**Imagex™ TGi**
(Model number IMX-TM-3)

**Imagex™ nanoCCD**
(Model number IMX-NM-3)

**User Guide**

14-bit Nanosecond Time-Gated Imaging System
*USB 2.0 Version for Windows™ Operating Systems*

Photonic Research Systems Ltd
Web: www.prsbio.com  e-mail: info@prsbio.com
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a) Electromagnetic and Electrostatic Interference

The normal functionality of the product may be disturbed by strong electromagnetic interference and/or electrostatic discharge and/or interruption to the mains power supply.

In the event of any problems of this nature you should carry out the following actions:

1) Attempt to stop the software by clicking on the STOP button or pressing the ESC key on the keyboard.

2) Unplug the USB connector and reconnect. This will solve the majority of problems.

3) If actions 1) and 2) do not resolve the problem please halt the Imagex application from the Windows Task Manager (Use CTRL+ALT+DEL keys) and restart the Imagex software.

4) If the problem persists please try using the product in a different location. In the event that this does not work please contact Photonic Research Systems for further assistance.

iv) FCC Statements (United States)

This Product is a Class A Digital Device intended for installation and use within a commercial, industrial or business environment. It is not intended for use within a home/household environment.

This product has been tested to ensure compliance with the Federal Code of Regulation (CFR Part 15, Subpart B) for Unintentional Radiators. Copies of the test reports for this product are available for inspection upon request.

The Federal Communications Commission (United States) (FCC CFR47 Part 15 Subpart B (Unintentional Radiators) has specified that the following notices be brought to the attention of users of this product.
‘This device complies with part 15 of the FCC rules. Operation is subject to the following two conditions:

(1) this device may not cause harmful interference, and

(2) this device must accept any interference received, including interference that may cause undesired operation.’

Use of Cables

This product is supplied with the correct shielded cables for use to comply with the Class A limits of Part 15 of the FCC Rules.

To comply with class A limits only shielded BNC cables should be used to connect external triggered lightsources to the product.
v) Disposal of waste equipment the European Union (WEEE)

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vi) Correct Ventilation

This product contains a fan-assisted Peltier CCD cooling system. To ensure efficient cooling is achieved DO NOT cover the ventilation grills on the back panel of the Imagex main unit or on the end panels of the Imagex PSU box.

Failure to observe this precaution may lead to impaired signal-to-noise characteristics on the gated image data and in extreme circumstances could lead to damage to the CCD sensor within the main system unit.
EC Declaration of Conformity

Manufacturer: Photonic Research Systems Ltd.
Unit 38, Newhaven Enterprise Centre,
Denton Island, Newhaven,
BN9 9BA, United Kingdom

Type of Equipment: Laboratory Equipment: Time-Gated CCD Imaging System

Model Number: IMX-TM-3
Product Name: Imagex-TGi

We declare under our sole responsibility that the device mentioned above complies with the following EU Directives:

Electromagnetic Compatibility Directive (EMC) 2004/108/EC,
RoHS Directive 2011/65/EU

Standards Applied for Demonstration of Compliance

EN61326-1:2013
Electrical equipment for measurement, control and laboratory use -
EMC requirements - Part 1: General requirements
Group 1, Class A equipment - (emissions section only)

EN61000-3-2:2006 (+A1/A2)
Electromagnetic compatibility (EMC) - Part 3-2:
Limits - Limits for harmonic current emissions
(equipment input current <=16A per phase)

EN61000-3-3:2008
Electromagnetic compatibility (EMC) - Part 3-3:
Limits - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current <16A per phase and not subject to conditional connection

EN61326-1:2013
Electrical equipment for measurement, control and laboratory use -
EMC requirements - Part 1: General requirements
Basic Environment - (immunity section only)

Design & Technical File Maintained at:
Photonic Research Systems Ltd
Unit 38, Newhaven Enterprise Centre,
Denton Island, Newhaven,
BN9 9BA, United Kingdom

Date of Validity: 25th April 2016

Authorised Signatory: Dr Andrew Mitchell
Position Held In Company: Managing Director
Declaration of Conformity

Manufacturer: Photonic Research Systems Ltd.  
Unit 38, Newhaven Enterprise Centre,  
Denton Island, Newhaven,  
BN9 9BA, United Kingdom

Type of Equipment: Laboratory Equipment: Time-Gated CCD Imaging System

Model Number: iMX-NM-3

Product Name: Imagex-nanoCCD

We declare under our sole responsibility that the device mentioned above complies with the following EU Directives:

Electromagnetic Compatibility Directive (EMC) 2004/108/EC,  
RoHS Directive 2011/65/EU

Standards Applied for Demonstration of Compliance

EN61326-1:2013 Electrical equipment for measurement, control and laboratory use -  
EMC requirements - Part 1: General requirements  
Group 1, Class A equipment - (emissions section only)

EN61000-3-2:2006 (+A1/A2) Electromagnetic compatibility (EMC) - Part 3-2:  
Limits - Limits for harmonic current emissions  
(equipment input current <=16A per phase)

EN61000-3-3:2008 Electromagnetic compatibility (EMC) - Part 3-3:  
Limits - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current <16A per phase and not subject to conditional connection

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EMC requirements - Part 1: General requirements  
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Design & Technical File Maintained at: Photonic Research Systems Ltd  
Unit 38, Newhaven Enterprise Centre,  
Denton Island, Newhaven,  
BN9 9BA, United Kingdom

Date of Validity: 25th April 2016

Authorised Signatory Dr Andrew Mitchell

Position Held In Company Managing Director

Signature
Statement of FCC Verification (Class A Unintentional Radiators)

Product Name : Imagex TGi
Product Model number/Unique Identifier : IMX-TM-3
Manufacturer : Photonic Research Systems Ltd, UK

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

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Changes or modifications to this product not expressly approved by the Photonic Research Systems or its Distributors could void the user's authority to operate this equipment.

The product was tested and found to be compliant with Class A emission limits for Unintentional Radiators (CFR 47 Pt 15 B (Emissions)) at dB Technology Ltd, Cambridge, UK, between 4th and 5th April 2016. Copies of the test report are available on request.

Summary of Test Results

<table>
<thead>
<tr>
<th>Test</th>
<th>Port</th>
<th>Method</th>
<th>Limit</th>
<th>Pass/Fail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conducted Emissions</td>
<td>AC power</td>
<td>ANSI C63.4 :2003</td>
<td>FCC(A)</td>
<td>PASS</td>
</tr>
<tr>
<td>Radiated Emissions &lt;1GHz</td>
<td></td>
<td>ANSI C63.4 :2003</td>
<td>FCC(A)</td>
<td>PASS</td>
</tr>
<tr>
<td>Radiated Emissions &gt;1GHz</td>
<td></td>
<td>ANSI C63.4 :2003</td>
<td>FCC(A)</td>
<td>PASS</td>
</tr>
</tbody>
</table>
Statement of FCC Verification (Class A Unintentional Radiators)

Product Name: Imagex nanoCCD
Product Model number/Unique Identifier: IMX-NM-3

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

This product is a Class A digital device that is marketed for use in commercial, industrial or business environments. It is not intended for use by the general public or to be used in the home.

Changes or modifications to this product not expressly approved by the Photonic Research Systems or its Distributors could void the user's authority to operate this equipment.

The product was tested and found to be compliant with Class A emission limits for Unintentional Radiators (CFR 47 Pt 15 B (Emissions)) at dB Technology Ltd, Cambridge, UK, between 4th and 5th April 2016. Copies of the test report are available on request.

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<td></td>
<td>ANSI C63.4 :2003</td>
<td>FCC(A)</td>
<td>PASS</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

The Imagex family of CCD Imaging Systems system offers direct CCD gating and Light source triggering to give the user a complete ‘turnkey’ solution with the following integrated features:

- Slow-Scan 14-bit Camera readout system with Correlated Double Sampling
- Continuously variable Gate/Delay generator for gating of camera sensitivity
- 3 Light Source Pulse/Delay Generators for triggering of lamp/laser pulses
- Broad range of frequencies for camera/light source gating and triggering
- Fan-Assisted 3-Stage Peltier Cooling System to allow long ‘On-Chip’ Integration times
- Flexible X-Y Binning control for improved sensitivity at lower resolutions

The system also comes complete with a comprehensive software package. The software is designed to offer a flexible but simple-to-use approach to time-gated imaging. These are just some of its features:

- Multiple Image Banks each with its own programmable gating properties
- Automated Image Sequence Collection. Take a series of images with a single mouse click.
- Programmable Pixel ‘Binning’ for ultrahigh sensitivity
- Automatic Image processing after data collection
- Automatic Dark Image Subtraction
- Data Export to 16-bit TIFF
- Comprehensive Image Visualisation features to allow you to combine intensity and time-resolved images
- Comprehensive Area of Interest (AOI) system for measurement of area intensities

The purpose of this section is to explain a little about time-gated imaging and to introduce some of the main features of the Imagex system. The Imagex-TGi range of products (TGi="Time Gated Integrating") was designed for the measurement of a wide range of fluorescent and phosphorescent labels with lifetimes ranging from sub-microsecond to milliseconds. Because of its excellent dark noise performance and pixel-binning capabilities it is also suitable for standard ‘ungated’ imaging applications involving very weak fluorescent and chemiluminescent samples.
Time-Gated Imaging

Fluorescent labels are widely used as highly detectable markers in a number of fields, particularly in cell biology. Often the limit of detection for such labels is set by background signals such as scattered exciting light and unwanted fluorescence and phosphorescence from the surroundings. One way to deal with this is to use a label with relatively long-lived emission and excite this with a pulse of light. Most of the background signals disappear a few nanoseconds after the light pulse, while the long-lived emission persists.

Pulsed excitation of a long-lifetime sample in the presence of high levels of short-lifetime ‘background’ fluorescence

Time-gated imaging allows a camera to be triggered by the light source, but remain insensitive whilst the light source is illuminated. After a predetermined delay the camera becomes sensitive to light and images the signal emitted after this point until the end of user-programmable time known as the ‘gate’ period. The Imagex system allows the user to set up a wide range of delay and gate periods to suit labels having a very wide range of decay times.

Gated Detection allows the longer-lifetime emission to be detected selectively

The Imagex system integrates the signal collected from multiple light source triggers/gate periods on the CCD chip. This means that you do not have to waste time reading out the image signal for each event until you have enough signal to use. This approach is very useful where the light source is designed to pulse repeatedly at high speed, but where each individual pulse may not be very intense.

Time-gated imaging is not only useful to collect long-lived signals in the presence of shorter-lived emission, but can also be used to reject long-lived signals. For example, if a normal fluorescent
label with a short lifetime is to be detected it might be necessary to reject some ambient light or possibly long-lived phosphorescence (often seen from optics or filters in the light path). Imagex can take a pair of images with different delay and gate settings and process them mathematically to separate long- and short-lived contributions to the image.

The imaging modes described so far have been used to reject a long- or short-lived signal to emphasise a particular contribution to the image. Imagex can also be used to measure the decay time of signals of interest, and where appropriate a decay-time map can be generated as a 'lifetime image'. This mode is particularly useful for sensor applications, where the sensitivity of fluorescence and phosphorescence to environmental influences is used for measurement purposes. For example, long-lived labels such as ruthenium complexes and porphyrin derivatives have been used in ‘paint’ formulations to measure oxygen. ‘Pressure-Sensitive Paint’ takes advantage of the ‘quenching’ of long-lived emission by oxygen in air, and has been widely used to coat aircraft models for wind-tunnel measurements of air pressure distribution across surfaces. As air pressure increases, so more oxygen dissolves in the ‘paint’ and the fluorescence intensity and decay time are both decreased. Imagex cameras can produce images that reflect the pressure distribution across structures coated with ‘pressure-sensitive paint’, either using normal intensity-based detection or by calculating a ‘map’ showing fluorescence lifetime across the sample. The lifetime-based approach is often superior to the measurement of intensity alone, because it is relatively insensitive to variations in paint thickness and to uneven illumination. These factors must be corrected if intensity-based imaging is to be used and the correction process requires a reference image, which is not always easy to obtain. Similar paint formulations have been used to measure temperature distribution across surfaces, and the same arguments apply in this case.

Although Imagex cameras were designed primarily for measurements of fluorescence and phosphorescence, they are also very well suited to measurements of chemiluminescence and bioluminescence, where long integration periods are sometimes needed for adequate sensitivity. Imagex cameras use thermoelectric-cooling of the CCD to provide a very low thermal noise build-up, and hence they can achieve long integration periods. Because the Imagex camera in ungated mode is so quiet it is also very suitable for imaging low levels of luminescence using high degrees of pixel binning.

The Imagex trigger outputs can be used to generate pulses to initiate an action, for example to trigger a pulsed light source. These triggering facilities are very useful for imaging any signals that are phase-locked to a stimulus, which need not be a pulsed light source. Thus, for example, Imagex is well suited to imaging electroluminescence triggered by a pulsed voltage source, or for sonoluminescence that is phase-locked to an ultrasonic stimulus.
Features of the Imagex Camera

- The Imagex range now features comprehensive 'On-Chip' binning control allowing any pixel binning factor from 1x to 40x to be controlled independently in both the horizontal and vertical dimensions for versatile control over resolution and sensitivity.

- The Imagex application software now has unique data visualisation capabilities to allow different kinds of image data to be combined within a single image. You can also view multiple images simultaneously using the new multiview setup system.

- Imagex now features 1nsec resolution, 0-255nsec programmable nanosecond delay generators on two of its lightsource trigger outputs allowing the system to be used with pulsed light sources for nanosecond gating experiments. The Imagex software allows the user easy access to these features. (This feature is available only on the Imagex nanoCCD model).

- The Imagex range features a USB 2.0 Interface for faster readout and ease of interfacing. All functions required for gated imaging are controlled via this single 'plug and play' interface. The USB 2.0 also allows for faster readout offering 8x faster readout when compared to the original parallel port Imagex system.
Chapter 2: Installation

● Camera Installation
The Imagex Time-Gated Imaging system is now integrated into a single easy to connect unit. This unit contains the CCD, associated cooling system and cold-chamber and all electronics required for time-gating and lightsource triggering.

● Connections
NB: The Imagex Power Supply should only be connected to the Imagex system when it is switched OFF. Failure to observe this may result in damage to the computer and/or camera system

Before installing the Imagex System for the first time, you should install the QuickUSB Driver. Please refer to Appendix I for instructions.

Once the QuickUSB Driver has been installed the Imagex System Unit can be connected to the computer by a single USB cable (supplied with the system). You can use any USB port on your computer as long as it is not connected through a USB hub device.

One or more Light Sources may be attached to the Camera Output Port using the SMB to BNC cables provided with the system. There are 3 Light source outputs in total.

Up to 4 TTL lamps may be driven from each of the light source outputs using appropriate ‘T’ adapters.

The Imagex system is supplied with a switched mode 24V DC. If you wish to use a different power supply it is essential that you contact Photonic Research Systems to confirm compatibility (e-mail us at info@prsbio.com). Photonic Research Systems will not be held liable for damage to the system by use of a power source that has not been approved by us.

To connect the supplied power supply simply insert the DC plug into the socket at the back of the Camera marked “24V DC”. Do NOT switch on the power supply or connect it to the mains supply until it is plugged into the camera.

● USB Port Specification
To run the Imagex system correctly the host computer must be equipped with at least one free USB2.0 port. As previously stated this port should NOT be connected via a USB Hub.

● Computer Specification
Most modern PCs will run the Imagex software without a problem. The suggested minimum specification for the computer that runs the Imagex software is 1Gb free memory, 2GHz Pentium or equivalent processor and 24-bit colour VGA graphics card/chipset with Windows XP, Vista or 7/8/10. Using a faster computer with more memory will usually translate into faster image readout and display performance. Any Windows-supported pointing devices can be used, but the system is usually controlled with a standard mouse pointer. The software is designed to run on 32-bit versions of Windows XP, Vista and 7/8/10.
Software Installation

The Imagex System is supplied with an installation disc for the system software. Please see Appendix II for instructions on installing the System software.

Once installed Imagex can be launched by double-clicking on its icon or selecting Imagex from the START>Programs menu.

Remember that before running the software you must have the QuickUSB driver installed. Instructions for this are provided in Appendix I.

Important: Windows Features that you need to turn OFF

Certain Features of the Windows operating system may impair the performance of the Imagex software. You should turn OFF the following features:

- Screensavers-This can be performed by right clicking on the Desktop and selecting the Screensaver Tab.

- Power Saving Schemes-Please ensure that all power saving schemes are switched off. These options are accessible from the Windows Control Panel.

- E-mail and Messenger-Type Programs-Any Internet connection that periodically polls the Internet connection and transfers data might affect some of the timing-critical functions of the Imagex system. In general it is safe to run these programs at the same time as Imagex as long as you are not performing data collection operations.

The Imagex software will still work with these features enabled but they may interfere with your measurements if left on.
**Optical Installation**

To correctly record fluorescence images it is important to ensure that the only light source is the fluorescence excitation source; i.e. ambient light should not be present. Whilst the background subtraction feature can remove contributions from ambient light, this signal will reduce dynamic range and compromise noise performance. If the ambient light is unstable with time then background subtraction *cannot* correct the images and you will obtain unrepresentative values for your sample. You should take particular care that light from indicator LEDs (which are often red in colour) does not enter the camera optics.

**Correct Use of Filters**

For any type of fluorescence imaging experiment the camera should be fitted with an appropriate C-Mount camera lens and a bandpass filter selected to transmit the fluorescence of the target fluorophore whilst blocking light from the excitation light source. The light source should also be fitted with a filter to block any wavelengths emerging from the light source which overlap the wavelengths of the fluorescence emission.

For microscopy, filters and dichroic mirrors are often sold as sets suitable for a particular range of fluorescent probes. These filter sets usually guarantee efficient rejection of the excitation source output and efficient detection of the target fluorophore.
Chapter 3: Getting Started

- Setting up the Light Source

To perform time-gated imaging you will require a *pulsed or modulated* light source. This can be either a laser, arc source or diode source. The only limitation for use with the Imagex system is that the light source must be controlled by a TTL signal. TTL stands for 'Transistor Transistor Logic' and is a standardised way of exchanging signals between devices. TTL has two recognised logic levels: '0' and '1'. Pulsed light sources are usually triggered by a transition from one TTL level to the other whilst TTL-modulated sources will follow the state of the TTL signal: '1'=Light ON, '0'=Light OFF. To use Imagex it is very important that you have characterised the operating limits of your light source. You need to consult the documentation supplied with the light source and be able to answer the following questions before attempting to use the system.

1) What is the maximum repetition frequency of the light source?
2) Is the light source a ‘pulsed’ source or a TTL modulated source?
3) If the source is of the pulsed type, is it triggered on a positive-going (logic '0' to logic '1') or a negative-going (logic '1' to logic '0') transition.
4) Does the light source have a fixed frequency or can it be run at a range of frequencies?

Some light sources such as pulsed laser systems and flashlamps have an optimal repetition frequency. Running them at other frequencies may lead to instability or even damage. Other light sources (such as pulsed LED sources) can be run at a larger range of frequencies and may be programmed to have varying pulse lengths. For these sources it is useful to have a range of frequencies available.

- Running Imagex for the first time

To begin using Imagex for the first time we suggest following these simple steps:

1) Double click on the Imagex icon or select Imagex USB from the Start>Programs menu.
2) When the Imagex software has loaded select a frequency that is suitable for your lightsoure

- Taking your first Time-Gated image

This may be the first time that you have used Time-Gated Imaging, so it is useful to discuss the approaches you might take to start using the technique and consider some of the problems you might encounter. Because the information in a time-gated image is often recorded AFTER the light source has gone out, it can look very different to the scene as viewed by the human eye or by a normal camera system. Sometimes, if your sample contains only short lifetime fluorophores then you see nothing at all! As you learn more about the system you will become more familiar with the effects of time-gating on your imaging. To begin with we suggest the following approach to familiarize yourself with the main Imagex controls.

1) Select the correct light source!
Consider the lifetime of your sample and its excitation and emission wavelength properties.

2) Use a test sample:
If you use a sample which contains only short lifetime material, using time-gated imaging will simply make the sample appear dark. It is best to start off with a test sample which contains long and short lifetime fluorophores. This will help you to see some relatively obvious effects of time-gating.
3) Start with Ungated Imaging in Low Res Mode:
When you are attempting to adjust the focus and exposure time, it is usually best to use ungated imaging mode. This is the mode where you will always see the biggest signal from your sample because it collects ALL the light. Because you are collecting more signal you can reduce the exposure time and thus increase the frame update rate. This makes it much easier to adjust the focus and position of the sample. Selecting Low Res mode speeds up imaging even further by pooling signal from several camera pixels into one readout event. This means that there are less pixels to read out and each pixel contains more signal for a given exposure time. Using the Low-Res readout mode is the closest you will get to video rate readout.

To use Ungated imaging click on the Ungated Image tickbox
This makes it easier to set focus and sample position.
(Note: The nanosecond delay control is only available on the Imagex nanoCCD model)

Once you are happy with your focus and field of view you can switch to time-gated imaging by unchecking the Ungated Image check box.

4) Set the Gate Width to approximately one third of the interpulse period:
If you are going to change the gate delay setting it is important that the gate period does not overlap the light pulses, otherwise you will collect unwanted short lifetime emission. A useful rule of thumb when experimenting with the gate delay is to set the gate period to approximately one third of the interpulse period. The example below shows a system with pulse repetition frequency of 300Hz and an interpulse period of 3333microseconds. Here, the gate width has been set to 1000 microseconds. This allows the user to set the gate delay anywhere between 0 and 2000 microseconds without having to worry about the gate period wrapping round to the next light pulse.

Setting the gate width to a third of the interpulse period allows you more freedom to experiment with the gate delay.
(Note: The nanosecond delay control is only available on the Imagex nanoCCD model)
5) Experiment with the gate delay!
You can get very different results depending on the gate delay setting. If you are trying to detect a long lifetime probe against a short lifetime background, experimenting with the gate delay setting can help you achieve the best results. You will usually see the biggest difference when you move from a negative delay to a positive delay. A negative delay simply means that the sensitive period or 'gate' period starts before the light is extinguished. In this case you will be detecting prompt fluorescence which in many cases is the dominant source of fluorescence. It is normal for the integration time to increase with delay time, simply because more of the signal is being rejected.

Click on the delay control to adjust the time between the end of the light pulse and the beginning of the gate period...

5) If you don't see anything...
If you have you system set up but you are still not getting an image, here are a few things to check:

- Have you opened the light source shutter/camera port/lens aperture?
- Are you using the correct filter set for your sample?
- Can you see any fluorescence by eye?
- Have you installed the QuickUSB Driver?
- If you are using short wavelength UV, do your optics transmit?
Chapter 4: Basic Operation

The Imagex software can collect image data into 16 Image ‘Banks’. The banks are numbered 1 to 16 and a thumbnail representation of the contents of each bank is shown to the left of the main image. Each Image Bank has a set of associated experimental parameters which is controlled by you through the Imagex software interface (shown below)...

When you left-click on the thumbnail for an image bank you will see the image that is currently loaded into that bank and the set of image collection parameters that will be used to collect data into that bank. The control interface is divided into logical sections down the left-hand side of the main program window. Here will explore the role of each of these sections.
● The Exposure Manager - Controlling Exposure Time and Image Resolution

One of the features of the Imagex software is that you can select a different image resolution for each individual bank. It may seem strange to want to use a lower image resolution, but in CCD imaging accepting a lower resolution means that you can achieve much higher sensitivity.

Imagex systems use ‘On-Chip’ pixel-binning to increase sensitivity at the cost of reduced resolution. Using increased pixel-binning can be very useful if you want to record images relatively quickly from low-light level samples. Imagex offers four ‘standard’ image capture resolutions and one ‘User-defined’ resolution whose X and Y binning factors can be controlled from the user interface. The table below shows how sensitivity changes for each of the standard resolution and for some examples of user-defined resolutions.

<table>
<thead>
<tr>
<th>Resolution</th>
<th>Pixel-Binning Factor (X*Y)</th>
<th>Effective Pixels (W*H)</th>
<th>Relative Sensitivity</th>
<th>Image Size (W*H)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Full’</td>
<td>1*1</td>
<td>782*580</td>
<td>0.5</td>
<td>782*580</td>
<td>Requires 2 exposures for interlaced readout</td>
</tr>
<tr>
<td>‘High’</td>
<td>1*1</td>
<td>782*290</td>
<td>1</td>
<td>782*580</td>
<td>Single frame readout</td>
</tr>
<tr>
<td>‘Medium’</td>
<td>2*1</td>
<td>391*290</td>
<td>2</td>
<td>782*580</td>
<td>Single frame readout</td>
</tr>
<tr>
<td>‘Low’</td>
<td>4*2</td>
<td>195*145</td>
<td>8</td>
<td>782*580</td>
<td>Single frame readout</td>
</tr>
<tr>
<td>‘User’</td>
<td>4*4</td>
<td>195*72</td>
<td>16</td>
<td>782*580</td>
<td>Single frame readout</td>
</tr>
<tr>
<td>‘User’</td>
<td>8*8</td>
<td>97*36</td>
<td>64</td>
<td>782*580</td>
<td>Single frame readout</td>
</tr>
<tr>
<td>‘User’</td>
<td>10*10</td>
<td>78*29</td>
<td>100</td>
<td>782*580</td>
<td>Single frame readout</td>
</tr>
<tr>
<td>‘User’</td>
<td>20*20</td>
<td>39*14</td>
<td>400</td>
<td>782*580</td>
<td>Single frame readout</td>
</tr>
</tbody>
</table>

Note that for the standard resolution Imagex system the Full Resolution readout has a relative sensitivity of 0.5. This is because it is read out as two interlaced ‘fields’. Each readout out has a relative sensitivity of 1 but because the complete exposure process takes twice as long the overall process is assigned a relative sensitivity of 0.5.

● Using the Exposure Manager to change Exposure Time

The Exposure Time Control determines for how long the camera collects signal before the image is read out. For a given sample the signal level is proportional to the exposure time, so extending the exposure time will increase sensitivity. You should take care that you do not set the exposure time to be too long as this will result in saturation of the camera with unpredictable results for your image. In ‘Gated’ Imaging mode it is important to remember that a small change in Gate Delay can lead to a significant change in signal level and that this must often be compensated for by adjusting the exposure time.

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One example where this situation might be encountered is in a sample with high levels of short lifetime fluorescence and very low levels of long lifetime fluorescence. With a gate delay time of zero or a negative value the bright short lifetime fluorescence signal will be dominant and a short exposure time will be adequate. When the gate delay is increased the short lifetime signal is gated out, but the short exposure time will be insufficient to detect the long lifetime component efficiently. Therefore, it may be necessary to increase the exposure time by several orders of magnitude to successfully image the long lifetime component.

The Imagex software uses standard 'up-down' controls to change the exposure time: To increase the exposure time, simply click on the top-half of the control situated to the right of the exposure time...

Click on the top half of the control to increase the exposure time...

Click on the bottom half of the control to decrease the exposure time...

Click on the Exposure Time value to enter a new exposure time using the keyboard...

Alternatively, if you already have a good idea of what exposure time you would like to use you can simply click on the exposure time value itself and enter the new time into the input box. You will have to delete the old time and the new time only becomes active when you press the RETURN key.

Note: Remember that when you change the exposure time you are only changing it for the Current Image Bank. If you wish to change the exposure time for all banks simultaneously you should ensure that the 'sync' box next to the exposure time control is checked

● Using Keystrokes to Change Exposure Time

In some circumstances it is easier to use keystrokes to change the exposure time. The Imagex software uses the F1 to F4 function keys (at the top left of a standard keyboard) to implement a handy control for the exposure time. The F1 to F4 keys can be thought of in the same way as set of controls on a media player, Fast Reverse, Reverse, Forward, and Fast-Forward.
Examples of using Exposure Time Function keys

Pressing F1 repeatedly when the initial exposure time is set to 500msec will have the following effect:
500msec (F1) ->400msec->(F1)-> 300 msec (F1)->200msec (F1)->100msec (F1)->90msec->(F1) 80msec etc....

Pressing F4 repeatedly when the initial exposure time is 500msec will have the following effect:
500msec (F4) ->600msec->(F4)-> 700 msec (F4)->800msec (F4)->900msec (F4)->1000msec->(F4) 2000msec etc....

Pressing F2 repeatedly when the initial exposure time is 500msec will have the following effect.
500msec (F2) ->490msec->(F2)-> 480 msec (F2)->470msec (F2)->460msec (F2)->450msec->(F2) 440msec etc....

Pressing F3 repeatedly when the initial exposure time is 500msec will have the following effect.
500msec (F3) ->510msec->(F3)-> 520 msec (F3)->530msec (F3)->540msec (F3)->550msec->(F3) 560msec etc...

Note: Even when you change the exposure time using keystrokes you are still only changing it for the Current Image Bank. If you wish to change the exposure time for all banks simultaneously you should ensure that the ‘sync’ box next to the exposure time control is checked.

Using the Exposure Manager to Change Image Capture Resolution

Imagex can capture images in a variety of resolutions. At lower resolutions you get lower detail but you get higher sensitivity. That is, you can capture lower intensity signals more quickly at lower resolutions but they won’t look as detailed. As you will see later you can combine the high sensitivity low-resolution data with lower-sensitivity high-resolution data to get the best of both worlds. You can select the resolution for the current image bank by clicking on any of the Preset or ‘Standard’ image resolution buttons. These will capture images using pre-defined image resolutions settings defined in the Imagex software.
Click on any of the video resolution buttons to change the speed and resolution of image capture.

For more flexibility there is a User Resolution (or ‘User bin’) control. When this setting is selected you can define your own X and Y resolution settings for image capture. Furthermore you can have different X and Y image resolutions for each image bank giving you maximum flexibility.

**User Resolution**

This option allows you to design your own image resolution setting by varying the X and Y pixel binning factors. Increasing the X and Y pixel-binning factors decreases spatial resolution but increases sensitivity.

Remember that sensitivity is proportional to the product of the X and Y binning factors so, for example, by increasing the X and Y binning factors both to 10 you can increase sensitivity by a factor of 100!

Adjust the X-Slider to change the X-Pixel Binning factor

Adjust the Y-Slider to change the Y-Pixel Binning factor

Note: Remember that when you change the Capture Resolution you are only changing it for the Current Image Bank. If you wish to change the Capture Resolution for all banks simultaneously you should ensure that the 'sync' box next to the Resolution control is checked

● **Time-Gating Settings-Controlling Frequency, Gate Width, Delay, Lamp Pulse Width and Nanosecond Delay**

As you will already know Imagex is a **Time-Gated Imaging System**. This means that the sensitivity of the camera changes periodically at a predetermined frequency and for a user-defined period. The term ‘Gate’ originates from electronics but if we imagine the ‘Gate’ as being a shutter in front of the camera then when the ‘Gate’ is open, photons reach the camera; when the ‘Gate’ is closed, photons are prevented from reaching the camera. If we then imagine this ‘Gate’ opening and closing in time with a flashing light source then we have the essence of the Time-Gated Imaging system.

The Imagex camera does not use a mechanical shutter but the analogy of a ‘Gate’ is still useful for descriptive purposes. If you examine the Exposure Manager you will see the **Time-Gating Key**. This small graphic shows the relationship between the Lamp Pulses and the ‘Gate Open’ Period. The lamp pulses are shown in blue whilst the ‘Gate Open’ period is shown in red. As you may have noticed, during Ungated operation the red region covers the whole of the period between lamp flashes to denote that the ‘Gate’ is open all the time. When you switch to Ungated mode the red region changes in size to match the whole length of the interpulse period.
Time-Gating Controls

Each of the parameters associated with the time-gated exposure has an associated software control. You can change the control either by clicking on the up and down arrows next to the button displaying the control value or by clicking on the button itself and simply typing in new value. There are no specific limits on the individual values of lamp pulse width, gate delay and gate width except that the sum of these values must fit within the time which is 95% of the interpulse time period (i.e. the inverse of the trigger frequency). The Exposure Manager has Five main controls associated with Time-Gated Imaging:

**Frequency Selector**
This pull down list allows the user to select any of the available frequencies. This control is sometimes 'Locked' as a safety feature to protect the light source. To Lock/Unlock the Frequency Selector click on the More Options... button (see later in this Chapter)

**Gate Width Control**
The Gate Width Control affects the length of time within a single interpulse period during which the camera is sensitive to light. A longer Gate Width will usually result in higher sensitivity.
Gate Delay Control
The Gate Delay is the time between the end of the light pulse and the start of the Gate 'Open' Period. As you increase this value you will collect less short lifetime fluorescence.

Lamp Pulse Width
The Lamp Pulse width control changes the width of the TTL signal supplied to the Pulsed or Modulated light source. It is important to note that if you are using a lightsource with a fixed pulse width, changing the width of the signal applied to the light source will not change the length of the light pulse but will change the delay between the end of the real light pulse and the start of the gate period. Ideally for fixed pulse width light sources you should always set the light pulse signal to be the same as the real pulse width. However, some fixed pulse light sources require a trigger signal which is longer than the real pulse.

Nanosecond (ns) delay
The nanosecond delay control allows you to delay the pulse for lamp output number 1 and 2 with nanosecond precision. As you increase this value the delay between the light pulse and the beginning of the camera gate 'ON' period increases. The nanosecond delay control can be varied from 0-255 nanoseconds in one nanosecond increments. However the way this is displayed will depend upon the selected imaging mode. (Please see the nanosecond imaging section for more information) This feature is only available on the Imagex nanoCCD model.

The Time-Gating key is only a guide to what is happening. If you are using Gate Widths and/or Gate delays which are much shorter than the interpulse period you will have to rely on the Gating controls to follow what is happening.

The Exposure Manager also has a series of checkboxes providing additional control over image collection.

Sync Controls
Each Time-Gating control has a SYNC checkbox associated with it. When this box is checked the corresponding control value for each of the image banks is made to be the same as the current bank. Subsequent changes to the control will then affect all other banks until the sync control is unchecked.

Ungated Image
This control determines whether images are collected in Time Gated mode using the controls described above or in Ungated mode. In ungated mode the camera is sensitive ('ON') all the time. The lamp still flashes at the selected repetition frequency and with the selected pulse width. Because ungated mode collects the most signal it is useful for focusing and positioning of the sample. It will also produce the image that is most similar to that viewed by the human eye.

Dark Image
When this box is checked Imaging takes place in either gated or ungated mode (depending on the setting of the ungated image control) but the light source is not triggered. This option is useful for measuring thermal noise on the CCD and any
sources of ambient light (i.e. light not emanating from the sample or the light source)

Checking the Ungated Image box results in the camera being sensitive for the whole of the interpulse period

● Imaging Commands-Triggering Exposures, Setting up Light Sources and Saving/Loading Settings

Once you have set up you Exposure Time, Resolution and Time-Gating Settings it is time to actually collect the image. The Imaging Commands Section is the place where you can trigger single and multiple exposures.

Imaging Commands-Controls

The Single Expose Button

This button triggers a ‘Still Image’ exposure into the current image bank. The image will be collected at the default capture resolution with time-gating parameters set up for that bank.

The MultiExpose Button

This button triggers a series of ‘Still Image’ exposures. Images in each bank included in the MultiExpose cycle will be collected with its associated time gating parameters. After the MultiExpose cycle is complete. The current Maths Script will be executed (if this option is selected from the More Options.. dialog) To interrupt the MultiExpose cycle click on the STOP button.

The Emergency Stop Button

The ‘Emergency Stop’ button is used if you want to immediately stop an imaging operation or data collection cycle. It will abandon any current exposure, MultiExposure, or Multiple Integration. If you wish to complete your current imaging operation and then stop you should use the ‘toggle Repeat checkbox’ approach. (See Below)
Setting Up Multiple Exposures

As you will have seen above there are two ways to trigger data collection. Clicking on the Single Expose button will trigger the collection of an image into the currently selected Image Bank with the associated imaging parameters.

Clicking on the MultiExpose Button will trigger collection of all images into all the Image Banks which currently have their ‘Include in MultiExpose’ boxes checked. You can set any number of the banks to be included in the MultiExposé cycle. If no images are currently selected then clicking on the MultiExpose button will have no effect.

Subtracting Background Images

When this control is checked each time an image is recorded a Background image is automatically recorded and subtracted from the main image. A background image has exactly the same imaging parameters as the normal image except that the light source is not triggered. This option is useful for removing unwanted signal components such as ambient light, A-D offset and fixed pattern CCD noise, but doubles the time taken for image acquisition.

Continuous Imaging-The Repeat Checkbox

Normally when you click on the Single or MultiExpose Button the software will collect an image or set of images and then stop and wait for your next command. You may wish to monitor the image continuously for focusing and positioning. To do this you need to check the ‘Repeat’ box within the Imaging Commands section. Then, when you click on the Single or MultiExpose button it will continue to collect images until you uncheck the ‘Repeat’ box (You can also toggle the ‘Repeat’ box by pressing the ESC button on the keyboard).

Clicking on the Single Expose will trigger an exposure into the current Image Bank whilst clicking on the MultiExpose will trigger collection of all images which have their ‘Include in MultiExpose’ boxes checked. If the ‘Repeat’ box is not checked the program will then stop collecting data...

With the ‘Repeat’ box checked clicking on the Single Expose will continuously trigger exposures into the current Image Bank whilst clicking on the MultiExpose will continuously trigger collection of all images which have their ‘Include in MultiExpose’ boxes checked. To stop data collection click on the ‘Repeat’ box again to uncheck it. Data collection will then terminate once the current set of data has been successfully collected. (The Repeat box can also be toggled by pressing the ESC key on the keyboard)
More Options-Lightsource, Maths Script, Triggering and Multiple Integration Controls

The 'More Options' button is contained within the Imaging Commands Section. Whilst it is a single button it leads to a number of useful functions. When you click on the 'More Options' button the following window is displayed...

'More Options' Controls...

- **Frequency is Locked**
  When this option is selected you cannot change the pulse repetition frequency from the Exposure Manager main window. This option protects the lamp from accidentally being driven at an inappropriate frequency.

- **Use Maths Script After MultiExpose**
  When this option is selected the current Maths Script will be executed after the collection of the MultiExpose images. If this option is NOT selected the Maths Script can still be executed by selecting 'Run Maths Script' from the Maths menu.

- **Select Gating Mode (nanoCCD only)**
  This option allows you to select one of several special gating modes available with the Imagex nanoCCD. More information is available in the Appendix.

- **Lamp Output**
  This option allows you to select the combination of lamps to be used for the current image. All selected lamps are run synchronously.
<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use same lamp combination for all images</td>
<td>This option forces the lamp combination to be the same for all images.</td>
</tr>
<tr>
<td>Pre-Expose Pause</td>
<td>This slider lets you set a pause between clicking on the Single Expose button and the data actually being collected. This can be useful if you wish to leave the room before collecting the data. (e.g. if you are conducting a particularly long exposure or are collecting a large number of images)</td>
</tr>
<tr>
<td>Pre-MultiExpose Pause</td>
<td>This slider lets you set a pause between clicking on the MultiExpose button and the data actually being collected. This can be useful if you wish to leave the room before collecting the Multiple Exposure data. (e.g. if you are conducting particularly long exposures or are collecting a large number of images)</td>
</tr>
<tr>
<td>Expose Integration Count</td>
<td>This slider allows you to integrate a number of images when you click on the Single Expose Button. The resulting image stored in the current Image Bank is the average of all the images collected. Integrating images can improve signal-to-noise by averaging out readout noise but obviously has an impact on the data collection time. It is most useful where you know that your sample is stable over the data collection period and the signal-to-noise is particularly important.</td>
</tr>
<tr>
<td>MultiExpose Integration Count</td>
<td>This slider allows you to integrate each of the images in the Multiexpose cycle a number of times when you click on the MultiExpose Button. The resulting images stored in their respective Image Banks are the average of each of sets of MultiExpose images collected. Please note that when performing image integration in MultiExpose mode, the system completes a full MultiExpose cycle for each step of the integration. This approach minimizes affects due to photobleaching and long-term drifts in the light source.</td>
</tr>
</tbody>
</table>

**The Zoom Window - Zooming and Scrolling your images**

The Zoom and Scroll control is located at the bottom left hand of the main image. It is a relatively simple control, but at the same time it is quite powerful. The control consists of a set of buttons and a small area called the Console that displays a miniature version of the main image.

It is important to note that the Console always shows the whole image, whereas the Viewport often shows only a section of the image (for example, when we are looking at a zoomed-up portion of the main image in the viewport).

As well as a representation of the whole image the Console also features a blue rectangle called the Locator. This shows the part of the main image currently being displayed in the viewport.
The Zoom Control at Zoom level 1. The Viewport Locator is showing that the Viewport is displaying the whole image.

As we click on the Zoom-in button the Locator shrinks in size because the viewport is displaying a smaller section of the main image.

The Zoom Control at Zoom level 3. The Locator is showing that the Viewport is displaying a small section of the whole image...

As we zoom back out again the Locator increases in size again. Although, we might expect that when we return to Zoom level 1 the locator would match the size of the whole image, this is not always the case. Sometimes, particularly when working with large, high-resolution images on a relatively small screen the whole image cannot be displayed at once and we must use the Locator to move the Viewport around the image even at Zoom level 1.

- **View Options-Viewing Single Images, Matrix Images and Multi-Images**
  The View Options menu allows you to display image data in a number of ways. The simplest is the Single Image View which simply displays the image contained in the currently selected Image Bank with the current Zoom, Scroll and Look-Up Table settings.
  The Matrix View allows you to combine two images (often an intensity image with a ratiometric image) whilst the MultiView options allows you to display various arrays of images from 1 by 2 to 4 by 4.
Using the View Setup Option

By default, when using any of the MultiView display modes Imagex will display the current Image Bank and enough consecutive images to fill the selected array of images for the selected MultiView. For example, if you are using the 2x2 MultiView and the current Image Bank is 5 then the software will display Images 5,6,7 and 8 in a 2 by 2 array.

You may find this approach a little restrictive and Imagex therefore provides a View Setup system which allows for a more versatile display of multiple images. To use the View Setup system you must open the Setup MultiView Editor for the MultiView Mode that you wish to use. These can be found in the View menu.
The Setup MultiView Menu

There is a separate setup for each MultiView mode. You only need to edit the one(s) that you are interested in using. The Setup MultiView Editor allows you to place either a Single Image from any of the image banks, the Matrix Image or a blank rectangle in each of the image positions of the MultiView display. You can also annotate the images with either the bank number or your own comment.

The Setup MultiView Editor for the 2 by 2 MultiView mode

To use the View Setup you must check the ‘Use View Setup’ box in the View Options Section…

Using the View Setup in 2 by 2 display mode

When you use the View Setup the display will change to show your chosen arrangement of images and comments like this…
Display using the 2 by 2 View Setup shown above

The Image above contains three single images (from Banks 1, 12 and 15) and the matrix image and each image has a user defined comment added. If you compare the image with the Setup Editor settings you should be able to see how they relate. Once you are satisfied with the way that your images are being displayed you can use Export the image as a 24-bit bitmap from the file menu. You can use the View Setup with each of the MultiView modes but you will have to edit the appropriate View Setup for each mode. As setting up some of the larger arrays can take some time, it is a good idea to save your View Setups from the View Menu.

You can find more about the details of setting up the Matrix Image in Chapter 11: The Tools Menu
Chapter 5: Storing and Retrieving Images

Loading and Saving of images is accomplished from the **File**... menu as shown below:

Files can be saved either as single frames or as a group file containing image data from several or all of the banks in the framestore.

**Saving the Current Image Bank**

To save the image in the currently selected image bank:

1) Click on the **File**... menu.

2) From the **File**.. menu select "SAVE 16-Bit FILE"

3) The Save Dialog Box will appear. This allows the user to select a disk drive, scan through available directories on that drive and enter a filename, before finally saving the image file, in **Imagex** format (with an .imw suffix). The Save dialog is a standard part of the Windows interface. It also allows you to delete, rename, cut and paste files as well as creating new directories. When an image is saved its time-gating settings, frequency and exposure time are saved with it. It is useful to use filenames which are suitably descriptive so that the file can easily be retrieved at a later date.
Loading an Image into the Current Image Bank
Images that have been stored on disk can be retrieved into the current data bank using the file Open Dialog Box

To load an image from disk into the current image bank:

1) Click on the FILES... menu.

2) From the FILES... menu select "LOAD 16-Bit FILE"

3) The File Load Dialog Box will appear. The file Open Dialog allows the user to select an .IMW file from any of the active disk drives.

4) The Imagex software allows different image sizes to be produced depending on the value of the Default Capture Resolution. However, Imagex only supports one image size at a time, within a given framestore. Therefore if you load an image whose size does not match the current framestore frame size, then that image will be stretched or shrunk to fit into the framestore. If that image is subsequently saved again from that framestore then it will be saved with its new frame size.

Saving the Entire Framestore
Sometimes it is useful to save the whole of the framestore within a single file. This process keeps closely associated files together and saves time when loading.

To save all the images in the framestore:

1) Click on the FILES... menu.

2) From the FILES... menu select "SAVE ALL 16-Bit FILES".

3) The Dialog Box for saving all files is very similar to that for saving a single image. The user can select the directory and disk drive onto which the file will be saved. The group file is saved as a single file with an .IMS suffix.

Loading a Set of Images
To load a saved set of images:

1) Click on the Files.. menu.
2) From the **Files**... menu select "Load all 16-bit Files".
3) The Dialog Box for loading a group file is very similar to that for loading a single image. The user can select the directory and disk drive from which the images will be loaded.

**NB** The process of loading the files will begin by loading the first file in the image set into the current bank. The program will not load past the last bank. Therefore if you are loading a complete file set, your current bank should be set to BANK 1.

**Saving Selected Images**
Sometimes it is useful to save some, but not all of the images in the framestore within a single file. This process allows you to save important images together whilst preserving disk space.

To save all the images in the framestore:
1) Click on the **FILES**... menu.
2) From the **FILES**... menu select "SAVE SELECTED IMAGES".
3) A dialog box is displayed which allows you to select specific images to save to a multi-image file. Tick on the check boxes corresponding to the images that you wish to save (TIP: try using the multi view to review which images you want to save)
4) The Dialog Box for saving the selected images is very similar to that for saving a single image. The user can select the directory and disk drive onto which the file will be saved. The group file is saved as a single file with an .IMS suffix.

**Exporting Image Data to a 16-bit TIFF file**
To allow the user to process Imagex data in other software packages the Imagex software can export its 16-bit data to a TIFF format file. This is one of the more common 16-bit image formats and is supported by a wide range of image processing packages.

To save the current framestore bank as a 16-bit TIFF image:
1) Click on the **FILES**... menu.
2) From the **FILES**... menu select "Export 16-bit TIFF File"
3) Use the File Save dialog to save your image in monochrome 16-bit TIFF format.
Chapter 6: The Viewport-Displaying Data

● Using Display Look-Up Tables- LUTs
Beneath the main image or Viewport you will find the Look-Up table control block. This area consists of a pair of controls, a colour bar and a pair of buttons. These controls are used to adjust the way in which 16-bit image data is displayed by controlling the display look-up tables. If a series of images have very different intensity values the display look-up tables can be adjusted for each image to change the way the images are displayed without affecting the nature of the data stored in the framestore.

The *Imagex* software has a sophisticated set of 16-bit display functions that can be used to control the contrast levels of the displayed images. The program uses a data structure called a 'look-up table' or LUT to define how an image will be displayed on the screen. The LUTs provided by *Imagex* can be either monochrome (shades of grey) or pseudo-colour (bright colours with similar brightness values across the range). Each image is passed through its own individual LUT before it is displayed on the screen. The LUT is constructed by setting all values below the black level to the first colour in the LUT palette (the underflow colour) and all the values above the white level to the last colour in the LUT palette (the overflow colour). The intermediate pixel values are spread linearly across the colours contained within the palette.

The *Imagex* LUT control panel

● Mouse Control of LUT Levels
Mouse control of look-up tables is achieved using the Black and White level controllers:

The *Imagex* level controller offers complete control over the black or white level of the look-up table by using a set of 4 clickable buttons. These buttons can be clicked with the left or right mouse button to obtain even greater levels of control:

<table>
<thead>
<tr>
<th>Button</th>
<th>Left Mouse Click</th>
<th>Right Mouse Click</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;&lt;</td>
<td>Decrease Level by 1000</td>
<td>Decrease Level by 100</td>
</tr>
<tr>
<td>&lt;</td>
<td>Decrease Level by 10</td>
<td>Decrease Level by 1</td>
</tr>
<tr>
<td>&gt;</td>
<td>Increase Level by 10</td>
<td>Increase Level by 1</td>
</tr>
<tr>
<td>&gt;&gt;</td>
<td>Increase Level by 1000</td>
<td>Increase Level by 100</td>
</tr>
</tbody>
</table>

Thus for coarse level control use the outer buttons of the control and for fine control use the inner buttons. If you already know the black or white level you would like to use you can simply click on the black or white value itself and an input box will be displayed which allows you to type in a new value for the level.
Keyboard Control of LUT levels

Look-Up tables can be changed interactively during any of the Setup modes using either the level controllers described previously or the Function keys F5 to F8.

<table>
<thead>
<tr>
<th>Function Key</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>Decrease Black level for current image by 500</td>
</tr>
<tr>
<td>F6</td>
<td>Increase Black level for current image by 500</td>
</tr>
<tr>
<td>F7</td>
<td>Decrease White level for current image by 500</td>
</tr>
<tr>
<td>F8</td>
<td>Increase White level for current image by 500</td>
</tr>
</tbody>
</table>

As contrast controls are adjusted the new black and white levels are updated underneath the Look-Up table colour bar (displayed beneath the main image). The LUT level controls define the black and white levels for a given image. By setting the black level to a high value any pixels in the image with a value lower than that level will appear black (or whatever the underflow colour is). By setting the white level to a low value a very dim image can be made to appear bright.

It is possible to set different black and white levels for each image. This makes parallel viewing of widely differing images easier. Of course, this can also cause confusion by removing the apparent differences in brightness between different images. To overcome this problem there is a ‘Sync’ checkbox next to the look-up table controls.

When ‘sync’ is checked all changes to the LUT of the current bank will also occur in parallel for the rest of the images in the framestore. When this menu item is unchecked, LUT changes will be confined to the current image (i.e. each image can have its display settings changed independently).

If you wish to quickly return to the default display setting use the View... menu option, “Reset Look-Up Tables” This command sets white levels for all images to 16000 and black levels for all images to 0.

Swapping Palettes

Images can be displayed in monochrome (greyscale), pseudocolour (false colour), or in an alternate (User Defined) palette. Monochrome images tend to look more photorealistic, whilst pseudocolour images can enhance contrast between areas of similar pixel value. To select a palette click on one of the palette selector buttons adjacent to the colour bar.
If you wish to load a new palette you can use the ‘Alt’ (alternate) palette button. Right click on this to show a selection of User Palettes to load. As with other changes to the display look-up tables, if the 'Sync' checkbox is checked in the View... menu then any changes to the palette will affect all images.

**Setting the Look-Up Table Underflow and Overflow Colours**

When the black level of a look-up table is set to a non-zero value it is possible for image pixels to hold a value that is below the black level value. It is normal practice to set this colour to black but any colour can be selected if the user wishes to particularly highlight these pixels. To set the underflow colour either click on the left-hand end of the colour bar or select “Set Underflow Colour” from the View Menu.

A colour selector will be displayed and you will be able to select a new colour to represent pixels whose intensity values fall below the Black level.

**The Colour Selector used to set the Underflow and Overflow colours**

Similarly by clicking on the right-hand end of the colour bar or selecting “Select Overflow Colour” from the View menu you can change the overflow colour. This colour is used to display pixels whose intensity values are greater than the LUT white level. This colour is commonly used to detect camera saturation or near saturation. The maximum possible value from the camera A-D converter is 16383. If the LUT white level is set to 16000 and the LUT overflow colour is set to red then you know that any red pixels image are either saturated or close to saturation.
Chapter 7: Area Measurements

The Imagex software has a comprehensive set of controls for area averaging and intensity monitoring within images. Up to 500 separate areas (sometimes referred to as 'Regions of Interest') can be defined. Areas can be square, rectangular, circular or elliptical. The controls for area averaging can be found directly above the Viewport in the 'Cursor & Area Information' panel. Areas defined for one bank are used automatically with the other banks and this allows easy comparison of intensity values between different images.

The Cursor & Area frame shows the value of the pixel under the cursor and statistics about the 'Active' areas which have been defined on the image.

- Monitoring the Mouse Cursor
  As the mouse cursor is moved across the Viewport the X-Y coordinate of the cursor and the Intensity value of the corresponding pixel are displayed in the Cursor & Area Information panel. The intensity value displayed corresponds to the pixel immediately below the tip of the mouse cursor. It is independent of the current LUT levels.

- Defining an Area
  The Imagex software uses the concept of an 'Active Area' to help the user define areas on the image. The 'Active' area is indicated by the number in the Area Selector pull-down menu. When the user drags the mouse across an area of the image this 'Active' area is redefined and the average value within that area is displayed next to the corresponding area button. Other areas on the image are unaffected. To change the current Active Area select an area number from the Area pulldown list. Further Area redefinitions will now be applied to the newly selected area.
Select Area no. 3 from the Area Selector....

Left Click and Drag on the Viewport to redefine Area 3....

Release the Mouse key and the new Position of Area 3 is displayed.

- **Changing the Area Type**

There are four basic area shapes available: Rectangle, Ellipse, Square and Circle. All are defined in the way described above. The shape associated with the current active area can be changed by selecting from the Area Shape Selector. After the shape of an area has been changed it will be redisplayed and its area statistics updated.
Changing the shape associated with area 3

**Showing or Hiding Area Components**
Each area has 3 components associated with it: The Area Tag, the Area Value and the Area Shape.

- **Area Tag**
  This is the number of the area and helps identify which area you are working with.

- **Area Value**
  This is the average value of the pixels contained within the Area.

- **Area Shape**
  This shows the boundaries and shape of the area.

The components of an *Imagex Area*

You can choose to Hide or Show any or all of these components on the Viewport depending on how cluttered you want the display to be. The Cursor & Area Information panel has tickbox controls for each of the Area components. Clicking on these will only affect the Active Area. If you wish to Show or Hide a particular component on all the areas then this can be done quickly from the Area Tools Menu. This feature is particularly useful if you have a large number of areas.

*The 'Show all' Submenu...*
To completely clear the Viewport of areas, tags and values simply select **Area Tools>Hide All>Areas**. You will find that when an area is hidden its Tag and Value are also hidden automatically. However, the statistics for the current Active Area are still shown in the Cursor & Area Information panel.

### Changing the Area colour

It is often useful to use colour coding to help identify the areas that you are working with. The Area Colour is used for displaying the area itself and its associated Tag and Value. The Area Colour is also used to display the Area Statistics in the Cursor & Area Information panel. To change the colour associated with the Active Area click on the Area Colour Selector.

Although the Imagex software does not place any restrictions on the use of area colours it is a good idea to avoid using the colours Black, White and Grey as these can sometimes be difficult to see on the image and on the Cursor & Area Information panel.

### Fine Control of Area parameters

Areas are usually defined by dragging the mouse on the Viewport. This allows the user to place the area on a particular feature of the image. Sometimes it is difficult to position the mouse pointer accurately. For higher precision control Imagex provides 4 controllers for area positioning and sizing.

These controls allow the user to apply a fine adjustment to the position and size of the active area. There are 4 controls with the following functions.

- **X**: Changes the X co-ordinate of the Left-Hand side of the of Active Area
- **Y**: Changes the Y co-ordinate of the Top of the Active Area
- **W**: Changes the Width of the Active Area
- **H**: Changes the Height of the Active Area

Where the Active Area is a square or circle the Height and Width always remain the same as each other. Thus, attempting to change either one of these parameters with the fine control will result in both being changed.
Exporting Area Data

If you wish to export data from a defined area across a number of banks you can select the Export Area Data option from the Area Tools menu.

This console allows you to export data as an ASCII X,Y data file suitable for import into popular spreadsheet programs. For the x-coordinate of this data set you can select any one of the parameters associated with image collection. These include Exposure Time, Gate Delay, Gate width, Lamp Pulse Width and Nanosecond Delay. The corresponding y-coordinate will be the area average value from the currently selected (‘active’) area. The ‘From Bank’ and ‘To Bank’ slider controls allow you to select a range of banks for the export operation. In the example above a file will be created with two columns. The x-column will contain the nanosecond delay values for banks 1 to 16 whilst the y-column will contain the active area average value for banks 1 to 16. Clicking on the ‘Export Data’ button will prompt you for a filename for the data file. Nanosecond Delay data can only be exported on the Imagex nanoCCD software.
Chapter 8: Nanosecond Imaging (nanoCCD models only)

● Overview & Terminology

*Imagex nanosecond imaging* is a new innovation in time-gated imaging. It builds on the *Imagex-TGi* time-gated imaging system but now allows nanosecond timescale measurements to be made. The technique used by the *Imagex nanoCCD* system relies on the fast switch-on time of the CCD gate (i.e the fast switch-on time of camera sensitivity). Each pixel has a switching time of the order of a nanosecond. There are variations in the switching time of each pixel relative to the gate signal which result in a characteristic ‘irising’ pattern which ultimately limits the time response of the camera. The time response of the *Imagex* camera is currently approximately 5nsec.

In this chapter we will refer to relative delays being either ‘positive’ or ‘negative’. The delay in question is the time difference between the end of the light pulse and the switching ON of the sensitivity of the camera.

Where the light pulse ends BEFORE the sensitivity of the camera is switched ON, the programmed delay in the interface unit is referred to as a POSITIVE DELAY.

Where the light pulse ends AFTER the sensitivity of the camera is switched ON, the programmed delay in the interface unit is referred to as a NEGATIVE DELAY.

If we use a NEGATIVE DELAY we will see both prompt and longer-lived fluorescence because the camera is sensitive while the light source is emitting a pulse.

If we use a POSITIVE DELAY we will see ONLY the longer-lived fluorescence emitted after the light pulse has ended. By increasing the value of the POSITIVE DELAY by a few nanoseconds we will selectively collect fluorescence whose fluorescence lifetime is a few nanoseconds longer.

The built-in circuitry of the *Imagex* system allows the delay between the triggering of the light source and the beginning of this fast switching of the gate to be programmed with nanosecond precision.

● Selection of Light Sources suitable for nanosecond Imaging

It is important to select the correct type of light source for nanosecond imaging with the *Imagex* system. In general you should select an **actively-triggered pulsed light source** with repetition rate of 20kHz or less.

All light sources in this class can be specified with respect to their pulse length and trigger jitter. (The trigger jitter is a measure of the variation in time between the trigger signal and the actual light pulse.) Ideally these parameters should be of the order of a few nanoseconds or less, to match the specification of the *Imagex* camera.

Some examples of light sources which have been used with the *Imagex* camera are described below:
<table>
<thead>
<tr>
<th>Source</th>
<th>Pulse width</th>
<th>Trigger Jitter</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen Laser</td>
<td>0.5-5nsec</td>
<td>0.5-50nsec</td>
<td>A good source of UV light (337.5nm) and often used in association with a laser dye module to</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>give a large range of wavelengths. Cheaper models tend to have relatively poor trigger jitter</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>performance (&gt;10nsec). Thyratron-triggered models have the best timing performance.</td>
</tr>
<tr>
<td>Pulsed LED</td>
<td>0.1-10nsec</td>
<td>c. 1nsec</td>
<td>A low-cost but highly stable light source which does not offer problems of laser speckle.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pulsed diodes are relatively weak sources when used at repetition rates suitable for the</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imagex system.</td>
</tr>
<tr>
<td>YAG laser</td>
<td>c.10nsec</td>
<td>c. 4nsec</td>
<td>High power/low repetition rate. Suitable for very weak fluorescent samples.</td>
</tr>
<tr>
<td>DPSS laser (Diode</td>
<td>0.5-10nsec</td>
<td>c. 1nsec</td>
<td>Medium repetition rate with good timing. A good source of UV (355nm). Very suitable for use</td>
</tr>
<tr>
<td>Pumped Solid State)</td>
<td></td>
<td></td>
<td>with the Imagex system.</td>
</tr>
</tbody>
</table>

● Setting up *Imagex nanoCCD* for nanosecond gating
The *Imagex* system has two gating modes: normal and nanosecond. To set up the nanosecond gating mode you need to use the normal gating mode. Therefore, you should open the Nanosecond Imaging menu and ensure that both ‘Use nanosecond Setup for Current Bank’ and ‘Use nanosecond Setup for All Banks’ are UNCHECKED.

![Image of Nanosecond Imaging menu](image)

The Nanosecond Imaging menu when in normal gating mode

To set up the *Imagex* system for nanosecond work you should follow these steps.

1) **Ensure that you are working safely!** If you are using a laser source the system should be in a suitable housing, preferably with a fibre-optic output stage to couple the light to your sample. Correct eyewear should be used to block laser emission and the room where the laser is in use should be fitted with a suitable interlock facility to prevent unprotected user accidentally entering the room whilst the laser is emitting. If in doubt about the precautions that you need to take you should consult your on-site laser-safety officer.

2) Set up your field of view and camera so that you are looking at a *short lifetime* sample. This can be either a selected fluorescence probe or a scatterer. If you are using a scatterer you will probably need to stop down your camera lens or use neutral density filters to prevent saturation of the camera. If you are using a short lifetime fluorescent sample you will need to use the correct filter set in order to reject direct emission from the light source.

3) From the light source documentation find the correct/optimum pulse repetition rate and select this in the *Imagex* software. Use the *sync* option to ensure that the same frequency is selected for all banks.
Now click on the ‘more’ button and select light output 1 or 2 and check ‘Use Same Lamp Combination for All Images’. Now select ‘Save Current Setup as Default’ to ensure that when the software is loaded it will automatically default to a lamp output and pulse repetition frequency that is suitable for your light source.

Connect the trigger input of your light source to one of the nanosecond light output connectors on the back of the Imagex unit. (L1 or L2)

Set Lamp Pulse Width to 1μsec and Gate Width to 1μsec.

If your light source has a specified trigger-output delay which is greater than 1μsec you should set the Gate Delay to this value. Otherwise you should set it to –1μsec.

Set the ns delay (nanosecond delay) to 0 (zero) nsec.

Ensure that the ‘Ungated’ check box is checked and press ‘ESC’ to Use Repeat Imaging.

Click on the Single Image Expose button to start collecting data.

Use the integration time controls to obtain a reasonable signal level and allow for camera focusing.

Now UNCHECK the ‘Ungated’ check box to begin Time-Gated imaging.

Adjust the ns delay (nanosecond delay) until you see a transition from light image to dark image. This will be a more gradual transition than seen in the microsecond adjustment due to the combination of light source pulse width, Trigger jitter and camera irising. If you are using a sub nanosecond pulsed light source with low jitter then you may be able to observe CCD ‘field irising’ as the gating signal crosses the CCD on the nanosecond timescale and CCD ‘interpixel irising’ arising from variations in individual pixel switch on time.

Once you have found the nanosecond delay where the image is just extinguished, open the ‘Nanosecond Imaging’ menu and select ‘Copy Current Setup to nanosecond Setup’.

Now, to save these values for future use, click on ‘Save Current nanosecond Setup as Default’.
16) Next, click on ‘Use nanosecond Setup for all Banks’ to start using nanosecond gating mode.
17) Finally click on the ‘More Options’ button once again and select ‘Save as Default Imaging Setup’ so that the next time you start the Imagex software you will be ready to use the Nanosecond gating setup.

● Working in nanosecond mode
Once you have set up nanosecond gating you will find that the \textit{ns delay} (nanosecond delay) parameter has both negative and positive delays. By setting this value to Zero you will set your laser pulse to occur immediately before the start of gate-ON period. If you find that you have only a small range of positive nanosecond delays to work with you can try inserting a hardware delay (e.g. a delay box or long length of coaxial cable) into the lightsource trigger path. This will then allow you to setup the nanosecond mode again but with a greater range of positive delays. (It may be necessary to select a different microsecond delay value when you have inserted the hardware delay).

Once you have established a suitable nanosecond setup you should ensure that the cables and lightpaths associated with your experiments do not change substantially. It is a good idea to label the cable(s) and make a note of any external delays used, so that you can set the system up again if you need to disassemble it for any reason.

The Imagex software allows you to set up experiments with multiple Image Banks where each bank has its own time-gating parameters. When you are in nanosecond mode the only parameter that needs to be varied from bank to bank is the \textit{ns delay} (nanosecond delay) setting.

There are two tools to help you work with nanosecond delays across multiple banks:

1) The nanosecond sync control
This option located next to the nanosecond control will synchronize the nanosecond value for all image banks whilst it is checked. Normally we don’t leave this control checked as it would force all banks to have the same nanosecond delay value all the time. However, it is useful to check it then uncheck it if you wish to set all banks to have the same delay before going on to set each one up individually.

2) The nanosecond ‘Spread’ option
This option is available from the ‘Nanosecond Imaging’ menu and is usually used when you have selected bank 1. The Spread function will automatically adjust the nanosecond delay value in regular increments for successive banks. You can choose an increment of 1, 2 5 or 10ns. When used in conjunction with the MultiExpose command this option allows you to quickly obtain a comprehensive set of nanosecond decay data.
The choice of nanosecond delay value depends upon the lifetime of the fluorophore that you are trying to selectively image. You should also remember that if this fluorophore is very long (e.g. >= 1 microsecond) you might be able to use the standard Time-Gated Imaging mode together with a longer pulsed light source.

In the example below a 10nsec Pulsed Blue Light Emitting Diode has been imaged in nanosecond mode. 16 Images have been collected with nanosecond delays ranging from –10 to 65 nanoseconds.

For this experiment the camera lens was defocused to blur the distribution of light from the LED which is normally highly directional. As can be seen from the figure the intensity of the light collected from the diode reduces as the nanosecond delay value increases. This is because at higher delay values the camera is becoming sensitive after the LED pulse has been triggered as shown in the figure below.
Notice that the full emission from the LED is seen at all negative delays and at the crossover point (Zero delay). If we were specifically trying to image long lifetime emission in the presence of a short lifetime background then we would use a positive delay where the short lifetime emission is rejected.
Chapter 9: Mathematical Image Functions

The images collected by the Imagex system can be considered as very large arrays of numbers arranged into lines and columns. Corresponding pixels on separate images can be subjected to the normal range of mathematical operations to create new images that may have new forms of contrast. This is demonstrated with the calculation of FLIM or Fluorescence Lifetime Images. Several inter-image mathematical operations are used in fluorescence imaging for ratio imaging, flat fielding and background subtraction. Imagex provides an intuitive and easy-to-use interface for performing simple arithmetical operations on images in any of the framestore banks. To perform inter-image arithmetic select any of the maths options from the Maths... menu. Once you have selected one of the maths operations from the Maths menu you can switch to any of the other maths operations by selecting within the 'Image Maths' dialog box.

- Image Addition

Image addition is achieved by selecting the Maths... menu then selecting the "Add Images..." option. The Add Images dialog box allows the user to select two images to add together and a target bank in which the resultant image should be stored. As an example, to add Image 1 to Image 2 and put the result into Image 3 then you would set up the Add Images Dialog as shown below:

The 'Add Images' Dialog: Image 3=(Image 1 + Image 2) * 1

Notice that as well as selecting the images to add you can also specify a multiplication factor. This can be any number, but it should be remembered that the result of the calculation will consists of 16-bit integers and that the maximum value that can be stored in the framebanks is 32,000. Images to be used in a maths operation are selected using buttons arranged in groups of eight on either side of the 'equals' sign. The images selected to be used in the maths operation are highlighted in red. There are no specific restrictions on which image banks are used a given mathematical operation. For example, the user may set all three image banks for the addition process to be the same. In this case the image under question will be added to itself and stored back into the same bank. The source image will have effectively been multiplied by two. A more usual application for image addition is where the user wishes to perform image integration to improve signal to noise by adding together sequential images of the same scene. After each operation the 'result' image (on the left-hand side of the 'equals' sign) becomes the currently
selected image effectively showing the result of the maths operation. Pressing the 'Close' button closes the Image Addition Control. The Image Addition settings when the window is closed are stored and will be the new starting values when the Image Addition Control is next opened. It should be noted that once a mathematical operation has been performed it cannot be undone. Therefore, if your source data are important you should back them up before using the Maths Dialog.

● Image Subtraction
Images can be subtracted to form a new (difference) image. Image subtraction is particularly useful for removing fixed pattern noise and is used to great effect to reduce thermal noise from the CCD chip. The Subtract Images dialog box is displayed by opening the Maths menu then selecting the "Subtract Images..." option. The Image Subtraction Control allows the user to select the two images to be subtracted and a target bank in which the resultant image should be stored. As in 'Add Images' the choice of images to be processed is totally under the control of the user.

The 'Subtract Images' Dialog: Image 2=(Image 2 - Image 6) * 1

● Image Division
Images can be divided to form a ratio image. The Divide Images dialog box is displayed by selecting "Divide Images..." from the Maths menu. The 'Divide Images' dialog allows the user to select the two images to be ratioed and a target bank in which the resultant ratio image should be stored. As the ratioing of two images with similar intensity values will tend to produce small floating point numbers it is important to multiply the resultant ratio by a large number to yield an integer value suitable for storage in the 16-bit integer framestore. The Divide Images dialog allows the user to set a multiplication factor that will be used to multiply the ratio before storage in the target image bank. For ease of use it is recommended that the gain factor should be set to 100 or 1000 so that the original ratio can easily be calculated from the ratio image pixel values.
**Add Constant to Image**

To add a constant value to a selected image choose "Add Constant to Image" from the Maths menu. This dialog is simpler than those previously described because there is only one source image involved.
● **Subtract Constant from Image**

If there is a constant level of background illumination across a sample or other source of positive signal offset then this can be simply corrected for by using "Subtract Constant from Image" from the **Maths**... menu. This is not as rigorous a correction procedure as the background image subtraction but can have a signal-to-noise advantage in that the subtraction does not contribute noise to the resulting image. It is most appropriate where there is a fixed offset in the measurement system, as is often found in video digitization.

![Image Maths Operations](image)

The 'Subtract Constant from Image' Dialog: Image 2=Image 1- 100

To perform background subtraction with a constant the user should first monitor the background illumination level in the absence of light using one of the areas averaging commands. This averaged value may then be used as the constant to subtract from the image.

● **Multiply By Constant**

It is often useful to scale the intensity of an image prior to another maths operation. One example is where you wish to add several images together and there is a risk of the resultant image overflowing the frame store bank. The 'Multiply Image by Constant' dialog box allows you to scale an image by any value to achieve this scaling effect. Once again the dialog box is relatively simple because there is only one source image.
The 'Multiply Image by Constant' Dialog: Image 2 = Image 1 * 1.35

● Clear Bank
The simplest of the Maths controls is the 'Clear Image' dialog box. This control has no source images, but simply a Result image and a number to put into that image. It is one way to clear an image, but the choice is yours as to whether you fill the image with zeros. A faster way of clearing image banks is to use the 'Clear Bank' command from the Tools Menu.

The 'Clear Image' Dialog: Image 1 is filled with a constant value of 300
Transform Image
The Transform Image function is a sophisticated and flexible way of performing an imagewide transformation from one set of numbers to another. It is particularly useful for applying calibration data to real samples. An example is where you have measured a set of image ratio values at say, a range of oxygen concentrations using an oxygen sensitive probe. In general it is unlikely that there will be a simple linear relationship between the measured ratios and the known oxygen concentrations. If you have measured these data over your range of interest then you can use the Transform function to perform this calculation.

Creating a Transform Table
Let us say that you have measured a number of known samples with varying oxygen concentrations using a fixed set of gating parameters throughout the experiment. If we now want to perform the same Time-Gated ratiometric measurement on a series of unknown samples then we can use our previously-measured calibration data together with the Transform function to measure the oxygen concentrations of the unknown.

The Imagex Transform function uses a set of X-Y data points to convert the ‘Y’ data contained in one image to X-data in a new image. The normal application for this process is for the implementation of a calibration curve to create images of real-world values such as temperature, pH calcium ion concentration and oxygen activity.

To create a new Transform table you should first have collected a set of data over the range of interest for your measurement. The X-data is normally a concentration value. For it to be used with the Imagex transform function your X-data should be in the range of 0 to approximately 10000 or 0 to 1000. Therefore if your X-Data has a maximum value of say 0.1 mM then you should multiply it by 1000 and express it in uM units. If this process yields X-values that are still too low you can multiply your data by a further factor of 10 or 100 and set the ‘X-Axis Multiplier’ accordingly. The software will then correct the data by this factor to show the correct concentration value. You can control how the units are displayed underneath the image by
entering the appropriate text into the ‘X-Axis Units’ text box. The Y-axis data must also cover a similar range of values. This is relatively simple to control for ratio values by setting the gain factor (e.g. to 1000 or 10000 for ratio values which lie between 0 and 1).

Because the Transform function converts ‘Y’ value into ‘X’ value, the ‘X’ values are entered into the ‘Transform To’ column on the right whilst the ‘Y’ values are entered into the ‘Transform From’ column on the left. It is also important to ensure that the ‘Y’ values are entered in ascending order as shown below and that the data set is monotonic. If your data is arranged in order of your ‘X’-axis values you may need to re-order it in favour of the ‘Y’-axis values.

The Transform Look-Up Table Editor

In the above example we want to convert ratios back into oxygen values so the ratio values are entered into the ‘Transform From’ column whilst the oxygen values are entered into the ‘Transform To’ column. To enter your data you simply type in the pairs of data points into the ‘From:’ and ‘To:’ and then click on ‘Insert Before Current Data Pair’ to add the pair to the list. If you make a mistake you can delete a data pair by selecting the unwanted data pair and clicking on ‘Delete Current Pair’. The success of the transform operation depends upon a number of factors: You should ensure that your calibration data are reproducible and cover the full range of the physical parameter that you are interested in. It is a good idea to test your Transform Table using known samples.

NOTE: The Transform Function cannot predict values outside of the range of the Look-Up table that you have entered!
Chapter 10: The Maths Script Editor

If you have read the previous chapter you will understand how you can perform simple arithmetic operations on images loaded into the Imagex framestore. If you frequently perform the same series of image collection operations and arithmetical functions it can sometimes save time to develop 'Scripts' using the Maths Script Editor. The Maths Script editor allows you to create a list of mathematical operations and imaging commands which can then be performed automatically either with a single mouse click or at the end of a MultiExpose event. To use the Maths Script Editor select 'Edit Maths Script' from the Maths menu:

Opening the Maths Script Editor...

The Maths Script Editor looks very much like the normal Maths dialog box...

The Script Editor has additional controls to allow you to select Mathematical Operations, Display Commands and Imaging Commands and build them up into a 'Script'. If you look on the right hand side of the dialog box you will see the new Script controls. The Script itself is shown in a box as a series of simple mathematical expressions and imaging commands. Values entered by the user are shown in brackets. The Script is always terminated with an 'End' command which cannot be deleted.

Note: When you save an Imagex Setup file the Script that is currently defined in the Script Editor is embedded in that Setup file and will be used when you use that Setup again.
Maths Script Editor controls

Add Maths Operation before current Entry
To build a Script, first set up the desired mathematical operation by selecting the appropriate source and target bank and the desired mathematical operator, then click on this button. This will insert the maths operation before the current selection in the script list.

Delete Current
If you wish to remove one of the maths operation that you have added, first select that operation then click on this button.

Clear Script
To remove all entries from the Script click on this button.

Test Script
Clicking on this button will test the effect of your script on the current set of images. Please note that if your script overwrites raw data you will not be able to do this more than once!

Load Script
This button allows you to load a previously saved script.

Save Script
This button allows you to save your script for later use.

Although the Exposure Manager allows a Maths Script to be executed automatically after a MultiExpose event this may not always be desirable. If for example you wish to save the raw data and then run a Maths Script you may wish to switch off the automatic triggering of the maths script from the More Options.. dialog in the Exposure Manager and instead use 'Execute Maths Script' from the Maths… menu. This will allow you to save both raw data and processed data.

The Maths Script is triggered automatically after you trigger a MultiExpose and you have ‘Use Maths Script after MultiExpose’ box checked in the ‘More Options’ Dialog. Even if you have no Image Banks included in the MultiExpose sequence you can still trigger the Maths Script by clicking on the MultiExpose button.
Chapter 11: The Tools Menu

The Tools... menu contain miscellaneous commands for clearing image memory, palette design and Matrix Image Visualisation.

- **Clear Current Bank**
  This option simply deletes the currently selected bank, setting all its pixels and properties to their default value (Zero).

- **Clear All Banks**
  This option simply clears all the banks in the Framestore and should therefore be used with caution!

- **Show Matrix**
  This option opens the Matrix Visualisation tool. For many types of ratiometric imaging there are at least two type of image produced: One is a normal intensity map of the scene i.e. a typical image. The other image is the result of a ratiometric operation between two or more images and contains no intensity information. The Matrix tool allows the user to map the ratio image onto the intensity image thus creating a hybrid 'Matrix' image.

  The Matrix Image Dialog be can be found in the Tools Menu. It allows you to select an ‘Intensity’ Image and a ‘Chroma’ image. The Intensity image then provides intensity data to the matrix image whilst the ‘Chroma’ image provides colour data. As you select different ‘Intensity’ and ‘Chroma’ image the source images and resultant ‘Matrix’ Image are updated in the dialog box in real time. You can also adjust the look-up table values for the images you have selected for greater control over the final image.

  The Matrix Image Dialog offers two ways of combining Intensity and colour data:

  - **Overlay-Multiply** mode Multiplies the colour in the ‘Chroma’ Image by the intensity values in the ‘Intensity’ image. This mode is particularly suitable for the combination of Intensity data with ratiometric data.

  - **Overlay-Add** mode Adds the colour in the Chroma Image to the intensity values in the Intensity Image. This mode is best suited to the combining of two intensity images.
The Matrix Image Dialog: Overlay-Multiply Mode

The Matrix Image Dialog: Overlay-Add Mode

One of the advantages of the matrix image is that it suppresses the parts of the ratio image which represent areas with low signal. In these areas the ratio calculation is usually most noisy and will often dominate the image.

● **Palette Designer**

The palette designer allows you to produce new palettes for the alternate palette. It works by allowing the user to specify up to 16 'set points' each of which can be assigned a 24-bit colour. The palette designer then generates a palette using one of two modes; 'spread' or 'fill'.
The spread mode shown below creates a smooth transition between each of the set points to fill the palette with 254 unique colours.

![Image of the Palette Designer in 'spread' mode]

The ‘fill’ mode shown below partitions the palette into discrete blocks of colours to give a palette with a small number of individual blocks of colour.

![Image of the Palette Designer in 'fill' mode]

Once you have chosen the number and colour of your setpoints you can save the palette by clicking on the ‘Save Palette’ button. If you click on either the ‘Save Palette’ or ‘Use Palette’ button then the new palette will become the alternate palette, selected when you click on the ‘Alt’ palette selector button.

If you wish to use your new palette as the default alternate palette every time you load the Imagex software then open the View... menu and select ‘Save Current Alternate Palette as Default’. It is a good idea to also save it under another name first (from the Palette Designer) so that you have an easily identifiable copy.

- **Clear Current Bank**
  This option simply deletes the currently selected bank, setting all its pixels and properties to their default value (Zero).

- **Reset All Sync Controls**
  This option sets all Sync Controls to ‘OFF’ for all of the Image Banks. This saves the user having to go through every Image Bank looking for Sync Controls to switch off.
Chapter 12: Saving and Loading your Experimental Setups

There are many parameters to adjust in a time-gated imaging system! Obviously you would not want to set the system up every time so we have provided a method for users to save the complete set of time-gating settings for the full set of image banks and to recall them at a later time.

The time-gating settings for all of the 16 image banks can be saved in a so-called 'Setup' file with file extension '.stx'.

The Setup file stores all of the time-gating parameters for all 16 image banks together with the Current Maths Script and various options such as the 'Use Maths Script' setting and the Lamp Combination settings.

Setup Files can be Saved and Reloaded from the Setup Menu as shown below.

In addition to saving a Setup file with a filename of your choice you can also opt to Save your current setup as the 'Default' setup (filename 'Default.stx'). This special file is loaded automatically when the software is started. So this an ideal way of setting up the system for highly automated use with minimal user interaction. The option for saving the Current Setup as the Default is also available from the More Options Dialog for your convenience.

Changing Gain and Offset
From the Setup Menu you can also change The Camera Gain and Offset values and save them for later use.
Chapter 13: Measuring Lifetime with the Imagex System

The Imagex System can be used to generate Fluorescence Lifetime Images using the Rapid Lifetime Determination method also known as ‘RLD’.

The Rapid Lifetime Determination method is suitable for two types of fluorescent sample:

1) A homogeneous single lifetime sample where the lifetime of that single lifetime component changes as a response to some environmental factor such as temperature or oxygen activity.

The decay of a single lifetime sample is outlined in the figure below:

![Diagram of single exponential decay](image)

| ![Diagram of single exponential decay](image) |

For a single exponential fluorescent sample with fluorescence lifetime $\tau$, the Intensity decays with time according to the relationship:

$$I(t) = Ae^{-t/\tau}$$

where $A$ is the 'pre-exponential' or starting intensity $e$ is the mathematical constant ‘$e$’ equal to approximately 2.7183 and $t$ is time.

NB: This relationship is correct for single exponential systems after the excitation pulse is completely extinguished.

Note that this describes the decay after the lightsource pulse has been completely extinguished. In cases where some of the fluorescence is collected whilst the lightsource pulse is still exciting the sample a different (and generally more complex) analytical approach should be taken.

2) A dual or multi-lifetime sample can also be measured where there is one 'Long' lifetime component and one or more 'Short' lifetime components. For the purposes of this description the 'Long' lifetime component should be at least 10x longer than any of the 'short' lifetime components. Once again, the 'Long' lifetime component might change in response to environmental factors. Because the Imagex System is a gated imaging system it can effectively reject and therefore not detect the short lifetime signals when the gate delay is set accordingly. In this mode this second type of sample appears to be identical to a single exponential long lifetime sample.
As shown below the RLD method requires the collection of two 'Source' Images, $S_P$ and $S_D$.

$S_P$ is the 'Prompt' Source Image or that with the shortest gate delay after the triggering of the light source.

$S_D$ is the "Delayed" Source Image or that with the longest gate delay after the triggering of the light source.

In the scenario above we can see the gates are exactly consecutive; the 'Delayed' gate begins when the 'Prompt' gate ends. All of the Lifetime Imaging Setup files supplied with the Imagex application software use this consecutive gates approach for collecting RLD images. However, the RLD calculation implemented in the Imagex software does allow the two gates to overlap or indeed to be separated as shown in the figures below:

\[ t = \frac{\Delta t}{\ln(S_P/S_D)} \]
RLD image collection with overlapping gates

$$t = \text{time (seconds)}$$

$$I = \text{Intensity (Arbitrary Units)}$$

- $G_p$: Gate width setting for "prompt" gated image.
- $G_d$: Gate width setting for "delayed" gated image.
- $\delta t$: Time delay between start of $G_p$ gate and $G_d$ gate.
- $S_p$: Integrated signal collected for "prompt" gated image pixel.
- $S_d$: Integrated signal collected for "delayed" gated image pixel.

Using the Rapid Lifetime Determination Method:

$$\tau = \frac{\delta t}{\ln(S_p/S_d)}$$

RLD image collection with non-overlapping gates

$$t = \text{time (seconds)}$$

$$I = \text{Intensity (Arbitrary Units)}$$

- $G_p$: Gate width setting for "prompt" gated image.
- $G_d$: Gate width setting for "delayed" gated image.
- $\delta t$: Time delay between start of $G_p$ gate and $G_d$ gate.
- $S_p$: Integrated signal collected for "prompt" gated image pixel.
- $S_d$: Integrated signal collected for "delayed" gated image pixel.

Using the Rapid Lifetime Determination Method:

$$\tau = \frac{\delta t}{\ln(S_p/S_d)}$$
The Imagex system is supplied with a number of Setup files for different lifetime ranges. These Setup files all follow the same rules:

- Both Source Images should have the same Gate width
- Both Source Images should have the same Lamp Excitation Pulse width
- Both Source images should have the same Lamp Trigger Frequency
- Both Source Images should use 'Background Subtraction' and not be 'Dark' images.

In addition to these rules we recommend that both source images are recorded with the same X and Y pixel-binning resolution. Recording the source images with different resolutions may produce strange gain effects because pixel-binning tends to increase the per-pixel signal and this may lead to incorrect lifetime calculations.

Because we are investigating a decaying signal the delayed source image, $S_D$ is generally less intense than the 'Prompt' $S_P$ source image. Therefore, to improve signal to noise on the calculated lifetime image it is permissible to increase the integration time of the delayed image so that it produces a more balanced signal. The relative integration times of the two images require some experimentation to get right for your system but as a general 'rule of thumb' we have provided Setup files where the integration time of the delayed image is 1.5 greater than that of the 'Prompt' image.

The Setup file controls the collection of the Source images. The calculation of the lifetime image is implemented by a 'Rapid Lifetime Calculation' routine that can be selected from the Maths... menu and that can also be added to a Maths Script for automated Lifetime Image collection.

**Imagex Lifetime Imaging Setup Files**

The Imagex Lifetime Imaging Setup files provides the application software with a set of instructions for the automated collection of Gated Source Images and subsequent processing and calculation of those files to generate a single lifetime image.

PRS has created a number of Setup files to cover different lifetime ranges. The imaging parameters arises from a number of factors including the desired lifetime range of the measurement and limiting factors such as average light intensity.

*Deciding upon the parameters for the Rapid Lifetime Source Images*

Here, we give one example of how we selected the imaging parameters for a given Setup file. There is no single correct way of generating these parameters; instead we must use our knowledge of the exponential decay process and any other factors that might affect sensitivity or signal-to-noise ratios. We believe that experimentation with known samples is an important part of the process of developing effective lifetime Setup files.

We start by considering the lifetime range we are interested in for our measurement. It is important to have some rough idea of the lifetimes we are expecting in order to correctly decide which trigger frequency, gate width and gate delays to use. For this example we will assume that our lifetime range of interest is 0 to 10 microseconds.

Ideally, we would like to select a frequency range where, on a cycle to cycle basis the emission triggered by one cycle has essentially decayed away by the beginning of the next cycle.
In the figure below we see a single exponential decay for a sample with a lifetime of 10μsec.

\[ I(t) = Ae^{-t/\tau} \]

\[ \tau = 10\mu\text{sec} \]

\[ A = 100 \]

Looking at this curve we can see that the decay has substantially disappeared by 70μsec or 7 times the lifetime. For simplicity we have chosen to use the trigger frequency with cycle time of 10 times the high end of the lifetime range as this allows us to include the time for the light pulse within the cycle. There is a disadvantage in this approach in that we are clearly using a relatively low duty cycle and in doing so extending the collection time for the data. Obviously users can choose to use a less conservative (i.e. higher) frequency if they want to get higher average excitation intensities and shorten measurement times.

The Imagex system has a prescribed list of allowed frequencies that can be used for data collection, so we must choose the frequency that gives the best match to the '10 times lifetime' rule we have created. The available frequency list is 10Hz, 20Hz, 50Hz, 100Hz, 200Hz, 300Hz, 500Hz, 1000Hz, 2000Hz, 3000Hz, 5000Hz, 10000Hz, 20000Hz, 30000Hz, 50000Hz and 100000Hz.

Therefore, for our example, we have established a lifetime range of 0 to 10μsec, and ascribed a trigger frequency of 10kHz (i.e. a cycle time of 100μsec or 10 times the lifetime of 10μsec).

Next, we should establish the Gate Delay setting for the first or 'Prompt' gated source image. This is often both sample- and lightsource-dependent and can be optimized by experimentation. However, a good rule of thumb is to assume that most short lifetime (i.e. nanosecond range) background fluorescence and scatter will approximately follow the profile of the light source. If we are using a light source with a switch-off time of say 100nsec we would recommend a delay of 10 times this value for the Gate Delay for the 'Prompt' Source Image. So, for this imaginary imaging system we would use a use a gate delay of 1 microsecond for the 'Prompt' Source image. Keeping the first delay relatively short will offer increased sensitivity for both the 'Prompt' and the 'Delayed' Gated Source images.

The next parameter we need to decide upon is the gate width for the two gated source images. For our Setup files we chose to use the a gate width value of 2.5 x the median value for the
range. For a 0 to 10 microsecond range the median value is 5usec and the gate width is therefore 12.5μsec. Once again the user can change this rule based on their own experimental results.

The delay difference between the 'Prompt' gated source image and the 'Delayed' gated source image is obtained simply if we are using the 'consecutive' gates model (see above) as it is the same as the gate width for both of the gated source images, namely 12.5μsec. We will also use this rule for the lamp pulse width for both source images. For a single exponential decay the lamp pulse width should not affect the calculation of the lifetime as long as the gates for the two source images are set to begin after the light source is completely extinguished.

Without having an idea of sample concentration and optical efficiency of filters and lenses used for the measurement we cannot ascribe meaningful integration times for the two Gated Source images, but to allow us to generate our setup files we will ascribe integration times of 1 second to the 'Prompt' Gated Source image and 1.5 seconds to the 'Delayed' gated source image. The Imagex Rapid Lifetime calculation corrects for differences in integration times when calculating lifetimes.

In summary therefore we have generated the following parameters for measuring lifetimes in the range 0 to 10usec.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trigger Frequency for both images</td>
<td>10kHz</td>
</tr>
<tr>
<td>'Prompt' Source Image Gate Delay</td>
<td>1μsec</td>
</tr>
<tr>
<td>'Prompt' Source Image Gate Width</td>
<td>12.5μsec</td>
</tr>
<tr>
<td>'Prompt' Source Image Lamp Pulse Width</td>
<td>12.5μsec</td>
</tr>
<tr>
<td>'Prompt' Source Image Integration Time</td>
<td>1 sec</td>
</tr>
<tr>
<td>'Prompt' Source Image is collected into Bank 1</td>
<td></td>
</tr>
<tr>
<td>'Delayed' Source Image Gate Delay</td>
<td>13.5μsec</td>
</tr>
<tr>
<td>'Delayed' Source Image Gate Width</td>
<td>12.5μsec</td>
</tr>
<tr>
<td>'Delayed' Source Image Lamp Pulse Width</td>
<td>12.5μsec</td>
</tr>
<tr>
<td>'Delayed' Source Image Integration Time</td>
<td>1.5 sec</td>
</tr>
<tr>
<td>'Delayed' Source Image is collected into Bank 2</td>
<td></td>
</tr>
</tbody>
</table>

Background subtraction ON for both Source Images
'Ungated' Option switched OFF for both Source Images
Resolution setting 'High'
Include in MultiExpose selected for Image Banks 1 and 2

Also

In the 'More Options' Dialog
'Use Maths Script after Multiexpose' is selected
In the embedded Maths Script the Lifetime Image is calculated into Bank 3
'Select Gating Mode' is set to 'Standard Imagex Gating Mode'
'Lamp Output' is selected for Lamp1, Lamp 2 and Lamp 3.
'MultiExpose Integration Count' is set to 1

The Rapid Lifetime Method Setup files supplied by default with the Imagex application software are designed to be optimized for given lifetime ranges. You may find that the lifetime range of your samples do not match well with any of the supplied Setup files. You should feel free to experiment with creating new Rapid Lifetime Setup files with new Trigger frequencies, Gate Widths and Gate Delays to optimize your measurements. The important thing to remember when creating new Setup files is to follow the rules shown above. It is often a good idea to use one of the supplied Setup files as a starting point and to then change one of the allowed parameters to see if it delivers improved performance for your sample. Remember to save your new Setup file with a new filename. If you require further help please contact PRS by e-mail at info@prsbio.com.
The Imagex Rapid Lifetime Calculation

The Imagex Rapid Lifetime Calculation can be selected either by creating a Maths Script that calls the RLC function or by selecting the function directly from the Maths menu. If you want a greater degree of automation and versatility then the Scripting approach is recommended. However if you simply want to familiarize yourself with what the function does then it is a good idea to click on the Maths Menu to try it out.

Select the 'Rapid Lifetime Calculation' option from the Maths... menu thus:

You will then be given the option of setting the Image Bank locations for the 'Lifetime' image and the 'Prompt' Source and 'Delayed' Source Images. Once you have set these then you can click on 'Apply Maths Operation' to calculate the lifetime image. Notice that you do not need to enter any other numeric data because the software calculates the delay difference from the image parameters in the selected Source banks.
In the example figure shown above the Lifetime Image will be calculated into Bank 3 whilst the 'Prompt' Source image and the 'Delayed' Source image are located in Banks 1 and 2 respectively.

Before performing the calculation the software checks that the Source Images comply with the rules set out for the Rapid Lifetime Method.

These rules are as follows:

- Both Source Images must have the same Gate Width and the Gate Width should have a positive value.
- Both Source Images should have the same Lamp Excitation Pulse width
- Both Source Images must have the same Trigger Frequency
- The Gate Delay of the 'Delayed' Source Image should be greater than the Gate Delay of the 'Prompt' Source Image.
- Both Source Images should be 'Gated' (i.e. not Ungated)
- Both Source Images should use 'Background subtraction' and NOT be 'Dark' Images.

If one or more of these rules are not met the system will display a message with information about the error and the calculation will be not proceed. At this point the Image Collection Parameters should be adjusted so that they match the requirements of the Rapid Lifetime Method. If the rules are met then the next time the calculation is requested it will proceed and the lifetime image will be generated in the selected target bank (Bank 3 in the example figure shown above).

An Error Dialog will be displayed if your Source Image Parameters are not appropriate for the Rapid Lifetime Calculation.

For the Rapid Lifetime Calculation to produce a result you will, of course, need some Source Images. These need to be a pair of Time-Gated Source Images with parameters that comply with the rules outlined earlier. To get you started we have included a special Setup file called 'RLCpair.stx'.

To load this Setup File click on the Setup Menu and Select 'Load Experimental Setup'.

Assuming your system is all set up, switched on and connected to a PC with the Imagex application running you can now simply click on the Multiexpose button to collect your pair of Source Images into Banks 1 and 2.

If you now revisit the Maths menu and Select 'Rapid Lifetime Calculation' you can generate your lifetime image. You may have to adjust the integration times for Bank 1 and 2 to get the exposure right but if you are new to the Imagex system then this is a good way to introduce yourself to the software. Remember that if you are using this step-by-step approach to creating your lifetime
images then you will need to recalculate the lifetime image each time you change one of the imaging parameters.

**Automating the Lifetime Imaging Process with Scripting**

You will probably find that using this step-by-step approach to lifetime imaging is rather cumbersome. Fortunately, the Imagex software is not really supposed to be used this way. Instead we would normally use a MultiExposure capture sequence in combination with a Maths Script.

The Imagex MultiExposure function allows you to automatically collect a sequence of images, but it also allows you to automatically run a Maths Script after the sequence has been collected. The Maths Script allows you to carry out a sequence of mathematical operations on your source data and one of those Mathematical operations is the Rapid Lifetime Calculation.

The Imagex software is supplied with a number of Setup files to perform lifetime imaging at different lifetime ranges. These are named 'RLC0-10usec.stx', 'RLC0-20usec.stx', 'RLC0-50usec.stx', RLC0-100usec.stx, 'RLC0-200usec.stx' and 'RLC0-1000usec.stx’. All of these Setup files automatically collect a pair of source images and then calculate the Rapid Lifetime Image.

The following Table shows the parameters used for each of these Setup files:

<table>
<thead>
<tr>
<th>Setup Filename</th>
<th>Lifetime Range (usec)</th>
<th>'Prompt' Gate Delay (usec)</th>
<th>'Delayed' Gate Delay (usec)</th>
<th>Gate width for 'Prompt' and 'Delayed' Image and Lamp Pulse Width (usec)</th>
<th>Trigger Frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLC0-10usec.stx</td>
<td>0-10</td>
<td>1</td>
<td>13.5</td>
<td>12.5</td>
<td>10,000</td>
</tr>
<tr>
<td>RLC0-20usec.stx</td>
<td>0-20</td>
<td>1</td>
<td>26</td>
<td>25</td>
<td>5,000</td>
</tr>
<tr>
<td>RLC0-50usec.stx</td>
<td>0-50</td>
<td>1</td>
<td>63.5</td>
<td>62.5</td>
<td>2,000</td>
</tr>
<tr>
<td>RLC0-100usec.stx</td>
<td>0-100</td>
<td>1</td>
<td>126</td>
<td>125</td>
<td>1,000</td>
</tr>
<tr>
<td>RLC0-200usec.stx</td>
<td>0-200</td>
<td>1</td>
<td>251</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>RLC0-1000usec.stx</td>
<td>0-1000</td>
<td>1</td>
<td>1251</td>
<td>1250</td>
<td>100</td>
</tr>
</tbody>
</table>

The Script that is associated with the RLC Setup files is very simple. It calculates the Lifetime Image and then prompts the user to save that file. This simple script is shown below as it appears within the Script Editor.
The Maths Script embedded within the RLC Setup Files

To find out how to create more complex scripts please see Chapter 10 of this user manual. If you find that you use a particular Setup file very often then it is a good idea to save that Setup file as the Default Setup file, also called ‘Default.stx’. This file is loaded automatically when you start the Images application. You can save the current Setup as the Default Setup from the More Options Dialog or from the Setup Menu.

Please note that the above files are generated at the beginning of every software session. If you are creating new setup files you should take care to create files with new and unique filenames otherwise they could be overwritten by the system-generated RLC setup files.

● Scaling Factors for Imagex 16-bit TIFF files created using the Rapid Lifetime Calculation.

Imagex can save data in two formats: its native 'IMW' format and an industry standard 16-bit TIFF format. Both types of files contain pixel data as an array of 16-bit integers. Whilst this is quite flexible it is not able to represent every sort of fluorescent lifetime that you might encounter.

.IMW files and 16-bit TIFF files both hold signed 16-data. This data has a possible range of -32768 to +32767. If we were to say that a pixel value of 1 represented a lifetime of 1 nanosecond then we could have an image representing lifetimes from 0 nanoseconds to 32767 nanoseconds. This is quite a good range for many applications but certainly does not cover everything.

The Imagex software solves this problem within its own file format by embedding a number of parameters within the file. These factors define the temporal units associated with the pixel array (either nanoseconds, microseconds or milliseconds) and a scaling factor. The scaling factor is the number that you multiply the raw pixel value by to get the actual lifetime value of the sample you have measured. You do not need to worry about how these parameters are used within the Imagex environment because they are handled automatically to show you lifetime values on the main display.

When you export the 16-bit data as a TIFF file you can only export the 16-bit values. You will then need to know the scaling factor and time unit associated with that file so that you can convert the 16-bit data into lifetime values. When Imagex exports a TIFF file it adds an Image Description tag that can be read by most image readers. You can use this information to get the correct lifetime information from any Imagex Lifetime TIFF file.

The tag is composed of two parts the first part features the temporal unit enclosed within brackets. Immediately to the right of this, without a space is the scaling factor.

Such a tag would appear as for example, (usec).1 This tag would mean that the when multiplied by 0.1 the pixels represent the lifetime in microseconds.

Another example would be (nsec)1 This would mean that when multiplied by 1 the pixels represent the lifetime in nanoseconds.
Chapter 14: Imagex Automation

The Imagex multi-expose button allows you to collect up to 16 images with User-programmed exposure times, and gating settings with the click of a single button. Furthermore, the multi-expose feature allows you to automatically run a maths script after image collection and then prompt the user for a filename to save the resulting data.

Imagex Automation takes the process of automatic image collection one stage further: multi-exposures, maths scripts execution and data saving can be set to run unattended for a user-defined length of time and number of repetitions.

- Imagex Automation Features

  Imagex Automation uses a Setup just like when you perform an multi-expose. The Setup will usually include the selection of banks to be exposed together with their respective gating settings and optionally a maths script to be executed automatically after image collection is complete. These functions are described earlier in this manual. Although it is not a requirement to save your settings in a setup file, it is strongly advised, particularly if you have a complicated set of image collection parameters.

  The purpose of the Imagex Automation function is to as follows:

  1) Allows a multi-expose/script execution to be repeated a user-defined number of times without user input.
  2) Allows the user to define a pause in between multi-expose/script executions
  3) Automatically saves the generated data without a user prompt.

This combination of functions allows the system and its associated PC workstation to be left unattended for many hours. With this in mind the user should ensure that the PCs energy-saving features are set correctly to prevent the computer automatically switching OFF or going into sleep mode during the automation run.

- Imagex Automation Controls

  If you have developed your multi-expose setup you can quickly move on to setting up your Automation Run. To do this you should go to the Automation menu and select Edit Automation Controls.

  ![Automation Menu]

  You will now see the dialog for the Automation Control.
Imagex Automation has 5 main controls as follows:

i) Interval (min)
   This is the time in minutes that the system waits in between each multi-expose cycle. It is used in conjunction with the Interval (sec) control. Thus the pause between multi-expose cycles is the sum of the Interval (min) value in minutes and the Interval (sec) value in seconds.

   e.g. if Interval (min) = 10 and Interval (sec) = 30 then the pause between multi-expose cycles will be 10 minutes and 30 seconds or 630 seconds.

ii) Interval (sec)
    This is the time in seconds that the system waits in between each multi-expose cycle. It is used in conjunction with the Interval (min) control. The pause between multi-expose cycles is the sum of the Interval (min) value in minutes and the Interval (sec) value in seconds.

iii) Repeat Number
    Once you have started an Imagex automation run by clicking on the 'Auto' button Imagex Automation will continue to trigger multi-expose cycles for the number of times selected in the 'Repeat Number' control.

    An automation run can be ended (not paused) by clicking on the STOP button on the main screen.
iv) Root Filename

*Imagex* Automation automatically saves data. To save disk space it only saves data classed as 'Unsaved Data'. It does not save blank/empty images. Unsaved data includes any recorded image data and any data produces as a result of automatically running the maths script after each multi-expose image collection cycle.

*Imagex* Automation always uses the *Imagex* 'data' directory as a working directory, but to improve file organization and prevent the chance of overwriting of previous data sets it creates a new sub-directory within the *Imagex* 'data' directory to store all the data for that given automation run.

For example if you have set the Root Filename to 'test' then *Imagex* Automation will take the following actions:

1) Create a directory in the imagex data directory thus

\data\test_on_11-7-17_at_13h22m

Note that the system creates the directory filename by combining the Root Filename and the date and time at which the Automation run was initiated. This feature prevents accidental overwriting of data when repetitively using the same Root Filename.

2) As the Automation Run progresses it will save *Imagex* files with .ims file extensions containing all the unsaved data from each multi-expose cycle. The filename is generated by combining the Root Filename with the time in seconds after the start of the run.

\data\test_on_11-7-17_at_13h22m\test0sec.ims
\data\test_on_11-7-17_at_13h22m\test3600sec.ims
\data\test_on_11-7-17_at_13h22m\test7200sec.ims
\data\test_on_11-7-17_at_13h22m\test10800sec.ims

etc...

v) Automatically Save TIFF files

If you have checked the Automatically Save TIFF files box then, in addition to the native *Imagex* files, the system will also save corresponding TIFF files for each Image bank containing 'Unsaved data'. Thus, in the above example the following files would be saved automatically.

\data\test_on_11-7-17_at_13h22m\test0sec.ims
\data\test_on_11-7-17_at_13h22m\test0sec_Bank_1.tif
\data\test_on_11-7-17_at_13h22m\test0sec_Bank_2.tif
\data\test_on_11-7-17_at_13h22m\test0sec_Bank_3.tif
\data\test_on_11-7-17_at_13h22m\test3600sec.ims
\data\test_on_11-7-17_at_13h22m\test3600sec_Bank_1.tif
\data\test_on_11-7-17_at_13h22m\test3600sec_Bank_2.tif
\data\test_on_11-7-17_at_13h22m\test3600sec_Bank_3.tif
\data\test_on_11-7-17_at_13h22m\test7200sec.ims
\data\test_on_11-7-17_at_13h22m\test7200sec_Bank_1.tif
\data\test_on_11-7-17_at_13h22m\test7200sec_Bank_2.tif
\data\test_on_11-7-17_at_13h22m\test7200sec_Bank_3.tif
\data\test_on_11-7-17_at_13h22m\test10800sec.ims
\data\test_on_11-7-17_at_13h22m\test10800sec_Bank_1.tif
\data\test_on_11-7-17_at_13h22m\test10800sec_Bank_2.tif
\data\test_on_11-7-17_at_13h22m\test10800sec_Bank_3.tif

etc...
Starting an Imagex Automation Run

Once you have set up your multi-expose sequence, your automated maths script and set your automation controls, then you can trigger an Automation Run.

To do this simply click on the 'Auto' Button on the main screen.

![Clicking the 'Auto' Button starts an Imagex Automation Run](image)

To end the automation run you should click on the STOP button.

![Clicking the 'STOP' Button ends the Imagex Automation Run](image)

If you choose to end the automation run you will still have all the complete data sets up until you chose to end the run. So if you are not sure how long you want your run to be it is safe to set the number of cycles to more than your actually need and then simply interrupt it when you consider you have enough data.

If the Root Filename has not been set or has been set to a blank string then the system will replace the Root Filename with the string "temp" to allow legal filenames to be created.

Tips when using Imagex Automation

*Imagex* Automation is intended for long-term experiments. We therefore recommend that you check the following items before starting a long experiment:

i) If your maths script includes a rapid lifetime calculation you should ensure that the data collection matches the specifications for this calculation. The best way to do this is to trigger a multi-expose with a test sample to confirm that the system does not flag up any errors. In any case you should ensure that your setup performs the intended functions by at least running the multi-expose sequence once before your main Automation run.

ii) Ensure that you have enough disk space for the data you will generate. Potentially, Imagex Automation can generate a lot of data.

iii) Ensure that the power saving features on your system are set appropriately for a computer to be left unattended for many hours.
APPENDIX I: Installing the QuickUSB Driver

To Install the QuickUSB Driver:

1) On the Imagex installation disk navigate to the QuickUSB folder

2) Double click on the Setup.exe application

3) Follow the Instructions in the Setup program

4) You can now plug in the Imagex system using the USB cable provided. Your computer should recognize the interface and start the hardware installation.
APPENDIX II: Installing the *Imagex Software*

To Install the *Imagex* software insert the installation CD into the drive then:

1) Use Windows Explorer to navigate to the folder 'Imagex Application Software 32-bit' or 'Imagex Application Software 64-bit' (depending on your operating system) on the Imagex Installation disk.

2) Double-Click on the 'Setup' Icon

3) Follow Instructions in the Setup program

4) The *Imagex* Software is now installed
APPENDIX III: Troubleshooting

Hopefully, you will find the Imagex system easy to use. If you have problems try this quick troubleshooting guide. It solves some of the most common problems that you might encounter. If this doesn't work contact PRS at info@prsbio.com.

**Problem: The Image is saturated very bright....**

*Possible solutions*

  **Maybe there is simply too much light!** This is not always as obvious as it sounds. If you are conducting a fluorescence measurement, check that your filter set is appropriately blocked otherwise emission from your light source might be getting straight into the camera. Also remember that ultraviolet and infrared light will also be seen by the camera, so just because the scene looks dark doesn't mean that there is no light there....

  **Is everything Plugged in?** This might be a time to check your connections from computer to interface box and from interface to camera head. Remember that your computer and camera system should be switched OFF before plugging or unplugging any connectors

**Problem: The Image is black/very dark....**

*Possible solutions*

  **Maybe there is simply too little light!** If you are using narrow band filters or low transmission filters on your camera you may have to open the aperture of your lens or increase your exposure time. Remember also that lenses vary widely in their efficiency of light collection

  **Is everything Plugged in?** This might be a time to check your connections from computer to interface box and from interface to camera head. Remember that your computer and camera system should be switched OFF before plugging or unplugging any connectors

  **Are you using a standard USB 2.0 port?** Using Imagex connected to a USB Hub may cause unexpected problems. Please ensure that you use a standard USB 2.0 as installed in the main body of your computer.

  **Are you using a low duty cycle?** If you combine a low frequency with a short gate frequency (e.g. 300Hz with a 1-microsecond gate) then the time when the camera is actually sensitive to light will be much shorter than the exposure time. This may not matter if you are using a pulsed light source such as a flashlamp with a relatively high peak power.
APPENDIX IV: Lens and Microscope mounting

The Imagex camera is provided as a standard C-Mount camera for use with a wide variety of lenses and microscope camera ports. However, there are situations where the distance between the lens or microscope mounting and the CCD sensor needs to be adjusted.

The user can add C-mount extenders or install spacer rings between the camera front plate and the lens to extend the distance between CCD and lens. This approach is often used when trying to reduce working distance and field of view for standard C-Mount lenses. (Spacer rings and C-mount extenders are widely available from video lens suppliers. See Edmund Scientific Inc for examples)

The camera sensor is thermoelectrically cooled within a sealed 'cold chamber'. The optical aperture to the CCD cold chamber is protected by a double anti-reflection window system. The outer window is usually colder than the ambient temperature. Therefore, to ensure that condensation on the outer window does not occur the camera should not be operated without an appropriate C-Mount lens in place. This is particularly important when using the system in conditions of high humidity and warm ambient temperature. In addition you should allow the camera to return to ambient temperatures before removing or changing the C-Mount lens.

If condensation occurs on the front window in the absence of a C-Mount lens you should ensure that the system power is switched OFF and then allow the system to return to ambient temperature before fitting another lens. Mounting a lens whilst there is condensation on the front window can lead to trapping of condensed water between lens and outer window and thereby cause deterioration in image quality.
APPENDIX V: System connections:
The Imagex system is supplied with a special power supply box to generate the correct voltages and currents for the Main Camera Unit. This power supply unit is powered by a single universal switched mode power supply that plugs directly into your mains supply.

To help you set up your system the various connections to the Imagex main camera unit are shown below. Please follow these connection instructions carefully.

The 24V switched Mode Power Supply
Your Imagex system is supplied with a 24Volt Switched mode power cable that can be used around the world. We recommend that you use only this power supply with your Imagex system. You should use an appropriate mains cable to connect the power supply to your local mains supply.

The Imagex Power Supply Box
Shown below is a diagram of the Imagex Power Supply Box. The purpose of this box is to take the 24V DC power and generate the correct voltages for the different components of the main Imagex Camera Unit.

The Imagex Power Supply Box is very simple to use:
1) Connect the 24V Switch Mode Power Supply to the 24V DC Input socket on the Imagex Power Supply Box
2) Plug the supplied 4-Core Cable into ‘Camera PSU’ socket on the Imagex Power Supply Box and then plug the other end of the cable into the ‘PSU’ Socket on the main Imagex Camera Unit.

The Imagex 4-Core Power Supply Cable
Your Imagex system is supplied with a a 4-Core Shielded Power Supply Cable to connect the output of the Imagex Power Supply Box to the PSU Input on the Main Camera Unit. The cable and how it is connected is shown below.
How to connect the *Imagex* Power Supply Box
The **Imagex Main Camera Unit**

The back panel of the Imagex Main Camera Unit has a number of connections:

1) The PSU connector is used to connect to the Imagex Power Supply Box and has a 4-Core Bayonet fitting
2) The USB 2.0 connector should be used to connect the Camera a USB 2.0 port on your host computer.
3) Trigger Outputs L1, L2 and L3 supply TTL control signals to appropriate light sources using channels 1, 2 and 3 as defined in the system software. These outputs are BNC 50Ohm terminated TTL.

You may notice that both the *Imagex* Main Camera Unit and the *Imagex* power Supply Unit have Ventilation Inlets and Outlets. It is very important that these are not blocked or covered in any way. Failure to observe this could lead to damage to the camera.
APPENDIX VI: Imagex Specifications

Sensor Type: Interline CCD 1/2" format-Monochrome

Image Resolution: 752 x 582 in 2 interlaced frames

Spectral Sensitivity: Maximum@500nm, 62% of maximum@400nm, 93% of maximum@600nm, 62% of maximum@700nm, 32% of maximum@800nm

Readout System: 14-bit A/D Convertor with Correlated Double Sampling

Interface: USB 2.0

Light Output Triggers: 3x (L1 and L2 with 1 nanosecond resolution control on Imagex nanoCCD), 50 Ohm matched TTL Level 5V=ON 0V=off. BNC Socket.

Horizontal Binning: 1 to 40 pixels 'On-chip' under software control

Vertical Binning: 1 to 40 pixels 'On-chip' under software control

Lamp Trigger Frequency: 10Hz to 100kHz

Nanosecond Delay Unit: 2 Channel 0-255nsecs with 1 nsec resolution on Imagex nanoCCD only

Power Supply: 24V DC >2Amp

Full Frame Readout Time: 0.4 seconds

Minimum Gate Width: 1 microsecond

Tripod/Optical Mount: Standard 1/4 20 UNC mount

Lens Mount: C-Mount

Cooling System: Fan-assisted 3 Stage Peltier Cooler

Cold Chamber: Sealed Neoprene/ABS Block system with Double Antireflection coated windows.

Operating System: Windows XP, Vista, Windows 7/8/10 operating Systems

Standard Systems supplied with:
1 x System Unit containing CCD, Delay Generators, Gating Circuitry, lamp trigger units and Image readout system.

1 x Imagex Power Supply Unit

1 x 4-Core Imagex Power Supply Box-to-Camera Main Unit Power Cable

1 x USB 2.0 Cable for computer connection.

1 x BNC to BNC Cable to connect a Light source.

1 x Software Installation Disk

1 x Imagex User Manual

we also supply

1 x 24V Switched Mode Power Supply. (Customer must supply mains lead for local mains supply)
APPENDIX VII: *Imagex* nanoCCD fixed and custom gating modes

The *Imagex* nanoCCD has a number of special modes for higher resolution control of the lightsource trigger output signal.

These modes are selected from the 'More Options' Dialog and are only available for the *Imagex* nanoCCD. You may notice that whilst any of these modes are selected the normal gating controls are dimmed out and cannot be used.

It is important to understand that in each of these modes the delays arising from cable lengths, optical pathlengths and lamp electronics may be significant. It is therefore the responsibility of the user to find the nanosecond delay setting required for the light source pulse to correspond with the onset of sensitivity of the camera gate.

25nsec Lamp Pulse with fixed 1usec Gate Mode

This mode operates at the selected trigger frequency. However both the Lamp pulse width and Gate width are fixed at 25nsec and 1usec respectively. The 0-255 nanosecond delay can be used to vary the position of the short lamp pulse with respect to the Switch ON of the sensitivity of the camera (the beginning of the gate period).

50nsec Lamp Pulse with Fixed 1usec Gate Mode

This mode also operates at the selected trigger frequency. The Lamp pulse width and Gate width are fixed at 50nsec and 1usec respectively. The nanosecond delay can be used to vary the position of the lamp pulse with respect to the Switch ON of the sensitivity of the camera (the beginning of the gate period)

Custom Modulation Profile

This mode is provided for future expansion. The software loads a pre-defined modulation profile from a file in the application root directory and this pattern is applied to the camera and light source at the selected trigger frequency. The Lamp pulse width, delay and Gate width are defined in the Custom Modulation File.

Once again the 0-255 nanosecond delay is applied to Lamp channels 1 and 2.

At present Custom Modulation Profiles are only developed by Photonic Research Systems as it is necessary for us to confirm that any proposed modulation profile is valid for correct camera gating functionality. If you wish to use a new Custom Profile please contact us and we will test it for you.
APPENDIX VIII: The Imagex Test Report

As part of our quality control process we put each Imagex camera through a number of tests designed to check each aspect of the system.

These tests are performed at board level before the camera is installed in its case and are repeated once the camera is complete. The results of the tests performed at this final stage are compiled into a short report and provided to the customer. An electronic copy of the report is also included on the installation disk provided with the camera.

The Imagex test report contains the serial number of the camera and this should be used as a reference in case of any problems found with the camera.

In addition to the serial number the test report also contains a recommended video gain and offset level that is specific to that camera. We recommend that when you set up your camera you set the gain and offset levels to match the values recommended in the report. This also applies to any software written with the Imagex Programmers Library.

These settings should be seen as a starting point for using the camera and it is always open for the user to experiment with these values for themselves.