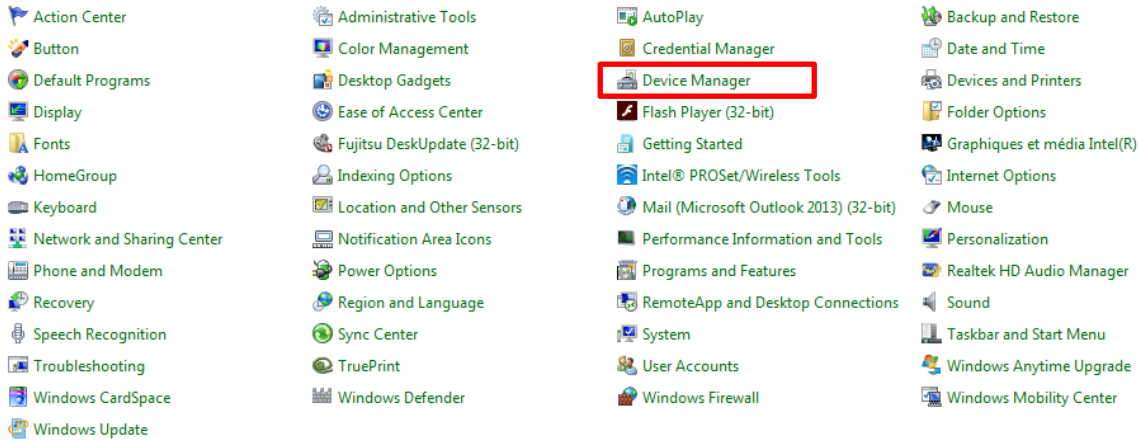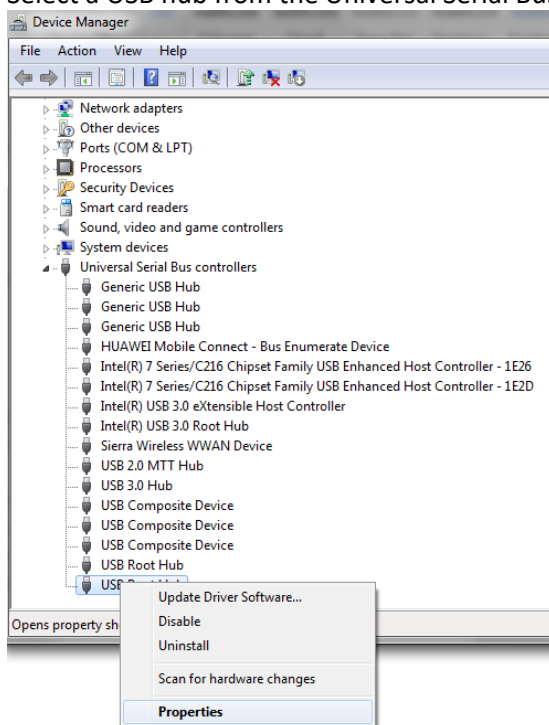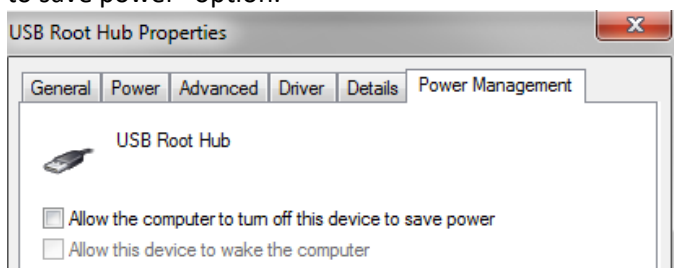
Animal care

Animal's eye protection

Animals' eyes have no blinking or nictitating function under anesthesia, and are prone to drying out across their surface with anesthesia lasting over 15 minutes.

We recommend Optixcare™, a veterinary product specifically for providing eye lubrication for routine protection provided to eyes during anesthetic. This product is an economical and effective tool for use as part of the multi-pronged approach to dealing with "dry eye".

The key ingredient in Optixcare™ Eye Lube is the carbomer gel. Carbomer gel brings a viscosity to the gel which causes it to remain on the cornea for an extended period of time. This viscosity allows for control of the drop applied which makes it easy to apply. Optixcare™ Eye Gel becomes more liquid immediately on contact with the salt in the tears of the eye. Unlike mineral oil or petroleum-based eye ointments, Optixcare™ Eye Lube is formulated with a sterile water base. Because of the water base and the gel viscosity only one drop per eye of Optixcare™ Eye Lube is needed.

Animal depilation

Not required, but sometimes very helpful is an exfoliant. Dependent on fluorochrome emission and signal strength, the animals' hair may interfere with signal acquisition. Shaving and / or a depilatory cream may be used to denude the area to be scanned to mitigate signal loss.

Nair™ Hair Remover – this is known for its depilatory action that works by breaking the disulfide bonds of the keratin molecules in hair. This reduces the tensile strength of the keratin so greatly that the hair can be wiped away. Two of the active ingredients are calcium hydroxide (lime) and sodium hydroxide (lye), which raise the pH of hair, chemically breaking it down. Some formulations also contain potassium thioglycolate, which breaks down the disulphide bonds in the hair's keratin thus weakening it enough to be simply wiped away. Products such as Nair™ often combine softening agents such as mineral oil to help offset the harsh active ingredients.

Isoflurane Anesthesia

Anesthesia protocol: recommended vaporizer & flowmeter Settings

Principles of the anesthetic machine

The anesthetic machine dispenses the gases that are necessary to induce sleep and prevent pain to animals during imaging manipulations.

The basic anesthetic delivery system consists of a source of oxygen (O2), an O2 flowmeter, a precision vaporizer, which produces a vapor from a volatile liquid anesthetic, a patient breathing circuit (tubing, connectors and valves), and a scavenging device that removes any excess anesthetic gases. This is critical, since room pollution with anesthetic gases may lead to health problems in animals as well as in humans.

During delivery of gas anesthesia to the patient, O2 flows through the vaporizer and picks up the anesthetic vapors. The O2-anesthetic mix then flows through the breathing circuit and into the patient's lungs, usually by spontaneous ventilation (respiration).

Oxygen supply may be provided by a central distribution system or by pressurized tanks. The two most common Oxygen tanks are portable "E" tanks, which contain about 660 liters of O2 at 1,900 PSI (pounds per square inch), and large stationary "H" tanks, which hold 6,900 liters of O2 at 2,200 PSI.
Portable "E" tanks are connected to the anesthetic machine by one or two hanger yokes. Proper connection is essential to avoid leaks. The "E" tank assembly on the anesthesia machine contains various pins whose position is peculiar for each gas. This is a safety device to prevent connection of the wrong cylinder to the anesthetic machine. The pins on the machine must match and fit snugly into the holes on the head of the cylinder by use of a single plastic gasket or 0-ring (supplied with each new tank).
Since Oxygen tanks store gas at very high pressure (roughly 2,000 PSI in standard), a pressure-reducing valve or regulator is required to reduce the pressure to about 50 PSI, which is a level that can easily be handled by the flowmeter Further reduction in pressure is achieved by adjusting the flowmeter control knob for safe delivery of gas to the patient. Central pipeline pressure is usually set at about 50 PSI of pressure for delivery to multiple anesthesia machines.

As O2 is consumed with regular use, the pressure within the tanks declines linearly. The regulator adjusts automatically when the pressure inside the cylinder falls as the gas is used. When the pressure reaches 500 PSI, an "E" tank contains approximately 175 L of O2 and should be replaced.

The purpose of gas-scavenging systems is to eliminate waste anesthetic gases (WAGs) from the work area to minimize breathing by personnel. WAGs are eliminated via an active (vacuum driven) activated charcoal canister system. Pollution may occur during chamber induction, maintenance of anesthesia by loose facemask or nose cone, discharge of waste gases from a breathing circuit into the room, and spillage or vapor escape when filling the vaporizer. To reduce pollution when using an induction chamber, scavenge the chamber adequately. When the animal is anesthetized, quickly remove it from the chamber and replace the lid.

The digital oxygen flowmeter uses an adjustable valve to deliver the desired flow in ml or liters per minute to the patient circuit. Flows of around 0.5-2 liters ofO2 per minute are commonly used with rodent anesthesia machines.

The vaporizers produce an accurate gaseous concentration from a volatile liquid anesthetic. The dial or knob on the vaporizer can be adjusted so that the precise percentage of anesthetic gas leaving the vaporizer is known. These precision instruments must be serviced and calibrated every 3 years.

The patient breathing circuit is the highway for anesthetic gas delivery to the patient. The goals of an anesthetic breathing circuit are to:

A. Deliver oxygen to the patient
B. Deliver anesthetic to the patient
C. Remove carbon dioxide that is produced by the patient
D. Provide a method for assisting or controlling ventilation, if needed

The Vilber's Biosthesia circuit uses very high fresh gas flows that deliver the anesthetic gas and washes out the exhaled CO2. This setup contains an exhaust hose to scavenge and evacuate excess anesthetic gases. The basic design of the circuit comprises a narrow bore tubing that delivers fresh gases (oxygen and anesthetic) to the patient; a wide bore tubing that collects exhaled gases, and its continuation into a corrugated tubing that leads to the scavenging activated charcoal canister. The scavenging systems is designed to absorb or eliminate waste anesthetic gases (WAGs) to prevent or minimize room pollution; WAGs are exhausted to the absorbent materials (charcoal filter).

The weight of the charcoal canister must be recorded upon installation and the canister should be replaced after a weight increase of 200 grams; this indicates that its capacity to absorb halogenated anesthetics such as the Isoflurane has been exhausted. Alternatively, the canister should be replaced after twelve hours of anesthesia time at an O2 flow rate of 2 liters per minute. When in use, the charcoal canisters should be placed in a position so as not to occlude the bottom, e.g., on a wire holder or on their side.

When the oxygen is turned on, the gas flow is continuous, as follows:
- O2 Cylinder
- Regulator Flowmeter
- Vaporizer
- Patient Breathing Circuit (Induction Chamber and/or Imaging System)
- Scavenging System

Getting started - inducing and maintaining anesthesia

Anesthesia may be induced in a single animal or in several rodents at the same time by use of an induction chamber.

1. Anesthetizing a single mouse

An individual mouse is anesthetized by placing it in an induction.

1. Verify that there is sufficient O2 in the tank(s) to last until the end of the planned procedure. Replace the "E" tank if the pressure gauge reads < 500 PSI. Centrally supplied O2 should be monitored. Ensure a pressure-reducing valve or regulator is in placed to reduce the pressure to about 50 PSI.

2. Verify that there is sufficient isoflurane in the vaporizer by checking the fluid level through the glass window.

When using an induction chamber:
3. Check all connections and make sure that the inflow to the chamber (from the vaporizer's common outlet) and its outflow (to the scavenging system) are in the open position. Make sure the back of the patient breathing circuit is also connected to the scavenging system.

Note: the connections between the anesthetic machine and the patient breathing circuit are color coded for ease of operation, to avoid potentially dangerous consequences to the animal and to minimize room pollution.

4. Close the imaging system flowmeter.

5. Place the animal inside the chamber and close the lid tightly.

6. Turn on the O2 source and set the fresh gas flow into the chamber at l to 2 liters per minute (LPM) using the induction chamber flowmeter control knob.

7. Turn on the vaporizer and adjust the Isoflurane concentration to 4-5% (lower % for sick or old animals; use 3.4% when in doubt)
8. When the animal is in a moderately deep plane of anesthesia (lying on its side and breathing rhythmically) remove it from the chamber and close the lid. Position the animal on the MousePad, the mouth next to the nose breather.

9. Open the gas flow to the imaging system nose breather.

10. Adjust the imaging system flowmeter to 0.5 LPM and reduce the Isoflurane concentration to 2.5-3.0%

11. Close the Induction box flowmeter.

12. Monitor the depth of anesthesia.
Anesthesia must be monitored from the time of induction to full recovery. An animal is considered to have recovered from anesthesia when it is fully awake and able to rest on its belly. The effectiveness of monitoring depends on the physiologic parameters chosen. Commonly used variables include: respiration rate and pattern, heart rate, color of the footpads, and response to toe-pinch reflex and painful manipulations. Respiratory rate changes from fast during induction to slower and more rhythmic as the depth increases. Heart rate follows a similar pattern. In addition to respiration and heart rate, the operator must monitor other parameters, such as toe-pinch reflex (leg withdrawal) and color of the footpads (pink in most rodents).

13. When finished with the procedure, shut off the Isoflurane vaporizer and maintain the O2 flow to the animal until it wakes up.

14. Shut off O2 flow at the tank if using an E tank; or disconnect from the O2 supply if centrally provided.

15. Return the animal to its cage.
Rodents recovering from anesthesia should be placed by themselves or with other similarly anesthetized animals in a clean cage lined with gauze or paper towels to prevent injuries and accidental inhalation of bedding material (aspiration pneumonia).

16. Check the digital flowmeter to verify that the O2 flow has ceased and turn the flowmeter control knob to the OFF position (fully closed clockwise).

2. Anesthetizing a group of mice

Several mice may be anesthetized as a group in the induction chamber and maintained individually on a nose cone during the desired procedure. One animal at a time may be removed from the induction chamber and connected to the Bain circuit. By use of the dual diverter valve the anesthetic flow may be diverted to either the chamber or the individual animal, or be allowed to flow in both directions simultaneously.

1. Verify that there is sufficient O2 in the tank(s) to last until the end of the planned procedure. Replace the "E" tank if the pressure gauge reads < 500 PSI. Centrally supplied O2 should be monitored. Ensure a pressure-reducing valve or regulator is in placed to reduce the pressure to about 50 PSI.

2. Verify that there is sufficient isoflurane in the vaporizer by checking the fluid level through the glass window.

When using an induction chamber:
3. Check all connections and make sure that the inflow to the chamber (from the vaporizer's common outlet) and its outflow (to the scavenging system) are in the open position. Make sure the back of the patient breathing circuit is also connected to the scavenging system.

Note: the connections between the anesthetic machine and the patient breathing circuit are color coded for ease of operation, to avoid potentially dangerous consequences to the animal and to minimize room pollution.

4. Close the imaging system flowmeter.

5. Place the animals inside the chamber and close the lid tightly.

6. Turn on the O2 source and set the fresh gas flow into the chamber at 2 liters per minute (LPM) using the induction chamber flowmeter control knob.

7. Place the mice inside the chamber and close the lid tightly.

8. Turn on the vaporizer and adjust the isoflurane concentration to 4-5% or lower, if indicated.

9. When the mice are moderately anesthetized remove one mouse from the chamber and immediately close the lid. Position the animal on the MousePad, the mouth next to the nose breather.

10. Open the gas flow to the imaging system nose breather.

11. Adjust the imaging system flowmeter to 0.5 LPM and reduce the Isoflurane concentration to 2.5-3.0%

12. Maintain gas flow at 2.5 LPM from the digital flowmeter to supply both the nose cone and the chamber.

13. Reduce the vaporizer selling to a maintenance level of 2.5 to 3 % isoflurane (or lower).

14. Continue to remove one mouse at a time from the chamber and proceed as described above for the first mouse.

14. When all mices are on the MousePad, close the Induction chamber flowmeter.

15. Monitor the depth of anesthesia.
Anesthesia must be monitored from the time of induction to full recovery. An animal is considered to have recovered from anesthesia when it is fully awake and able to rest on its belly. The effectiveness of monitoring depends on the physiologic parameters chosen. Commonly used variables include: respiration rate and pattern, heart rate, color of the footpads, and response to toe-pinch reflex and painful manipulations. Respiratory rate changes from fast during induction to slower and more rhythmic as the depth increases. Heart rate follows a similar pattern. In addition to respiration and heart rate, the operator must monitor other parameters, such as toe-pinch reflex (leg withdrawal) and color of the footpads (pink in most rodents).

16. When finished with the procedure, shut off the Isoflurane vaporizer and maintain the O2 flow to the animal until it wakes up.

17. Shut off O2 flow at the tank if using an E tank; or disconnect from the O2 supply if centrally provided.

18. Return the animal to its cage.
Rodents recovering from anesthesia should be placed by themselves or with other similarly anesthetized animals in a clean cage lined with gauze or paper towels to prevent injuries and accidental inhalation of bedding material (aspiration pneumonia).

19. Check the digital flowmeter to verify that the O2 flow has ceased and turn the flowmeter control knob to the OFF position (fully closed clockwise).

The main problem associated with chamber induction is the potential for room pollution and personnel exposure to inhalation anesthetics. This occurs when the chamber is opened to introduce or remove animals. For this reason, it is advisable to keep the chamber in a well-ventilated location. When using the nose cone, it is important that it fit snugly over the rodent's muzzle to prevent leakage of anesthetic gases.

Potential problems with the anesthetic circuits
The O2 flow rate should be around 3 times the patient's minute ventilation. In practical terms, O2 flow should be no less than 500 ml (0.5 liters) per minute. When anesthetizing mice in the induction chamber, the O2 flow must be increased to approximately 1.5-2 liters per minute. Insufficient flow rates may allow CO_2 to be re-breathed and cause respiratory acidosis

The circuit deliver high flow of dry cool gas to the patient, which causes significant body heat and humidity loss. Small rodents are therefore prone to becoming hypothermic and dehydrated. It is therefore essential to place rodents on a heating surface such as the MousePad, especially during prolonged procedures. When using a heating blanket or heat lamp, the operator must ensure that the animals are not exposed to excessive heat, which may drop the blood pressure and/or cause skin burns. In addition, anesthetized animals have impaired thermoregulation. A humidifier may be used to add moisture to the gases in the patient breathing circuit.

Minute ventilation is the volume of air that ventilates the lungs in one minute. It is calculated by multiplying the tidal volume (air moved in and out of the lungs with each breath) by the respiratory rate (200-300 breaths/minute in the awake mouse). For example, the tidal volume of a 25-g mouse is approximately .25 ml (1% of its body weight). The minute ventilation for this mouse would be approximately 50 ml (500cc per minute = O2 flow rate). During isoflurane anesthesia, the respiratory rate is markedly decreased.to about 25-50 breaths/min and the depth of each

respiration is increased. Much of the gases that flow into the patient never reach the lung alveoli, i.e., where the actual exchange of O2 and CO2 occur. Much air occupies the space between the nose and the alveoli in the lungs: this is known as "anatomical dead space". Another significant portion of the fresh gases occupy the tubes and adapters between the anesthetic machine and the nose: this is called 'mechanical dead space'. Both the anatomical and the mechanical dead spaces are excluded from the gas exchange process and therefore must be added to the calculation of tidal volume and minute ventilation.

Troubleshooting the anesthetic machine

The most common problem with anesthetic machines is leaks. Leaks commonly occur around tubing connections, flow valves and O2 yokes. It is imperative that any leaks be corrected since they can waste gas and/or expose the operator to high levels of anesthetic vapors.

Excerpts and references

Excerpts from Cornell Institutional Animal Care and Use Committee

- Mouse anesthesia - Induction Chamber Isoflurane
- Place the animal in the induction chamber
- Adjust the oxygen flowmeter to 0.8–1.5 L/min
- Adjust the isoflurane vaporizer to 2%–3%.
- For maintenance, use adjust the flowmeter to 0.4-0.8 L/min.

Isoflurane is a vasodilator, and its use can result in fatal hypotension. Administer isoflurane with caution in animals that may be dehydrated or otherwise at risk for hypotension. Maintaining animals with isoflurane concentrations in excess of the recommended levels may result in death of both healthy and compromised animals.

General considerations
a. Heat loss is rapid in anesthetized rodents. Keep animals warm by wrapping/covering them (e.g., drape or towel), and/or providing a heat source (e.g., heat pad, Grabber® hand warmer) until they have fully recovered from anesthesia. Ensure that all heat sources are thermostatically controlled and/or carefully monitored, so to prevent burns while maintaining thermal support.
b. Apply sterile ophthalmic lubricant (e.g., Puralube) to eyes once animal is anesthetized.
c. Ensure adequate depth of anesthesia prior to performing procedures (e.g., monitor pedal withdrawal and palpebral reflexes).
d. Ensure animal safety: ° Minimally, monitor respiration, and skin/mucous membrane color. ° For long procedures, or procedures that restrict direct observation (e.g., CT scan) utilize a heart rate monitor or respiratory and/or oxygen saturation monitor.
e. Anesthetized rodents must not be placed in contact with loose bedding or similar materials. Recover animals in a bare cage or on top of a paper towel (or similar barrier) to prevent aspiration of bedding.
f. Monitor until fully recovered from anesthesia.
g. Clearly document all procedures (including anesthesia) with specific procedure cards or detailed notes on cage cards, see ACUP 542: Maintaining Clinical Records for Animal Research Models.

Excerpts from Univ. of Pennsylvania

Since Isoflurane is extremely potent and fast acting (due to its high volatility and low tissue solubility with highly calibrated precision vaporizers), it is essential that the animals be closely observed by the researchers while induction of anesthesia occurs, to allow for appropriate removal of the animals following induction to prevent over anesthetization and death.

Begin by attaching the anesthesia supply, and return lines to the induction chamber using the appropriate fittings. Tape is not an appropriate fitting. The scavenger canister should then be attached to the return line. The animal is then placed in the induction chamber and the gas turned on at 2-3% with a flow rate of 0.8-1.0 liter/min. Once the animal is under, the gas supply to the induction chamber should be turned off. The supply and return lines should be moved to the nose cone. The animal should then be removed from the induction chamber and mounted with the nose cone. The gas flow should be restored when the animal is fully mounted with the nose cone. Once under, Isoflurane levels of 1-1.5 % at a flow rate of 0.4-0.8 liter/min is an appropriate level of anesthesia for rats and mice. Larger flow rates may be necessary for larger animals.

NOTE: To avoid waste gas contamination in the environment, always use the oxygen flush valve for approximately 90 seconds to purge the induction chamber with oxygen before opening the lid.

References:
http://www.research.cornell.edu/care/documents/ACUPs/ACUP101.pdf

http://www.uphs.upenn.edu/radiology/research/labs/saif/docs/SOP-2.04_isoflurane.pdf

Helpful Tips

Oxygen flowrates and Isoflurane concentrations in this manual are meant only as guidelines. Operators should determine their own values based on observation.

During long procedures, it is important to continually monitor the animals in the imaging system for proper depth of anesthesia. It may be necessary to reduce the Isoflurane concentration by 0.5 (%) during hours-long procedures.

If preparatory work is required before placing animals into the imaging system, mice will remain anesthetized for approximately one minute once removed from the induction chamber.

FAQ

Animals are waking up:
Be sure that the oxygen regulator is turned to 2 liters per minute (lpm) or higher.

Be sure that the vaporizer dial is turned to the appropriate Isoflurane concentration.

Be sure that the quick connect fittings leading to the breather and chamber are completely mated. The collar on the female fittings must be completely pushed forward for complete mating to occur.

Be sure that none of the hosing is kinked.

If a particular species or strain of animal is consistently waking up on the anesthesia machine, an adjustment may need to be made to the SOP for that species to reflect a higher working concentration of Isoflurane.

Animals are waking up in the induction chamber:
Be sure that the induction chamber flowmeter is open.

If everything is connected properly and oxygen is flowing into the system, try increasing the Isoflurane concentration to 4-5% for several seconds until the animals appear to be anesthetized, then reduce concentration to the species appropriate level.

Animals are waking up on the breathing device:
Be sure that the imaging system flowmeter is open.

The animal may simply need a higher concentration of Isoflurane, try increasing the concentration on the vaporizer by 0.5%.

If the animal was not properly anesthetized in the chamber prior to moving to the breathing device, or if the animal was out of the chamber for too long prior to moving to the breathing device, the animal may wake up. If this occurs place the animal back into the chamber and restart the anesthesia process from step one. Be sure that the animal is completely anesthetized before removing from the chamber.

Animals are too deeply anesthetized:
Usually in this case the Isoflurane concentration on the vaporizer is too high. If an animal is observed to be in danger of dying, reduce Isoflurane concentration to zero and let pure oxygen flow until the animal returns to a normal state of anesthesia, then turn the Isoflurane concentration to a lower value than previously used. Breathing should be even and not appear labored and limbs should appear pink, not blue.

If an animal is anesthetized on the breathing device and oxygen supply is suddenly cut off or drastically reduced, the animal may suffer from a lack of oxygen. Be sure that your oxygen tank contains adequate oxygen for the length of your procedure and that the gas supply to the breather is not obstructed in any way.

During hours-long procedures anesthetic can build up in the animal and it may be necessary to reduce Isoflurane concentration over time.

During long procedures, animals may become hypothermic. Be sure that the MousePad warming plate is on and set to the appropriate temperature.

If a particular species or strain of animal is consistently dying on the anesthesia machine, an adjustment may need to be made to the SOP for that species to reflect a lower working concentration of Isoflurane.

The vaporizer inlet/outlet cap popped off during operation:
The inlet/outlet caps connecting to the vaporizer are designed to pop off when pressure builds up in the system. This occurs when gas entering the system has insufficient outlet.

At least one of the flowmeter must be open at all times while the system is in use.

Be sure that the quick connect fittings leading to the breather and chamber are completely mated. The collar on the female fittings must be completely pushed forward for complete mating to occur.

Be sure that none of the hosing is kinked.

I can smell Isoflurane outside of the Biosthesia:
Be sure the EVAC 2 is on.

The charcoal filters may be saturated with Isoflurane. Weigh the filters and discard any that have increased in weight by 200 grams. Replace any filters that have been used but were never weighed.

Be sure that the induction chamber flowmeter is closed prior to removing the animals from the chamber.

I can smell Isoflurane inside the imaging system:
Be sure the EVAC 2 is on.

Be sure that any nosecones not in use are plugged with the included black threaded nosecone plugs.

Be sure that all of the hosing to and from the breather is connected and that the exhaust hose coming from the side of the imaging system is connected.

You may have the hoses connecting the breather reversed, if this occurs, gas will exhaust directly into the imaging system. Refer to the installation instructions on how to properly connect these items. If the labels on the breathing device are missing, the breather inlet is the hose connected to the white silicone duckbill valves inside the breather port. The breather exhaust is the hose connected to the black holes inside the breather port.

If all of the instructions have been followed and problems are still arising, the vaporizer or oxygen regulator may be out of calibration. Please contact Vilber Lourmat for instructions on how to have these items serviced at a factory authorized service center.

How often should I have my vaporizer serviced?
Vaporizers should be on a regular service schedule that reflects usage and the individual institution's requirements on maintaining the vaporizer. We recommend having the vaporizer serviced every 2 years.

Can I maintain animals in the chamber while animals are on the breathing device?
No. Only one component, the chamber or the breather may be used at a time, not both.

How often should I replace the charcoal filters:
After a weight increase of 200 grams

Care and Cleaning

Vaporizer

Every two weeks, the vaporizer should be drained into appropriately marked containers and disposed of properly. Less frequent intervals may be used when the anesthetic agent does not contain additives or a stabilizing agent.
WARNING: Do not drain the vaporizer into a container that is not properly marked.
Please refer to the Draining procedure described in this manual.

The vaporizer must be fully serviced and calibrated at least every three years. Servicing must be done by an authorized service technician. Due to the permanent usage, it is important to maintain regular servicing of the vaporizer to avoid malfunctioning. Deposits can affect seriously the function and concentration delivery. Vaporizers must be disassembled for servicing. This should be done only by a certified service center with proper test equipment. When assembling, it is necessary to replace some spare parts to secure longevity of vaporizers. Finally, the Vaporizer goes through a strictly defined test program with concentration examinations under different temperature and flow conditions and thermostat must, if necessary, re-calibrated.

WARNING: DO NOT MODIFY, TAMPER WITH OR DISASSEMBLE THE VAPORIZER. THIS UNIT MUST ONLY BE SERVICED BY AUTHORIZED PERSONNEL.

Digital Flowmeter

The device does not contain any repairable elements. The device is calibrated during production. Open the device only to the extent required to replace the battery. Any attempt to further dismantle the device will affect its accuracy.

Replacing the Battery

If the display shows «low batt» or if is blank, it is necessary to replace the battery.

Remove 4 screws (Torx screwdriver, size TX 8), then push back cover carefully backwards. Open back cover and remove battery. If the battery contacts are fouled or corroded, clean with a dry paper towel. Replace the battery with a Lithium, AA, 3.6 V one. Insert new battery observing the + and – symbols in the battery compartment. The device is activated automatically.

Installation is the reverse of the disassembly procedure.

Finally perform a function check.

Disposal: Ensure that the battery is properly disposed. Observe the disposal regulations applicable in your country!

Please refer to the Drager user manual for complete information and procedure in regards of the digital flowmeter.

Cleaning

Disinfect the induction box, the operation table, etc. with 70% Ethanol and rinse with water.

Long Inactivity

The instrument does not require any particular maintenance after long inactivity, except cleaning.

Before putting your system in operation again, first check all connections and parts to make sure there is no leakage.

Software operation

Introduction

This chapter describes how to operate the Newton imaging system.

Before to operate the Newton system, it is important that you have read and understood the safety information. Make sure to read this User Guide relevant safety instructions before you start to operate the system.

⚠	**WARNING** Do not operate Newton system in any other way than described in the User Manual.

The Newton instrument is a high-end ultra-sensitive scientific camera platform, designed to extract the lowest level of detection from your sample. Our superior sensitivity, resolution and dynamics provide optimal performance across a large array of applications. The Newton system is ready to work for your most demanding bioluminescence, multiplexing or fluorescence applications, according to your system configuration.

The standard imaging process is as follows:

Start the Newton system & software

Position your sample

Capture an image

Visualize your image

Save and print

Analyse or post-process
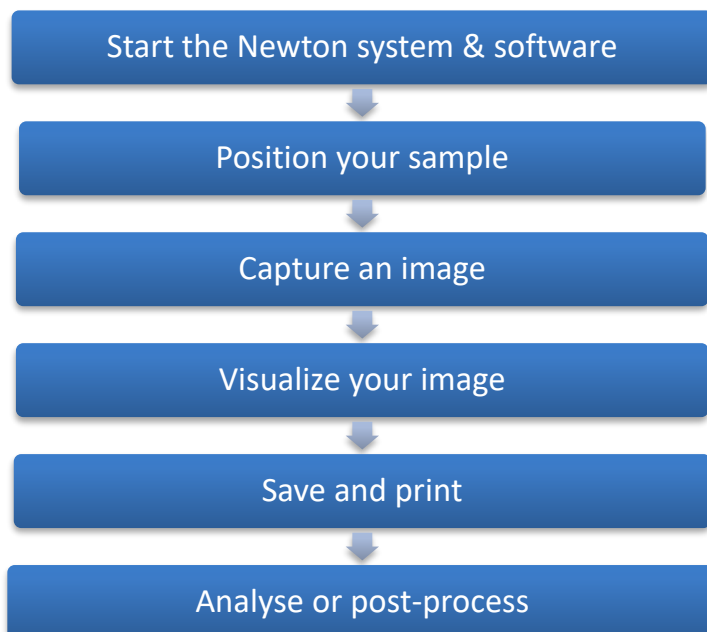
Start the Newton system & software

Switch on the Newton system
On the right hand side of the imager, press the power button to the position I, to turn it on.

Launch the software
Power on the computer. After the computer has booted up completely, switch on the Newton system. Wait for 10 seconds and double click on the Newton icon: ▪. The software opens on the image acquisition window.

The following messages are displayed until the Newton system is ready for image acquisition:
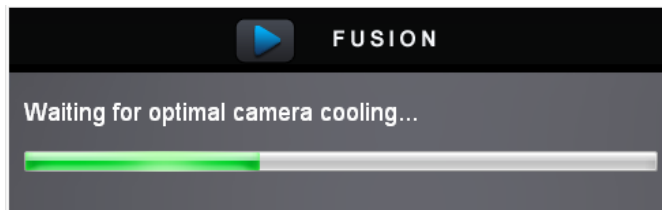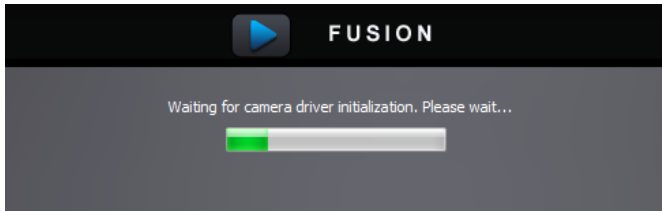
NOTICE

The Notification window provides information on the system settings and status.
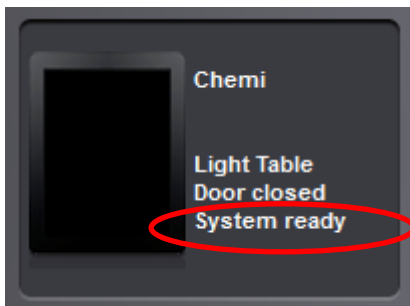
The message "System ready" indicates the camera has reached the appropriate temperature for image acquisition.

Position your sample

Select a tray corresponding to the sample to be detected.
Each application must be used with a specific MousePad. The Newton imaging system supports the following sample pad, according to your system configurations:
- Heated MousePad
- Hepa filtered animal isolation chamber MousePad

Open the door of the Newton system.
Position the sample on the appropriate position, on top of the MousePad.
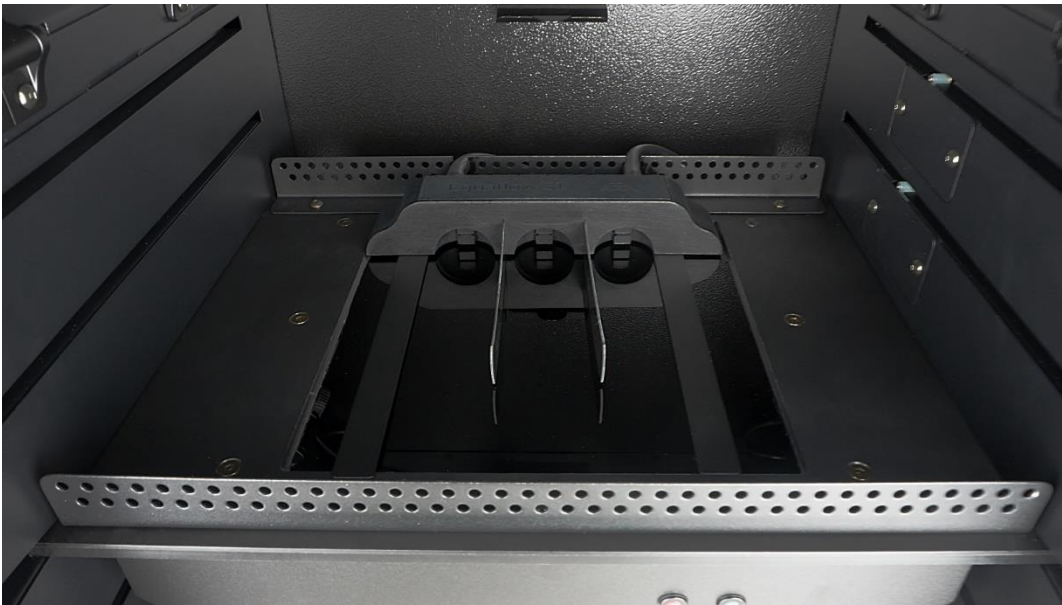
For the Newton FT500 and BT500 systems, the fields of view at each preset position are as followed
- Position 1: 200x200mm (5 mice)
- Position 2: 180x180
- Position 3: 160x160 (4 mice)
- Position 4: 140x140
- Position 5: 120x120
- Position 6: 100x100
- Position 7: 100x100
- Position 8: 80x80
- Position 9: 60x60 (macro)

For the Newton FT100 and BT100 systems, the fields of view at each preset position are as followed followed
- Position 1: 120x120

You can pull out the MousePad drawer for easier access to the positioning surface.
Push the tMousePad drawer to the maximum and close the main door.

Capture an image

This chapter describes the steps to acquire an image. It is organized around the following workflows:
• Methods for bioluminescence image acquisition
• Methods for fluorescence image acquisition

Bioluminescence image acquisition

The bioluminescence image capturing method is available in all Newton systems configurations.
Bioluminescence imaging is based on the endogenous production of light by the expression of the enzyme luciferase. This enzyme, found in fireflies and other bioluminescent organisms, produces light upon reacting with the substrate luciferin in the presence of oxygen and ATP. Like GFP, luciferase has been used extensively in vitro to study cellular processes. In small animal imaging, luciferase have both been employed to study transgene expression, tumor growth/treatment/metastasis, and infectious disease processes
Since bioluminescent imaging does not require an excitation light source, there is extremely low background, which is often caused by autofluorescence. Hence, bioluminescent imaging enables the imaging of processes that produce minimal signal, such as in the case of lymphocyte trafficking in vivo or the detection of a small number of cancer cells in vivo.

Fluorescence image acquisition

Fluorescence is a major method used for gene expression and protein detection. It results from a process that occurs in molecules known as fluorophores. Typical of fluorescent imaging, acquisition of fluorophore signal relies on excitation by an external light source. The fluorophore absorbs the excitation light, reaching a higher energy state. By returning to its former state, it emits fluorescent light.
The Newton system separates the emitted light from the excitation light in order to obtain an optimum sample image. According to the configuration, the Newton can accommodate up to 8 excitation and emission channels in the IR, NIR, visible area and is ideal for a large array of applications.

Image capture workflow

The basic steps to acquire an image are:

Choose the application type

Define the exposure mode

Acquire the image pressing the Start button
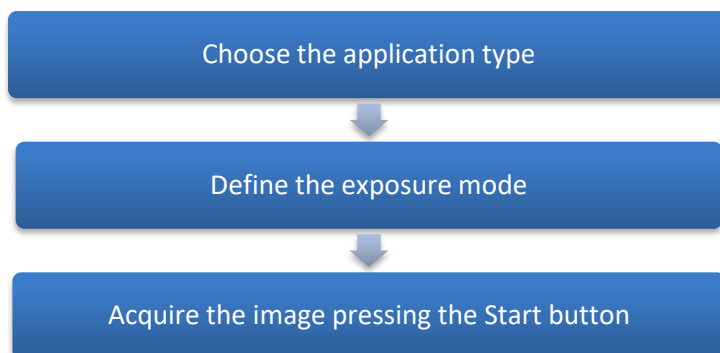
Choose the application type

The Application selector
The Newton runs with pre-defined image acquisition parameters to facilitate the use of the system for a specific application. These pre-defined parameters are gathered in the Newton Application Protocol and can be selected from the Application Selector:
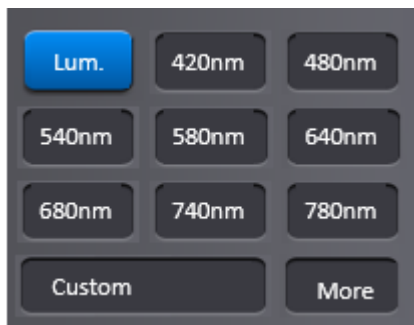
The Newton Application protocol will ease the imaging workflow by automatizing the exposure parameters adjustment. The Application protocol automates a task or a set of tasks that you perform repeatedly or on a regular basis. It stores the following information:
• The image exposure pre-defined set-up;
• The live preview pre-defined parameters;
• The image display pre-defined set-up;
• The image printing pre-defined set-up;
• The image file pre-defined set-up.

The benefits of the Application protocol are as follows:
• Time saving
• Reproduction of image acquisition parameters
• Protocols are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

The factory settings include several pre-defined Application protocols according to the configuration of your system. You can also create your own Application protocol. Please refer to the Application protocol chapter of this User Guide to have more details on the way you can create your own Application.

For a bioluminescence sample, select the Luminescence application from the Application Selector window:
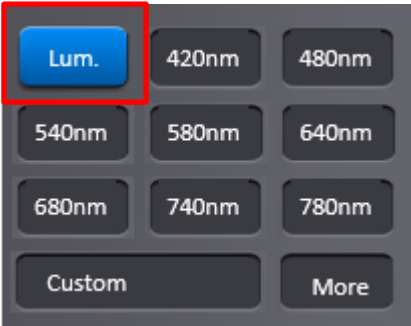
Define the exposure mode

The Newton system has three exposure mode:
• The Auto-exposure mode
• The Manual mode
• The Serial mode

The exposure modes are gathered in the exposure mode menu:
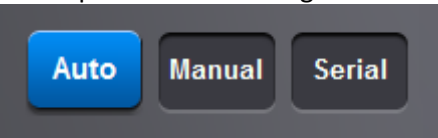
The Auto mode

The Newton system calculates automatically the optimum exposure time (Auto-exposure). When you select the Auto-Exposure option, the system samples the light levels and uses the values to calculate the final exposure time. To set the auto-exposure mode, select Auto from the exposure mode menu:
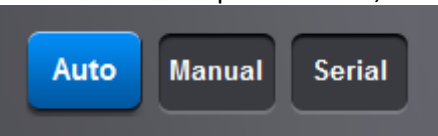
The Manual mode

The Newton system requires the user to specify the exposure time.
To set the exposure time, select Manual from the exposure mode menu:
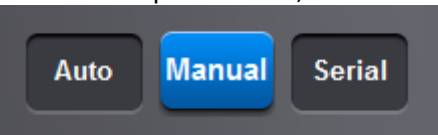
For Bioluminescence application, the selection of the manual mode will open the following menu:
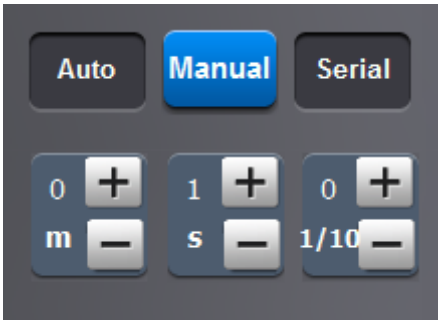
For Fluorescence or Visible applications, the selection of the manual mode will open the following menu:
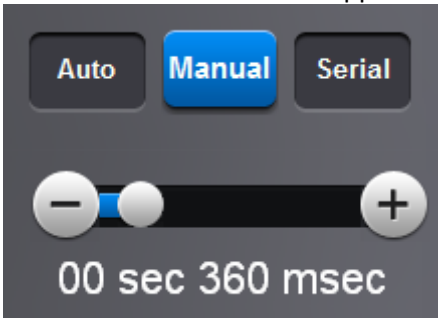
Enter the duration of the exposure.
Define your exposure time using the scroll bar for the fluorescence mode:

Note: The integration time increase or decrease by 40 milli-seconds.

Note: With long integration time, a delay could be necessary before an image is displayed on the monitor (up to twice the selected Exposure time).

Define your exposure time using the table for the bioluminescence mode:
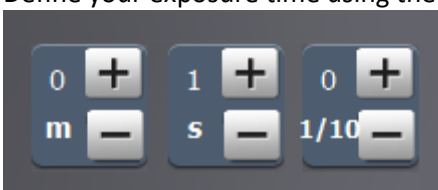
Note: When the specified exposure time is reached, the last captured image is displayed. The camera continues to integrate the image on the CCD sensor, updating the display whenever the specified Exposure time is reached. The Stop exposure button stops the exposure process. The last full exposure is displayed.

The Serial mode
The Newton system captures a series of image with a range of exposure times to be configured by the user.
The Serial exposure mode allows repetitive image acquisition with or without image accumulation. You can then select the image you prefer from the series of images.
To define a series of image capture, select Serial from the exposure mode menu:
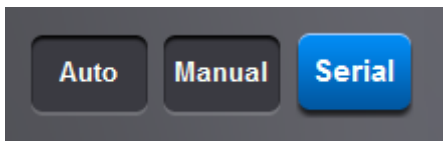
Acquire the image

Select Start to process the image acquisition.

The Auto mode

After pressing the Start button in the Auto mode, the system will propose the optimum exposure time based on a test image previously taken automatically.
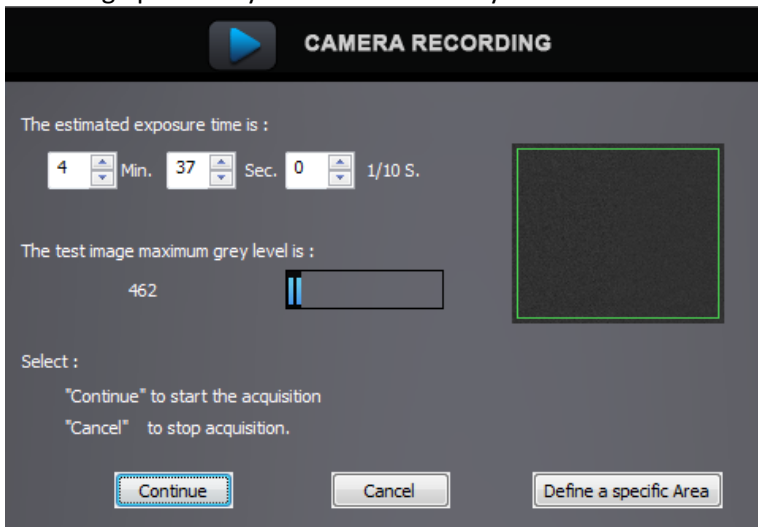
Select Continue to process with the indicated exposure time.
Define an area on the test image to calculate the autoexposure time from a part of your sample.
Or modify the estimated exposure time and select Continue to process.

Note: The auto-exposure is controlled by a set of parameters described in the Application protocol chapters of this manual.

Note: The maximum grey level of the test image provide an indication of the sensitivity obtained in the final image. With a low grey level (80 to 220), the output image is expected with a low sensitivity. With a higher grey level, the image is expected to have a better sensitivity.

Note: A progress bar shows the progress of the image acquisition.

Note: With long integration time, a delay could be necessary before an image is displayed on the monitor. The acquisition will stop automatically at the end of the exposure time.

Note: When the specified exposure time is reached, the sample image is displayed.

Note: You can define a specific area from the test image for the calculation of the autoexposure time. To proceed, click on Define a specific area. A predefined green window will appear on the test image:
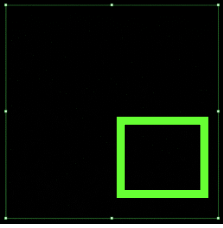
Define your area of interest. Then, click on the Continue button:
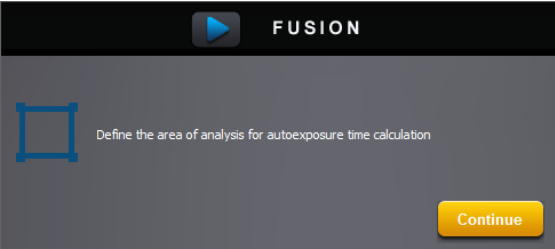
The Manual mode

In a bioluminescence mode, once the image is acquired, it is automatically displayed on the screen.

In a Fluorescence application protocol with an exposure time inferior to 4 seconds, the image is displayed when the specified exposure time is reached. The camera continues to integrate the image on the CCD sensor, updating the display whenever the specified Exposure time is reached. The Stop exposure button stops the exposure process. The last full exposure is displayed.

Note: In both modes a progress bar shows the progress of the image acquisition.

Note: For the fluorescence mode, the software has two exposure time scales:
- One for short times: 40 milli-second to 4 seconds
- One for long times: 4 sec to 1 minute
The exposure time scale is defined in the Application protocol.

Note: With the short integration time scale, the integration time increases or decreases by 40milli seconds.

Note: With long integration time, a delay could be necessary before an image is displayed on the monitor (up to twice the selected Exposure time).

The Serial mode

The Serial exposure mode allows you to acquire a user defined number of images (frame). This set of frame defines a video sequence. Each image has an exposure time. The software will wait a user-defined time elapse prior to acquire the subsequent image.

The number of images defines the number of frames of the Serial exposure sequence.

In the Serial mode, all the images are saved in a specify directory. After clicking on the Start button, a pop-up window displays the following menu:
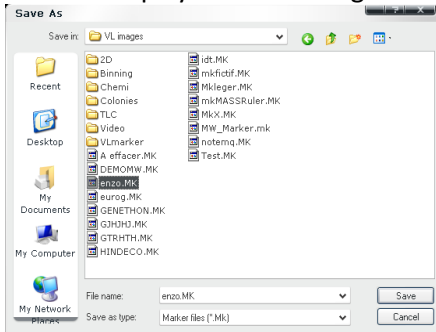
Browse to specify the directory. Type the file name and click on Save.

Note: After acquiring image N°1 the following files are created
IM0000xx_2.tif: native image acquired
IM0000xx_2Sum.tif: sum of pixel value of images xx_1 and xx_2

After acquiring image N°3
IM0000xx_3.tif : native image acquired
IM0000xx_3Sum : Sum of Im0000xx_2Sum and image Im0000xx_3

The Newton asks you then to define the serial image acquisition parameters:
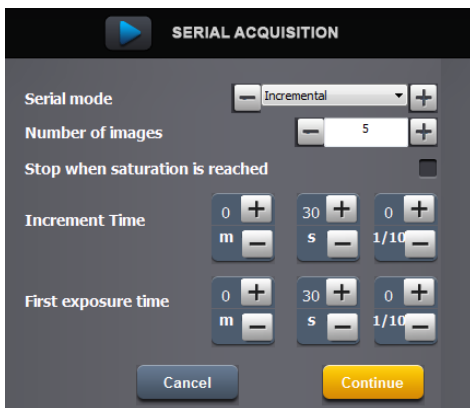
The serial exposure has 4 different modes:
• Incremental
• Accumulation
• Repetitive
• Programmed

• **The incremental mode**
In the incremental mode, you define the exposure time of the first image of the series, then the time to be added to this increment for each subsequent image:

Image 1	20s	20second is the first exposure time

Image 2	20s Image B: 30s + = Image 2 The increment time is 10s. Image B has the exposure time of the previous image (20s) plus the increment time (10s). Image 2 is the result of the addition of image 1 plus Image B.
Image 3	Image 2 + Image C=40s = Image 3 The increment time is 10s. Image C has the exposure time of the previous image (30s) plus the increment time (10s). Image 3 is the result of the addition of image 2 plus Image C.
n image	The number of images of the serie is defined by the Number of images parameter.

Note: the series could stop automatically before the Number of images parameter is reached if the Stop when the saturation is reached is selected. If this option is on, the serial process is stopped automatically when the saturation pixel is reached on the obtained accumulated image.
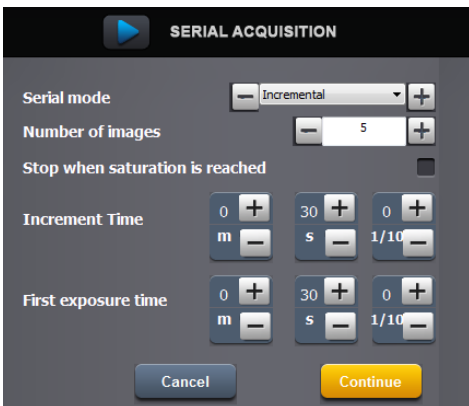
The number of images decides on the total number of frames to be taken within the serial mode.
The first exposure time defines the duration of the exposure for the first image of the process.
The increment time is the time to be added to the exposure time of the previous image.
Select stop when the saturation is reached if you wish to stop the process when the saturation level is obtained on a particular frame.

• The Accumulation mode
In the accumulation mode, you define a same exposure time for each image of the series. Then from the second image acquired, each new image is added to the previous one.
Example:

Image 1	20s 20second is the Time per image
Image 2	20s + Image B : 20s = Image 2

	Image B has the exposure time of the previous image (20s) Image 2 is the result of the addition of image 1 plus Image B.
Image 3	 Image C has the exposure time of the previous image (20s). Image 3 is the result of the addition of image 2 plus Image C.
n image	…The number of images of the serie is defined by the Number of images parameter.

The number of images decides on the total number of frames to be taken within the serial mode.
The time per image defines the duration of the exposure for all images of the process.
The time between images define a time elapse between the images. The software will wait for the defined time elapse prior to acquire the subsequent image.
Select stop when the saturation is reached if you wish to stop the process when the saturation level is obtained on a particular frame.

• The Repetitive mode
In the accumulation mode, you define a same exposure time for each image of the series.
Example:

Image 1	20second is the Time per image
Image 2	
Image 3	
n Image	… The number of images of the serie is defined by the Number of images parameter.
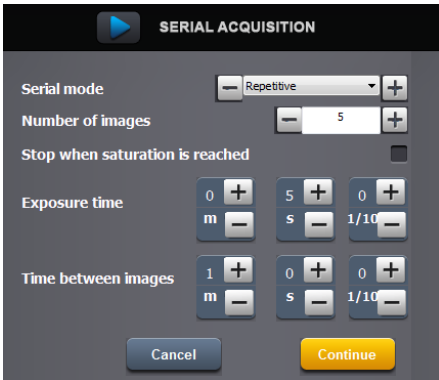
The number of images decides on the total number of frames to be taken within the serial mode.

The time per image defines the duration of the exposure for all images of the process.

The time between images define a time elapse between the images. The software will wait for the defined time elapse prior to acquire the subsequent image.

Select stop when the saturation is reached if you wish to stop the process when the saturation level is obtained on a particular frame.

• The Programmed mode
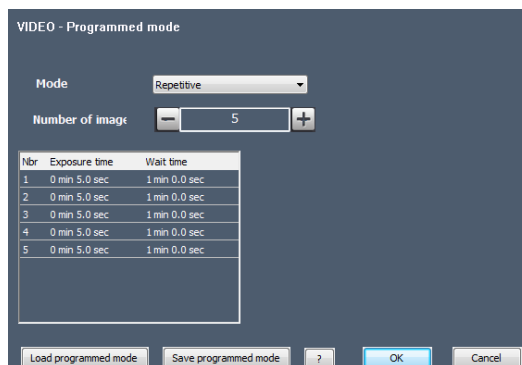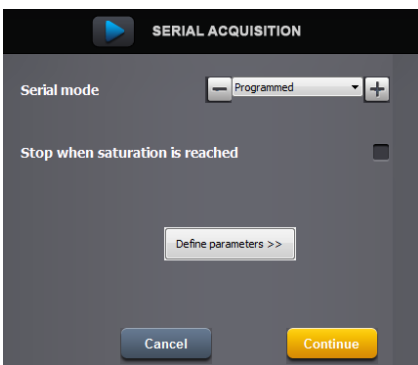
The programmed mode is the same as the repetitive or the accumulation mode in which you can define a specific exposure time for every single image of the serial process.

• Catalogue function

You can open a previously saved image series using the catalogue function. To proceed, click on Catalogue. This will open the Catalogue menu:
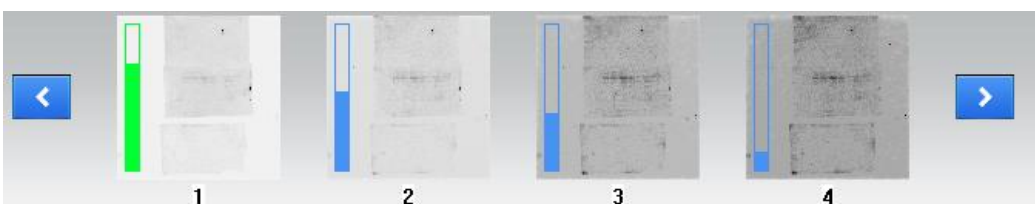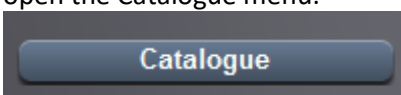
Note: The blue graph on the top of the image thumbnail indicates the image dynamic

Note: The green graph on the top of the image thumbnail indicates the best image from the image series from the dynamic point of view

You can either display the summed images or the native images.

• **Bioluminescence kinetic signal**

The Western blot bioluminescence process involved an enzyme-conjugated antibody to cleave a chemiluminescent substrate. Chemiluminescent substrates emit light due to an enzymatic reaction. As a reporter signal for the chemical reaction, light allows for the visualization and depiction of proteins in membrane-based immunoassays such as Western blots and dot blots. Light is emitted during the reaction when a molecule, excited by the enzymatic reaction to a higher energy level, returns to the ground state. The enzyme/substrate kinetics can be measured thanks to the system Kinetics function from the Serial catalog menu:
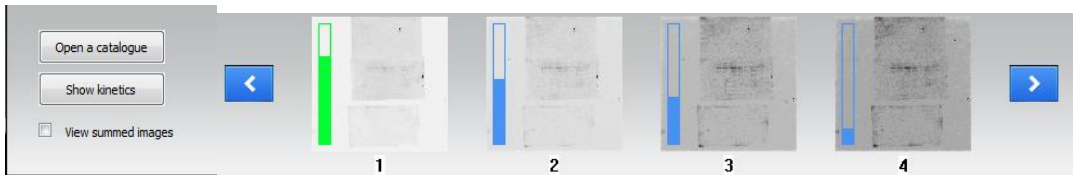
In order to study your protocol signal kinetics, we suggest to set a serial process, repetitive mode for approximately 10 images. The Kinetics graph calculates the signal intensity for each images and sort the images according to the time spend from the beginning of the serial process.
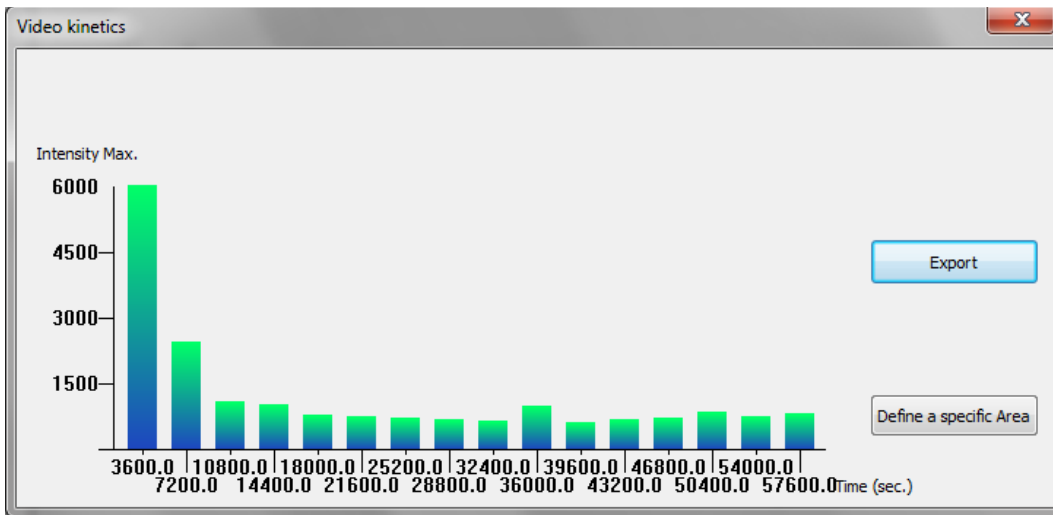
Note: The Kinetics graph could only be obtained from non-summed images. We recommend to set the same time for each image and to select the repetitive mode.

Additional exposure parameter

• **Preview**

You can also preview your sample using the preview function (live mode):

Preview mode allows direct visualization of the image. This mode enables you to adjust the aperture and the focus, and to position your sample.

Note: A live image means the image displayed is refreshed every 1/20th of a second. This short exposure time (or frame) is adequate for a variety of white light samples including protein gels and autoradiography. A live image, however, is not sufficient for most samples, which are visualized and photographed over a relatively dim light source. A feature called integration compensates the low light situation by allowing the CCD camera to obtain a timed exposure.

Note: After 2 minutes, the software will automatically stop the live preview.

Note: The Preview mode is designed to help for sample positioning and focusing. To this extend, the Preview mode is always with white light EPI illumination.

The stop function captures the last image. To proceed, click on the stop preview button.

• **Adjust**
The Adjust button gives you access to the main exposure parameters. These parameters could be changed independently from the Application protocol.
To proceed, click on the Adjust button.

This will open the set-up menu window:
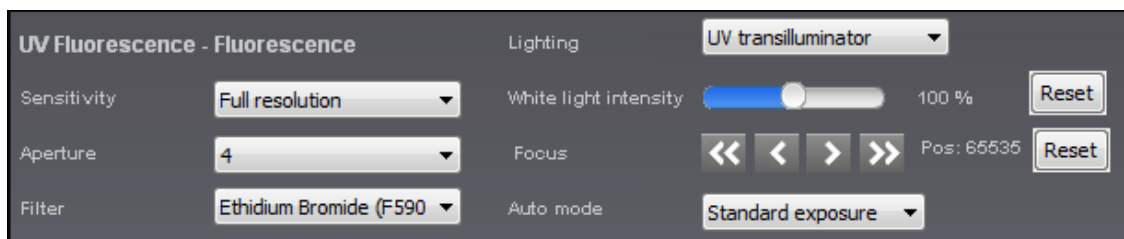
Note: An asterisk is displayed after the application protocol name if the set-up is modified

• White Light adjustment
In the Live preview mode or in the White Light Epi mode, you can adjust the light intensity by clicking on the white light intensity cursor:

You can reset the white light intensity to its original 100% position by clicking on Reset.

• Focus adjustment (Newton system with motorized lens only)
You might need to adjust the focus to adjust the sharpness of the image. Turning the ring clockwise or counter clockwise changes the focal point of the lens. To proceed, click on the << or >> button to access correct the Focus adjustment. Each time you press on one of those buttons the zoom moves of one step the focus settings. It is not

necessary to keep the button pressed, press as many times as necessary to get a fine focus adjustment. You can then save the position in your Application profile if necessary.

• Newton SL6 Xpress zoom lens control
The SL6 Xpress Adjust button gives you access to the zoom lens parameters. These parameters could be changed independently from the Application protocol.
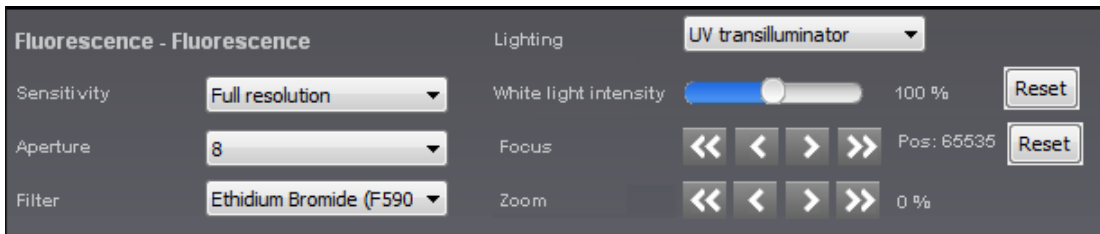
You might need to modify the focus to adjust the sharpness of the image or the zoom to zoom in or out on the sample:
- Zoom. Zoom allows you to change the size of your sample, on the image. To proceed, click on the << or >>. The zoom automatically moves to the new value. The zoom control will not affect the focus.
- Focus. Focus is needed to adjust the sharpness of the image. Turning the ring clockwise or counter clockwise changes the focal point of the lens. To proceed, click on the << or the >> button to access the Focus adjustment. Each time you press on one of those buttons the zoom moves of one step the focus settings. It is not necessary to keep the button pressed, press as many times as necessary to get a fine focus adjustment.

Note: "<<" and ">>" are for fast adjustment and "<" and ">" are for step-by-step adjustment.

Note: You can save the focus or zoom positions in a specific application protocol by clicking on the Save as button of the Set-up menu.

To close the Adjust menu, click on the orange Adjust button:

Visualize your image

The Image Dynamic

The Image Dynamic informs you of the obtained image amplitude compared to the potential image depth. The largest the dynamic you have without reaching saturation, the better is your image as you obtained more quantitative data.

Access the Image Dynamic from the main software window:

The image dynamic refers to the range of grey levels in between the minimum and the maximum pixel intensities obtained in an image. Image depth is expressed as gradation level. In an image, the density range between white and black is divided into a number of gradation levels. For instance:

A 16-bit image has 65536 gradation levels.
A 12-bit image has 4096 gradation levels.
An 8-bit image has 256 gradation levels.

Case 1:	Case 2:	Case3:
The Image Dynamic indicates a fairly full dynamic. This image has a maximum of quantitative data compared to the capability of the camera. This image is highly recommended.	The Image Dynamic indicates a very poor dynamic. This image has only a portion of the quantitative data that it could have obtained. You can extend the dynamic by increasing the exposure time.	The Image Dynamic indicates that this image contains saturated portion which could not be suitable for quantification.

Note: by moving the mouse over your image, the pixel value and the coordinates of each pixel is displayed in the Image Dynamic window.
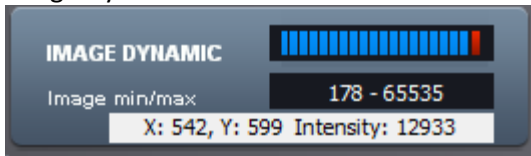
Note: by clicking on More from the Display menu, you could have access to the image histogram:
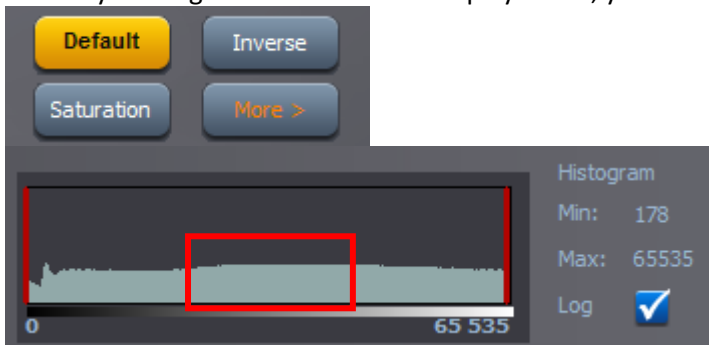
A histogram is a graphical representation of the number of pixels at each brightness level. It is a point of reference for examining the distribution of brightness levels in an image. The readings in the histogram window can be converted to log scale using the checkbox.

The Image Display

Access the Image Display from the main software window:
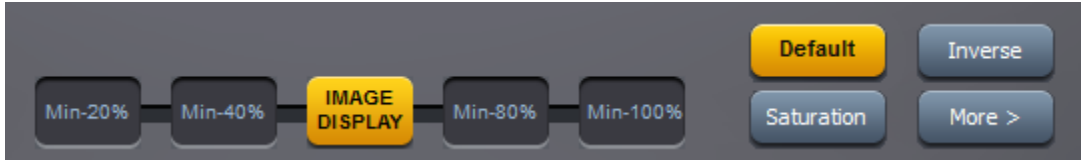
The optimum display window is helpful to enhance the image display by modifying the image greyscale selection to be displayed.

In standard, the Newton images have 16-bit format and Windows® can only display 8-bit images (256 grey levels). Due to this limitation, the software handles two images:
A "memory" image corresponding to the 16-bit format (65 536 grey levels)
A "displayed image" corresponding to the image displayed on the screen (256 grey levels)

The easiest way to calculate the "display image" would be to translate the full grey scale each time an image is acquired: the x grey levels values of the "memory" image corresponds to 256 values in the displayed image. In that case, it won't be possible to visualize faint spots on a dark image.
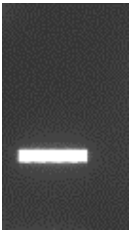
The software offers the possibility to select the grey level range to translate for the display image calculation. All the grey levels under the "Min value" defined will be converted to 0 (Black) in the displayed image. All the grey levels upper the "Max Value" defined will be set to 255 (White) in the displayed image. The grey levels between those two limits will be converted in an intermediate grey level value following a linear rule.

You can select one from the 5 image display presets:

The image displays presets goes from the minimum image grey levels to 20%, 40%, 60% or 100% of the image depth.

Note: The optimum display has no impact on the analysis. Only the display of the image is modified.

Case 1:	Case 2:
Default optimum display	Optimum display enhancement. The image appears brighter. The faint bands are more visible.

Click on "Default" to calculate the ideal pixels values to be displayed according to the defined parameters in the application protocol.

Default

Note: Click on Default to reset the display to the default display settings:

Note: the Display button shows an asterisk as below when the default image display settings is changed:

Default *

Click on "Inverse the image" to inverse the grey level of the image. This makes a negative image.

Inverse

Case 1:	Case 2:
Positive image (white spot, black background)	Inverted image - Negative image (black spot, light background)
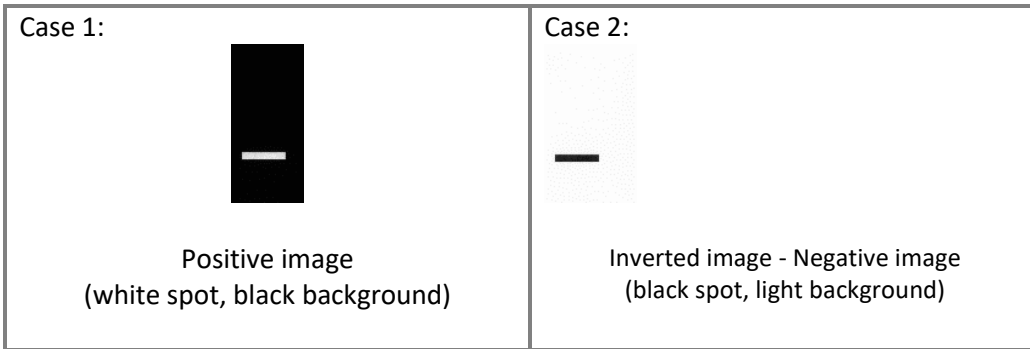
Click on "Saturation" to displays the saturated pixels in red.

Saturation

A saturated image is inappropriate for image quantification with image analysis software. The saturation option allows you to visualize in red, pixels that have the maximum grey level in order to avoid flattened peaks.
The maximum grey level depends of the bit-depth. For instance:
A 16-bit image has a maximum grey level of 65535
A 8-bit image has a maximum grey level of 255.

Note: If an image is being acquired and the «Saturation» option is checked, the modification is applied to the current acquired image

Note: A saturated image creates quantification error when studied by an image analysis software. Decrease the exposure time or increase the f aperture value to avoid saturation.

Case 1:	Case 2:
Original display	Same image with the saturated pixels indicated in red
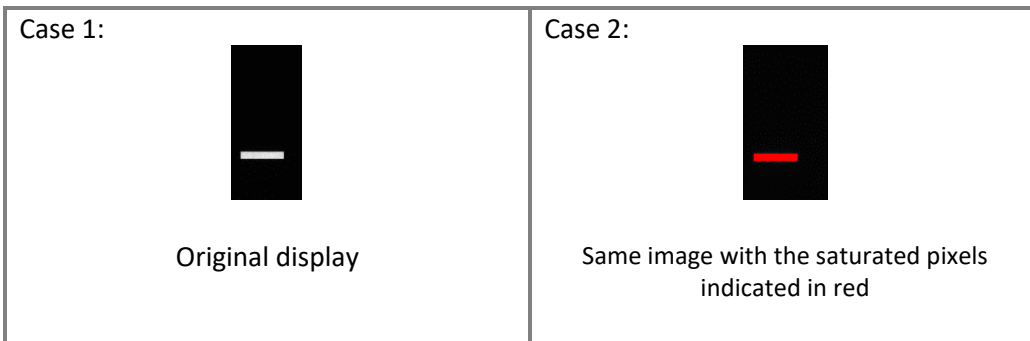
By clicking on More from the Display menu, you could enter manually the lower and the upper display range values.

More >

Min grey level:	Max grey level:	Gamma:
512	65408	1.00

GFP   Coomassie   Multicolor   Red
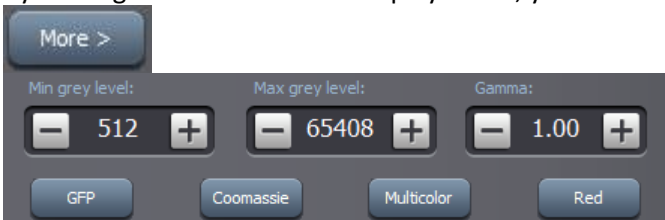
You can then edit manually the lower and the upper display range values in the corresponding edit field:

Min grey level:	Max grey level:
512	65408
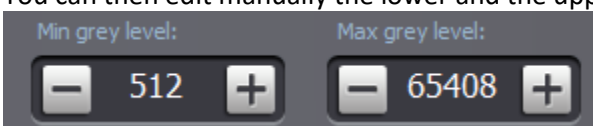
• Gamma

Gamma adjustment corrects an image display by creating a new version of the original. To create the new displays range, the Gamma Adjust function reassigns the grey values of each pixel in the image according to the curve in the following graph:

The above graph demonstrates the basic principles of gamma adjustment:
- Black (pixel value = 0) remains black at all gamma values.
- White (pixel value = full scale) remains white at all gamma values.
- Gamma values greater than one lift the darker areas of the original image into the brighter areas of the new image.

A gamma curve is smooth: there are no unexpected jumps or cut-offs. This means that when viewing a gamma adjusted image, you will be able to see the details (intensity differences) in both the black and white areas of the image.

When the bright areas of these types of images are correctly exposed, the darker areas can be so dark that they are in effect invisible. Gamma Adjust can remedy this problem. The gamma adjustment results in a better display of detail by lightening the darker areas without burning out bright areas or lightening black areas:

• Pseudo colors

The pseudo colors can display different types or levels of fluorescence in an image. It replaces the original grey levels of the image by another palette color. The software has several predefined palette designs. Select your palette design from the followings:

Original image	GFP painted image	Coomassie painted image	Multicolored painted image	Red painted image

The Image Tab

Several images could be opened at the same time. You can easily switch from one image to another image using the arrows of the image tab.

With the , you can close all images.

With the , you can navigate from one image to another.
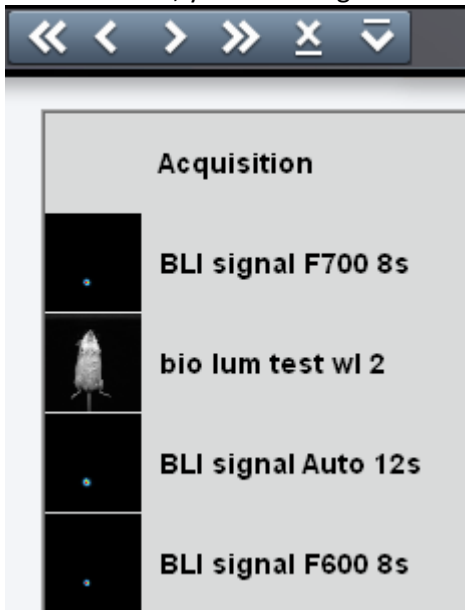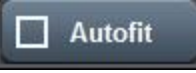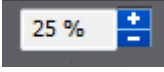
With the , you can close the image.

The lower Image Tab

The lower image bar contains the following features:

	Model in 3D your sample image
	Compare two or several images
	Access the Good Laboratory Practice (GLP) file which track all the image treatments performed with the software

Copy	Copy an image, a table or a graph onto the clipboard for insertion into another Windows program
Autofit	Resize the image to fit the size of the monitor
25 %	Zoom in or out the image

• **3D scan**

The Newton 3D Scan images your sample in real time and reconstructs the data to create live three-dimensional model. The 3D reconstruction provides direct information regarding the image dynamic, background level and protein or DNA quantity. A little change of exposure time will refresh the 3D view automatically. The saturation effect can be controlled live before the image is frozen.
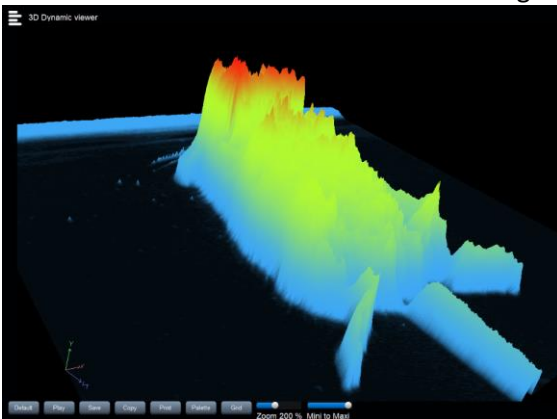
Click on Default to reset the display to the default display settings

Play: make the 3D view turning automatically.

Save: saves a 3D view to a new file or file location.

Copy: copy a 3D view onto the clipboard for insertion into another Windows program.

Print: prints a 3D view as it appears in the image window.

Grid: with the grid option, you can display a grid on the screen to visualize your 3D image gel according to horizontal and vertical axis. To proceed, select the grid option.

Zoom: zoom in or out the 3D view

Mini to maxi: select the scale of 3D visualization

Palette:

You can define and apply a color palette to your 3D image, allowing you to apply specific RGB values to monochrome images. The pseudo colors can display different types or levels of fluorescence in an image. It replaces the original grey levels of the image by another palette color. To proceed, click on the Pseudo-color button. A window displays the following menu:
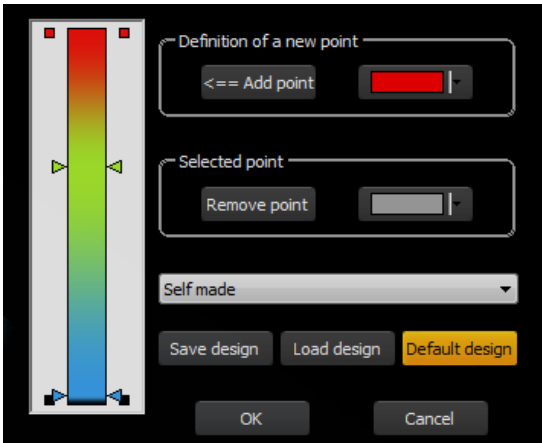
There are seven pre-defined color options: Red, Green, and Blue, Greyscale, Inverted greyscale, Yellow and Multicolor. Click on the pre-defined option icon. The image is then displayed with the default pseudo-colors settings. For instance, the image could be as follows with the Multicolor option:
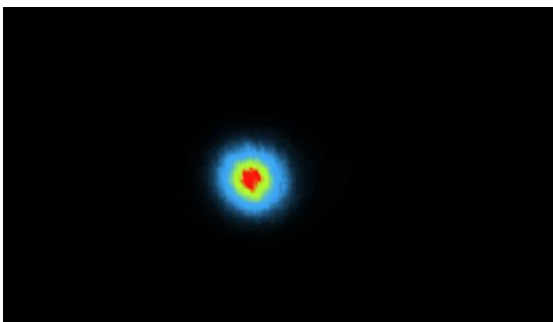
You can define custom palettes using the Add a point option:
Click on Add a point to add a color on the pseudo colors list. Select the color from the Add a point palette:
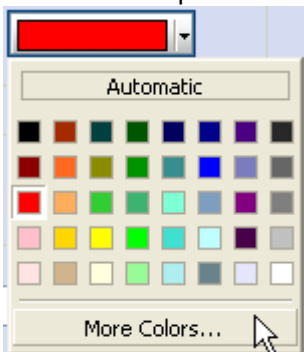
For the bicolor selection, click on the arrow to define the value of the color you want to modify. While keeping the mouse button pressed, move the arrow to its new value. Release the mouse button when value is satisfactory, the image is automatically updated. You can repeat these operations as many times as necessary for all pseudo colors.
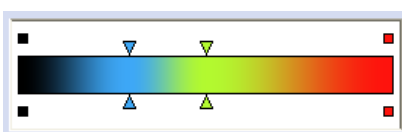
If needed, select the point to remove and click on Remove point to remove a color from the pseudo colors list.

User defined palette design

You can also save and load your own palette design. Define the set of colors you want to apply and click on Save to save the palette design. Click on Load to open your palette design.

• **Compare**

This function is used to compare two or several images in a single view.
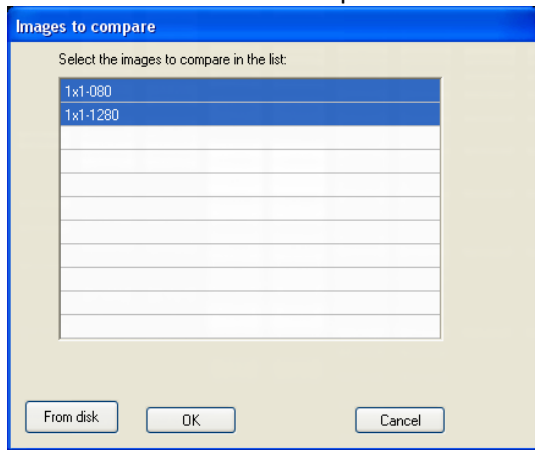
A list displays the image already opened. You can also add an image to the list by opening an image from your computer. To proceed, click on the "From disk" button. A pop-up window displays the following menu:
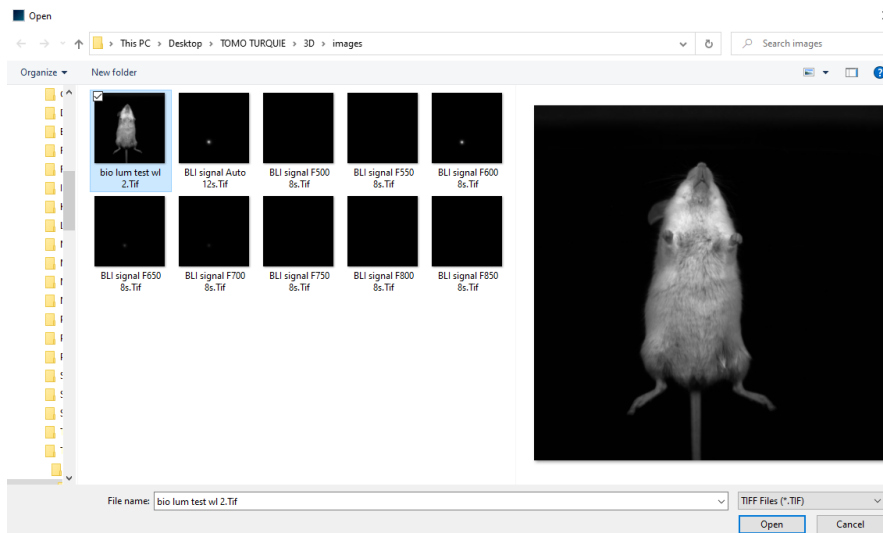
Browse to specify the image directory

Double click on the image name you want to load

When all the images to be compared are gathered in the list, click on the image you want to compare: and validate by clicking on OK:

A new window opens with the compared image:

To close the compare view, click on the "Close" button:

Click on normalize to normalize the display of the second image to the one of the first image:

Click on overlay to overlay the two opened imaged one on each other. *Image Overlay* lets you combine 2 images in the same display view.

● **GLP**

The Good Laboratory Practice (GLP) file is made to track all the image treatments performed with the software. Click on the "GLP" icon. A pop-up window displays the following menu:
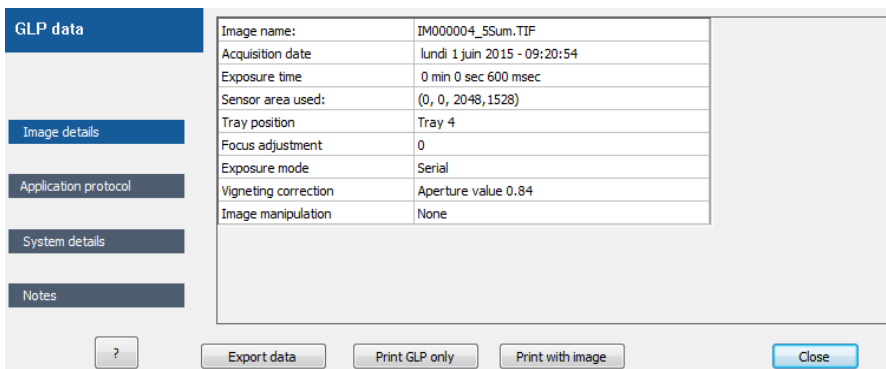
The GLP file has 4 different topics:

- the Image details which provide information on the way the image has been taken;
- the Application protocol which provide information on the Application protocol used to take the image;
- the System details which provide details on the system used to take the image;
- the Notes which provide you space to add information to your image.

● **Copy**

Copy the image as displayed onto the clipboard for insertion into another Windows program.

● **Autofit**

Click on the "Autofit" to resize the image to fit the size of the monitor.

The full resolution of the acquired may be larger than the screen resolution. The navigation requires the Windows scroll bar. The Autofit allows you to view the whole image, regardless of the window size. Typically, reducing the size of a window also cuts off part of the image. The Fit to Window option solves this problem by resizing the image so that it is always the same size as the window.

The Autofit proportions the display of the image to the screen resolution.

• **Zoom**

Click on the "+" or "-" to zoom in or out the image

Case 1:	Case 2:
100%	150%
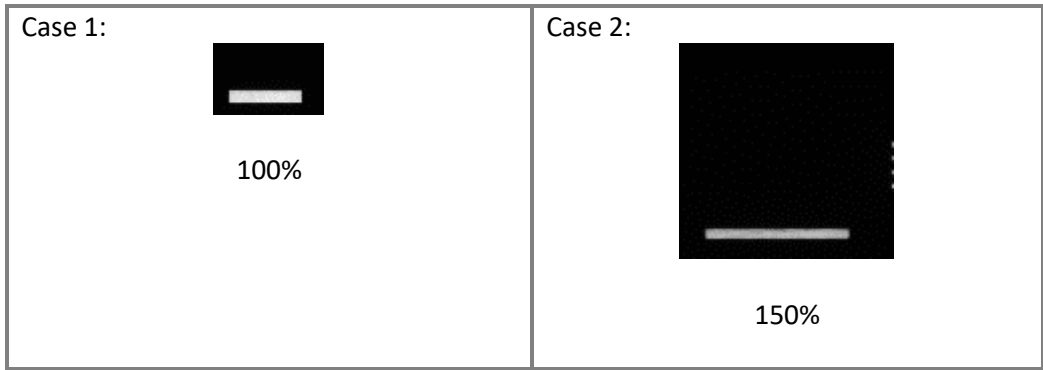
Save and print

Save

To save the image select Save. This will allow you to reopen the image later to use the image tools, to perform an analysis or to print the image.
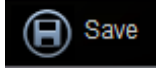
This function saves a previously unsaved image to a new file, or updates the changes to an existing image file, or save an image to a new file or file location. Click on the "Save" icon. A pop-up window displays the following menu:
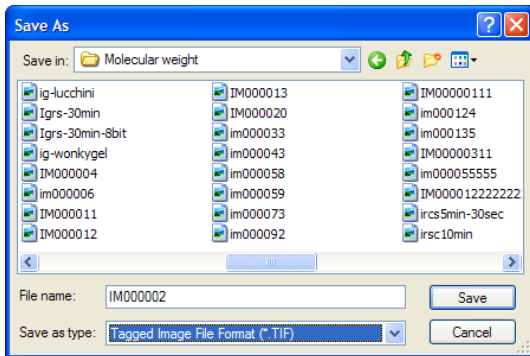
Browse to specify the image directory:
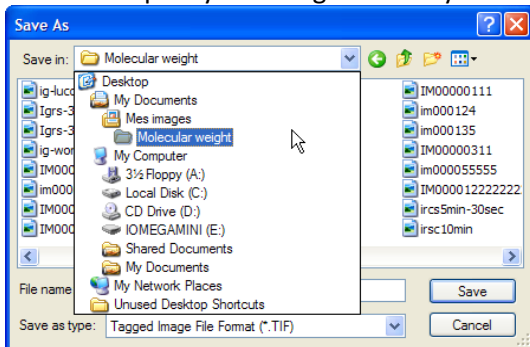
Enter the desired file name, select a file extension and validate
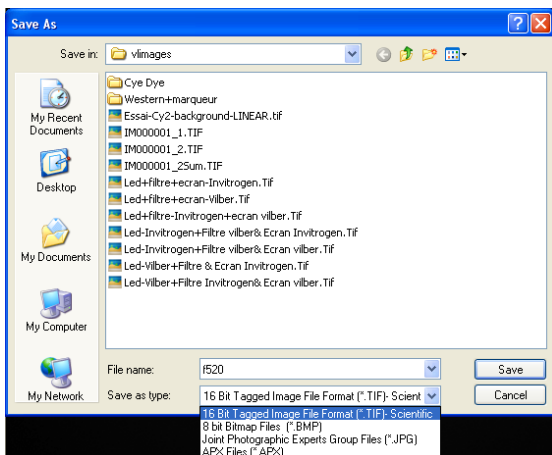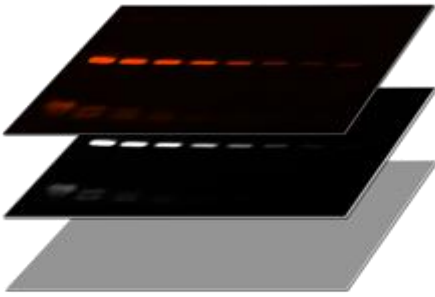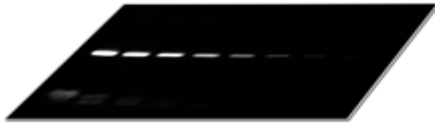
Note: the software proposes a default file name (im00000x). If the default file name is selected, it will be incremented by one each time an image is saved.

Images can be saved in CompatibilityPlus 16-bit TIFF scientific image format (recommended), 16-bit TIFF scientific image format, BMP (8-bit format only), JPEG (compressed) or APX (proprietary file format – 16-bit format).

The CompatibiliyPlus file format is a multi-layer based format which contained the image as displayed in the software, the raw data image, the image settings and the GLP data.

In the standard 16-bit TIFF file format, the TIFF image contained only the raw data and the GLP data. The image is showed according to the software display preference. Other software use other displays settings. Thus, the image showed in the image acquisition software could look like different if opened in a Microsoft software for instance. The CompatibilityPlus file format provides an alternative to this process thanks to a multi-layer approach.

CompatibilityPlus format with: one layer containing the displayed image, one layer containing the RAW image and one layer containing the image data	Standard 16-bit TIFF image and GLP image data

The first slice of the layer is a bitmap image of the image as displayed in the acquisition software.
The second layer contains the raw image data.
The third image layer contains the image information for the acquisition software: GLP, displays settings…

The CompatibilityPlus image format has the following advantages
• Respect of the image integrity
• Compatibility with other software platform
• Image information incorporated in the image file
• CompatibilityPlus image can be directly use for publication or reporting

Open
This function opens a previously saved image file of a specified format (i.e; TIFF, BMP, GIF, JPEG …).
Click on the "Open an image" icon to open an image. A pop-up window displays the following menu:
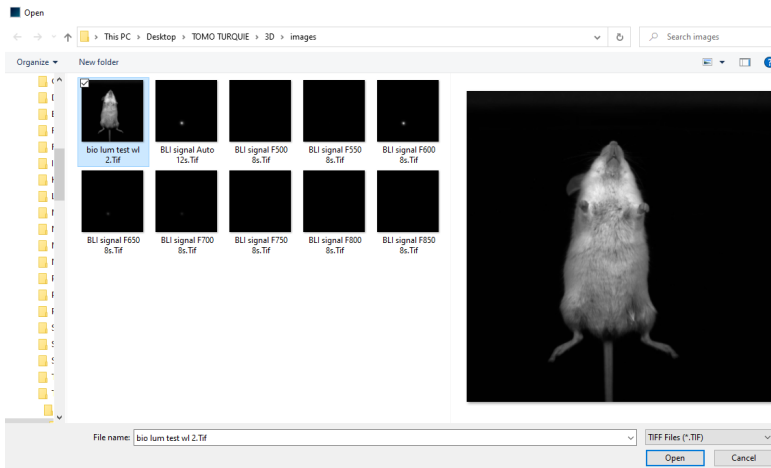
Browse to specify the image directory
Double click on the image name you want to load

You can narrow the image selected in the image list by selecting a specific image format:
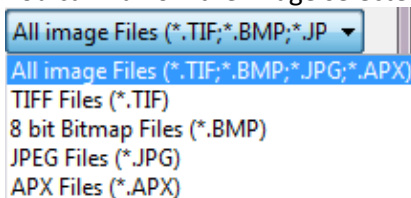
You can select multiple files to be opened at once. To proceed, hold down the Ctrl key on your keyboard, and select the first file. Then while still holding down that button, select the second, third, etc. file. You have more than one selected. Click on open to open the files.

Print

This function prints a previously opened image as it appears in the image window.

Click on the "Print" icon. A pop-up window displays the following menu:
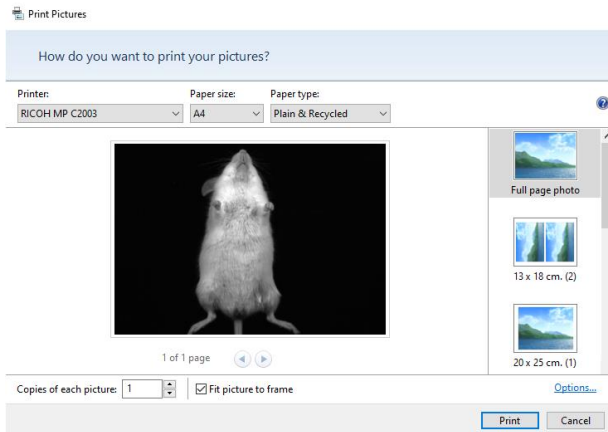
Click on Print to print as previewed.

Choose a printer
Click on Properties to modify the default setting of the printer, if necessary.
Select the number of copies.
Click on OK to validate your options.

Note: After you have installed and setup your printer, the procedure for setting up and configuring a printer is the same as in other Windows program.

Report

This function prints a report from your image acquisition:
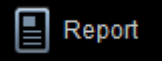
Click on the "Report" icon. A pop-up window displays the following menu:
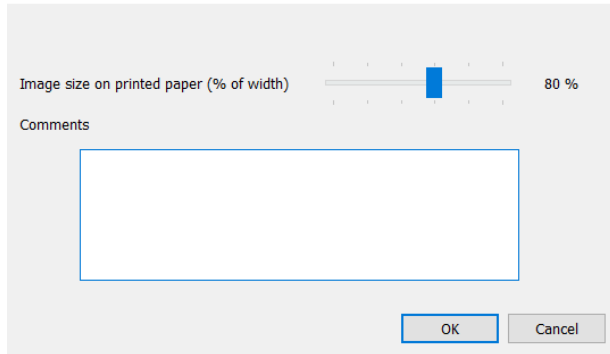
Select the image size. The image could be printed full page which 100% or smaller.
You could also add comments which will be displayed below the image.
GLP data such as image date and exposure time will also be printed with the image.

Example of report:
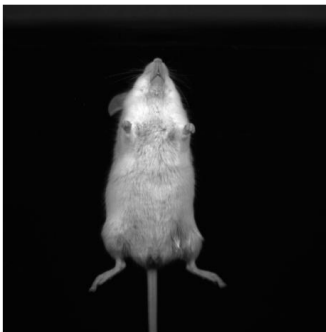
Image name:	bio lum test wl 2.Tif
Acquisition date	12 Kasým 2019 Salý - 15:41:01
Exposure time	Live mode (40 msec)
Tray position	Tray 3
Aperture	0.7
Sensitivity	High (1)
Filter position:	11: Bioluminescence (-20)
Lighting	White light epi low

Post process the image

Access the Edit post process menu

Select the Edit function from the menu bar to access the Edit menu:
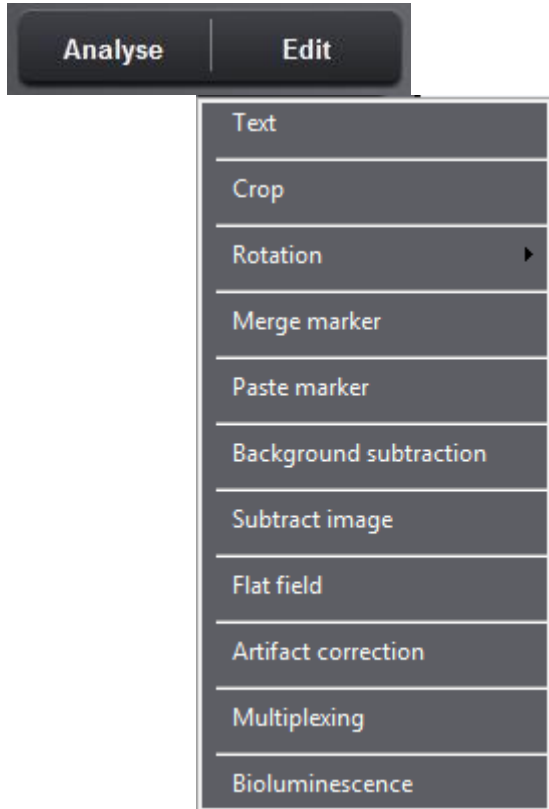
- Select Text to annotate your image with text and arrows.
- Select Crop to cut out a portion of an image.
- Select Rotation to rotate your image clockwise in 90° increments or mirror it vertically or horizontally.
- Select Merge marker to gather a blot image and its marker image into a new composite image.
- Select Paste marker to copy and paste a marker from the marker image into the signal image
- Select Background subtraction to separates foreground objects from their background clutter.
- Select Flat field to correct for uneven illumination intensity for both the background and the signal
- Select Artifact correction to remove dust or small defect from an image
- Select Multiplexing to combine two or three different fluorescent channels into a new colored composite image.
- Select Bioluminescence to combine a colored overlaid image and a monochrome image into a new colored composite image.

Add text

Click on the "Text" from the Edit menu. A window displays the following menu:
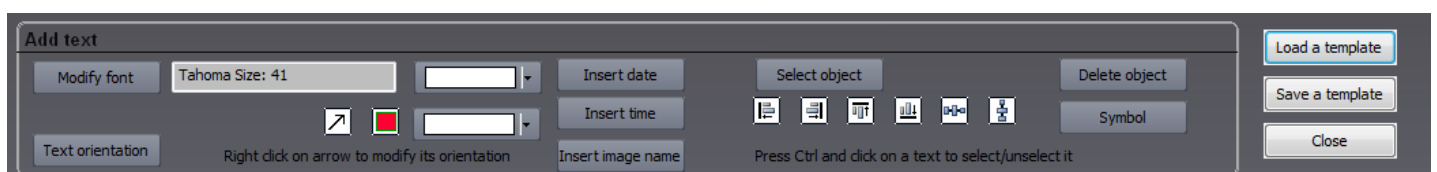
You can annotate your image with text and arrows.

•Text
Left click at the point where you want to insert the text. The Text window appears. Enter the text.
You can define the appearance of the text. Select the Modify font to modify the font, the font style and the text size.
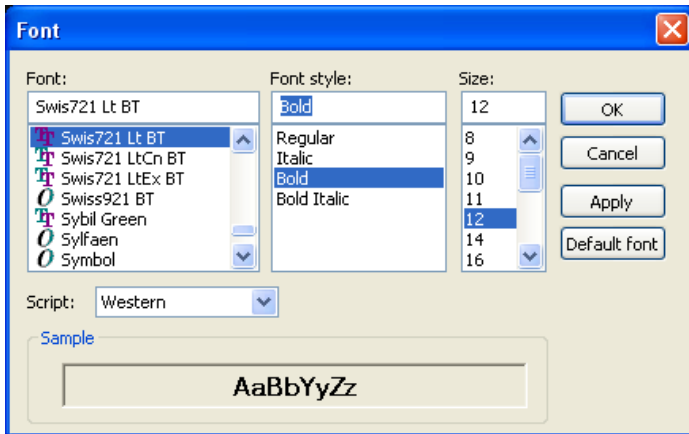
You can modify the font color by selecting the color option:
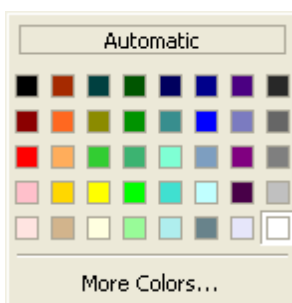
You can select the text orientation by clicking on the corresponding button:
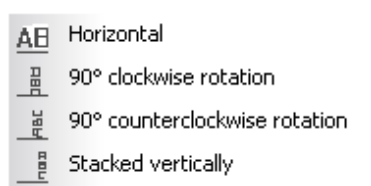
•Object insertion
You can use an arrow or a rectangle to emphasize a particular area in an image. To proceed, click on the following icon:

to draw an arrow

Note: You can turn the arrow direction by using the right click of your mouse.

to draw a rectangle

You can modify the object color by selecting the color option:

You can insert symbol by clicking on the Symbol button. You can also add the following items to the image:
- Date. Add the current date or the GLP date to the image. The current date is the date set on the computer you are using. The GLP date is the image acquisition date as recorded in the Good Laboratory Practice file (GLP).
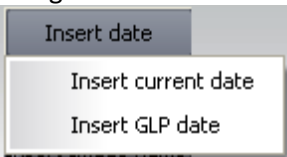
- Time. Add the current time or the GLP time to the image. This current time is the time set on the computer you are using. The GLP time is the image acquisition date as recorded in the Good Laboratory Practice file (GLP).
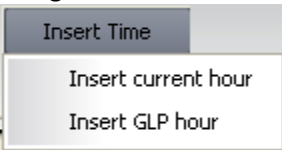
- Image name. Add the image title to the image. The full image name is the file name and location of the opened image. The short image name is the file name of the opened image.
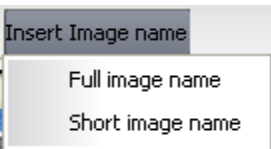
- Symbol. You can select a symbol from the symbol fonts and insert it on your image
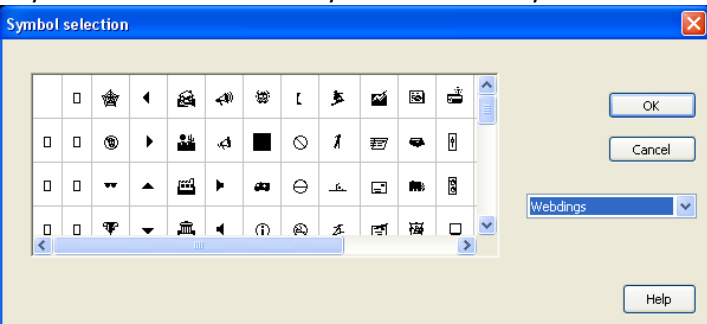
• **Text or objects position**
Select the objects that you want to align:
Use the "Select objects" button to select multiple objects of your choice and drag a dotted box over the objects using the on the image
Use the Select all objects" button to selects all the text objects from your document

Note: the selected objects are surrounded by an orange frame:

Note: the master object is surrounded by a bold orange frame:

Then align the text objects according to your desired layout:

Align left

Align right

Align top

Align bottom

Arrange horizontally objects equal distances from each other . Select at least three text objects you want to arrange and then click en click Distribute Horizontally

Arrange horizontally objects equal distances from each other . Select at least three text objects you want to arrange and then click en click Distribute Vertically

Note: text objects are aligned or arranged according to the master object position.

Note: you can delete n object by selecting the object and clicking on :

• **Template**
The text can be saved as a template and re-used for further analysis to facilitate routine text addition.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the text comments. You can save the template created on one image and / or load the template on another image.

The benefits of the template file are as follows:
- Time saving
- Reproduction of image analysis parameters
- Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

To close the text editor function, click on

Crop the image
You can cut out a portion of an image. Click on "Crop" from the Edit menu. A window displays the following menu:
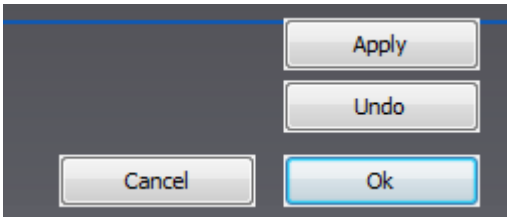
A default area is displayed. Click on the tags surrounding the area to modify its size. Drag and drop the area to modify its position.
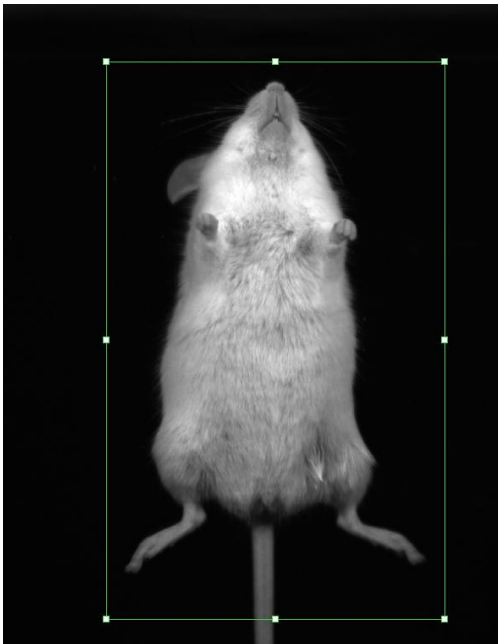
Note: To delete a previously defined area, click once again on the function.

2- Click on the "Preview" to crop the image:

Note: You can undo the cropping by clicking on "Undo"

Note: Exit the Cropping function by clicking on "Ok" to validate the selection or "Cancel" to discard it and return to the original image.

Note: the defined area can be moved with the keyboard arrows.

Rotate the image

You can rotate your image clockwise in 90° increments or mirror it vertically or horizontally. Click on "Rotation" from the Edit menu. A window displays the following menu:
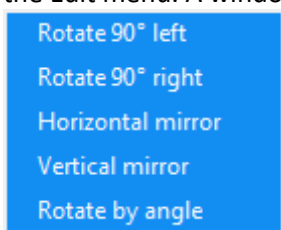
Click on the "Rotate" icon to rotate right the image. The image is rotated clockwise in 90° increments
Click on the "Horizontal mirror" icon to flip the image from top to bottom.
Click on the "Vertical mirror" icon to flip the image from right to left.

The "Rotate by angle" icon to rotate the image in increments other than 90°. Click on the rotation icon. A window display the following menu:
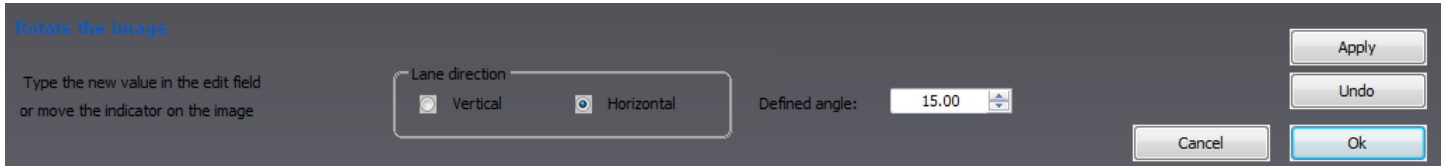
You can also define the angle of rotation in degrees. To complete the rotation, click on the Rotate button. Click on the Undo button to come back as previously.

Thanks to the axis, which appears on the image, you can also directly rotate the image using this overlay. To perform the rotation, position the cursor on the green square and drag in the yellow arrow direction. As you drag, the arrow will rotate and the angle in the box will change. Adjust the green lane to be as parallel as possible as the lanes. To complete the rotation, click on the Rotate button.

Adjust the green line to be as parallel as possible from the lane. Then, clock on Rotate.

Background subtraction

The Subtract background feature allows to separates foreground objects from their background clutter. Image background could interfere with quantification or signal visualization. Background could results from gel opacity, random signal noise, opacity of the carrier medium (film, gel matrix, or blot matrix) or film fogging.

The Subtract background applies to the active image. Click on the "Subtract background" button. A window displays the following menu:

Select the removal intensity and click on apply. The background is then removed from the entire image.

The intensity will determine how much background will be subtracted. A large intensity will more closely follow the profile trace, removing more background. A too small intensity will result in poor Subtract background. A disk radius that is too small may subtract actual data.

Note: few seconds could be necessary to perform the background subtraction.

Note: For a more accurate background subtraction, you can set the bands parameters: white bands / black background / black bands, white background. You can select the following options:
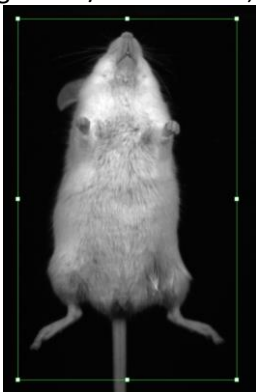
Dark background for images with white signals and black background
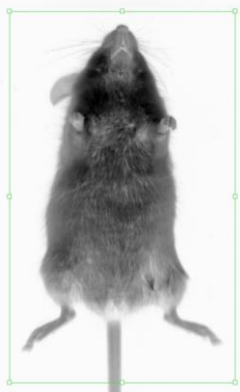
Light background for images with black signals and clear background

To close the function, click on or

Flat field

The Apply flat field feature correct for uneven illumination intensity for both the background and the signal.
Even under the best of imaging conditions, the illumination across a field of view isn't perfectly uniform. This is due to the light source imperfections (i.e. slight misalignments and additional physical properties) and to the optical elements constraints (bulb, filters, mirrors, objectives) within a light path.
Flat Field Correction is a commonly used approach for this problem. This process will compensate for different illumination intensity. In the flat fielded image, a uniform signal will create a fairly uniform output (hence flat-field).

The Apply flat field applies to the active image. Click on the "Apply flat field" button. A window displays the following menu:

Click on preview to get the flat fielded image.

Note: few seconds could be necessary to perform the flat fielding.
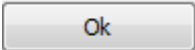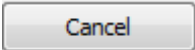
Note: For a more accurate background subtraction, you can set the bands parameters: white bands / black background / black bands, white background. You can select the following options:
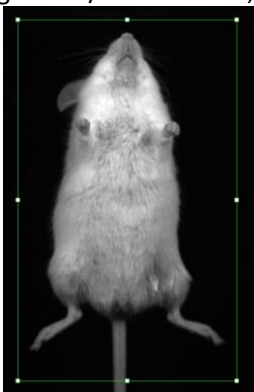
White bands on dark background for images with white signals and black background
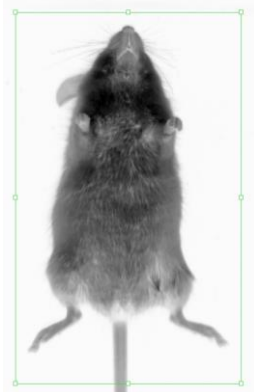
Black bands on light background for images with black signals and clear background

To close the function, click on [Ok] or [Cancel] button

Artifact correction

The Remove artifacts function removes dust or small defect from an image

This function replaces a pixel by the median of the pixels in the surrounding. The Remove artifacts applies to the active image. Click on the "Remove artifacts" button. A window displays the following menu:
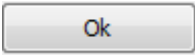
The radius defines the size of the artifacts to be removed. This value is given in pixel.
The threshold determines by how much the artifact pixel must deviate from the median to get replaced. This value is given in grey levels.
The type of artifact determines whether the brighter pixels or the darker pixels than the surrounding should be replaced.

Click on Preview to remove the artifacts from the entire image.

To close the function, click on [Ok] or [Cancel] button

Multiplexing

The multiplexing function allows combining two or three different fluorescent channels into a new colored composite image. The multiplexing option is used primarily in fluorescence imaging when specimens have been stained with more than one dye or in bioluminescence when you want to add in color a marker lane to your signal sample image.

Click on the "Multiplexing image" button. A window displays the following menu:
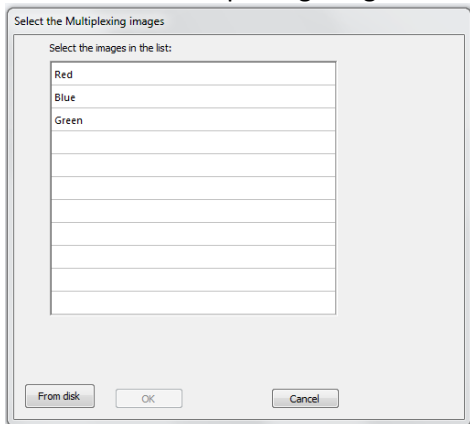
A list displays the image already opened. You can also add an image to the list by opening an image from your computer. To proceed, click on the "From disk" button. A pop-up window displays the following menu:

Browse to specify the image directory
Double click on the image name you want to load

When all the multiplexing images are gathered in the list, click on the first image. A pop-up window invites you to select the first color channel from blue, green, red:

When the first channel is selected, proceed in the same way for the one or two others channels. You will obtain an image list codified by the color channel.
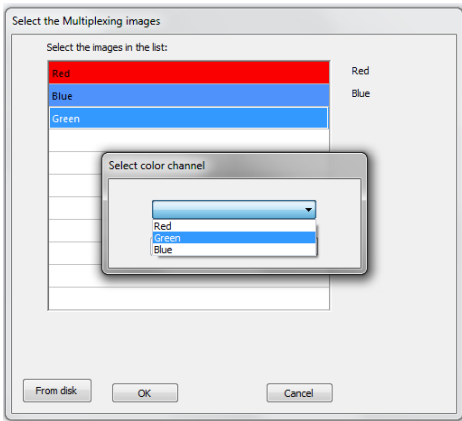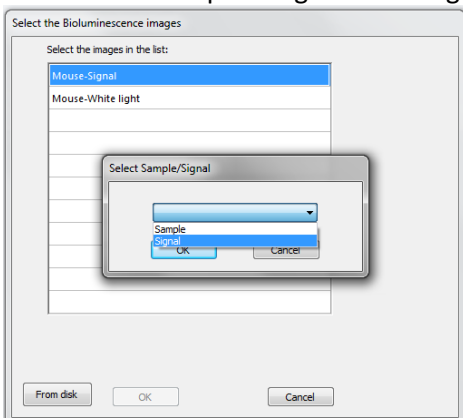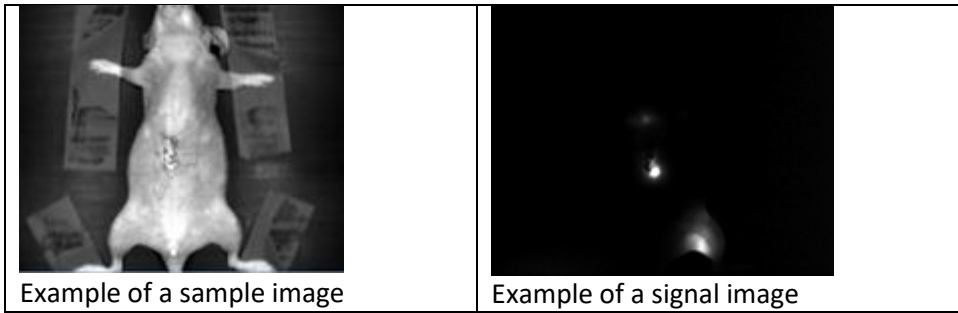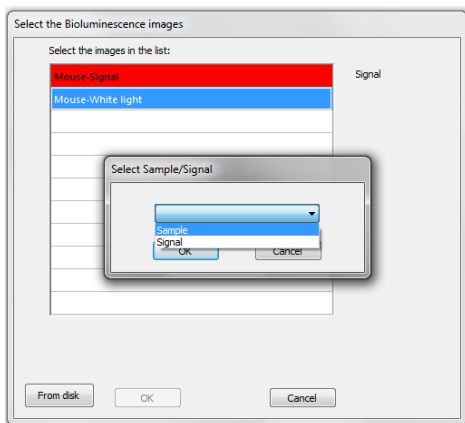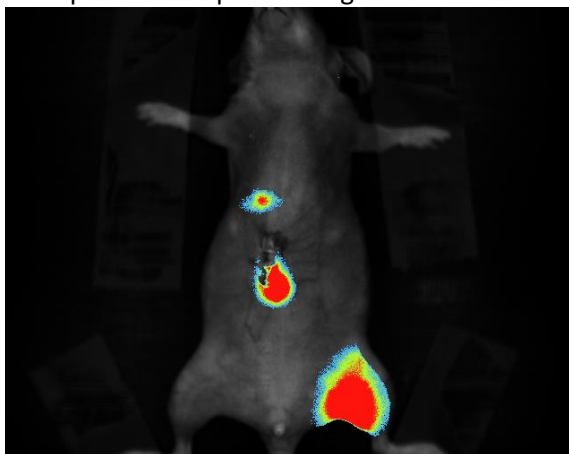

Click on the "OK" button. A window displays the following menu:

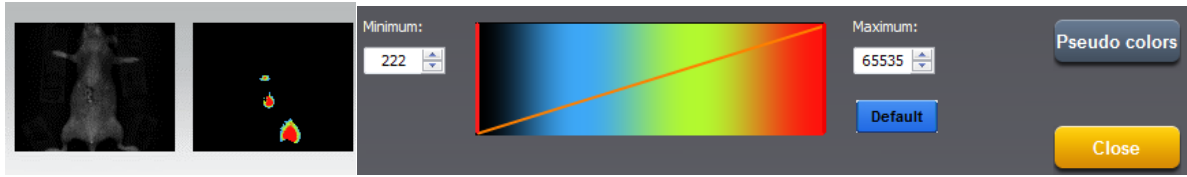A new color composite image appears in the main windows, gathering the multiplexing images, channel by channel. Example of a composite image elaborates from 3 different channels:
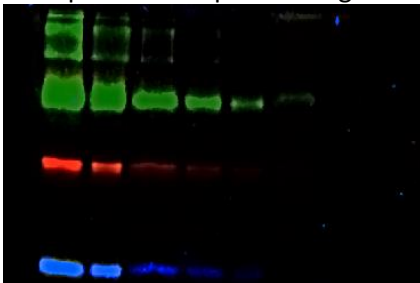
You can adjust the displays of the composite image. To proceed, click on the thumbnails you want to modify. The Display Adjustment graph for the channel you selected will appear in the menu:
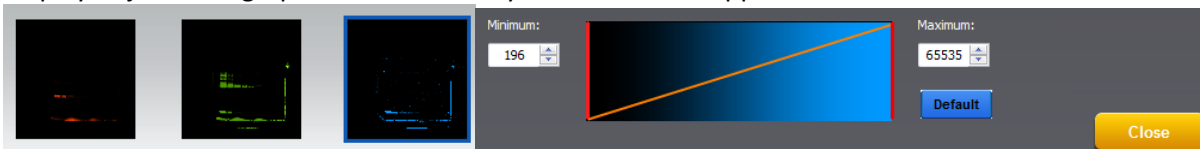
Use the Display Adjustments to optimize the display to enhance the features of interest in the image.
Adjust the display by moving the slider, or by typing a number in the value box.

To close the function, click on

Bioluminescence

The bioluminescence function allows combining a colored overlaid image and a monochrome image into a new colored composite image.

Click on the "Bioluminescence" button. A window displays the following menu:
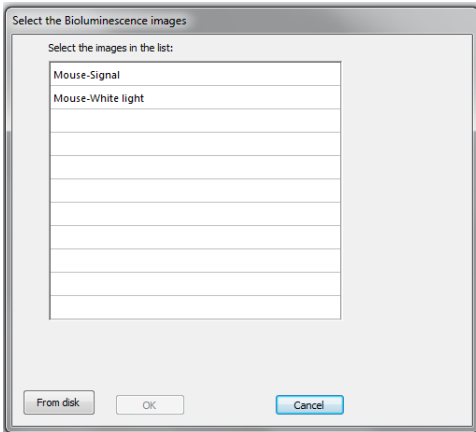
A list displays the image already opened. You can also add an image to the list by opening an image from your computer. To proceed, click on the "From disk" button. A pop-up window displays the following menu:

Browse to specify the image directory
Double click on the image name you want to load

When all the bioluminescence images are gathered in the list, click on the first image. A pop-up window invites you to select the sample image and the signal image:

Example of a sample image	Example of a signal image

When the signal image is selected, proceed in the same way for the sample image. You will obtain an image list codified by color, red for the signal image and grey for the sample image:

Click on the "OK" button. A window displays the following menu:
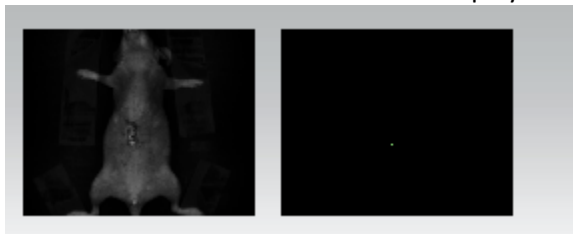
A new color composite image appears in the main windows, gathering the multiplexing images, channel by channel.

Example of a composite image:

You can adjust the displays of the composite image. To proceed, click on the thumbnails you want to modify. The Display Adjustment graph for the channel you selected will appear in the menu:

Use the Display Adjustments to optimize the display to enhance the features of interest in the image.
Adjust the display by moving the slider, or by typing a number in the value box.

To close the function, click on

The Application protocol

What is an Application protocol?

The software runs with pre-defined image acquisition parameters to facilitate the use of the system for a specific application. These pre-defined parameters are gathered in the software Application protocol.

The Application protocol automates a task or a set of tasks that you perform repeatedly or on a regular basis. It stores the following information:
- The image exposure pre-defined set-up;
- The live preview pre-defined parameters;
- The image display pre-defined set-up;
- The image printing pre-defined set-up;
- The image file pre-defined set-up.

The factory settings include several pre-defined Application protocols

You can also create your own Application protocol.

The benefits of the Application protocol are as follows:
- Time saving
- Reproduction of image acquisition parameters
- Protocols are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

How to access the application protocol?

Select an application protocol from the application menu. Click on the More button to open the application protocol menu.
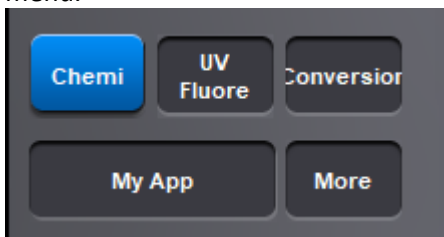
A window displays the following menu:
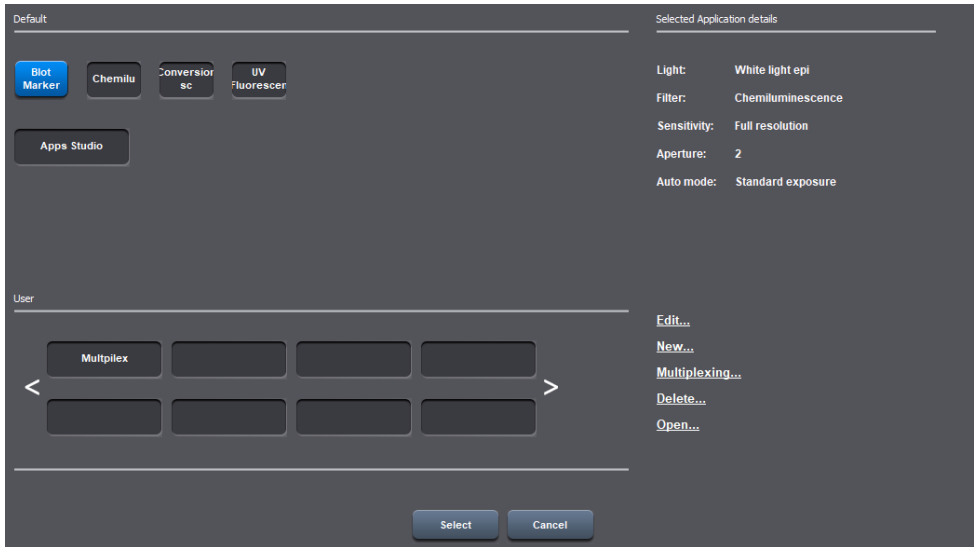
The list of the default Application protocols appear on the upper part of the Window. The list of user defined Application protocols appear in the lower part of the window. A summary of the main features of the selected Protocol is given in the right hand side of the window.

- Select Edit to modify an existing Application protocol.
- Select New to create a new Application protocol.
- Select Multiplexing to a group of protocols.
- Select Delete to erase an Application protocol.
- Select Open to create a new Application protocol.

New, Edit & Multiplexing – Create or modify a Single or Multiplexing applications types

A **single application** defines the set of parameters for a specific protocol. All the image exposures taken with this application follow the same protocol.

Advanced exposure – Exposure predefined set-up

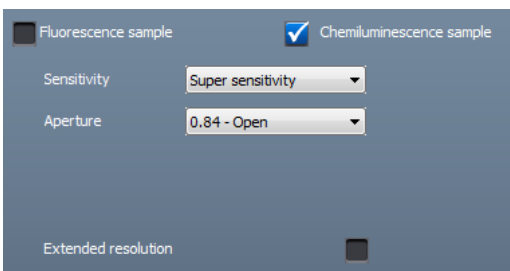Exposure folder contains the exposure pre-defined set-up:
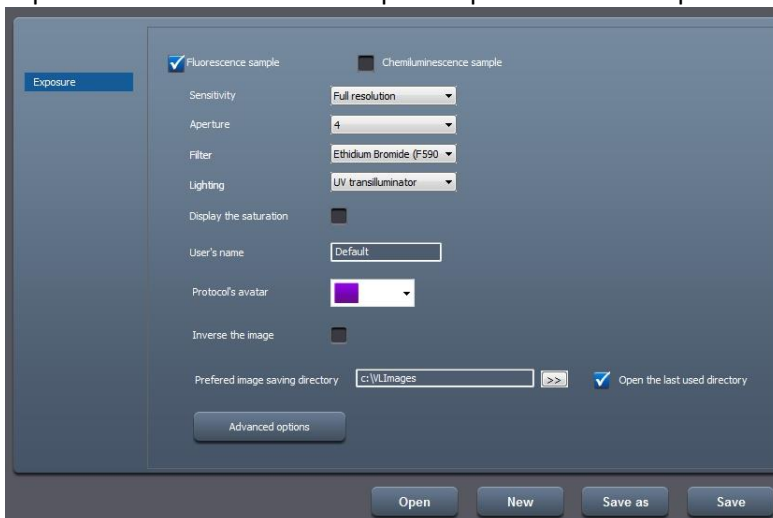
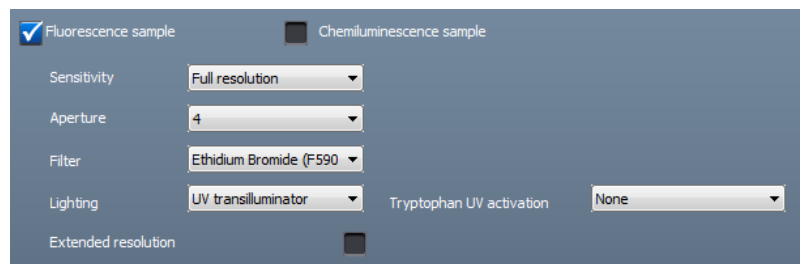Typical Exposure set-up for a fluorescence application
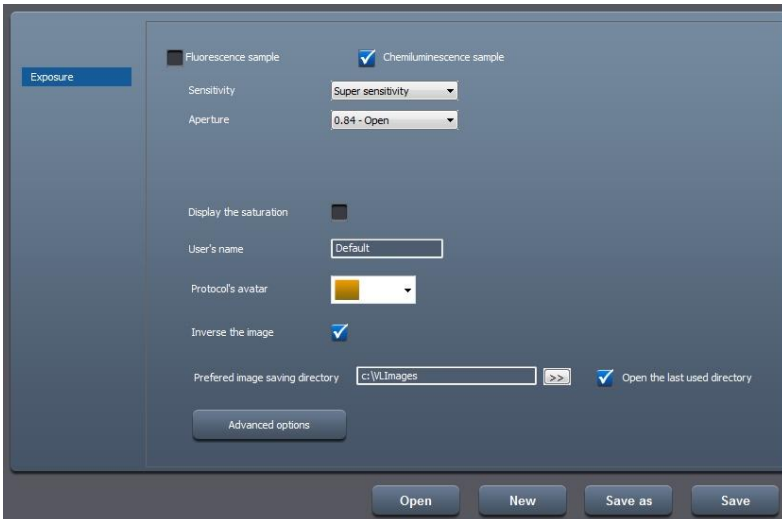
Typical Exposure set-up for a bioluminescence application

• Application mode

<div align="center">Manual exposure set-up
Bioluminescence mode</div>

<div align="center">Manual exposure set-up
Fluorescence mode</div>

You can select the application mode for the image exposure, between bioluminescence and fluorescence:
- Bioluminescence: no lighting and filter options
- Fluorescence: lighting and filter option

• Sensitivity

This option defines the image sensitivity for your image exposure. The Newton systems offer exquisite resolution and sensitivity to maximize quantifiable data. The system can be used at either its full resolution or with binning. Sensitivity options are as followed:
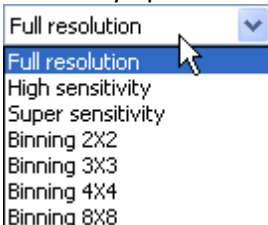
The full resolution is the native camera image.

The High sensitivity and Super sensitivity modes increase the sensitivity using the HSR-High Sensitivity Reading technology. The High sensitivity is combining full resolution and HSR reading technology. The super sensitivity mode combined the HSR-High Sensitivity Reading technology and binning 2x2.

The binning technique combines the charge from adjacent pixels so that the total charge can be read-out as a single pixel. The result is an increased signal and thus an improved sensitivity and a better signal-to-noise ratio. This allows reducing the exposure time. The reduction of the amount of pixels improved the frame rate of the image acquisition. However, the image resolution is decreased by the binning factor (i.e: 4 for a binning of 2 by 2).
A 2x2 binning factor means that pixels in two rows and two columns (a total of 4 pixels) are combined to be represented as one pixel. The sensitivity is heightened but the resolution is then divided by 4.

• **Aperture**
This option defines the aperture to be used for image acquisition. For Fluorescence, the aperture should be set from f4.0 to f0.7 for very dim sample. For bioluminescence, the standard aperture is f0.70.

• **Filter (Fluorescence mode only)**
This option defines the default filter used in the Application protocol. For instance, F590 for Ethidium Bromide applications with fluorescence or F999 SkyLight filter for Ethidium Bromide applications with SkyLight fluorescence.

Note: The filter options depend on your system configuration. In standard the position 6 is let for bioluminescence exposure (no filter).

• **Lighting (Fluorescence mode only)**
This option defines the lighting used for the image exposure. All Newton systems are equipped with LED Visible light. You can select between low and high intensity.

Note: The lighting options depend on your system configuration., blue or RGB Spectra lighting could be available. The selection of these lighting options will be done from this menu.

• **Extended resolution**
The effective pixels technology allows the extension of the camera native image resolution by software interpolation.

Note: This process requires important computer calculation and sometime could be necessary before the extended image is displayed.

• **Auto-exposure set-up**
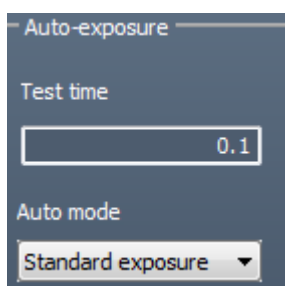
Test time

In order to calculate an ideal exposure time, the software needs to analyze a test image, taken in binning mode with a test exposure time. Then it searches for the minimum and maximum acceptable grey levels and calculate the exposure time so the highest grey levels values of the final image reach a maximum defined (Saturation Value).
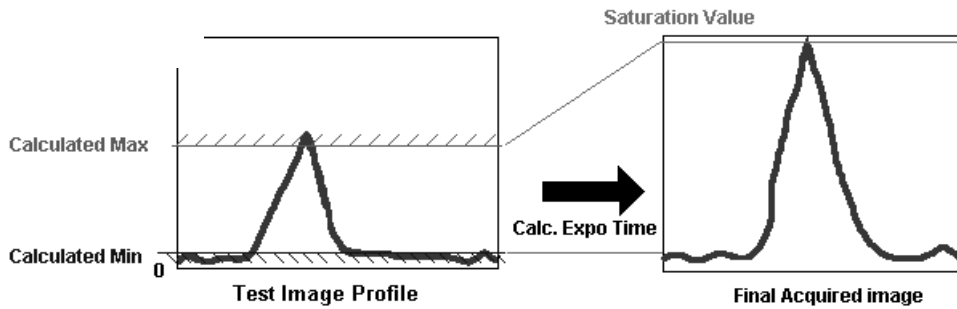

Calculated Min ("Lower Gray levels" parameter) : Nr of Lower Gray levels with at least 10 pixels in the image
Calculated Max ("Higher Gray levels" parameter) : Nr of Higher Gray levels with at least 1 pixel in the image

For bioluminescence, the standard test time is 2 seconds. For fluorescence, he standard test time is 0.2 seconds.

Note: in the case the au-exposure does not give you entire satisfaction for your specific application, we recommend increasing the test time.

Auto mode
The Auto mode options allow you to select between a standard auto exposure time, an extended auto exposure time or a reduced auto-exposure time.
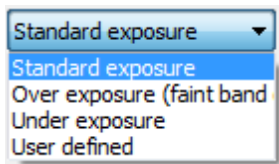

Standard exposure is optimized for all bands.
Over exposure (faint band) results in a longer exposure where faint bands are more visible, but more prominent bands may be over exposed.
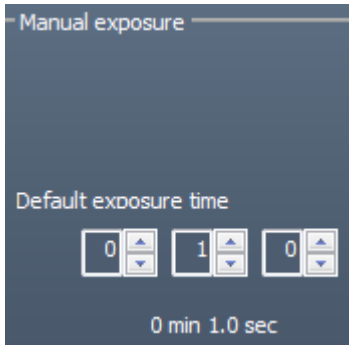Under exposure (bright band) results in a shorter exposure where bright bands are more visible, but more prominent bands may be under exposed.
User defined allows you to decide of the over exposure or under exposure settings. For instance, 150% will increase the standard exposure time by 1.5.
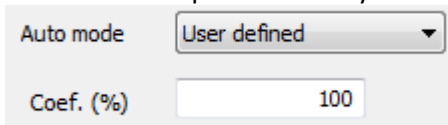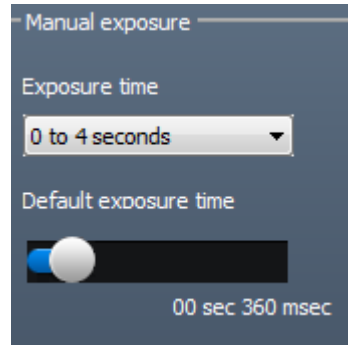

• **Manual exposure set-up**

Manual exposure set-up
Bioluminescence mode

Manual exposure set-up
Fluorescence mode

This option defines the default imaging exposure time.

For the fluorescence mode, the software has two exposure time scales:
- One for short times: 40 milli-second to 4 seconds
- One for long times: 4 sec to 1 minute

Note: With the short integration time scale, the integration time increases or decreases by 40milli seconds.

Note: With long integration time, a delay could be necessary before an image is displayed on the monitor (up to twice the selected Exposure time).

Preview – Live preview pre-defined parameters

Preview folder contains the live review pre-defined set-up:
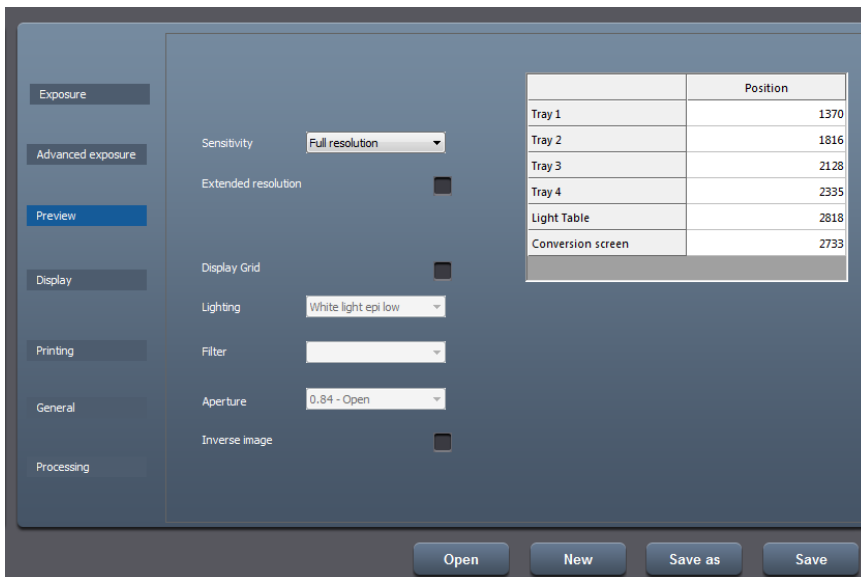
• **Sensitivity**

This option defines the image sensitivity for your image exposure. The Newton systems offer exquisite resolution and sensitivity to maximize quantifiable data. The system can be used at either its full resolution or with binning. Sensitivity options are as followed:
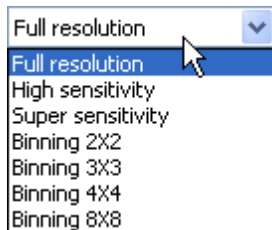
The full resolution is the native camera image.

The High sensitivity and Super sensitivity modes increase the sensitivity without affecting the image resolution using the HSR-High Sensitivity Reading technology. The High sensitivity is combining full resolution and HSR reading technology. The super sensitivity mode combined the HSR-High Sensitivity Reading technology and binning 2x2.

The binning technique combines the charge from adjacent pixels so that the total charge can be read-out as a single pixel. The result is an increased signal and thus an improved sensitivity and a better signal-to-noise ratio. This allows reducing the exposure time. The reduction of the amount of pixels improved the frame rate of the image acquisition. However, the image resolution is decreased by the binning factor (i.e: 4 for a binning of 2 by 2).
A 2x2 binning factor means that pixels in two rows and two columns (a total of 4 pixels) are combined to be represented as one pixel. The sensitivity is heightened but the resolution is then divided by 4.

• **Extended resolution**
The effective pixels technology allows the extension of the camera native image resolution by software interpolation.

Note: This process requires important computer calculation and sometime could be necessary before the extended image is displayed.

• **Focus correction**
In the case your sample height is not standard for the system – i.e.: a microtitration plate - you can set a specific focus level for your application.

Note: By default, the focus correction should be 0

Note: This focus correction adjustment is valid only for a specific tray position

• **Grid**
With the grid option, you can display a grid on the screen to adjust your gel according to horizontal and vertical axis. To proceed, select the grid option.
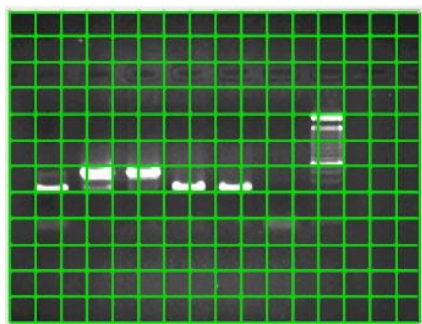
Note: The grid option is only available with the Preview mode and is not available for 8x8 binning mode.

Note: You can remove the grid option by deselecting this option.

• The lens prefixed position table

	Position
Tray 1	1198
Tray 2	1644
Tray 3	1956
Tray 4	2184
Transilluminator	2690
Conversion screen	2627

The lens prefixed position table summarizes the default focusing preset per tray position for the concerned Application protocol.

Note: The default positions are part of the system factory settings. Nevertheless, you can save your owned position using the Set-Up functions of the Exposure menu.

The Newton SL6 Xpress zoom and focus prefixed position tables

The zoom and focus prefixed position table summarizes the default focusing preset per tray position for the concerned Application protocol.

Zoom protocol preset

	Position
Tray 1	0
Tray 2	0
Tray 3	0
Tray 4	0
Transilluminator	0
Conversion screen	0

Focus protocol preset

	Position
Tray 1	65535
Tray 2	65535
Tray 3	65535
Tray 4	65535
Transilluminator	65535
Conversion screen	65535

Note: The default positions are part of the system factory settings. Nevertheless, you can save your owned position using the Set-Up functions of the Exposure menu.

Display – Image display pre-defined parameters

The Display folder contains the image display pre-defined set-up:

• Select display mode
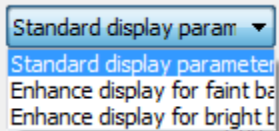
The Select display mode options allow you to select between a standard display, an enhance display for the faint band and an enhance display for the bright band.
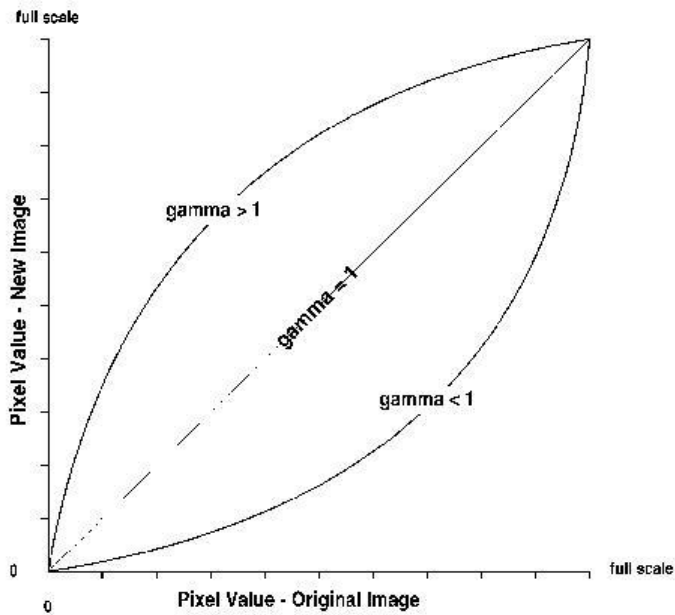
Standard display is optimized for all type of image.

Enhance display for faint band ensure the faint bands are more visible, but more prominent bands will look very strong. This is well adapted for bioluminescence sample.

Enhance display for bright band ensure the bright bands are more visible compared to their background. This is well adapted for fluorescence sample.

• Set the gamma value

Gamma adjustment corrects an image by creating a new version of the original. To create the new image, the Gamma Adjust function reassigns the grey values of each pixel in the image according to the curve in the following graph:

The above graph demonstrates the basic principles of gamma adjustment:
- Black (pixel value = 0) remains black at all gamma values.
- White (pixel value = full scale) remains white at all gamma values.
- Gamma values greater than one lift the darker areas of the original image into the brighter areas of the new image.

A gamma curve is smooth: there are no unexpected jumps or cut-offs. This means that when viewing a gamma adjusted image, you will be able to see the details (intensity differences) in both the black and white areas of the image.

When the bright areas of these types of images are correctly exposed, the darker areas can be so dark that they are in effect invisible. Gamma Adjust can remedy this problem. The gamma adjustment results in a better display of detail by lightening the darker areas without burning out bright areas or lightening black areas:

• **Inverse the image**
Select "Inverse the image" to inverse the grey level of the image. This makes a negative image.

• **Display the saturation**
A saturated image is inappropriate for image quantification with image analysis software. The saturation option allows you to visualize in red, pixels that have the maximum grey level in order to avoid flattened peaks.

The maximum grey level depends of the bit-depth. For instance:
A 16-bit image has a maximum grey level of 65535
A 8-bit image has a maximum grey level of 255.

Select the saturation option and the saturated pixels are displayed in red:

Note: If an image is being acquired and the «Saturation» option is checked, the modification is applied to the current acquired image

Note: A saturated image creates quantification error when studied by an image analysis software. Gel-doc systems have to indicate to the user if the image is saturated and if it is then necessary to modify the integration time.

• **Select the color palette**

The pseudo colors can display different types or levels of fluorescence in an image. It replaces the original grey levels of the image by another palette color. The Bio-1D software has several predefined palette designs. Select your palette design from the followings:

• Activate autofit for acquisition
Click on the "Autofit" to resize the image to fit the size of the monitor.
The full resolution of the acquired may be larger than the screen resolution. The navigation requires the Windows scroll bar. The Autofit allows you to view the whole image, regardless of the window size. Typically, reducing the size of a window also cuts off part of the image. The Fit to Window option solves this problem by resizing the image so that it is always the same size as the window.
The Autofit feature proportions the display of the image to the screen resolution.

• Clarity™

Clarity is a display filter which enhance the contrast and sharpness. Clarity does not affect the raw data.

• Automatic vignetting correction (SL and Solo systems only)
Vignetting is a reduction of an image's saturation at the periphery compared to the image center. Vignetting is an unintended effect caused by lens limitations. Optical vignetting is caused by the physical dimensions of a multiple element lens. Rear elements are shaded by elements in front of them, which reduces the effective lens opening for off-axis incident light. The result is a gradual decrease in light intensity towards the image periphery. Optical vignetting is sensitive to the lens aperture.

The automatic vignetting correction will correct the optical defect at the end of the image acquisition if selected.

Automatic vignetting correction	☑	aperture	0.84 - Open ▼
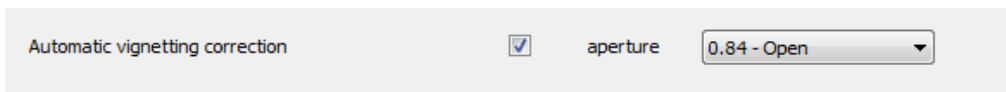
Printing – Image printing pre-defined parameters

The Printing folder contains the image printing pre-defined set-up:

Print with image name	☐
Print with date and time	☐
Print out text color	▢ ▼
Free text print out	▭
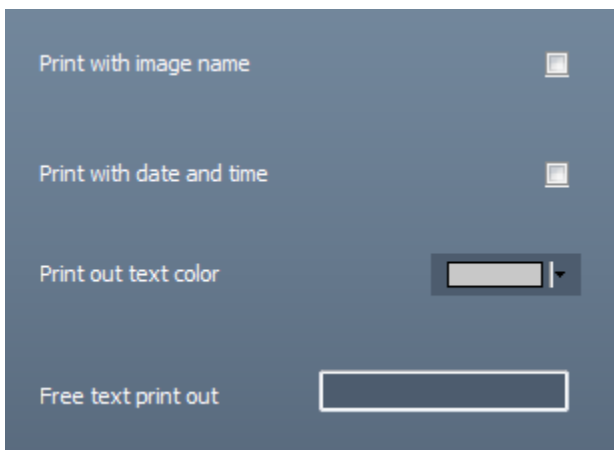
• Print with image name
Print the image with its name.

• Print with date and time
Print the image with the computer time.

**• Print out text color**
Select the color of the text printing.

• Free text print-out
You can select a free text (for instance the name of your institute), the image name (if the image has been previously saved) and date and time.
The text is then displayed on the image print out:

General – Image file pre-defined parameters
The General folder contains the image file pre-defined set-up
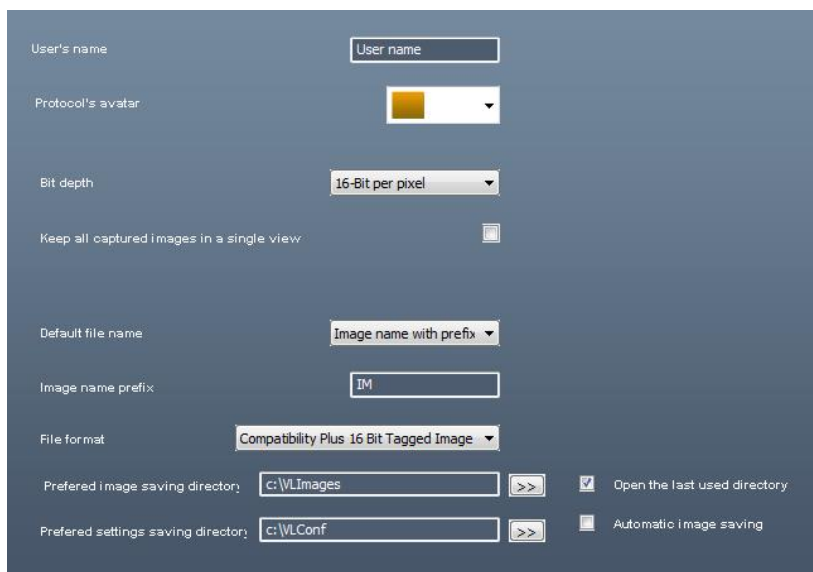
•User name
You can define the name of a user for an application protocol. The user could be the name of a working group, the name of a user or a subtitle to the name of the application.

•Protocol's avatar
To simplify the reconnaissance of the Application's protocol, an icon is provided to differentiate the application by a color.

•Bit depth
The Newton systems offer the possibility to select the bit-depth for more convenience, from 16-bit, 14-bit and 8-bit.
- 8-bit images have 256 grey levels;
- 14-bit images have 16384 grey levels;
- 16-bit images have 65 536 grey levels.
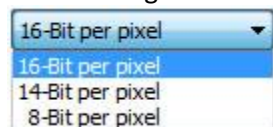
The default bit depth mode is 16-bit.

•Default file name / Image name prefix
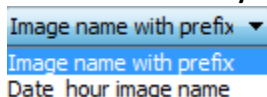
The default file name could either be based on the Date and Hour the image has been taken or by a prefix. You can decide of the default generic file name to simplify the file name generation. By default, the name is IM00000x. The image could also be named according to the time and hour it has been taken.

•**File format**

Images can be saved in CompatibilityPlus TIFF scientific image format (16-bit format recommended), TIFF scientific image format (16-bit format), BMP (8-bit format only), JPEG (compressed) or APX (proprietary file format – 16-bit format). This option allows you to select the default file format.

```
Compatibility Plus 16 Bit Tagged Image File
16 Bit Tagged Image File Format (*.TIF) - S
8 bit Bitmap Files  (*.BMP)
Joint Photographic Experts Group Files (*.J
APX Files (*.APX)
```

•**Preferred image saving directory**

This option defines the directory where the image is saved by default

Alternatively, you can select to always open the last used directory by clicking on the following option:

☑ Open the last used directory

•**Autosave**

Use the Image Auto-Save feature to automatically save images as part of the capture process, in the defined image saving directory.

•**Preferred settings saving directory**

This option defines the directory where are saved the configuration files.

Processing – Applying post process function to an acquired image

The Processing folder contains the image post process options:
- Subtract background
- Apply flat field
- Remove artifacts.

Select to automatically activate the post processing after the image acquisition.

**Note**: Please refer to the Processing menu to have more details on the post process working principles.

The Apps studio

The Newton Apps Studio is an innovative library of applications which contains more than 40 different protocols for your blot, gel and other bioluminescence samples. The protocols are easily accessible and gathered into four master protocols: DNA/RNA gels, Protein gels, Protein blots, Bioluminescence and others.

The Studio contains the excitation and the emission spectra of the main fluorophores used in modern molecular biology laboratory. It also suggests the best possible system configuration in terms of excitation light source, emission filter and sensitivity level. The Apps Studio ensures reproducibility and one click image acquisition for the best ease of use.

You can access the Apps Studio by clicking on the AppsStudio tab from the Application protocol menu:
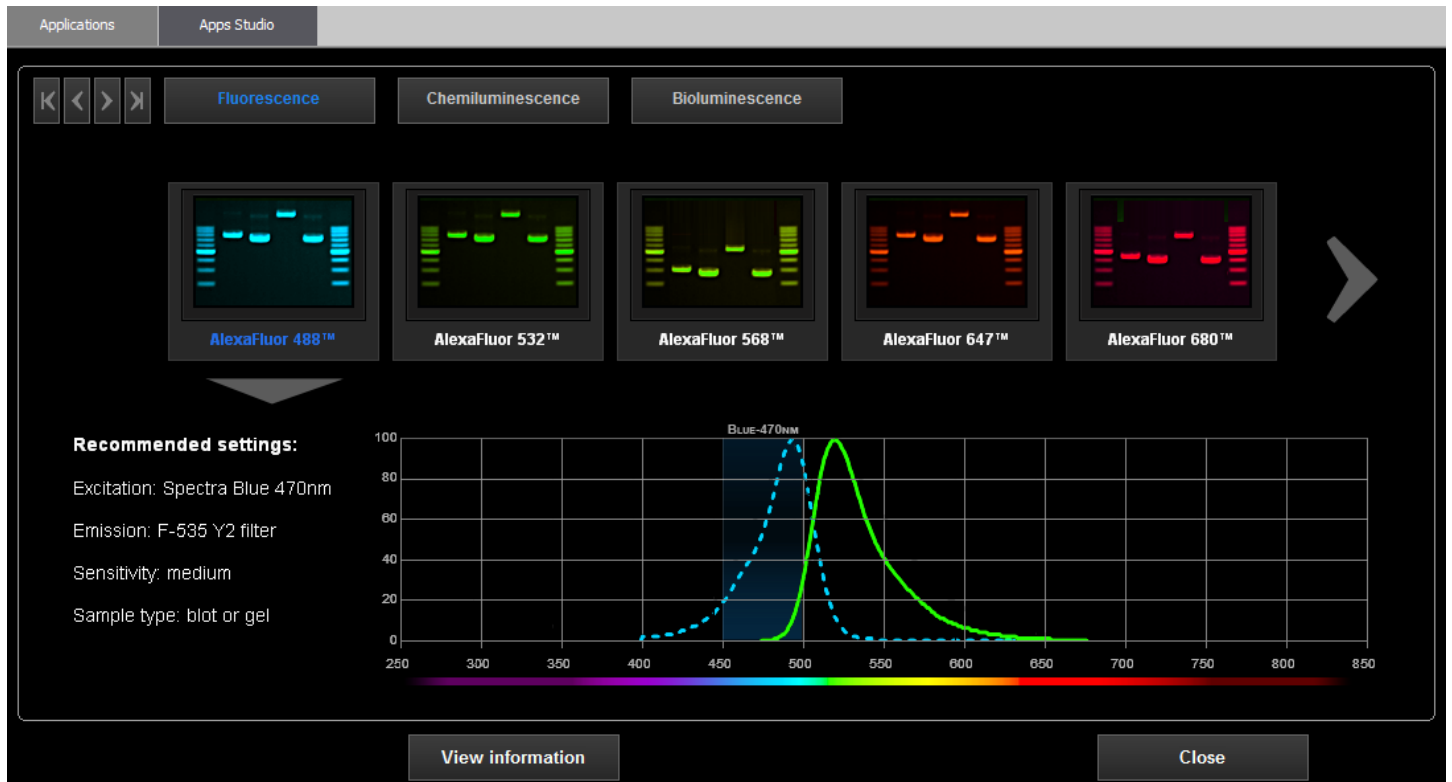
The applications library is organized into 3 chapters:
- Fluorescence
- Bioluminescence
- Bioluminescence

Select your chapter and then your application. If the configuration of your system allows it, you can load the application protocol and auto-expose your sample directly from the Apps Studio.
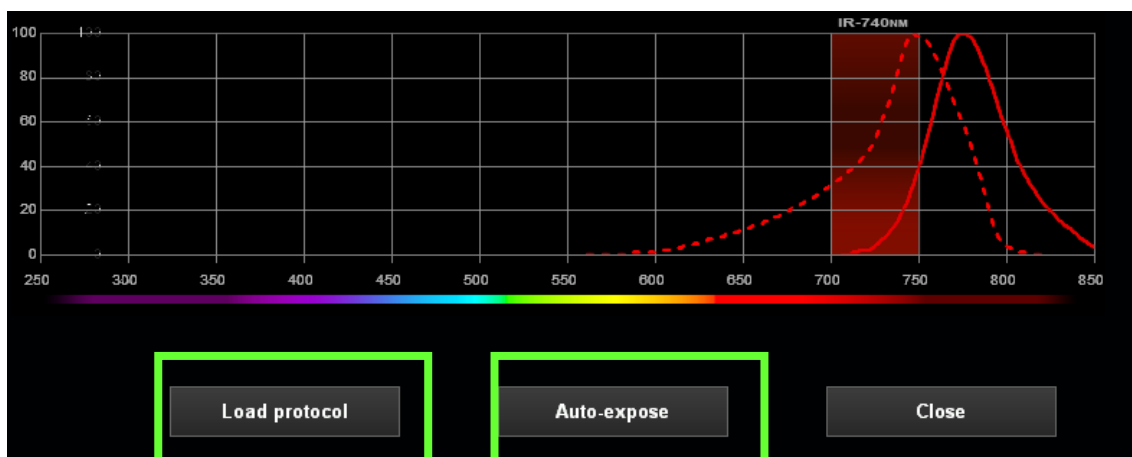

Multiplexing Application Protocol

Multiplexing applications types combine several single application protocols to facilitate the automatization of multiplex image capture.

Multiple application is a group of protocols. The group is a cluster of applications from which you can easily select one single application or another one within the group. It could gather the single application related to an imaging

mode: for instance the Spectra Capsule Blue, Green or Red. The user can select the single application within the list. Each single application runs independently of the other applications of the group.

Multiplexing images protocols allow you to group up to four different applications.
Select the applications to be grouped from the single image application protocols lists and save your Multiple protocol:
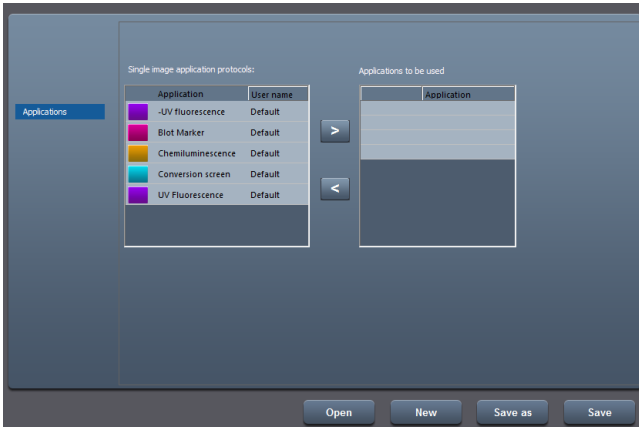
From the software main menu, the Sequence imaging is displayed when a Sequence application type is selected:

Click on start to launch the images acquisition process:

The system will automatically start the imaging workflow defines in the multiple images application protocol.

Note: With long integration time, a delay could be necessary before an image is displayed on the monitor. The acquisition will stop automatically at the end of the exposure time.

The System settings

Settings feature allows the adjustment of the system settings such as the filter controls or the lens calibration. Click on "Settings" from the icon barre.

A window displays the following menu:
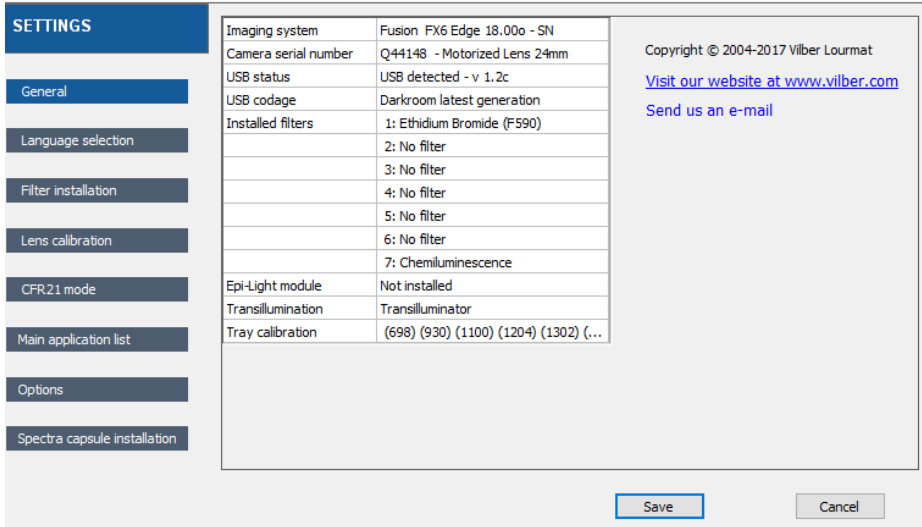
The Settings menu contains the following features, related to the Newton system:

General	Displays the Newton information such as the system option, the software version, the filters installed and the lens calibration state.
Language selection	Select the language you want the software to run
Filter installation	Install or remove a filter from the filter wheel
Lens calibration	Re-calibrate the lens for a perfect positioning of the focus for the preset tray and transilluminator position
CFR21 mode	Activate the CFR21 mode
Main application list	Manage the list of applications which appear in the application protocol folder.
Options	Manage the list of options .
Spectra Capsule Installation	Install or remove a capsule from the capsule holder.

The general system settings

The General header from the Settings menu summarizes the information concerning your system and its set-up. It gives you access to the following information:
⇨ Systems name & version
⇨ Camera and darkroom state
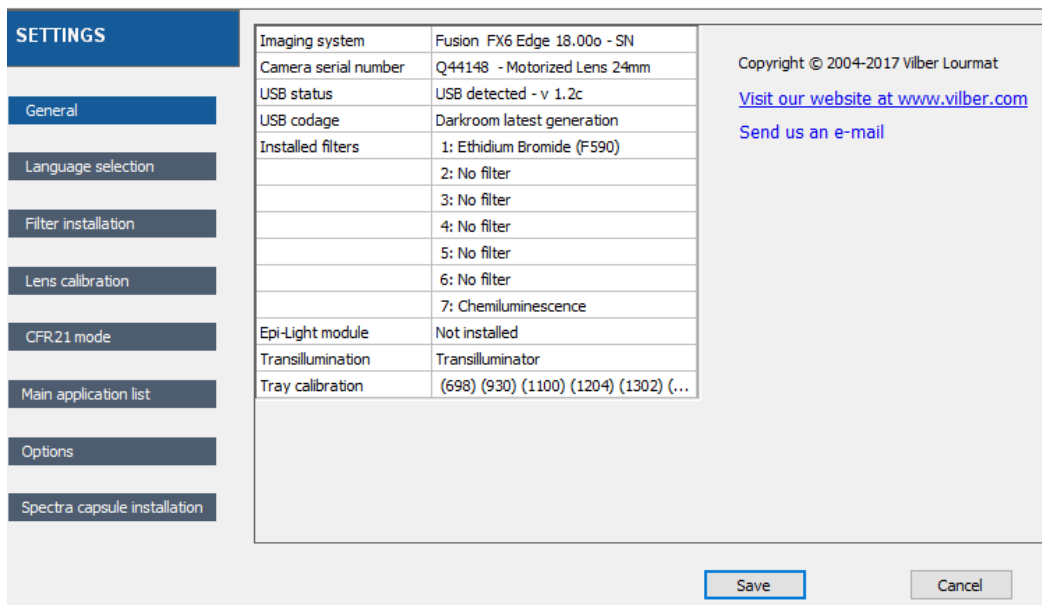
⇨ Options installed
⇨ Calibration stat

Imaging system	Fusion FX6 Edge 18.00o - SN
Camera serial number	Q44148 - Motorized Lens 24mm
USB status	USB detected - v 1.2c
USB codage	Darkroom latest generation
Installed filters	1: Ethidium Bromide (F590)
	2: No filter
	3: No filter
	4: No filter
	5: No filter
	6: ND-8
	7: Chemiluminescence
Epi-Light module	Not installed
Transillumination	Transilluminator
Tray calibration	(707) (950) (1114) (1221) (1293) (...

To proceed, select the General header from the Settings menu.

You can open the pdf manual of the system by clicking on the View pdf manual button.

To close the Settings menu, click on [Save] or [Cancel]

Language selection

The software software could run in English, in German, and in French. Select your language from the list. To proceed, select the Language selection header from the Settings menu:
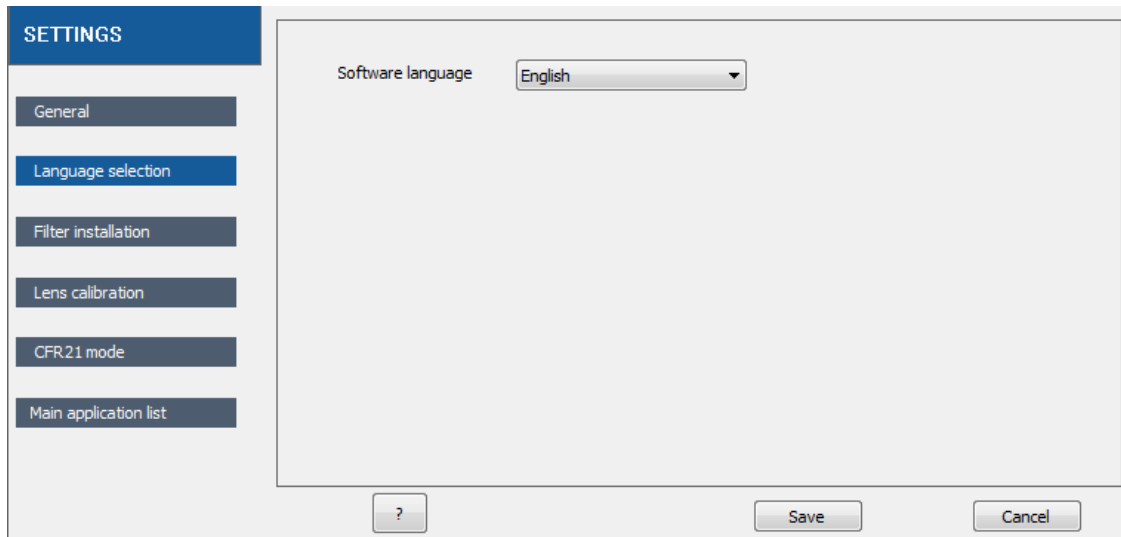
Then select the appropriate language from the pop-up windows:
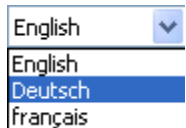
You will be prompt to restart the software for the change to occur.

To close the Settings menu, click on [Save] or [Cancel]

Filter installation

The Filter installation function is designed to facilitate the camera emission filter installation and its record in the software.
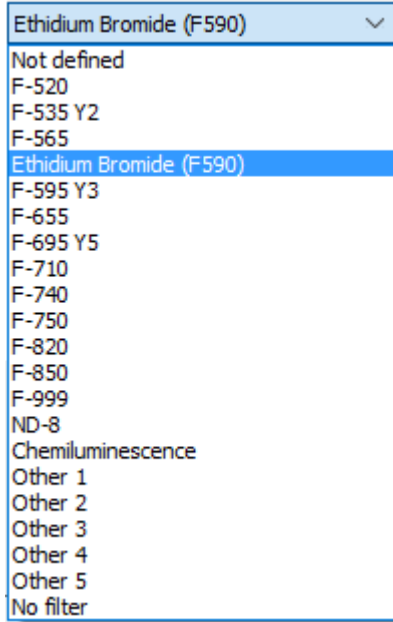
By default, the position 1 is kept for the Ethidium Bromide emission filter (F590 for the blue option, F999 for the SkyLight option). The position 7 is for bioluminescence applications.
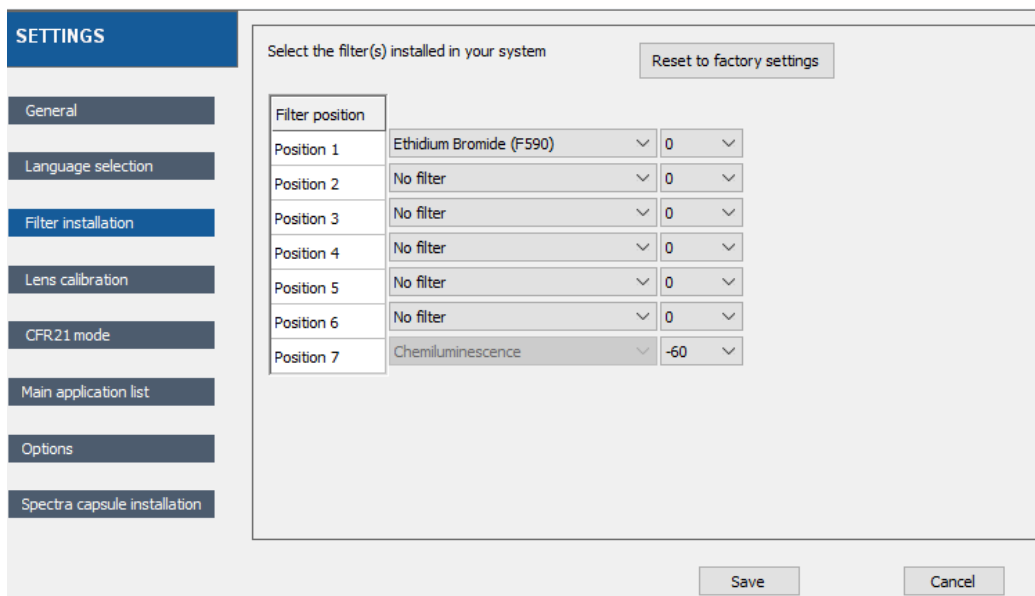
To add a filter:
1- Make sure the door of the Newton is closed
2- Select a filter position and click on the arrow to open the filter list

The filter list window displays the following filter option:

Ethidium Bromide (F590)
Not defined
F-520
F-535 Y2
F-565
Ethidium Bromide (F590)
F-595 Y3
F-655
F-695 Y5
F-710
F-740
F-750
F-820
F-850
F-999
ND-8
Chemiluminescence
Other 1
Other 2
Other 3
Other 4
Other 5
No filter

3- Select the filter n you want to add the filter wheel position by clicking on it:
4- Click on Set to filter position. The filter wheel is then conveniently positioned to the described position. Open the Newton door and position the filter.

Install the filter on the hole in front of you by turning clockwise in the thread groove on the inside surface of the filter wheel.

To close the Settings menu, click on [Save] or [Cancel]

Lens calibration – Newton FX series

The Newton FX series have a lens focusing pre-calibration for the standard sample height and for the following positions: tray 1, 2, 3 and 4 and transilluminator level.

You might have to recalibrate the system in the case your images are not sharp anymore for a standard sample height in any of the standard sample position: tray 1, 2, 3 and 4 and transilluminator level.

In the event you need to recalibrate the lens focusing, select the Lens calibration header from the Settings menu.
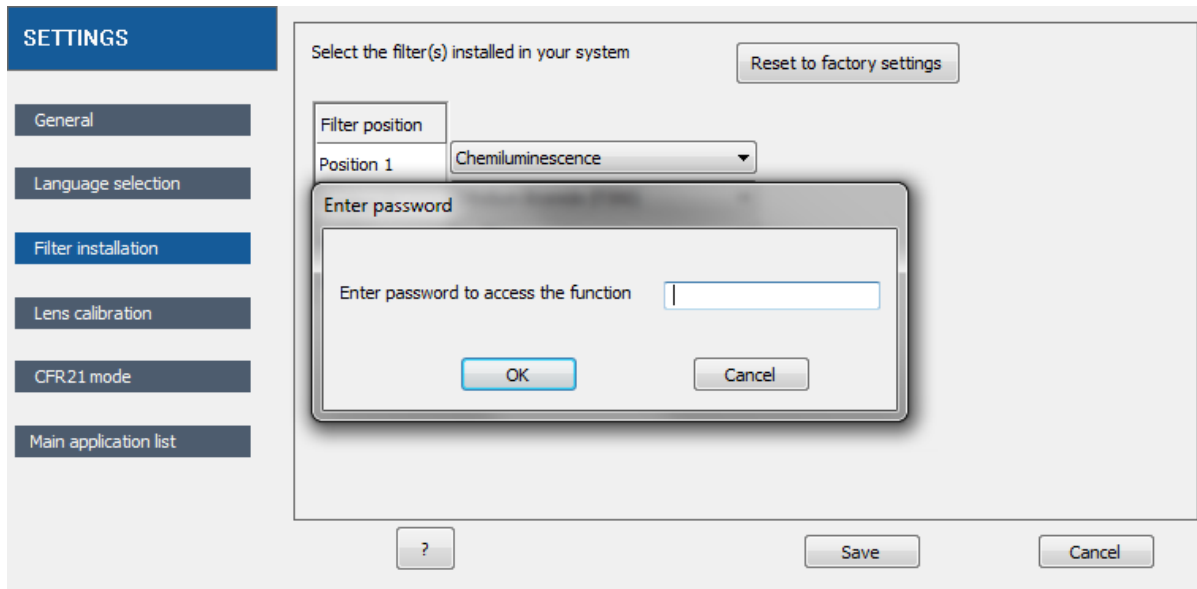
You will be prompted to enter a password to access the function. Please contact your local dealer to obtain this password. You will then access the following menu:
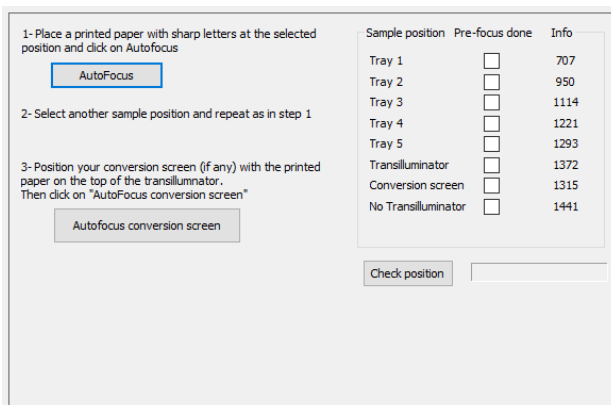
From the Newton installation CD-Rom, print the focal point test card BMP document (Focussing-target.bmp) in a white paper using the higher printer quality.

Install the focal point test card in the tray 1 and click on autofocus.
Repeat the same for the other positions (tray 2, 3 and 4 and transilluminator level)

To close the Settings menu, click on [Save] or [Cancel]

For the conversion screen position, install the conversion screen on the top of the transilluminator. Then, click on Autofocus conversion screen.

CFR21 mode activation

The 21-CFR mode is a closed system which ensures data security. These features are provided to ensure protection of records:
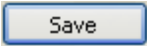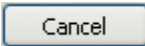- The image data is saved in a document which contains all the actions taken to results in this image

- The image document is saved is a proprietary file format which can be open only with the Vilber's software set in the 21-CFR mode
- The data are saved with the user security of the signed-in user
- Records created on a 21 CFR 11 system cannot be opened on a non-21 CFR 11 system

To activate the 21-CFR mode, you will need the CFR21-Administrator software. Please contact your local dealer for more information.

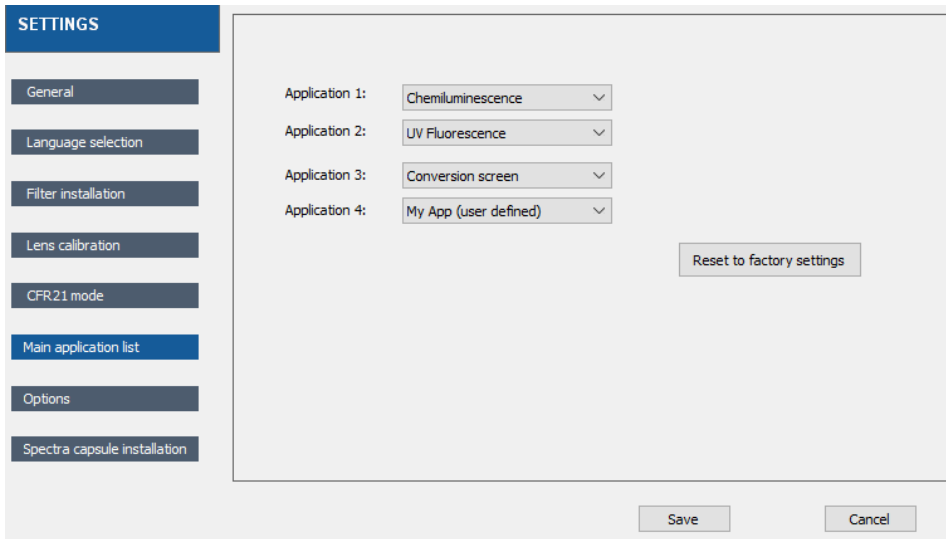From the software, select the CFR21 mode header from the Settings menu and click on Activate the CFR21 mode:
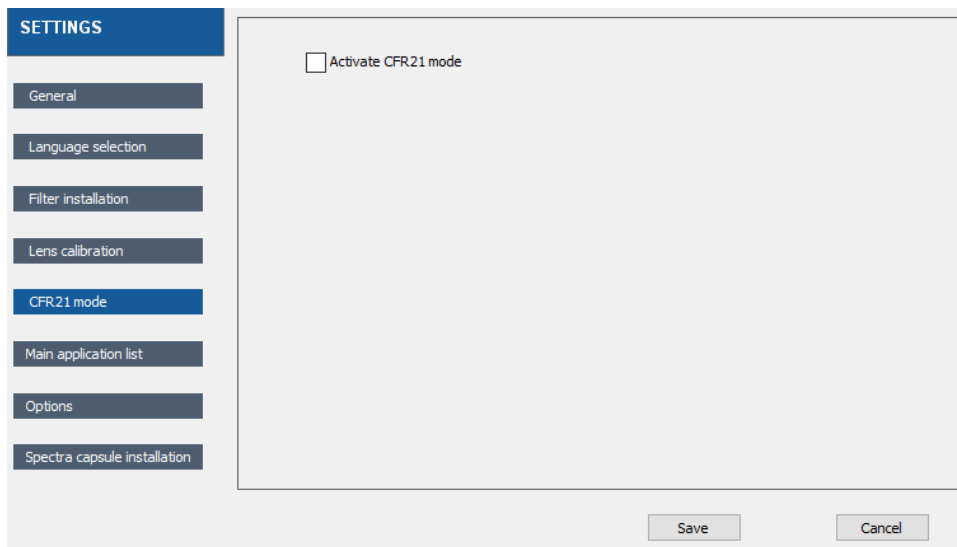
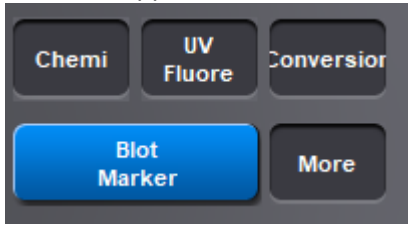To close the Settings menu, click on or

Main application list

The main application list lock the default applications protocol which appear in the image acquisition menu:


To unlock the list, select the My App (user defined) application. The user will then be able to change the application from the image acquisition menu.
If a specific profile is selected, this profile cannot be modified from the image acquisition menu.

Options

The Options folder contains the following system settings:
- Keep preview image in the list of images;
- Add marker function uses Paste Marker instead of Merge Marker;
- Disactivate laser lighting;
- Image DPI information
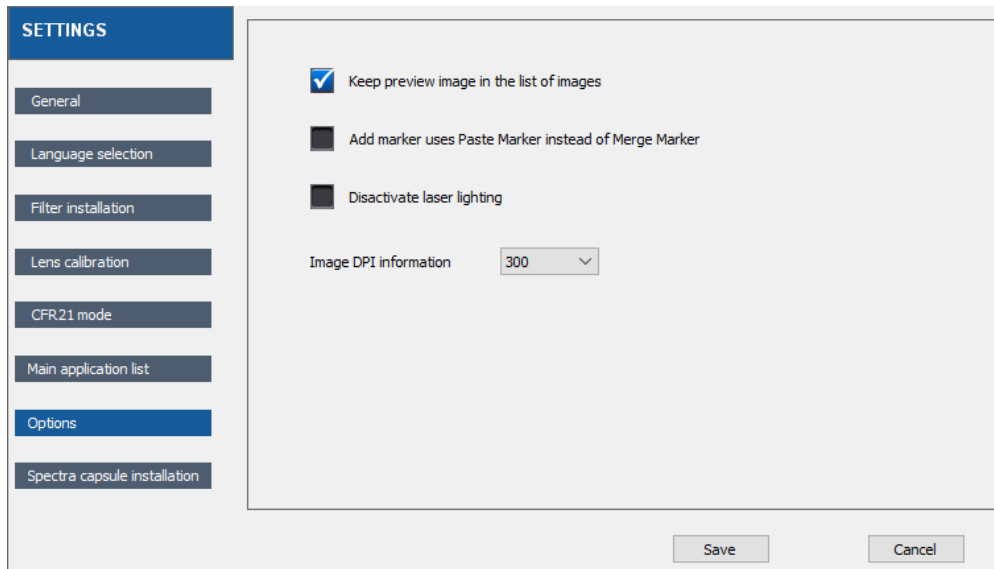
• Keep preview image in the list of images
The preview image could either appear in the list of images of the images tab or remain in the acquisition view.
If the option is selected, all preview image will open an image tab:

By default, this option is OFF.

• Add marker function uses Paste Marker instead of Merge Marker
Select from the Add Marker or the Marge Marker function the automatic merging function which appears on the main acquisition menu.
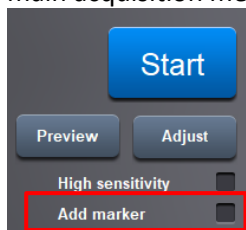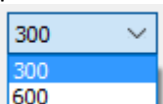

• Disactivate laser lighting
By selecting this options, the lasers remain OFF when the door is opened.

• Image DPI information
Dots per inch (DPI) is a measure of spatial printing dot density, in particular the number of individual dots that can be placed in a line within the span of 1 inch (2.54cm). You can choice from 300 to 600 DPI:

Spectra Capsule Installation

The Spectra Capsule installation function is designed to facilitate the installation of the Capsules and its record in the software. This function switch off the power supply of the capsule holder which is compulsory for installation.
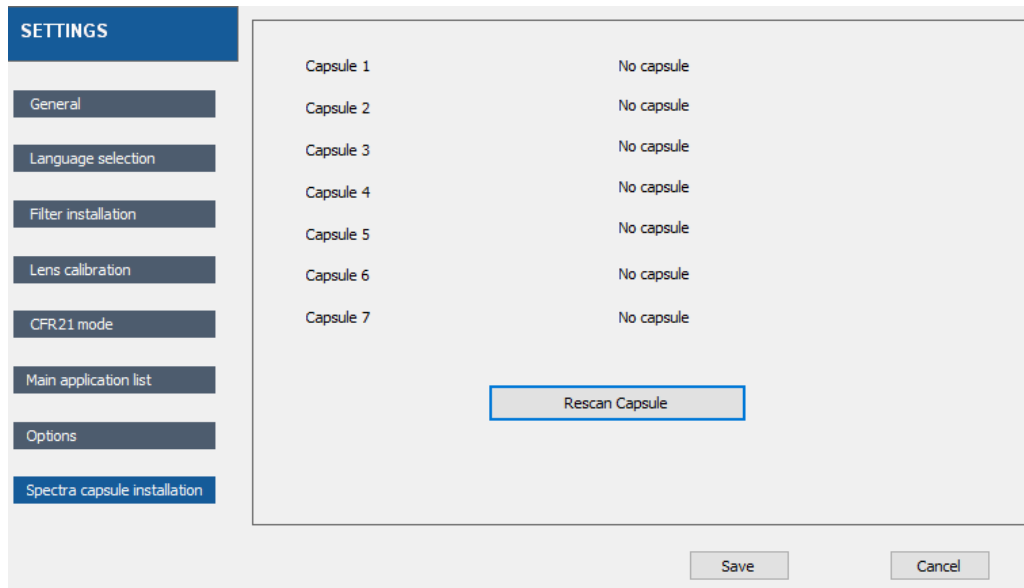
To add a Capsule:
1- Open the Spectra capsule installation menu
2- Open the door of the Newton
3- Insert the Capsule in its holder
4- Click on Rescan Capsule to record the installation in the software.

To close the Settings menu, click on [Save] or [Cancel]