Vilber is pleased to announce the release of a new Western Blot immunodetection process to enhance our current Western Blot and fluorescent documentation product portfolio, Fusion Purity.

This Sales Manual is intended to provide a thorough collection of product information for sales and training support.
Thank you

Dear Customer,

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

In order to learn the capabilities of your Fusion 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 Lourmat 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:
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   D-88436 Eberhardzell
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   T.: 49 (0) 7355 931 380

Do not hesitate to visit our website at www.vilber.com
### Table of contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notice</td>
<td>6</td>
</tr>
<tr>
<td>Trademarks</td>
<td>6</td>
</tr>
<tr>
<td>Introduction</td>
<td>7</td>
</tr>
<tr>
<td>Prerequisites</td>
<td>7</td>
</tr>
<tr>
<td>About the Fusion imaging system</td>
<td>7</td>
</tr>
<tr>
<td>Warranty</td>
<td>8</td>
</tr>
<tr>
<td>Safety instruction</td>
<td>10</td>
</tr>
<tr>
<td>Introduction</td>
<td>10</td>
</tr>
<tr>
<td>Safety notices</td>
<td>10</td>
</tr>
<tr>
<td>General precaution</td>
<td>11</td>
</tr>
<tr>
<td>Important UV safety information</td>
<td>13</td>
</tr>
<tr>
<td>Important electrical power safety information</td>
<td>15</td>
</tr>
<tr>
<td>Minimum computer configuration</td>
<td>18</td>
</tr>
<tr>
<td>Software installation</td>
<td>19</td>
</tr>
<tr>
<td>Prerequisite</td>
<td>19</td>
</tr>
<tr>
<td>Fusion-Capt software installation</td>
<td>19</td>
</tr>
<tr>
<td>Evolution-Capt software installation</td>
<td>29</td>
</tr>
<tr>
<td>Trouble shooting – Windows device manager</td>
<td>34</td>
</tr>
<tr>
<td>Configure your PC to improve the system performance</td>
<td>37</td>
</tr>
<tr>
<td>Bios settings – Disable the CPU EIST function</td>
<td>37</td>
</tr>
<tr>
<td>USB Advanced Power options</td>
<td>37</td>
</tr>
<tr>
<td>USB Hub Advanced Power options</td>
<td>39</td>
</tr>
<tr>
<td>Software operation</td>
<td>41</td>
</tr>
<tr>
<td>Introduction</td>
<td>41</td>
</tr>
<tr>
<td>Start the Fusion system &amp; software</td>
<td>42</td>
</tr>
<tr>
<td>Position your sample</td>
<td>43</td>
</tr>
<tr>
<td>Capture an image</td>
<td>45</td>
</tr>
</tbody>
</table>
Chemiluminescence image acquisition .................................................................................. 45
Fluorescence image acquisition .......................................................................................... 45
Image capture workflow ...................................................................................................... 45
Choose the application type .............................................................................................. 46
Define the exposure mode ................................................................................................. 48
Acquire the image ................................................................................................................ 50
Visualize your image .......................................................................................................... 62
Save and print ....................................................................................................................... 75

Analyse the image .............................................................................................................. 80
Access the analysis software.............................................................................................. 80
Analysis software interface ............................................................................................... 80
Molecular weight analysis module ..................................................................................... 90
Detect – Edit lane ................................................................................................................ 90
Detect – Edit bands ............................................................................................................ 93
Detect – Add marker values ............................................................................................. 96
Molecular weight results .................................................................................................. 101
Return to the image acquisition module .......................................................................... 102

Quantification analysis module ........................................................................................ 103
Detect – Lane definition .................................................................................................. 103
Background subtraction ..................................................................................................... 106
Spot separation .................................................................................................................. 111
Spot quantification ............................................................................................................ 114
Quantity of reference ....................................................................................................... 115
Return to the image acquisition module .......................................................................... 118

Post process the image ..................................................................................................... 119
Access the Edit post process menu .................................................................................. 119
Add text ............................................................................................................................... 120
Crop the image .................................................................................................................. 123
Rotate the image ................................................................................................................. 124
Merge marker ..................................................................................................................... 127
The System s

The Application protocol

New application – Single, Sequence or Multiple applications types

Advanced exposure – Exposure predefined set-up

Preview – Live preview pre-defined parameters

Display – Image display pre-defined parameters

Printing – Image printing pre-defined parameters

General – Image file pre-defined parameters

Processing – Applying post process function to an acquired image

The Apps studio

The System settings

The general system settings

Language selection

Filter installation

Lens calibration – Fusion FX series

Lens calibration – Fusion SL series

CFR21 mode activation

Main application list

Paste marker
Background subtraction
Flat field
Artifact correction
Multiplexing
Bioluminescence

The Application protocol

What is an Application protocol?

How to access the application protocol?

Paste marker ................................................................. 130
Background subtraction .................................................. 134
Flat field ........................................................................ 135
Artifact correction .......................................................... 136
Multiplexing .................................................................... 136
Bioluminescence ............................................................. 139
The Application protocol ..................................................... 143
What is an Application protocol? ....................................... 143
How to access the application protocol? .............................. 143
New application – Single, Sequence or Multiple applications types ......................................................... 144
Advanced exposure – Exposure predefined set-up .................. 149
Preview – Live preview pre-defined parameters .................... 153
Display – Image display pre-defined parameters .................. 156
Printing – Image printing pre-defined parameters ................. 158
General – Image file pre-defined parameters ........................ 159
Processing – Applying post process function to an acquired image ................................................................. 161
The Apps studio ................................................................ 162
The System settings .......................................................... 164
The general system settings ................................................ 164
Language selection .......................................................... 166
Filter installation ............................................................. 166
Lens calibration – Fusion FX series ..................................... 168
Lens calibration – Fusion SL series ..................................... 170
CFR21 mode activation ..................................................... 171
Main application list ......................................................... 172
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Introduction

Prerequisites

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

In order to operate the Fusion 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 UV 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 Fusion imaging system.
• Some Fusion configurations involve UV illumination. This instrument should be used only by trained personnel who know the health risks associated with the UV radiation normally associated with this instrument. Users should be trained on the appropriate personal protection gear for working with UV light to minimize UV exposure.

About the Fusion imaging system

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

The following systems are controlled by the Fusion-Capt software:
• Fusion FX7 and Fusion SL7.
• Fusion Pulse 7.
• Fusion Solo 7S.

The following systems are controlled by the Evolution-Capt software:
• Fusion FX6 and Fusion SL6.
• Fusion Pulse 6.
• Fusion Solo 6S and Solo 6X.
A cooled scientific CCD camera is used to capture high resolution digital images of protein and DNA bands in gels and on membranes obtained by electrophoresis or Western blotting separation methods. The instrument can capture images of chemiluminescent, fluorescent, and colorimetric samples, depending on the system configuration. The instrument can be used for research purposes in the academia and life sciences industry. The instrument cannot be used for diagnostic purpose.

Fusion 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 Fusion, you simply reach the lowest limits of detection on all of your samples.

Warranty

The Fusion 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, UV tubes ...), 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
The Fusion imaging system is powered by mains voltage, may be equipped with a UV light source, 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 and UV 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.

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

UV WARNING indicates an imminently hazardous UV radiation situation which, if not avoided, could result in 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 Fusion 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 Fusion imaging system in dangerous atmosphere or with dangerous materials for which the Fusion 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 not 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.
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 person 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 UV 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.

---

**Important UV safety information**

**UV WARNING**

In any UV WARNING situation, the operator should wear appropriate safety glasses or a protective mask and gloves. UV radiation can be dangerous for unprotected eyes and skin; therefore we recommend the user to wear UV protective goggles (LP-70) or face-shield (MP-80 or MP-800). Protect all skin surfaces (including the neck, ears, and hands).

Use of the UV transilluminator acrylic protective screen does not guarantee the user protection from UV radiation. The use of protective goggles, mask, and/or gloves is strongly recommended.

This instrument should be used only by trained personnel who know the health risks associated with the UV radiation normally associated with this instrument. Users should be trained on the appropriate personal protection gear for working with UV light to minimize UV exposure.

In its lowered position, the UV-Pad or UV transilluminator’s acrylic shield provides UV protection. However, it does not provide complete protection to the user. In its raised position, the acrylic shield does not protect others who are standing in the area around the imager.

**UV WARNING**

Do not touch the UV unit after UV exposure. There is a risk of skin burn.
UV WARNING
The instrument is equipped with a safety interlock. If the interlock is out of order or has been tampered with, UV and visible light may be emitted, which may cause skin burn and impair vision. Do not open the instrument door during imaging operation.

WARNING: The non-respect of these instructions can cause very serious burns to the user when the instrument is in use.
WARNING: Ultra Violet light is dangerous for unprotected eyes and skin, therefore the user must wear UV protective goggles (Ref. LP70) or a face-shield (Ref. MP-80 or MP-800)

SAVE THESE INSTRUCTIONS

AVISO: Durante el uso del instrumento, no seguir las instrucciones puede causar quemaduras graves al usuario.
PRECAUCION: La radiación ultravioleta puede ser peligrosa para los ojos y la piel expuestos sin protección. Para protegerse, es impérativo usar gafas o una máscara.

CONSERVAR CUIDADOSAMENTE ESTAS INSTRUCCIONES.

AVERTISSEMENT: Lors de l’utilisation de cet appareil, le non-respect des instructions peut provoquer de graves brûlures à l’utilisateur.
ATTENTION : Le rayonnement ultraviolet est dangereux pour les yeux et la peau exposée sans protection, il est impératif de porter des lunettes (Réf. LP70) ou un masque de protection (Réf. MP-80 ou MP-800).

CONSERVER SOIGNEUSEMENT CES INSTRUCTIONS

AVVERTIMENTO : Durante l’utilizzo di questo apparecchio, il non rispetto delle istruzioni può provocare bruciature gravi all’utilizzatore.
ATTENZIONE : I raggi Ultra Violetti essendo pericolosi per gli occhi e la pelle esposti senza protezione, è obbligatorio portare gli occhiali (Ref. LP 70) oppure una maschera di protezione (Ref. MP-80 o MP-800).

CONSERVARE CON CURA QUESTE ISTRUZIONI.

WARNUNG: während der Benutzung dieses Gerätes kann die Missachtung der Anleitung schwere Verbrennungen an Personen hervorrufen.
ACHTUNG: Ultraviolette Licht ist gefährlich für ungeschützte Augen und die Haut. Der Benutzer ist daher angehalten, eine UV-Schutzbrille (Art. LP-70) oder ein Schutzschild (Art. MP-80 oder MP-800) zu tragen.
### Important electrical power safety information

**WARNING**

Connect the Fusion imaging system to an appropriate AC voltage outlet that is properly grounded and protected by a circuit breaker. Connecting to ground constitutes an obligatory protection.

![Warning Icon]

Connect the Fusion imaging system to an appropriate AC voltage outlet that is properly grounded and protected by a circuit breaker. 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 Fusion 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 Fusion 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 the equipment 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.
Minimum computer configuration

**Main board**: Desktop computer type

**Processor**: Intel Corei5 and upper recommended.

**Operating system**: Windows 7, Windows 8.1

**Memory**: 4 GB ram and above

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

**CD-Rom**: CD player necessary to install the software

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

**USB port**: At least two USB ports available in the computer rear side. One USB-3 port is necessary for the camera.

---

**NOTICE**
Windows 7 and Windows 8.1 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 the Windows power options.

---

**NOTICE**
Windows 7 and Windows 8.1 must be installed in the computer intended to be used to control the Fusion imaging system.

Please run completely the Windows Update before installing the Fusion’s software.
Software installation

Prerequisite

**WARNING**
The Fusion system should be **switched off** before any software installation.

**WARNING**
Please run completely the Windows Update before installing the Fusion’s software.

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

Fusion-Capt software installation

**NOTICE**
The following systems are controlled by the Fusion-Capt software:
- Fusion FX7 and Fusion SL7.
- Fusion Pulse 7.
- Fusion Solo 7S.

Please refers to the CD-Rom software name to ensure you have the appropriate software for your system.
Please ensure the Fusion 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 7 and Windows 8.1 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 Fusion Setup window will appear, welcoming you to the Install Wizard for Fusion-Capt Setup.
Please click on the Install software icon.

A pop-up window displays the following Windows installation authorization message:

![Image of Windows Security pop-up window]

Click on the “Install” button. The setup wizard will then start to initialize. This could take up to 1 minute:

![Image of Software installation progress]

The setup wizard will then start the installation process:
Step 2. ➔ The welcome screen is displayed, click on NEXT to continue.

Click on NEXT to continue.
➢ Select the Destination directory ("c:\Program files\Vilber Lourmat\Fusion" by default).
Then click on NEXT. Program files are installed in the specified directory and group created:
When the software installation is complete, click on Finish. The system will then start the driver installation process:

The setup wizard will then install the USB controller driver.

Click on Install to install the system’s USB driver.

When finished, click on OK.

A pop-up window displays the following camera driver installation message:
Select the “I accept the terms in the License Agreement” option

Then, click on the “Install” button. The setup wizard will then install the camera driver.
A pop-up window displays the following camera software installation message:

```
Installing Scientific Camera Support Library

Please wait while the Setup Wizard installs Scientific Camera Support Library.

Status:
```

Then, click on the “Install” button. The setup wizard will then install the camera software.
Then, click on the “Install” button. The setup wizard will then install the camera software.

Once the software is installed, the system is updated. This can take a few minutes, depending on the computer speed. Then, you might be requested to restart your computer:
After the restart, switch on the Fusion system. Keep the Fusion-Capt CD-Rom in the CD-Rom drive of your computer. The darkroom driver is then installed by Windows:

![Installing device driver software](image)

This driver is related to the Fusion darkroom management.

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

The camera driver is then installed by Windows:

![Installing device driver software](image)

The following message should appear for the Fusion FX7 and SL4:

![Scientific USB Camera](image)

The following message should appear for the Fusion FX5 and SL2:

![Scientific FireWire Camera](image)

You can now properly use the Fusion software and hardware.
Evolution-Capt software installation

**NOTICE**
The following systems are controlled by the Evolution-Capt software:
- Fusion FX6 and Fusion SL6.
- Fusion Pulse 6.
- Fusion Solo 6S and Solo 6X.

Please refer to the CD-Rom software name to ensure you have the appropriate software for your system.

Please ensure the Fusion 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 7 and Windows 8.1 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 Fusion 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
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 Fusion system. Keep the Fusion-Capt CD-Rom in the CD-Rom drive of your computer.

The darkroom driver is then installed by Windows:

![USB Scientific Camera V2](image)

You must restart your system for the configuration changes made to USB Scientific Camera V2 to take effect. Click Yes to restart now or No if you plan to restart later.

When finished, click on Yes to restart the computer.

The darkroom driver is then installed by Windows:

![Installing device driver software](image)

Click here for status.

This driver is related to the Fusion darkroom management.

Wait for 10 seconds and double click on the Fusion icon: [image]

The camera driver is then installed by Windows:

You can now properly use the Fusion 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; For Windows 7 or Windows 8, select System and security, then system, then Device Manager:
The Device Manager should appear as below for the Fusion FX7, Pulse 7, SL7 and Solo 7:
The Device Manager should appear as below for the Fusion FX6, Pulse 6, SL6 and Solo 6:
Configure your PC to improve the system performance

This tech note outlines known good practices which are likely to improve camera performance. Our imaging system is a real-time streaming devices 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 Fusion 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.

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](#).

<table>
<thead>
<tr>
<th>Plans shown on the battery meter</th>
<th>Change plan settings</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Balanced (recommended)" /></td>
<td>Automatically balances performance with energy consumption on capable hardware.</td>
</tr>
<tr>
<td><img src="image" alt="High performance" /></td>
<td>Favors performance, but may use more energy.</td>
</tr>
</tbody>
</table>

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

Select a USB hub from the Universal Serial Bus controllers menu and right click to open its Properties.
From the Properties window, select Power Management and disable the “Allow the computer to turn off this device to save power” option:

Repeat for all the USB Hub found in the Universal Serial Bus Controllers menu of the Windows Device Manager.
Software operation

Introduction
This chapter describes how to operate the Fusion imaging system.

Before to operate the Fusion 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 Fusion system in any other way than described in the User Manual.

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

The standard imaging process is as follows:

1. Start the Fusion system & software
2. Position your sample
3. Capture an image
4. Visualize your image
5. Save and print
6. Analyse or post-process
Start the Fusion system & software

Switch on the Fusion 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 Fusion system. Wait for 10 seconds and double click on the Fusion icon: . The software opens on the image acquisition window.

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

![Fusion System Message 1](image1.png)

![Fusion System Message 2](image2.png)

NOTICE
The Notification window provides information on the system settings and status.

![Notification Window](image3.png)

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 sample tray. The Fusion imaging system supports the following sample trays, according to your system configurations:

<table>
<thead>
<tr>
<th>Application</th>
<th>Samples</th>
<th>Tray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemiluminescence</td>
<td>Blot</td>
<td>Black tray</td>
</tr>
<tr>
<td></td>
<td>Titer plate</td>
<td>Black tray</td>
</tr>
<tr>
<td></td>
<td>Plant</td>
<td>Black tray</td>
</tr>
<tr>
<td>UV fluorescence (transillumination)</td>
<td>Gel or any UV transparent sample</td>
<td>UV-Pad or UV transilluminator (1)</td>
</tr>
<tr>
<td>Blue fluorescence (transillumination)</td>
<td>Gel or any transparent sample</td>
<td>Sky-Pad or blue conversion screen (1)</td>
</tr>
<tr>
<td>Spectra fluorescence (EPI blue, green, red, IR)</td>
<td>Gel</td>
<td>Spectra-Pad or black tray (1)</td>
</tr>
<tr>
<td>Colorimetric (White light transillumination)</td>
<td>Gel</td>
<td>White-Light-Pad or White light conversion screen (1)</td>
</tr>
<tr>
<td>Colorimetric (EPI white light)</td>
<td>Blot</td>
<td>Black tray</td>
</tr>
</tbody>
</table>

(1) The type of tray depends of the Fusion series

Open the door of the Fusion system.
Position the gel or blot on the appropriate sample tray.

For blot or opaque samples, the black tray is removable from the Fusion’s cabinet to help you to position your sample and to eventually add the ECL substrate outside the darkroom.

The Fusion system has 4 alternative tray positions. The position to be used varies according to the size of the sample. Use the upper tray position for a smaller field of view of a blot and a shorter exposure time. Lower the tray position to get a larger field of view.
For the Fusion FX7, the Fusion SL7, the Fusion Pulse 7 and the Fusion Solo 7S, the estimated field of view at each tray position is as followed
- Tray 1: 100x100
- Tray 2: 120x120
- Tray 3: 140x140
- Tray 4: 160x160

For the Fusion FX6, the Fusion SL6, the Fusion Pulse 6, the Fusion Pulse TS6 and the Fusion Solo 6S, the estimated field of view at each tray position is as followed
- Tray 1: 85x70
- Tray 2: 95x75
- Tray 3: 110x90
- Tray 4: 130x100

For gels, you can pull out the transilluminator drawer for easier access to the UV filter. Push the transilluminator drawer in and close the main door. If you have a gel application, ensure the black tray does not obstruct the camera field of view before closing the door.
Capture an image
This chapter describes the steps to acquire an image. It is organized around the following workflows:
- Methods for chemiluminescence image acquisition
- Methods for fluorescence image acquisition

Chemiluminescence image acquisition
The chemiluminescence image capturing method is available in all Fusion systems configurations. Chemiluminescence is the main method used to detect proteins in Western blotting. The chemiluminescent reaction occurs when an enzyme such as horseradish peroxidase (HRP) or alkaline phosphatase reacts with a chemiluminescent substrate (such as luminol or dioxetane) to produce a weak signal. The emitted light has a wavelength between 400 and 500 nm.

The Fusion system captures the light which is emitted during enzymatic reaction on the blot. When a colorimetric marker is present in the sample, an additional image can be captured using the EPI white light source.

Fluorescence image acquisition
Fluorescence is the main method used for gene expression and protein detection. It results from a process that occurs in molecules known as fluorophores. The fluorophore absorbs the excitation light, reaching a higher energy state. By returning to its former state, it emits fluorescent light.

The Fusion system separates the emitted light from the excitation light in order to obtain an optimum sample image. According to the configuration, the Fusion can accommodate up to 7 excitation and emission channels in the IR, NIR, visible and UV area and is ideal for a large array of applications such as Western blot fluorescence, 1D DNA gel, 1D protein colorimetric samples, multiplexing, stain free gel and blot.

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 Fusion 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 Fusion Application Protocol and can be selected from the Application Selector:

The Fusion 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 chemiluminescence sample, select the Chemiluminescence application from the Application Selector window:

For a UV excited gel sample, select the UV fluorescence application from the Application Selector window:

For a colorimetric sample using white light conversion screen, select the Conversion screen application from the Application Selector window:
Define the exposure mode
The Fusion 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 Fusion 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 Fusion system requires the user to specify the exposure time.
To set the exposure time, select Manual from the exposure mode menu:

For Chemiluminescence 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 chemiluminescence 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 Fusion 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 a specific area](image_url)

Define your area of interest. Then, click on the Continue button:

![Continue button](image_url)

**The Manual mode**

In a chemiluminescence 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.

![Stop button](image_url)

**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 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).
**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 frame 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:

![Save As Menu](image)

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 Fusion asks you then to define the serial image acquisition parameters:

![Serial Acquisition Menu](image)

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:

Example:

<table>
<thead>
<tr>
<th>Image 1</th>
<th>20s</th>
<th>20 second is the first exposure time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Image 2</td>
<td>20s</td>
<td>Image B: 30s = Image B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The increment time is 10s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Image B has the exposure time of the previous image (20s) plus the increment time (10s).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Image 2 is the result of the addition of image 1 plus Image B.</td>
</tr>
<tr>
<td>Image 3</td>
<td></td>
<td>Image 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Image 2 + Image C=40s = Image 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The increment time is 10s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Image C has the exposure time of the previous image (30s) plus the increment time (10s).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Image 3 is the result of the addition of image 2 plus Image C.</td>
</tr>
<tr>
<td>n Image</td>
<td></td>
<td>The number of images of the serie is defined by the Number of images parameter.</td>
</tr>
</tbody>
</table>

**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:

<table>
<thead>
<tr>
<th>Image 1</th>
<th>20s</th>
<th>20second is the Time per image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Image 2</td>
<td>20s</td>
<td>+ Image B : 20s = Image 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Image B has the exposure time of the previous image (20s)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Image 2 is the result of the addition of image 1 plus Image B.</td>
</tr>
<tr>
<td>Image 3</td>
<td></td>
<td>Image 2 + Image C : 20s = Image 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Image C has the exposure time of the previous image (20s).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Image 3 is the result of the addition of image 2 plus Image C.</td>
</tr>
<tr>
<td>n Image</td>
<td></td>
<td>...The number of images of the serie is defined by the Number of images parameter.</td>
</tr>
</tbody>
</table>
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:

<table>
<thead>
<tr>
<th>Image 1</th>
<th>20s–Image 1</th>
<th>20second is the Time per image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Image 2</td>
<td>20s–Image 2</td>
<td></td>
</tr>
<tr>
<td>Image 3</td>
<td>20s–Image 3</td>
<td></td>
</tr>
<tr>
<td>n Image</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>
  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:
Chemiluminescence kinetic signal

The Western blot chemiluminescence 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 UV 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 (Fusion 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.

• Fusion 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:
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.

Case 2:
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.

Case 3:
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 Fusion 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.

<table>
<thead>
<tr>
<th>Case 1:</th>
<th>Case 2:</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Default optimum display" /></td>
<td><img src="image2.png" alt="Optimum display enhancement" /></td>
</tr>
</tbody>
</table>

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](default_button.png)

**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 *](default_button_asterisk.png)

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

![Inverse](inverse_button.png)
Click on “Saturation” to displays the saturated pixels in red.

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 on 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.
By clicking on More from the Display menu, you could enter manually the lower and the upper display range values.

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

• **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:

- **Clarity™**  
  Clarity™ is a display filter which enhances the contrast and sharpness. Clarity does not affect the raw data.

<table>
<thead>
<tr>
<th>Case 1:</th>
<th>Case 2:</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="With Clarity™ display" /></td>
<td><img src="image2" alt="Without Clarity™ display" /></td>
</tr>
</tbody>
</table>

- **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:

<table>
<thead>
<tr>
<th>Original image</th>
<th>GFP painted image</th>
<th>Multicolor</th>
<th>Red painted image</th>
</tr>
</thead>
</table>
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 an image, a table or a graph onto the clipboard for insertion into another Windows program

Resize the image to fit the size of the monitor

Zoom in or out the image

**3D scan**

The Fusion 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:

  ![GLP data](Image)

  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: |
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 CompatibilityPlus 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 display 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.
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:

| All image Files (*.TIF;*.BMP;*.JP | All image Files (*.TIF;*.BMP;*.JPG;*.APX) |
| TIFF Files (*.TIF) | 8 bit Bitmap Files (*.BMP) |
| JPEG Files (*.JPG) | APX Files (*.APX) |

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:

![Print window]

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.
Analyse the image

Access the analysis software
Access the analysis menu by clicking on the Analyse button:

![Analyse buttons](image)

- Quantification
- Molecular Weight
- Bio-1D

Select the Molecular weight icon to open the molecular weight analysis (MW) module
Select the Optical density - 1D icon to open the optical density (OD) analysis module based on a 1D detection
Select the Bio-1D menu to open the image in the optional Bio-1D software.

Analysis software interface
The analysis module opens on the following window:

![Analysis software interface](image)

The analysis software operating environment is organized into three areas:
The dash board is different for each analysis module. For molecular weight, it contains the following tabs:

1. Detect
2. Analyse molecular weight (MW)
3. Home

For the Quantification analysis module, the dash board contains three different tabs:

1. Window definition
2. Spot quantification
3. Home

The image window displays the active image. It also contains the image toolbar:

- Print the image
- Set the band parameters (white bands / black background; black bands/ white background)
- Copy to clipboard
- Display the molecular weight on the image
- Change the image display parameters
- Autoscale
- Zoom in or out the image
Export the table to Microsoft Excel

Print

The Print 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.

Copy to clipboard
The Copy to clipboard function copies an image, a table or a graph onto the clipboard for insertion into another program. This option is identical to the Windows® [Ctrl C] command.
To proceed, click on the Copy to clipboard icon. The image, the table or the graph is now ready to be pasted into another application.

Open the application that you want to paste the image into, and select from the available pasting options ([Ctrl V] command for Windows® software).

**Black / White bands**

This function sets the bands parameters (white bands / black background / black bands, white background). To proceed, click on the Black/White bands icon. A pop-up window displays the following menu:

You can select the following options:

- **Automatic detection.** Capt software will automatically detect the bands color scheme according to the defined percentage of tolerance

- **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

**Copy to clipboard**

The Copy to clipboard function copies an image, a table or a graph onto the clipboard for insertion into another program. This option is identical to the Windows® [Ctrl C] command.
To proceed, click on the Copy to clipboard icon. The image, the table or the graph is now ready to be pasted into another application.

Open the application that you want to paste the image into, and select from the available pasting options ([Ctrl V] command for Windows® software).

**Display the molecular weight**
Click on the “display the molecular weight” to display the calculated molecular weight on top of the image.

![Default display](image1.png)  
![Molecular weight displayed on the image](image2.png)

**Image display parameter**
Click on the “Image display” to displays the Image Master data as well as the Image Display set-up.
The Image Master data refers to the dynamic range of grey levels in between the minimum and the maximum pixel intensities obtained in an image.

Image depth is expressed as graduation level. In an image, the density range between white and black is divided into a number of gradation levels. For instance, a 12-bit image has 4096 gradation levels. The image dynamic refers to the number of grey levels in between the minimum levels obtained and the maximum level obtained on a specific image.

The Image Master data informs you of the obtained dynamic on your image compared to the potential image depth.

Access the Image Display from the main software windows:

The optimum display window is helpful to enhance the image display by modifying the image greyscale selection to be displayed.

In standard, the Fusion 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.

For both values, you can:
- Edit the value in the corresponding field
- Select the value by dragging and dropping the arrow
- Click on the “optimum display” button: the software will then calculate the ideal values to be selected according to the parameters defined

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

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

**Inverse**

**Case 1:**

Positive image
(white spot, black background)

**Case 2:**

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 on 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.

<table>
<thead>
<tr>
<th>Case 1:</th>
<th>Case 2:</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Original display" /></td>
<td><img src="image2.png" alt="Same image with the saturated pixels indicated in red" /></td>
</tr>
</tbody>
</table>

**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:

- **Clarity™**
  Clarity™ is a display filter which enhance the contrast and sharpness. Clarity does not affect the raw data.
• **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:

![Palette options]

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

• **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

![Image zoom cases]

**Case 1:**

**Case 2:**
• Excel
This function transfers the results table to Microsoft Excel™.
To proceed, click on the Send to Excel™ icon. The Excel software is automatically opened by the Fusion software and the table is transferred to Excel™.

Molecular weight analysis module

The software Molecular Weight module features the calculation of electrophoretic distances according to markers or standards:
in molecular weight (unit: KiloDalton)
in fragment sizes (unit: Kilobases)

A regression method is used to calculate the molecular weight/size of the unknown bands. The software will use the molecular weight values of the marker (standard) bands to calculate the standard curve. This standard curve is then used to calculate the values of the unknown bands.

At the end of the process, you can have the following outputs:
- Molecular weight marker’s profile and migration curve
- Molecular weight values

Detect – Edit lane

The molecular weight module opens on the Edit lane dashboard of the Detect process:
The dashboard details the lane edition parameters:

- **Area of interest**
  On the image, click and drag to define the analysis area and to overlap the lanes. You can easily adjust the size of the area by clicking on the tags surrounding the area and drag the selected border to the requested size.

The lanes are defined by green lines, overlaid on the gel image. The gel area is surrounded by square anchors:

To resize the entire lane frame, drag an anchor point in or out. The opposite anchor point will remain fixed while the frame expands or contracts. The frame will expand or contract from the center.

To move the entire frame to a new position, position the mouse on the frame to obtain a cross cursor:
Click and drag the cursor to move the entire frame.

**Note:** It is not necessary to include the well line in the area of interest window. The calculation of molecular weight does not require this reference line.

- **Lane direction**
  Select the direction of the lanes from horizontal or vertical

The lane direction is automatically modified on the image.

- **Number of lanes**
  Select the number of lanes:

  ![Number of lanes](image)

The number of lane is automatically modified on the image.

- **Gap between the lanes**
  Define the gap between the lanes:

  ![Gap between the lanes](image)
The gap between the lanes is automatically modified on the image.

The “Reset” button restores the default lane detection parameters.

• Next
The “Next” button validates your parameter and opens the following analysis step.

A - Edit lane

B - Edit band

• Help menu
Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function

Detect – Edit bands
The Edit band process follows the lane definition:

The edit band process automatically identifies all the bands for the defined lanes. You can also manually mark the bands on the image or on the lane’s profile. All bands will be automatically detected when you first access the band detection process, based on default parameters.

The bands are marked by green lines, overlaid on the gel image.

Note: you can either access the lane definition menu by clicking on the next button of the lane definition or directly on the band detection tab.
The dashboard details the edit band parameters:

- **Band detection on the image**
  
  You can add or remove bands by clicking directly on the image. Place the cursor at the chosen location and click. The band is immediately added or removed. The red line allows you to check band alignment between lanes.
• Band detection on the profile

The profile is calculated based on the average intensity of each row of pixels across the specified width of the lane. A lane profile provides a visualization of the intensity of the bands. Bands are represented by peaks.

You can add or remove bands by clicking directly on the profile. To proceed, select the cursor type:

![Cursor options](image)

The linear cursor has the shape of an arrow (↑ → )
The rectangular cursor has the shape of a square (□)
Place the cursor at the chosen profile location and click. The detection line is automatically added or removed.

**Note**: For arrow cursor, the band is added at the cursor position.
For rectangular cursor, the band is added at the highest position within cursor bounds.

• Profile visualization

In the profile parameter window, you can select the lanes’ profile to be displayed. To do so, just click on the profile name to select or unselect the profiles.
You can reset the detection by clicking on the “Reset” button. The detection lines will then be removed.

You can re-detect the bands by clicking the “Re-detect” button. The detection is based on the default parameters.

**Next**
The “Next” button validates your parameter and opens the following analysis step.

- B - Edit band  
- Next >>  
- C - Marker values

**Back**
The “Back” button validates your parameter and opens the following analysis step.

- B - Edit band  
- << Back  
- A - Lane definition

**Help menu**
Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function.

**Detect – Add marker values**
The marker values process follows the band detection. This function allows the assignment of the molecular weight marker’s values to the bands of the marker lane.

**Note**: you can either access marker value menu by clicking on the next button of the band detection or directly on marker value tab.
The dashboard details the marker values parameters:

• **Load a marker**
  Click on the “Load a marker” button to open a marker value file.

A pop-up window displays the following menu:

Browse to specify the marker directory. Double click on the file name you want to load

• **Create a marker**
  Click on the “Create a marker” button to create marker’s values.
Type your values, band to band, in a descending order. The OK button validates your data.

**Note:** A minimum of four values is necessary to validate the data.

**Note:** if an automatic calculation with immediate application of the standard values is carried out, it is not necessary to enter all the bands given by the manufacturer's specifications, but only those which are commonly found on the lanes of the gel.

You can save your molecular weights data and create your own marker library; To proceed, click on the “Save” button:

![Save button](image)

A pop-up window displays the following menu:

![Menu](image)

Browse to specify the directory. Type the file name and click on Save.

Select the lane corresponding to the molecular weight marker:

![Marker's lane](image)

The migration curve is automatically displayed next to the lane’s profile:
The migration curve allows to check the detection of value application errors, distortion errors, bad separation between the bands, or the quality of the standard itself.

**Note:** To delete the wrong data, you can either place the cursor arrow on the wrong value itself and click on it, or go for the manual assignment.

**Note:** The displayed migration curve is of the cubic spline type and must then include a minimum of 4 values.

**Note:** The minimum MW indicates the minimum molecular weight, which can be calculated, based on the marker’s value assignment.

**Note:** The maximum MW indicates the maximum molecular weight, which can be calculated, based on the marker’s value assignment.

Assign the marker values to the band  
Assign manually the marker values to the lane by selecting the appropriate option:

- Automatic assignment
- Manual assignment

For manual assignment, click first on the molecular weight’s value to be assigned. The value is highlighted in red. Then, click on the corresponding lane. The value is assigned to the lane:
• **Next**
The “Next” button validates your parameter and opens the following analysis step.

<table>
<thead>
<tr>
<th>C – Add marker values</th>
<th>Next &gt;&gt;</th>
<th>2- Analyse - MW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A- results</td>
</tr>
</tbody>
</table>

• **Back**
The “Back” button validates your parameter and opens the following analysis step.

<table>
<thead>
<tr>
<th>C – Marker values</th>
<th>&lt;&lt; Back</th>
<th>B- Band detection</th>
</tr>
</thead>
</table>

• **Edit the marker values**

Click on the “Edit the marker values” button.

A pop-up window displays the following menu on which you can modify the marker values:
You can add, remove, and save your marker values;

- **Help**

  Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function

**Molecular weight results**

The molecular weight results process follows the marker’s value assignment.

**Note**: you can either access the molecular weight results by clicking on the next button of the marker’s value assignment or directly by clicking on the Molecular weight tab of the 2-Analyse-MW folder.

The dashboard details the molecular weight results parameters:
The “Back” button validates your parameter and opens the following analysis step.

<table>
<thead>
<tr>
<th>2- Analyse – MW</th>
<th>&lt;&lt; Back</th>
<th>1- Detect</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Molecular weight</td>
<td>C- Marker values</td>
<td></td>
</tr>
</tbody>
</table>

• Help

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function.

Return to the image acquisition module

To return to the image acquisition module, select Home from the dash menu.
Quantification analysis module

The software Quantification module features the quantification of spot in volume, percentage.

At the end of the process, you can have the following outputs:
- Lane’s volume and concentration

Detect – Lane definition
The Quantification module opens on the Lane definition dashboard of the Window definition process:

The dashboard details the lane definition parameters:

• Define a new lane
On the image, click on the top left corner of the lane, then drag to define the size of the analysis area. You can easily adjust the size of the area by clicking on the tags surrounding the area and drag the selected border to the requested size.
The lane is defined by green lines, overlaid on the image. The area is surrounded by square anchors:

To resize the entire lane frame, drag an anchor point in or out. The opposite anchor point will remain fixed while the frame expands or contracts. The frame will expand or contract from the centre.

To move the entire frame to a new position, position the mouse on the frame to obtain a cross cursor: Click and drag the cursor to move the entire frame.

- **Copy a lane**
  To copy a lane, select the lane in the lane list:
Click on the Copy button. The lane is then duplicated:

The number of lanes is automatically modified in the lane list.
You can move the lane frame to a new position. In order to do so, position the mouse on the. Click and drag the cursor to move the frame.

• Delete a lane
To delete a lane, select the lane in the lane list:

Click on the Delete button. The lane is then deleted.

The number of lanes is automatically modified in the lane list.
You can move the lane frame to a new position. In order to do so, position the mouse on the. Click and drag the cursor to move the frame.
• **Next**
The “Next” button validates your parameter and opens the following analysis step.

| 1 A – Lane definition | Next >> | 1 B – Background subtraction |

• **Help menu**
Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function

Background subtraction
The background subtraction process follows the lane definition.
Image background interferes with quantification and data analysis such. Background could results from gel opacity, random signal noise, opacity of the carrier medium (film, gel matrix, or blot matrix) or film fogging.

As image background interferes with quantification and data analysis, we recommend performing a background subtraction before any peak volume quantification. The software has several approaches for subtracting background intensity from gel images. You can either subtract background for the whole image or for individual lanes.

Note: As background subtraction permanently changes the image, this is not possible to save the image with a processed background subtraction. However, the process can be saved by saving the complete analysis through the Save analysis process.

The dashboard details the noise subtraction parameters:
• Rolling ball

The rolling ball method is named for a hypothetical ball that rolls along underneath the lane profile, removing the intensity levels along the length of the lane. The rolling ball subtraction method removes background levels along the length of the lanes according to the size of a virtual ball. The ball is rolled under each profile of the image so its movement varies along the image. The ball follows the profile trace, touching fewer points along the trace and removing the lower part of the points which defined the subtracted background.
The centre of gravity of the ball describes a curve: This curve represents the noise to be subtracted. The curve depends on the size of the ball and on the size of the peaks.

The size of the ball affect the position and movements of the centre of gravity and thus it determined how much background will be subtracted. A smaller disk will more closely follow the profile trace, removing more background. A disk radius that is too large will result in poor Subtract background. A disk radius that is too small may subtract actual data.

The software calculates automatically the ideal parameter for background subtraction. This could be manually modified by adjusting the spot size:

To process the rolling ball background subtraction, click on the “Do rolling ball” button: The changes will be automatically applied to the image.

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

• Linear approach
The linear approach is a lane-based background subtraction. It allows to manually define the level of noise to be subtracted on the lane profile. This option gives you the opportunity to set different background subtraction levels for each lane.

Click on the “Linear” button: It opens the lane profile window:
In the profile parameters window, select the lane to perform the linear approach:

Lane number: [A]  Subtract noise  Apply to all lanes

On the profile, click to define the background linear level you want to remove:
Then, click on Subtract noise:

The changes will be automatically applied to the image and to the profile:

The linear approach is a lane-based background subtraction. You can set the same subtraction level for all lanes or specify an individual subtraction level for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button.

- **Next**
  The “Next” button validates your parameter and opens the following analysis step.

  1B – Background subtraction | Next >> | 1C-Spot separation

- **Back**
  The “Back” button validates your parameter and opens the following analysis step.

  1B – Background subtraction | << Back | 1A - Lane definition

- **Help**
  Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function.
**Spot separation**

In order to measure the volume of a particular spot, you need:

- To define the boundary around the spot;
- To compare the intensity data inside the boundary with the data of other spots or of a standard.

A volume is the sum of the pixel intensity inside a defined boundary. The purpose of the spot separation is to define this boundary. The spot separation process follows the background subtraction.

**Note:** you can either access the spot separation function by clicking on the next button of the background subtraction or directly by clicking on the spot separation of the Window definition folder.

The dashboard details the spot separation parameters:

- **Modify the spot separation**
  The software proposes by default an automatic predefined spot separation based on the band detection. You can modify the default spot separation by selecting the “Modify the spot separation” option.
The default separation is illustrated on the lane’s profile:

The brackets illustrate the bands boundaries:

You can easily reposition a band’s boundaries. In order to do so, click on the bracket and drag the cursor:
Drag the cursor until the area of the band that you want to define has been completely enclosed.

**Note**: When you release the mouse button, the band’s volume is automatically recalculated to take into account the new area of interest.

To ignore a band, select “Bands to ignore” from the profile’s parameter menu:

![Image showing the Bands to ignore option](image)

Then, click on the band you want to ignore:

![Image showing the band highlighted in grey](image)

The band is then highlighted in grey and discarded from the result table:

**Note**: you can ignore more than one band at a time.  
**Note**: to stop the process, click again on the “Bands to ignore” button.

To increase the gap in between the lane, select the “Separation gap” option from the profile’s parameter menu:

![Image showing limited and extended separation gap](image)

To ignore add a separation or delete a separation, select “Bands to ignore” from the profile’s parameter menu:

![Image showing separation options](image)

Then, click on the band where you want to add a separation or on a current separation you want to delete:
**Spot quantification**

You can use the Volume tools to quantitate bands, spots, arrays, and other image data.

The images taken by the Fusion imaging system are displayed by your computer screen in the form of grey scale images. Our systems convert the light signals from your gel or membrane samples into digital data. A digital image is a finite set of pixels. The digital image contains a fixed number of rows and columns of pixels. Pixels are the smallest individual element in an image, holding quantized values that represent the light intensity at any specific point. The light intensity is graded into a grey scale. The word pixel is based on a contraction of pix ("pictures") and el (for "element").

Volume is use in the band or spot quantification process. The volume is the sum of all the pixels intensities included in the defined area (window + separation). To measure the quantity of a particular band or spot, you need to define a band or a spot area.

Quantification is based on the image in pixels whose intensity is coded on a scale.
- The scale has 65,536 grey levels for a 16-bit image
- The scale has 256 grey levels for an 8-bit image

The quantity (or density) of a spot is calculated from its volume. This is made of the sum of all pixel intensities composing the spot.
In other words, the spot quantity then depends on:
- The number of pixels inside the area of the spot
- The intensities of these points

\[ V = \sum n_i I_i \]

Image analysis allows comparison in between concentrated intense spots and weaker but more diffused bands.

Results are given in volumes that may be recalculated according to an OD of reference or a concentration master-curve.

To measure the amount of a particular spot, you need to define the boundary around the spot and compare the intensity data inside the boundary with the data of other spots or of a standard.

**Quantity of reference**

A volume is the total signal intensity inside a defined boundary drawn on a lane. The purpose of the volume of reference is to use volumes of known concentration to calculate the unknown concentrations.

The volume of reference process follows the spot separation.

**Note:** you can either access the volume of reference function by clicking on the next button of the background subtraction or directly by clicking on the volume of reference of the 2-Spot Quantification folder.

The dashboard details the quantity of reference parameters:
\textbf{Quantity of reference}

The calculation of the unknown concentrations is based:
On the calculated volumes
On the known concentration. The known concentration is the quantity of reference.

The quantity of reference could correspond to one or several spots.
The purpose of the quantity of reference is to define the known concentration:

In the “Quantity of reference” edit field, type the quantity of known concentration you want to have as a reference:

\textbf{Lane of reference}

The lane of reference defines the lane of the known concentration.
Select the lane of reference from the list:

If a single lane is selected, only the volumes of this reference lane will be used to calculate the relationship between the volume and the quantity. The other concentrations are calculated based on the concentration/volume relationship of this specific lane.
Illustration 1: 100% / lane 1 / all bands. Total concentration lane 1 = 100%

If “All lanes” is selected, for each lane a new relationship between volume and quantity will be recalculated, according to the band’s lane selected. For instance, the defined parameters are 100% for all band all lanes; the results table could be as follows. Lane by lane, the total band concentration is 100%:

<table>
<thead>
<tr>
<th>Number</th>
<th>Lane 1</th>
<th>Lane 2</th>
<th>Lane 3</th>
<th>Lane 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>Volume</td>
<td>%</td>
<td>Volume</td>
</tr>
<tr>
<td>No 1</td>
<td>44.703</td>
<td>663551</td>
<td>70.582</td>
<td>2646172</td>
</tr>
<tr>
<td>No 2</td>
<td>25.475</td>
<td>3780989</td>
<td>25.504</td>
<td>9561786</td>
</tr>
<tr>
<td>No 3</td>
<td>14.264</td>
<td>2117062</td>
<td>3.913</td>
<td>1467075</td>
</tr>
<tr>
<td>No 4</td>
<td>9.304</td>
<td>1380926</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No 5</td>
<td>3.574</td>
<td>530607</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No 6</td>
<td>1.840</td>
<td>273100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No 7</td>
<td>0.635</td>
<td>123060</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No 8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No 9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Illustration 2: 100% / all lanes / all bands. Total concentration all lanes = 100%

- **Spot(s) of reference**

  The quantity of reference could correspond to one or several spots of the selected lane. Select one or several spots of the lane of reference from the list:

  ![Spot selection interface]

Example

Let’s consider the known concentration is 100% contains in all the spots of lane A. The settings should then be as follows:

![Settings interface]

The results table indicates the following for lane 3A:

<table>
<thead>
<tr>
<th>Number</th>
<th>Vol. %</th>
<th>Volume</th>
<th>Height</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>No 1</td>
<td>100.000</td>
<td>481,417</td>
<td>1,075</td>
<td>740</td>
</tr>
<tr>
<td>No 2</td>
<td>150.773</td>
<td>725,845</td>
<td>994</td>
<td>1,258</td>
</tr>
<tr>
<td>No 3</td>
<td>570.907</td>
<td>2,748,441</td>
<td>1,179</td>
<td>5,698</td>
</tr>
<tr>
<td>No 4</td>
<td>189.841</td>
<td>913,928</td>
<td>853</td>
<td>1,650</td>
</tr>
<tr>
<td>No 5</td>
<td>146.077</td>
<td>703,241</td>
<td>898</td>
<td>1,256</td>
</tr>
</tbody>
</table>
• **Results table**
In the result parameter window, you can select the lanes and the values to be displayed in the results tables:
- Concentration
- Volume

![Screenshot of Result Parameter Window]

**Note:** You can select or unselect the lanes to display in the results table.

**Note:** To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.

• **Back**
The “Back” button validates your parameter and opens the following analysis step.

```
1C – Spot separation << Back 2A – Quantity of reference
```

• **Help menu**
Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function.

Return to the image acquisition module
To return to the image acquisition module, select Home from the dash menu.
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.

  ![Font window](image)

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

  ![Color options](image)

  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:

![Arrow icon](image)
to draw an arrow

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

![Rectangle icon](image)
to draw a rectangle

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

![Color options](image)

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).

![Date options](image)

- 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).

![Time options](image)
- 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

![Symbol selection](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

  ![Text](image)

  ![Text](image)

  **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 an 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.

  ![Load a template](image1)
  ![Save a template](image2)

  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 **Close**

**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:

![Crop window](image3)

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:

- Rotate 90° left
- Rotate 90° right
- Horizontal mirror
- Vertical mirror
- Rotate by angle

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, click on Rotate.
**Merge marker**

The Merge marker feature allows to gather a blot image and its marker image into a new composite image. Merge marker is frequently used for colorimetric markers run together with chemiluminescent samples.

Click on the “Merge marker” button. A window displays the following menu:

![Image of Merge marker window]

Select the Marker image by clicking on “Select image”. A pop-up window displays the following menu:

![Image of Select Marker image window]

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:

![Image of File Explorer]

Browse to specify the image directory
Double click on the image name you want to load
**Note:** only two positive or two negative images can be added. Thus, it could be necessary to inverse the image one or the image two for having satisfactory results.

Then, select the signal image by clicking on “Select image” from the Second image paragraph. A pop-up 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

A new composite image appears in the main windows, gathering the marker and the signal images.

Adjust the weight you want to give to each image
You can adjust the proportion of the marker and of the signals images in the composite image.
Case 1: weight of 50% for each image

Case 2: weight of 75% for the Signal image

Case 3: weight of 75% for the Marker image
To close the function, click on **Close**.

**Paste marker**

The paste marker feature allows to copy and paste a marker from the marker image into the signal image. Paste marker is frequently used for colorimetric markers run together with chemiluminescent samples. This process will not affect the quantitation of the spots of the signal image.

Click on the “Paste marker” 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

**Note:** only two positive or two negative images can be added. Thus, it could be necessary to inverse the image one or the image two for having satisfactory results.

Select the Marker image by clicking on “Select image”. A pop-up window displays the following menu:

Then, select the signal image by clicking on “Select image” from the Second image paragraph. A pop-up window displays the following menu:
Click on the “OK” button. A window displays the following menu:

A default area is displayed. Click on the tags surrounding the area paste. Drag and drop the area to modify its position.

Click on the “Preview” to paste the marker lane. The marker lane is now paste inside the signal image.
You can adjust the threshold you want to apply to the marker lane so that the background level of the marker lane will be at the same level as the one of the signal image.

**Note:** You can rotate the area of interest when the mouse hand cursor is active

**Example 1:**

![Example 1 Image]

The marker lane background is at the same level as the one of the signal.

**Example 2:**

![Example 2 Image]

The marker lane background is at an upper level as the one of the signal.

**Example 3:**
The marker lane background is at a lower level as the one of the signal.

You can repeat this procedure to add a second or a third molecular weight marker on your sample image.

**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:

- 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

Example:

Original image

Image with Subtract background

To close the function, click on Ok or Cancel.

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 chemiluminescence 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:

![Multiplexing image menu](image)

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:

![Image directory pop-up](image)

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:

![Display Adjustment Graph](image)

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 **Close**.

**Bioluminescence**

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

![Bioluminescence Example](image)

Click on the “Bioluminescence” button. A window displays the following menu:

![Bioluminescence Menu](image)

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 Close.
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 right hand side of the application name to open the application protocol menu.
The Application protocol can either be displayed with a summary view or with a complete view. By default, the application protocol is displayed with the summary view. Click on Advanced options to have access to all the application protocol features.

**New application – Single, Sequence or Multiple 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.

Sequence and Multiple 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. For instance, a Multiple application can gather the single applications of a specific user: John in the example below. It could also gather the single application related to an imaging mode: the Spectra Red Green or Red in the example below. The user can select the single application within the list. Each single application runs independently of the other applications of the group.
To access Multiple application, open the Application protocol window and select New. A menu will then propose to create either a single application, a sequential application or a multiple application. Select Multiple:

Multiple-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:
Sequence application is a set of applications combined together automatically for multichannel imaging. For instance, an excitation color channel (red, green, blue, IR) can be assigned to an application protocol. Each single channel will be imaged one after the other.

To access Sequence application, open the Application protocol window and select New. A menu will then propose to create either a single application, a sequential application or a multiple application. Select Sequence:
Sequence images protocols allow you to group up to three different applications. Select the applications to be grouped from the single image application protocols lists and save your Sequence application 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.
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 chemiluminescence application

- Application mode
  - [ ] Fluorescence sample
  - [x] Chemiluminescence sample
You can select the application mode for the image exposure, between chemiluminescence and fluorescence:
- Chemiluminescence: no lighting and filter options
- Fluorescence: lighting and filter option

• Sensitivity
This option defines the image sensitivity for your image exposure. The Fusion 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:

<table>
<thead>
<tr>
<th>Sensitivity Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full resolution</td>
</tr>
<tr>
<td>High sensitivity</td>
</tr>
<tr>
<td>Super sensitivity</td>
</tr>
<tr>
<td>Binning 2x2</td>
</tr>
<tr>
<td>Binning 3x3</td>
</tr>
<tr>
<td>Binning 4x4</td>
</tr>
<tr>
<td>Binning 8x8</td>
</tr>
</tbody>
</table>

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 UV Fluorescence, the aperture should be set at f4. For chemiluminescence, the standard aperture is f0.84.
• **Filter (Fluorescence mode only)**
  This option defines the default filter used in the Application protocol. For instance, F590 for Ethidium Bromide applications with UV 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 chemiluminescence exposure (no filter).

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

  **Note:** The lighting options depend on your system configuration. UV, 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**

  ![Auto-exposure](image)

  **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).
For chemiluminescence, the standard test time is 2 seconds. For fluorescence, the standard test time is 0.2 seconds.

Note: in the case the auto-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**
- **Over exposure (faint band)**
- **Under exposure**
- **User defined**

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 on 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
Chemiluminescence 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 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).

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 Fusion 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:

<table>
<thead>
<tr>
<th>Option</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full resolution</td>
</tr>
<tr>
<td>High sensitivity</td>
</tr>
<tr>
<td>Super sensitivity</td>
</tr>
<tr>
<td>Binning 2x2</td>
</tr>
<tr>
<td>Binning 3x3</td>
</tr>
<tr>
<td>Binning 4x4</td>
</tr>
<tr>
<td>Binning 8x8</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Position</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tray 1</td>
<td>1198</td>
</tr>
<tr>
<td>Tray 2</td>
<td>1644</td>
</tr>
<tr>
<td>Tray 3</td>
<td>1956</td>
</tr>
<tr>
<td>Tray 4</td>
<td>2184</td>
</tr>
<tr>
<td>Transilluminator</td>
<td>2690</td>
</tr>
<tr>
<td>Conversion screen</td>
<td>2627</td>
</tr>
</tbody>
</table>

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 Fusion 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.
**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**

  - **Standard display parameter**
  - **Enhance display for faint band**
  - **Enhance display for bright band**

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 chemiluminescence 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.

- **Print quality settings**
  These settings control the resolution and quality of the printed images. The default settings are usually sufficient for most applications.

- **Print resolution**
  The print resolution determines the quality of the printed image. A higher resolution results in a sharper image.

- **Print orientation**
  Choose the orientation of the printed image. You can select portrait or landscape orientation.

- **Print margins**
  Set the margins around the printed image to ensure that the image does not extend beyond the edges of the paper.

- **Print options**
  These options allow you to customize the appearance of the printed image. You can specify the color mode, the page layout, and other settings.

**Printing – Image printing pre-defined parameters**
The Printing folder contains the image printing pre-defined set-up:
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 Fusion 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.

**CompatibilityPlus 16 Bit Tagged Image File**
- 16 Bit Tagged Image File Format (*.TIF)
- 8 bit Bitmap Files (*.BMP)
- Joint Photographic Experts Group Files (*.JPG)
- 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 \( \checkmark \) Apply after image acquisition 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 Fusion 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
- Chemiluminescence
- 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.
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 Fusion system:

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>Displays the Fusion information such as the system option, the software version, the filters installed and the lens calibration state.</td>
</tr>
<tr>
<td>Language selection</td>
<td>Select the language you want the software to run</td>
</tr>
<tr>
<td>Filter installation</td>
<td>Install or remove a filter from the filter wheel</td>
</tr>
<tr>
<td>Lens calibration</td>
<td>Re-calibrate the lens for a perfect positioning of the focus for the preset tray and transilluminator position</td>
</tr>
<tr>
<td>CFR21 mode</td>
<td>Activate the CFR21 mode</td>
</tr>
<tr>
<td>Main application list</td>
<td>Manage the list of applications which appear in the application protocol folder.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Imaging system</th>
<th>FusionCapt Advance FX7 16.09</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camera serial number</td>
<td>289142 – Firmware &gt;2.0.0&lt;</td>
</tr>
<tr>
<td>USB status</td>
<td>USB detected – v 4.3c</td>
</tr>
<tr>
<td>USB caddage</td>
<td>Enhanced darkroom Ep WL+U+Blue</td>
</tr>
<tr>
<td>Installed filters</td>
<td>1: Ethidium Bromide (F590)</td>
</tr>
<tr>
<td></td>
<td>2: No filter</td>
</tr>
<tr>
<td></td>
<td>3: No filter</td>
</tr>
<tr>
<td></td>
<td>4: No filter</td>
</tr>
<tr>
<td></td>
<td>5: No filter</td>
</tr>
<tr>
<td></td>
<td>6: Chemiluminescence</td>
</tr>
<tr>
<td>Epi-Light module</td>
<td>Spectra RGB Installed</td>
</tr>
<tr>
<td>Transillumination</td>
<td>UV Table</td>
</tr>
<tr>
<td>Tray calibration</td>
<td>(1393) (1828) (2129) (2361) (2842...</td>
</tr>
</tbody>
</table>

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 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:

- English
- Deutsch
- Français

You will be prompted 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 Capt Advanced software.

For 6 position filter wheel:
By default, the position 1 is kept for the Ethidium Bromide emission filter (F590 for the UV option, F999 for the SkyLight option).
The position 6 is for chemiluminescence applications:

For 4 position filter wheel:
By default, the position 1 is kept for Chemiluminescence, position 2 for Ethidium bromide filter.
To add a filter:
1- Make sure the door of the Fusion 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:

- Chemiluminescence
- 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-820
- F-999
- Chemiluminescence
- Other 1
- Other 2
- Other 3
- Other 4
- Other 5
- No filter

3- Select the filter 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 Fusion 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 – Fusion FX series**

The Fusion 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 Fusion 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.

Case of the Fusion SL2 Xpress
The Fusion SL2 Xpress has only 1 tray position. For calibration, proceed in the same way as with a standard system.
Lens calibration – Fusion SL series

In standard, the manual lens of the Fusion SL series is the VL38mm f0.84. For maintenance issue, it might be possible you will need to change this lens to the VL50mm f0.95.

In the event you need to change the lens from the 38mm to the 50mm lens, select the Lens calibration installation 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:

Select the appropriate lens from the menu.

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