

# **UltraVIEW ERS Getting Started Guide**

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## 2 . UltraVIEW ERS Getting Started Guide

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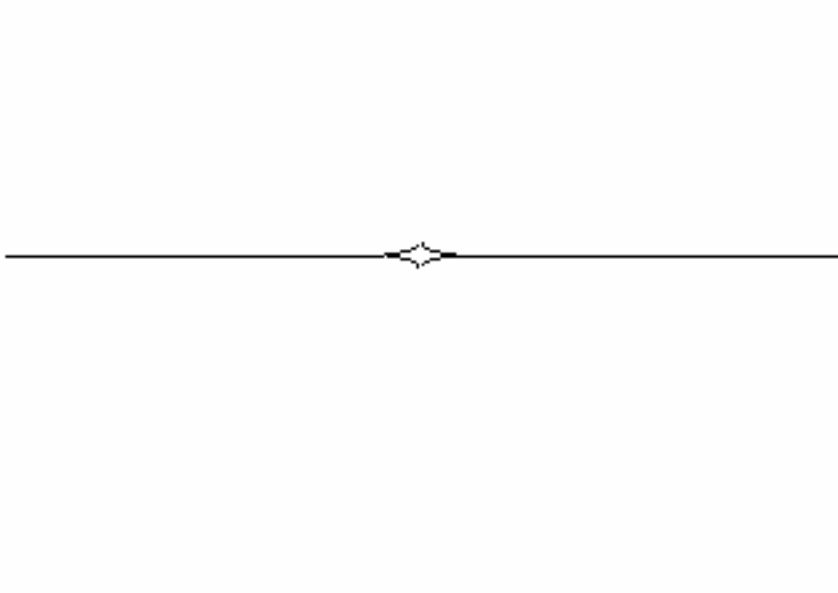
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# **Introduction**

## ***General Overview***

---

UltraVIEW ERS is a comprehensive, fast and low light level confocal image acquisition and analysis system for images generated individually, or in sequences, from a microscope.

UltraVIEW ERS differs from some other systems in that it does not use a separate image processing unit, but rather reads and writes from a hard disk using a fast disk controller with advanced data caching. Full integration with Microsoft Windows® at the system level gives high performance and seamless integration with existing laboratory systems.

To achieve high speeds and yet maintain superb image quality, the digital camera uses a cooled charge coupled device (CCD) detector.

### ***Image processing and analysis***

UltraVIEW ERS supports several key morphometric analysis techniques, to provide measurement of the changing state of the sample of interest. Images can be viewed in pseudo-color or monochrome or combined to composite color images on the high-resolution color graphics display. The color palette is user-selectable. Data can be output as a movie to include in presentations.

### ***Further information***

For further information about using the system, see the *Operation* section of this manual, starting on page 43, and the on-screen help system as described in *Help System* on page 76.

To familiarize yourself with UltraVIEW ERS we suggest that you work through the on-screen tutorials. The UltraVIEW tutorials can be opened from the Help menu in the software, or from the Windows Start menu by selecting Programs and then PerkinElmer Software.


**NOTE:** To display the tutorials correctly, ensure that the screen resolution of your monitor is set to 1920 by 1200 pixels.

For further information about the laser and camera systems, see the individual manuals supplied.



## ***A Quick Guide to Image Capture***

---

1. Turn on the UltraVIEW ERS system (laser, filter controller, synchronization box, confocal scanning unit, camera controller, synchronization controller, PC).
2. Start the UltraVIEW ERS Imaging Suite software, and then click **CAPTURE**.
3. In the Gallery Tree View, create a **New User** and **New Experiment** as required.
4. Focus the sample under microscope using Brightfield or epifluorescent illumination.
5. Switch lightpath to sideport (to confocal scanning unit).
6. In the Hardware Controller, select **Fast Sequential** for fast, dual color excitation discrimination experiments, or **Emission Discrimination** to use bandpass filters.
7. Select the appropriate **Dichroic** button.
8. Select the required laser line and then adjust the **Power**.
9. Select the appropriate filter from the **Emission** drop-down list (**Emission Discrimination** only).
10. Focus the sample.  
Use the **Autoscale** check box on the Grey Levels dialog if you need help finding the sample.
11. Optimize camera settings on the Image Control dialog: exposure, binning, gain, subarray.
12. In the Hardware Controller, select the required channel **Record** check boxes.
13. Define acquisition criteria: **z**, **t**, **delay**.
14. Click  on the Sequence Control panel to start acquisition.

## ***Conventions Used in this Manual***

---

Normal text is used to provide information and instructions.

**Bold** text refers to text that is displayed on the screen.

UPPERCASE text, for example ENTER or ALT, refers to keys on the PC keyboard. '+' is used to show that you have to click two keys at the same time, for example, ALT+F.

All eight digit numbers are PerkinElmer part numbers unless stated otherwise.

### ***Notes, cautions and warnings***

Three terms, in the following standard formats, are also used to highlight special circumstances and warnings.

**NOTE:** A note indicates additional, significant information that is provided with some procedures.

**CAUTION**

We use the term **CAUTION** to inform you about situations that could result in **serious damage to the instrument** or other equipment. Details about these circumstances are in a box like this one.

**D****Caution (Achtung)**

Bedeutet, daß die genannte Anleitung genau befolgt werden muß, um einen **Gerüteschaden** zu vermeiden.

**DK****Caution (Bemærk)**

Dette betyder, at den nævnte vejledning skal overholdes nøje for at undgå en **beskadigelse af apparatet**.

**E****Caution (Advertencia)**

Utilizamos el término **CAUTION (ADVERTENCIA)** para advertir sobre situaciones que pueden provocar **averías graves en este equipo** o en otros. En recuadros éste se proporciona información sobre este tipo de circunstancias.

**F****Caution (Attention)**

Nous utilisons le terme **CAUTION (ATTENTION)** pour signaler les situations susceptibles de provoquer de **graves détériorations de l'instrument** ou d'autre matériel. Les détails sur ces circonstances figurent dans un encadré semblable à celui-ci.

**I****Caution (Attenzione)**

Con il termine **CAUTION (ATTENZIONE)** vengono segnalate situazioni che potrebbero arrecare **gravi danni allo strumento** o ad altra apparecchiatura. Troverete informazioni su tali circostanze in un riquadro come questo.

**NL****Caution (Opgelet)**

Betekent dat de genoemde handleiding nauwkeurig moet worden opgevolgd, om **beschadiging van het instrument** te voorkomen.

**P****Caution (Atenção)**

Significa que a instrução referida tem de ser respeitada para evitar a **danificação do aparelho**.



**WARNING**

We use the term **WARNING** to inform you about situations that could result in **personal injury** to yourself or other persons. Details about these circumstances are in a box like this one.

**D**

**Warning (Warnung)**

Bedeutet, daß es bei Nichtbeachten der genannten Anweisung zu einer **Verletzung** des Benutzers kommen kann.

**DK**

**Warning (Advarsel)**

Betyder, at brugeren kan blive **kvæstet**, hvis anvisningen ikke overholdes.

**E**

**Warning (Peligro)**

Utilizamos el término **WARNING (PELIGRO)** para informarle sobre situaciones que pueden provocar **daños personales** a usted o a otras personas. En los recuadros como éste se proporciona información sobre este tipo de circunstancias.

**F**

**Warning (Danger)**

Nous utilisons la formule **WARNING (DANGER)** pour avertir des situations pouvant occasionner des **dommages corporels** à l'utilisateur ou à d'autres personnes. Les détails sur ces circonstances sont données dans un encadré semblable à celui-ci.

**I**

**Warning (Pericolo)**

Con il termine **WARNING (PERICOLO)** vengono segnalate situazioni che potrebbero provocare **incidenti alle persone**. Troverete informazioni su tali circostanze in un riquadro come questo.

**NL**

**Warning (Waarschuwing)**

Betekent dat, wanneer de genoemde aanwijzing niet in acht wordt genomen, dit kan leiden tot **verwondingen** van de gebruiker.

**P**

**Warning (Aviso)**

Significa que a não observância da instrução referida poderá causar um **ferimento** ao usuário.

# **Safety Practices**

## Chapter Overview

---

This chapter describes the general safety practices and precautions that must be observed when operating the UltraVIEW ERS.

This advice is intended to supplement, not supersede, the normal safety codes in the user's country. It is also a supplement to the PerkinElmer standard Safety and Health Policy. The information provided does not cover every safety procedure that should be practiced. Ultimately, maintenance of a safe laboratory environment is the responsibility of the analyst and the analyst's organization.

Carefully read the safety information in this chapter. When setting up the instrument or performing analyses or maintenance procedures, strictly follow the instructions provided.

**NOTE:** There is further safety information for the laser and camera sub-systems supplied in the specific manuals for those sub-systems.



**WARNING**

*This equipment utilizes a powerful source of laser radiation. Do not switch on the laser source until all optical connections are complete.*



**WARNING**

*Before using the system, ensure that the microscope has only a 100:0% switching slider between the camera port and eyepieces. Do not open the unit. Ensure that the correct filters for the light source used are fitted. Ensure that the laser source is disabled when changing the microscope optical path between eyepiece and camera port.*



**WARNING**

*Failure to follow the instructions given in this manual may invalidate your warranty, impair the safe functioning of your equipment or result in hazardous radiation exposure.*

## ***General Safety***

---

- Do not push anything through any ventilation holes.
- Do not allow ventilation holes to become blocked or covered.
- In the event of damage to any part of the system's casing, mains cables, voltage selectors or mains inlet connectors, do not allow it to be used. Have it checked by a suitably qualified person.
- If liquid enters any part of the system, do not allow it to be used and have it checked by a suitably qualified person.

### ***Periodic checks***

In order to ensure continuing safety of personnel using the system, you should perform the following checks at regular intervals:

- A visual check for damage to the casing, connectors or cables.
- An electrical safety test using a portable appliance tester (PAT). When performing an earth continuity test, connect only to the chassis metalwork, not to any of the connector casings.

### ***Static electricity***

The system is, in general, resistant to static discharge in normal use. However certain areas should be subject to extra care. Always follow good Electrostatic Discharge (ESD) procedures when touching sensitive parts in order to prevent damage to the system.

- Always make sure that the system is switched off before connecting or disconnecting cables, especially to the camera head.
- Do not touch the edge connector area of any PC plug-in card while they are not installed in the host computer. Keep the cards in their protective bags until you are ready to use them.
- The CCD inside the camera head is as sensitive to static discharge as it is to light. You cannot access the CCD itself if the camera has not been dismantled. However under some circumstances, a heavy electrostatic discharge to the lens mounting can cause a build up of charge in the CCD area. This has an effect similar to over-exposure of the image, and will dissipate naturally if the system is left running for a few minutes.

## ***General Operating Conditions***

---

The UltraVIEW ERS has been designed and tested in accordance with PerkinElmer specifications and in accordance with the safety requirements of the International Electrotechnical Commission (IEC). The UltraVIEW ERS conforms to IEC61010-1 (Safety Requirements for electrical equipment for measurement, control and laboratory use) as it applies to IEC Class 1 (earthed) appliances and therefore meet the requirements of EC directive 73/23/EEC.

Only use the UltraVIEW ERS indoors and under the following conditions:

Temperature                      15 °C to 25 °C

Relative Humidity                70% maximum at 25 °C

If possible, avoid any adjustment, maintenance and repair of the opened, operating instrument. If any adjustment, maintenance and repair of the opened instrument is necessary, this must be done by a skilled person who is aware of the hazard involved.

Whenever it is likely that the UltraVIEW ERS is unsafe, make it inoperative. The UltraVIEW ERS may be unsafe if it:

- shows visible damage;
- fails to perform the intended measurement;
- has been subjected to prolonged storage in unfavorable conditions;
- has been subjected to severe transport stresses.



## ***Environmental Conditions***

---

The instrument has been designed to be safe under the following conditions:

- Indoor use;
- Altitude up to 2000 m;
- Ambient temperatures of 5 °C to 40 °C;
- A maximum relative humidity of 80% for temperatures up to 31 °C, decreasing linearly to 50% relative humidity at 40 °C;
- Mains fluctuations not exceeding  $\pm 10\%$  of the nominal voltage.



**WARNING**

*If the equipment is used in a manner not specified herein, the protection provided by the equipment may be impaired.*

**CAUTION**

*Do not install the system where excessive heat, moisture, solvent vapor, dust, vibration or direct sunlight is expected to exist. The system must not be used out of doors or in any wet environment.*

**CAUTION**

*Do not touch any part inside the scanner. Any dust, dirt or minor damage on the optical parts inside the scanner may result in physical damage and/or loss of image quality.*

**CAUTION**

*Always cover unused ports of the scanner with the protective covers.*

## ***Laser Safety***

---

- The Ar ion or Ar-Kr laser comes with its own instruction manual. Take care to follow the safety instructions and any other recommendations contained in it.
- Access to the laser area must be limited to personnel who are required to be there, and who have been instructed in the safe operation of lasers.
- Warning signs must be posted in conspicuous locations near the laser area.
- The key(s) to the laser(s) must be kept safely, so that unauthorized persons are not able to operate the laser(s).
- Never look directly into the laser beam.
- Never remove or open any covers or paneling from the instrument.
- Before using the system, ensure that the microscope has only a 100:0% switching slider between the camera port and eyepieces, and that the microscope eyepiece cover is in place.
- Do not open the unit.
- Ensure that the correct filters for the light source used are fitted.
- Ensure that the laser source is disabled when changing the microscope optical path between eyepiece and camera port.

## ***Laser emission control***

- With the emission key at the OFF or MOTOR ON positions, the laser beam is not emitted from the confocal scanner.
- The interlock circuit must be closed before the laser beam can be emitted from the confocal scanner. This circuit is connected to the UltraVIEW ERS computer, so that the aperture is under software control.
- A mechanical shutter on the laser controls the emission of laser light through the optical fiber; see Figure 3 and Figure 6 on pages 26 and 29 respectively.

## ***Electrical Safety***

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The instrument has been designed to protect the operator from potential electrical hazards. This section describes some recommended electrical safety practices.



**WARNING**

*Lethal voltages are present at certain areas within the instrument. Installation and internal maintenance of the instrument should only be performed by a PerkinElmer service engineer or similarly authorized and trained person. When the instrument is connected to line power, opening the instrument covers is likely to expose live parts. Even when the power switch is off, high voltages can still be present. Capacitors inside the instrument may still be charged even if the instrument has been disconnected from all voltage sources.*

The instrument must be correctly connected to a suitable electrical supply. The supply must have a correctly installed protective conductor (earth ground) and must be installed or checked by a qualified electrician before connecting the instrument.



**WARNING**

*Any interruption of the protective conductor (earth ground) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous. Intentional interruption is prohibited.*



**WARNING**

*Make sure that the electricity supply inlets on the instrument are not obstructed i.e. leave a gap to allow easy disconnection from the electricity supply.*

## Power Requirements

The gas laser will require a 208-230 volts AC supply. Please refer to the laser manual for details of its power requirements. The other voltage requirements are listed in the table below.

ERS Control Box (voltage is auto-ranging)	105-125V AC	Fuse 4 AT, 250 V
	210-250V AC	Fuse 2 AT, 250 V
	50/60 Hz	
	100 VA	
Proscan II, Model H30-1	110/240 V AC	Fuse 2 AT, 250 V
	50/60 Hz	
	150 VA	
Hamamatsu AC Adaptor, A3472-07	100-240 V AC	Fuse 3.15 AT, 250 V AC
	50/60 Hz	
	Current: 2.2 A	
Laser Engine (Point Source) Model 01-2049	110/240 V AC	Fuse 5 AT, 250 V AC
	50/60 Hz	
	250 W	

Do not operate the system outside its supply ratings. Before switching on, make sure you know the mains voltage that is available and have selected the appropriate voltage setting and fuse rating on all parts of the system.

The system requires an electrical earth connection to ensure safe operation, and must be connected to a properly earthed (three pin) mains outlet. If a mains extension lead or replacement mains supply lead is used, care should be taken to use only a three core lead and to ensure the continuity of the earth conductor.

## ***Electrical Protection***

Insulation: Class I rating for external circuits. Only connect equipment that meets the requirements of IEC 61010-1, IEC 60950 or equivalent standards.

Installation Category: The instruments are able to withstand transient overvoltages typically present on the MAINS supply. The normal level of transient overvoltages is impulse withstand (overvoltage) category II of IEC 60364-4-443.

Pollution Degree 2: Normally only non-conductive POLLUTION occurs. Occasionally, however, a temporary conductivity caused by condensation must be expected.

## ***Working with the Instrument***

- Connect the instrument to a correctly installed line power outlet that has a protective conductor connection (earth ground).
- Do not operate the instrument with any covers or internal parts removed.
- Do not attempt to make internal adjustments or replacements except as directed in the manuals.
- Disconnect the instrument from all voltage sources before opening it for any adjustment, replacement, maintenance, or repair. If afterwards, the opened instrument must be operated for further adjustment, maintenance, or repair, this must only be done by a PerkinElmer Service engineer.
- Whenever it is possible that the instrument is no longer electrically safe for use, make the instrument inoperative and secure it against any unauthorized or unintentional operation. The electrical safety of the instrument is likely to be impaired if, for example, the instrument shows visible damage; has been subjected to prolonged storage under unfavorable conditions; or has been subjected to severe stress during transportation.

## ***Airflow***

The laser and camera head all need a continuous flow of air which must not be blocked. The system relies on this airflow, both for reliable running and to maintain its specified performance. Please refer to the laser manual for the specific airflow requirements of the laser.

In the event of overheating, various protection devices throughout the circuitry are designed to shut down to safeguard the user, and to avoid damage to the system. In this event the CCD cooling may cease, or the system may simply not image correctly. It should be switched off and allowed to cool before being used again.

The ERS control box must stand on its feet, not its side. Ensure the air gap above and below the unit allows adequate ventilation. Do not obstruct the grills on the top and the bottom of the box.

## ***EMC Compliance***

---

The system complies with the requirements of the following standards:

- EC directive - The UltraVIEW ERS has been designed and tested to meet the requirements of the EC directive 89/336/EEC and 93/68/EEC. The UltraVIEW ERS complies with the EMC standard EN61326 (EMC standard for electrical equipment for measurement, control and laboratory use).
- FCC rules and regulations - This product is classified as a digital device used exclusively as industrial, commercial, or medical test equipment. It is exempt from the technical standards specified in Part 15 of the FCC Rules and Regulations based on Section 15.103 (c).

To ensure that the system continues to comply with these standards, check that all connector retaining screws, where supplied, are secure; finger-tight is sufficient. Also check that all case fixing screws are in place and secure.

Only the interconnecting cables supplied with the system should be used. The use of any cable not manufactured to the correct specification, or a cable which is damaged, may result in the system failing to meet the above EMC standards. It may also degrade the system's performance. User supplied cables connected to the Trigger Port should be adequately screened.

If the system is exposed to high levels of power disturbance, electromagnetic radiation or electrostatic discharge some degradation in performance may be observed. The system will continue to function normally when the disturbance ceases. In exceptional cases it may be necessary to re-initialize the system.

## Safety Approvals

The following is a list of air cooled Class IIIb lasers, manufactured by Melles Griot or Point Source, with their associated CDRH accession numbers that are available for use with the UltraVIEW ERS system.

Name	Model number	Technology	Wavelength (nm)	Power (mW)	CDRH #
Melles Griot	543-A-A03	Ar ion	454 457 465 472 477 488 496 502 514	4 20 14 14 21 100 18 9 100	8620503
Melles Griot	643-Y-A02	Ar-Kr	568	20	8620503
Point Source	iFLEX2000	Diode	405	25	
Point Source	iFLEX2000	Diode	440	20	
Point Source	iFLEX2000	Diode	640	35	



**WARNING**

*The laser engine weighs 46 kg and should only be lifted by two people. Consult local codes of practice issued by safety advisors before attempting to lift the laser engine.*



# Warning Labels



**WARNING**

When this label is attached to an instrument it means refer to the manual in order to find out the nature of the potential hazard and any actions which have to be taken.

## Warning labels on the confocal scanner

The Confocal Optical Scanner belongs to a laser hazard Class 3B, which indicates that it is capable of producing skin or eye damage if used improperly. Direct viewing into the laser beam must be strictly prevented. Safety requirements concerning health and industrial protection for handling laser instruments must be strictly observed. Before operating the Confocal Optical Scanner, please read the following to become familiar with its safety control, markings and warnings.

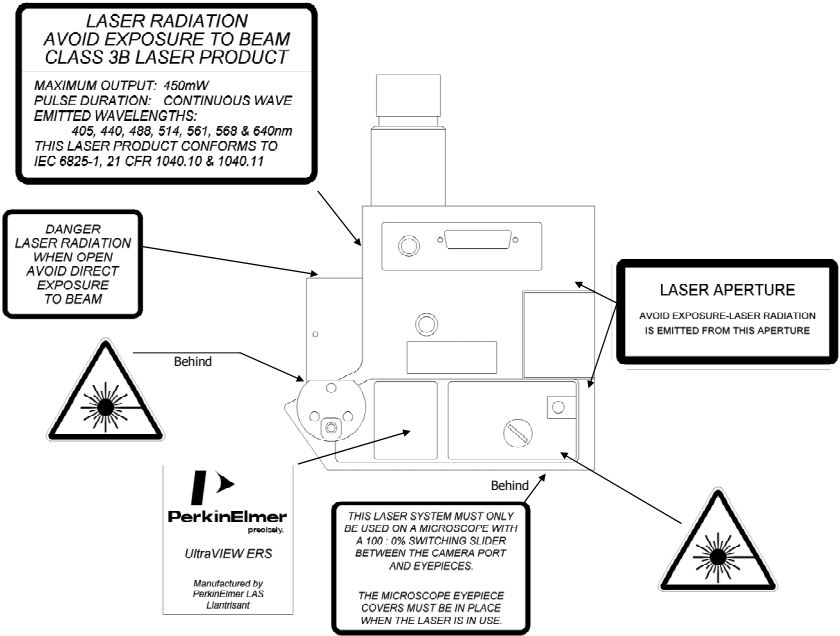


Figure 1 Labels on the scanner head

THIS LASER SYSTEM MUST ONLY BE USED ON A MICROSCOPE WITH A 1000% SWITCHING SLIDER BETWEEN THE CAMERA PORT AND EYEPIECES  
 THE MICROSCOPE EYEPIECE COVERS MUST BE IN PLACE WHEN THE LASER IS IN USE

These labels are placed close to the microscope eyepiece

AVOID EXPOSURE LASER RADIATION IS EMITTED THROUGH THIS APERTURE CLASS IIib LASER PRODUCT  
 MAXIMUM OUTPUT 80mW  
 PULSE DURATION CONTINUOUS WAVE  
 EMITTED WAVELENGTHS 488nm and 568nm  
 THIS LASER PRODUCT CONFORMS TO IEC 6825-1, 21 CFR 1040.10 AND 1040.11

**DANGER**  
 LASER RADIATION - AVOID DIRECT EXPOSURE TO BEAM  
 MAXIMUM OUTPUT 80mW  
 EMITTED WAVELENGTHS 488nm and 568nm  
 CLASS IIib LASER PRODUCT

These labels are placed in prominent positions on the microscope



Figure 2 Labels on the microscope

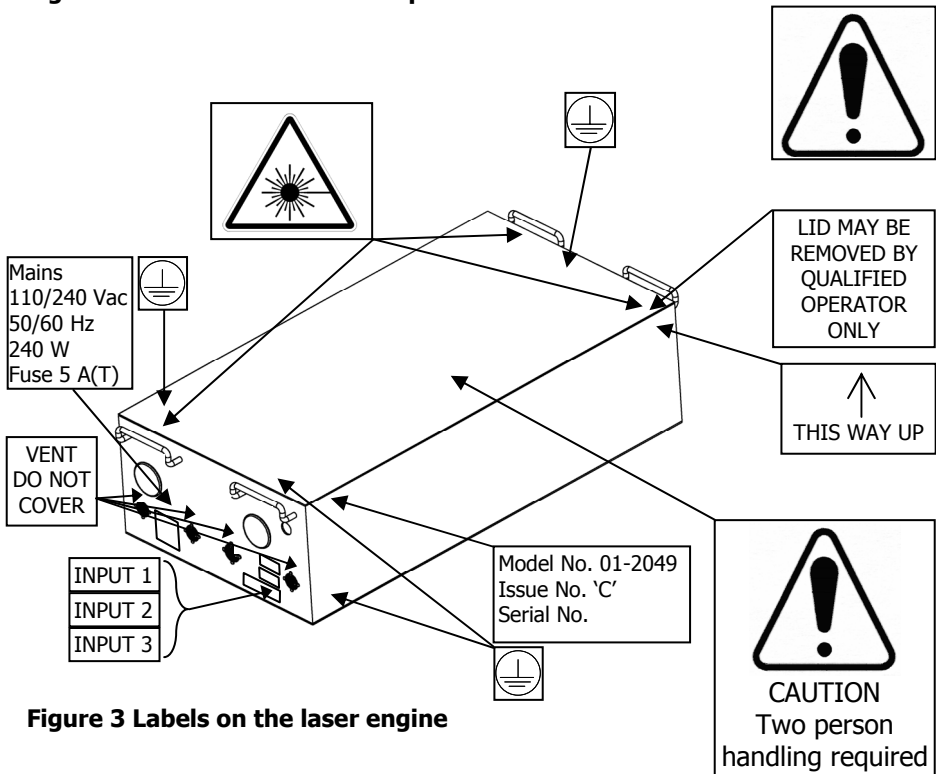


Figure 3 Labels on the laser engine

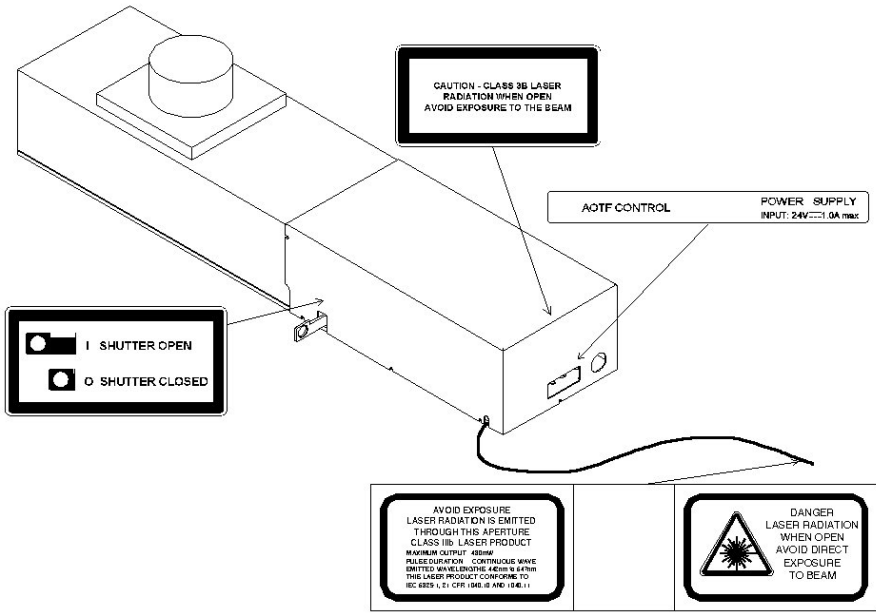


Figure 4 Labels on the three line laser system

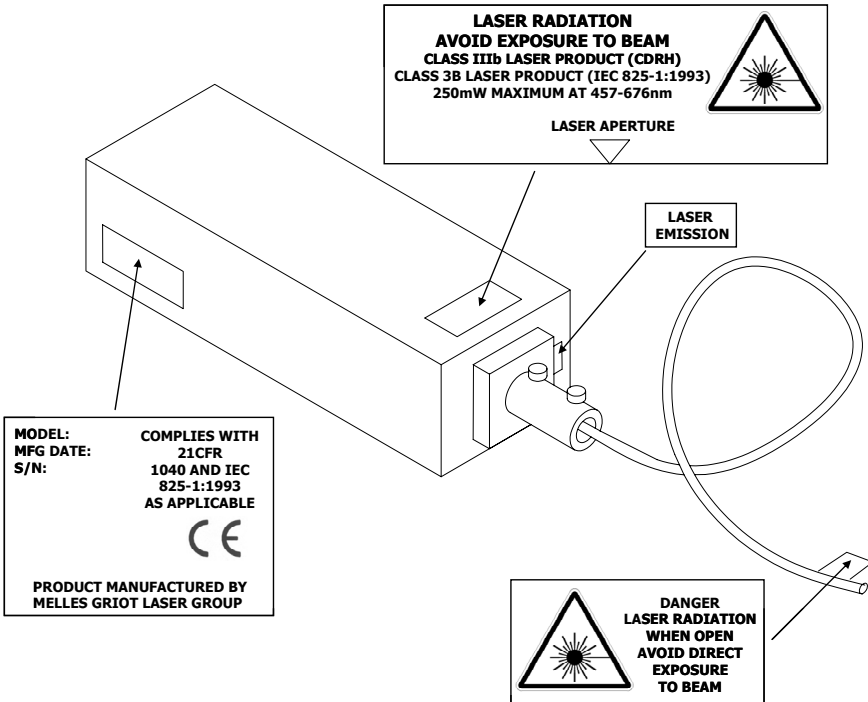


Figure 5 Labels on the gas laser

### Warning labels on the ERS Control Box

These labels are displayed on the rear of the ERS control box.



Caution, risk of electric shock  
(refers to Z axis drive voltage)



Refer to user documentation for a description  
of the connections on the ERS Control Box

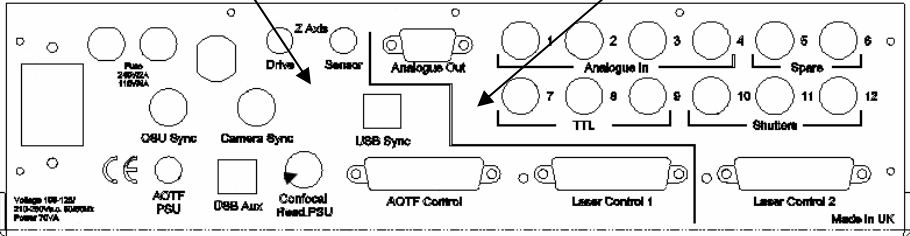


Figure 6 Labels on the ERS Control Box

## Fuses

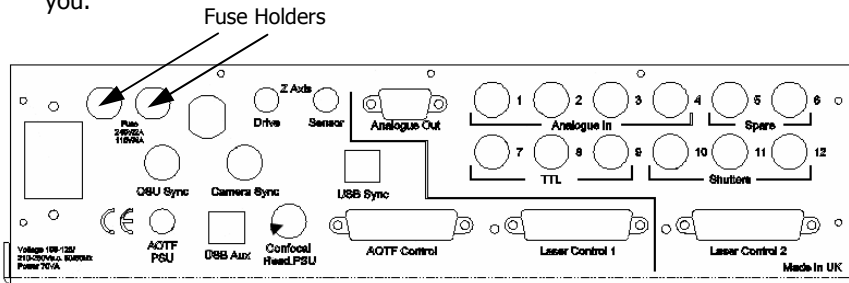
**NOTE:** There are two fuses accessible on the back of the ERS Control box, as detailed here. The details for replacing fuses for the camera or laser power supply are given in the separate manuals for those sub-systems.



### **Electrical hazard**

*To prevent potential injury to yourself and damage to the instrument, switch OFF all instruments in the system and disconnect them from the line power supply before you alter, or make any new, electrical connections.*

1. Switch off the instrument and remove the line power cord from the electrical supply.
2. Turn the fuse holder on the rear of the control box (see Figure 7) a quarter turn anticlockwise using a flat-headed screwdriver and gently pull out towards you.



**Figure 7 Rear view of ERS Control Box**

3. Replace the fuses with new ones of the same type and rating.  
The fuses are 32 mm x 6.3 mm 250Vac, 2 AT (2 amp time delay) on a 240V system and 4 AT, 250V on a 110V system.

**NOTE:** The module has two fuses. Always replace both at the same time, even if only one blew, as the other may have been weakened.

4. Replace the fuse holder by pushing it in and turning clockwise.

**NOTE:** If you use the correct fuses but the instrument still does not work correctly, or the fuses blow repeatedly, contact your PerkinElmer office or representative.

# **System Description**

## ***Overview***

---

UltraVIEW ERS is a system for microscopic analysis, aimed primarily at live cell imaging – revealing the structures and processes within cells. It consists of a confocal scanner, a camera, a laser light source, and a computer (including software). It is connected to a microscope.

The UltraVIEW ERS system helps in the study of cells by revealing and recording structures and processes within the cell. It does this by combining two key techniques: fluorescence imaging and confocal imaging.

By using fluorescent imaging, selected parts of a cell can be illuminated, while the rest of the cell remains dim. By using several different fluorescent dyes, parts of the cell can be displayed in different colors, revealing the structure clearly.

UltraVIEW ERS uses a confocal system, to resolve detail at a single plane. By taking two dimensional images over a range of planes, information in three dimensions can be captured and recorded.

By using a very efficient confocal system, this three-dimensional information can be captured in real time, so that processes can be studied and recorded as they happen.

Because UltraVIEW ERS will work with low levels of light, the images can be captured over long periods, without damaging the cells.

Once the images are captured and recorded, the UltraVIEW ERS analysis tools can be used to extract data from them, so that processes can be quantified and the data recorded.

Images, data and graphs can then be transferred to other programs for display.



## ***The UltraVIEW ERS System***

---

The UltraVIEW ERS system includes a confocal scanning unit, a camera, a laser light source, an Acousto-optical Tuned Filter (AOTF), and means of controlling the focus of a microscope.

The actual arrangement of the hardware will depend on the system that you have purchased; this list may therefore include items which are not in your system.

### ***Laser***

The laser or laser engine will produce the excitation light. The wavelengths of the light from the laser and the dyes used in the sample must be matched to produce an emission from the sample. The laser will produce two, three, five or six wavelengths depending on the particular system you have; an Acousto-optical Tuned Filter (AOTF) selects the correct wavelength for each image. An optical fibre links the laser to the confocal scanner. The laser combiner contains three lasers with the outputs merged into a single fiber.

### ***Confocal scanning unit***

The confocal scanning unit is based on the Nipkow spinning disc design in which laser light (excitation) is passed through pinholes and focused on to the sample, and the fluorescence (emission) from the sample is focused and passed through the same pinholes. This rejects light from the non-focal plane parts of the sample, producing a confocal image. The confocal scanner includes an eyepiece for direct viewing of the confocal image.

### ***Camera***

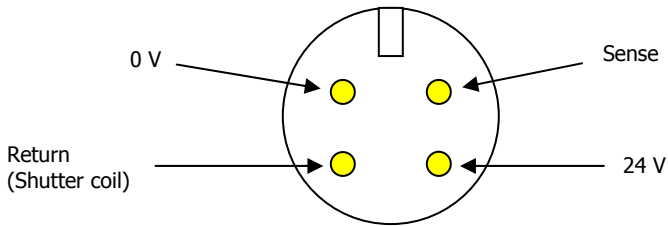
The ORCA camera uses a cooled digital CCD. The C9100-50 (EMCCD) camera uses a cooled digital CCD and an electron multiplier for greater sensitivity. The camera collects the images from the confocal head and sends them to the computer.

### ***Z-axis control***

The Z-axis control is fitted to the microscope. It consists of a small stage placed under the objective lens that moves the lens towards and away from the sample.

### ***Shutters***

In addition to the shutter built into the confocal head, you may have a Brightfield shutter. You can mix Brightfield images with confocal images in your UltraVIEW ERS experiment.

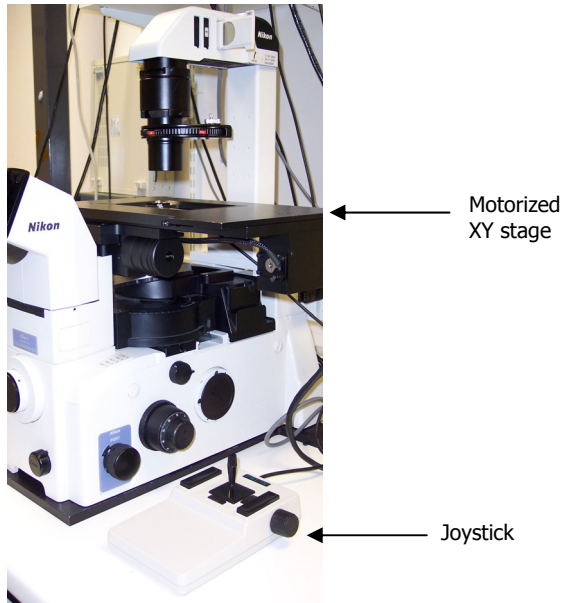


### ***Emission Discrimination Filter Wheel***

Your system may have a filter wheel. This provides emission discrimination.

### ***Motorized XY Stage***

The motorized XY stage enables you to accurately control the position of the sample slide in the XY plane using the UltraVIEW ERS software, or by using a joystick. In a typical experiment using the XY stage, you select a number of different positions on the slide, enter the experimental settings at each position, and then start recording your images. Image capture is fully automated.



**Figure 8 Motorized XY stage and joystick**



**WARNING**

*When you are using a motorized stage, do not place your fingers between the moving and fixed parts of the stage. The motors driving the stage are powerful and do not stall easily.*

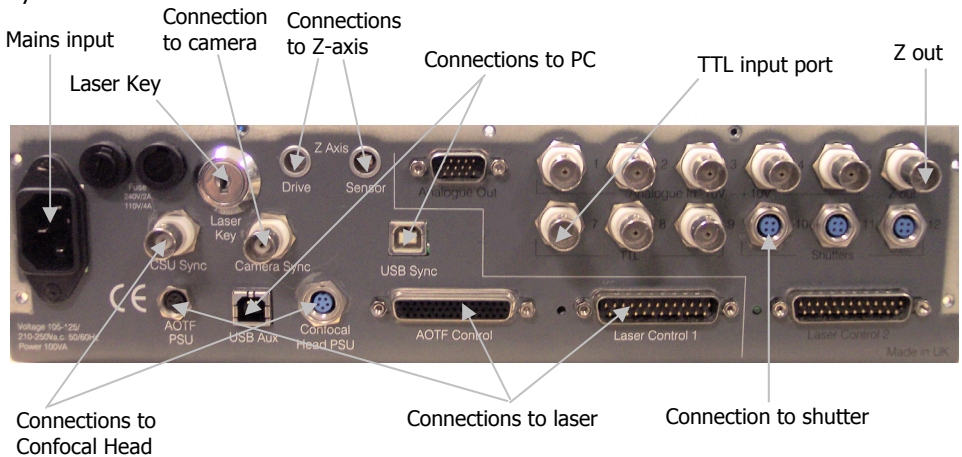
For a description how to use the XY stage see the UltraVIEW Help.

## **Computer**

The computer loaded with the UltraVIEW ERS software has extension cards fitted to provide, for example, the digital control to the confocal head, and to accept the images from the camera. For fast capture and processing of the images, UltraVIEW ERS is supplied with a fast computer, extensive RAM and large hard disk drive.

## **ERS Control Box**

The ERS Control box is the main hardware interface between the computer, laser, Z-axis control and shutters. It also keeps the confocal head and camera synchronized.



**Figure 9 Connections to the ERS Control Box**

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Your system will be fully connected at installation. However, if you need to check the connections or reconnect your system, see the table below.

<b>RS Control Box Connection</b>	<b>Output Voltage from RS Control Box</b>	<b>Input Voltage to RS Control Box</b>	<b>Connected to</b>
Z out: BNC socket: output for monitoring Z-stage	+5 V dc, 1.5 mA		To A to D card in Synchronizer Box
TTL: BNC socket: input pulse for system		+5 V dc, 1.5 mA	Optional: from user's control equipment
TTL: BNC socket	NU		NU
TTL: BNC socket	NU		NU
Shutter: 4 way TRIAD socket	+24 V*		Shutter
Shutter: 4 way TRIAD socket	+24 V*		Optional Shutter
Shutter: 4 way TRIAD socket	+24 V*		Optional Shutter
Analogue out (15-way HD D-type)	-10 V to +10 V		NU
AOTF Control	+5 V, 20 mA		Laser
Laser Control 1 (see below for details)	+5 V, -15 V (100 mA)		Laser
Laser Control 2 (see below for details)	+5 V, -15 V (100 mA)		Optional laser
Z-axis Sensor Lemo SA 0S 304	+5 V		Z-axis piezo actuator
Z-axis Drive Lemo SA 0S 250	-10 V to +150 V		Z-axis piezo actuator
USB Sync	+5 V		Any USB port on PC
Camera Sync: BNC socket	+5 V, 20 mA		Camera PSU with interface

CSU Sync: BNC socket	+5 V, 20 mA		Confocal Head
AOTF PSU: Binder 09 0074 00 02 panel socket	+24 V*		Laser
Confocal Head PSU: 5 pin TRIAD socket.	+12 V*		Confocal head
USB Aux	+5 V		Any USB port on PC

- \* The +12 V can deliver up to 1.5 A approximately externally  
The +24 V can deliver up to 2.4 A approximately externally

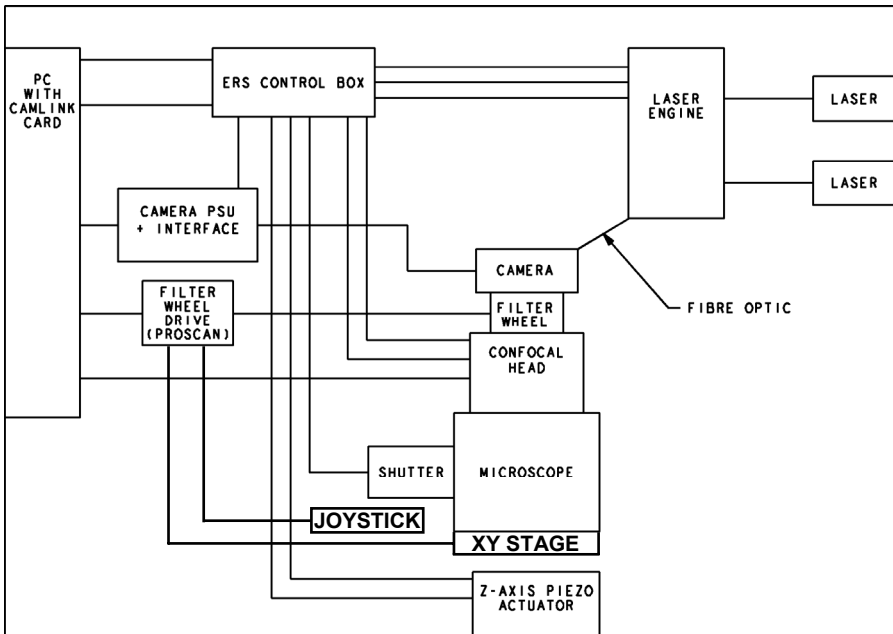
Laser Control 1 and Laser Control 2 Pin Connections: 25-way D-type

Pin	Description	Details
1	Key switch	Connection to back panel laser switch
3	Key switch	Connection to back panel laser switch
4	Standby: relay contact	Relay contact
6	Current Control	Output: 8 bits: 0 to 5 V from DAC
11	Ground	
12	-15 V @ 100 mA	Power in: connected to "Standby" via relay contact for controlling the laser
16	Ground	
23	Laser 1 (2) front panel LED, Laser LED back panel: LED low	Connects directly to LEDs
24	Laser 1 (2) front panel LED, Laser LED back panel: LED high	Connects directly to LEDs
25	Chassis Ground	

All other pins not used

Note that for full operation, both USB ports on the ERS Control Box must be connected to USB ports on your computer. Laser Control 1, shutter and TTL input are optional connections which may not apply to your system.

## System Connections

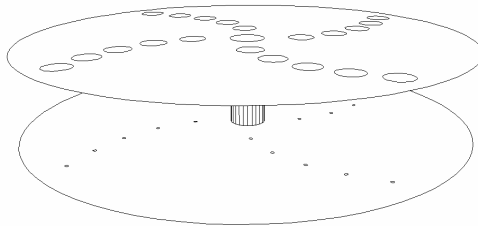


## Theory

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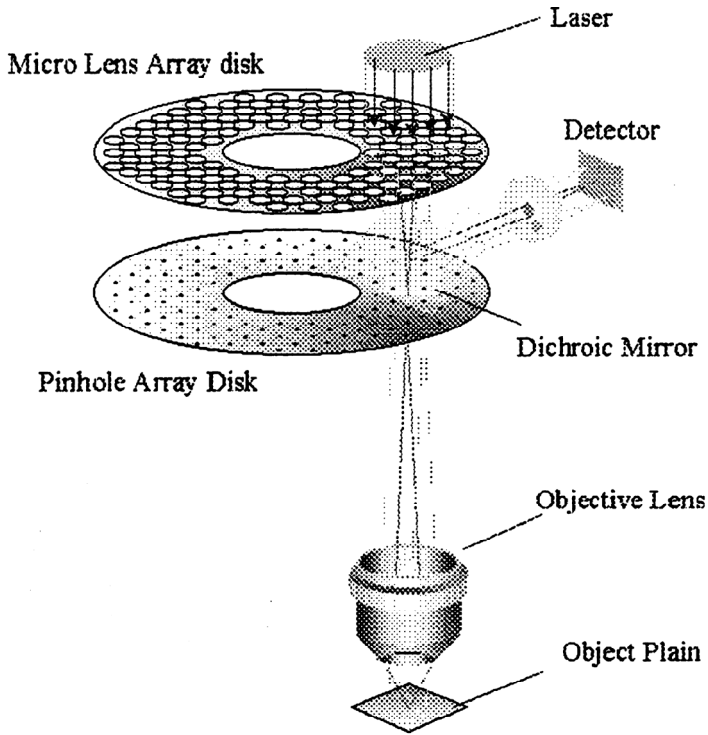
The confocal optical scanner forms part of the UltraVIEW ERS system. It is used in conjunction with a microscope, a light source, a digital camera and a computer workstation running UltraVIEW ERS software. The confocal optical scanner is mounted onto the microscope and is attached to the digital camera and light source. The UltraVIEW ERS confocal scanner is of the Nipkow disc type. In a Nipkow scanner, the specimen under study is illuminated by light that has been passed through a spinning pinhole disc. The image collected by the microscope objective lens is passed back through the same pinhole disc. This transmits in-focus light from one plane and rejects stray light, producing a sharp image of a single plane of the specimen.

The PerkinElmer confocal optical scanner increases light throughput and sensitivity by including a second disc with micro-lenses that are accurately aligned with the pinholes in the first. Light is gathered by the micro-lenses and focused on to the pinholes, producing more than fifty times increase in the illumination of the sample. Figure 10 shows twenty pinholes and micro-lenses; the discs in the UltraVIEW ERS confocal scanner have twenty thousand pinholes and micro-lenses.



**Figure 10 Twenty pinholes and micro-lenses**

Further improvement in the image signal-to-noise ratio is achieved by separating the illumination beams and the image beams between the two discs.



**Figure 11 Two discs separating the illumination beams and image beams**



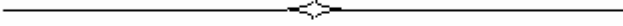
## ***Specifications***

---

This is the specification of the confocal scanning head.

CRDH accession number	9712021
Laser class	IIIb
Scanning mode	Nipkow disc with microlens array
Scanning speed	1000 frames/sec
Field size	7 × 10 mm (0.28 × 0.39 inches)
Excitation	See laser list, above
Observation	Direct through eyepiece or through camera port
Installation	At microscope C-mount on regular and inverted types (Zeiss, Olympus, Nikon or Leica)
Optical fiber connection	FC connector
Size (w × h × l)	150 × 210 × 210 mm (5.9 × 8.3 × 8.3 inches)
Weight	5 kg (11.1 lb)
Operation temperature range	15 – 25 °C (59 – 77 °F)
Operating humidity range	40 – 70%

Power supply is from the ERS Control box.



**Operation**

## ***Overview***

---

This section outlines the basic method of operating the system.

### ***Software as an upgrade***

If this version of UltraVIEW ERS is an upgrade to an older version, and you have a Hamamatsu Orca camera, you may need to upgrade your camera firmware. Please contact your local PerkinElmer Technical Support Representative.

### ***About other software***

UltraVIEW ERS places great demands on the computer on which it is run, especially when acquiring images at high speed. For this reason, we normally supply UltraVIEW ERS already installed and configured, and we advise you not to run any other software at the same time as UltraVIEW ERS, including a screen saver. Some software can adversely affect the system settings after installation, even though it is not actually running. In order to ensure that your system maintains its optimum performance, it is best to contact PerkinElmer before installing any other software on your UltraVIEW ERS workstation, and especially if you want to move it to a different computer for any reason.

### ***Administrator Password***

UltraVIEW ERS requires users to be logged in to the PC operating system as administrators when installing the software.

**NOTE:** No password is allocated when the computers are shipped from PerkinElmer.

**NOTE:** If a password is set and then forgotten, PerkinElmer have no way of unlocking the computer and will be unable to assist.

### ***Using the software under Windows "Restricted Users" Group***

**NOTE:** This section only applies when the underlying file system has been formatted as NTFS and not FAT16/FAT32. Windows XP by default is installed as NTFS.

## ***Installing the software***

- UltraVIEW ERS software should be installed under a Windows "Administrator" group account.

## ***Running the software***

If the UltraVIEW ERS software is intended to be run under a Windows "Restricted User" group account then please note the following:

- When exiting the Ultraview ERS software under a Windows "Restricted User" account, ensure that the current selected UltraVIEW user is one which has been created by the currently logged on Windows "Restricted User" account. Exiting the application with a selected UltraVIEW user created under another Windows user account will result in an "Access denied" message being displayed and the UltraVIEW user's template not being saved.
- An UltraVIEW user created under a Windows "Restricted User" account can read and create new experiments under another UltraVIEW user's account (regardless of whether the user was created under a Windows "Administrator" or "Restricted User" group account) but cannot delete their experiments or the UltraVIEW user. An attempt to delete an experiment will result in an access denied message being displayed  $n$  times, where  $n$  represents the number of series in the experiment plus one. Any single images in the experiment will additionally result in  $m*5$  access denied messages, where  $m$  is the number of single images.

These two points do not apply if the UltraVIEW ERS software is intended to be run under a Windows "Administrator" group account. An UltraVIEW user created and currently logged on under a Windows "Administrator" group account can read, write and delete other UltraVIEW users' experiments and also any UltraVIEW user.

## ***Start up and Shut down***

---

### ***Initial checks***

If the system has just been installed, has recently been moved, or is in an unknown state, you may want to refer to *Safety Practices* starting on page 13 and check the following areas:

- The correct mains voltage is selected on all units;
- All cables are connected correctly;
- The laser is set up correctly, and has provision for cooling;
- There is allowance for airflow around the camera and computer.

### ***System start up***

#### ***Ar ion and Ar/Kr lasers***

1. Switch on the Ar ion laser AC power supply.
2. Turn the key to the **On** position, and then turn further to the **Start** position. Hold the key in the **Start** position for three seconds and then release. The key returns to the **On** position.
3. Ensure the mechanical shutter is open.
4. Repeat steps 1 to 3 for the Ar/Kr laser (if applicable).

#### ***Laser engine***

If you have a laser engine:

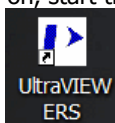
5. Turn on the power to the laser engine.
6. Turn the key 90° to the position marked **1**.
7. Ensure the shutter is open.  
The shutter is controlled by the silver knob labeled **Output** on the top of the laser engine. Turn the knob clockwise to the position labeled **1** to open the shutter.

**Other hardware**

8. Switch on the ERS Control box.
9. Switch on the ProScan box.
10. Switch on the camera power supply.
11. Switch on the CSU22 confocal scanner by turning the key to the **run** position. A green and a red light are displayed.
12. Switch on the Brightfield illumination.
13. Switch on the microscope.
14. Switch on the PC monitor.
15. Switch on the PC and log-on.

**NOTE:** The Camera Power Supply should be left switched on for the duration of experiments. This will maintain the camera's CCD cooling, which takes some time to stabilize.

16. Once everything has been switched on, start the software by double clicking



on the UltraVIEW ERS desktop icon

OR

From the **Start** menu select **Programs**, and then choose **UltraVIEW ERS** from **PerkinElmer Software**.

17. From the startup screen, click **CAPTURE**.

You may start taking images immediately, however optimum performance will only be obtained once the CCD has reached its final operating temperature. In general, about half an hour should be allowed before taking any demanding images.

**NOTE:** If you wish to run the UltraVIEW ERS software without using the camera or other hardware, then at the startup screen click **ANALYSIS**. The ERS Control Box, Camera Power Supply and the Laser may be left switched off. The program will then run, allowing you to review and analyze your existing experiments. If you subsequently wish to use the hardware, ensure that the hardware is switched on and then click the Enable Capture button on the toolbar.

## System shut down

1. Exit the UltraVIEW ERS software, and shut down the PC.
2. Turn the key of the gas laser to the **Off** position.



*Do not switch the power off. The power must be left on as this controls the fan used to cool the laser. The fan will automatically switch off after approximately one minute. Switching the power off prematurely will damage the laser.*

3. If you have a second gas laser, turn the key of the gas laser to the **Off** position.



*Do not switch the power off. The power must be left on as this controls the fan used to cool the laser. The fan will automatically switch off after approximately one minute. Switching the power off prematurely will damage the laser.*

**NOTE:** You do not need to turn the power to the gas lasers off after the fan has switched off. The next time you use the UltraVIEW ERS system, you will only need to turn the key on each gas laser as the power will be on.

4. If you have a laser engine, switch it off by turning the key anti-clockwise to the position labeled **0**.
5. Switch off the power to the laser engine.
6. Turn the CSU22 confocal scanner to the position labeled **0**.
7. Switch off the ERS Control box.
8. Switch off the ProScan box.
9. Switch off the camera power supply.
10. Switch off the Brightfield illumination.
11. Switch off the microscope.

**NOTE:** The laser shutters do not have to be closed during the shut-down procedure. They may be left open.

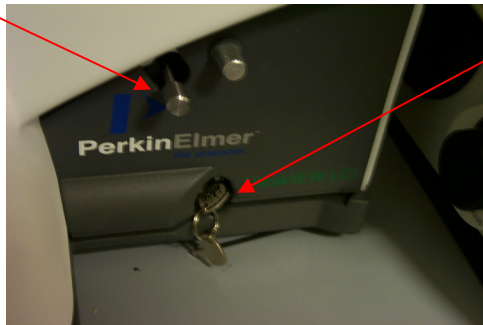


## Getting Your First Image

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1. Switch on your system.  
See *System start up* on page 46.
2. If not already on, switch on the microscope Brightfield illumination.
3. With a sample on the microscope slide, set the microscope to eyepiece viewing, and looking through the microscope eyepieces, focus the sample in the centre of the field of view.  
We recommend that you use the reference pollen grain slide for this.
4. Still using Brightfield illumination, switch the microscope viewing to side port.
5. Turn the key in the confocal head to the middle position, as shown in Figure 12.

Ocular (eyepiece) control

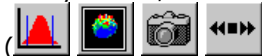






Key in the  
confocal head

**Figure 12 Confocal head**

6. In the UltraVIEW ERS software, make sure that the Grey Levels dialog, the Image Window, the Image Control panel and the Sequence Control panel are all displayed.


If they are not, click the appropriate icons on the toolbar



(   ) and arrange the panels as necessary so that they are visible.

7. On the Image Control panel, set the **Exposure time** to 300 ms and **Binning** to 1 x 1.
8. On the Grey Levels dialog, make sure the **Autoscale** check box is selected.

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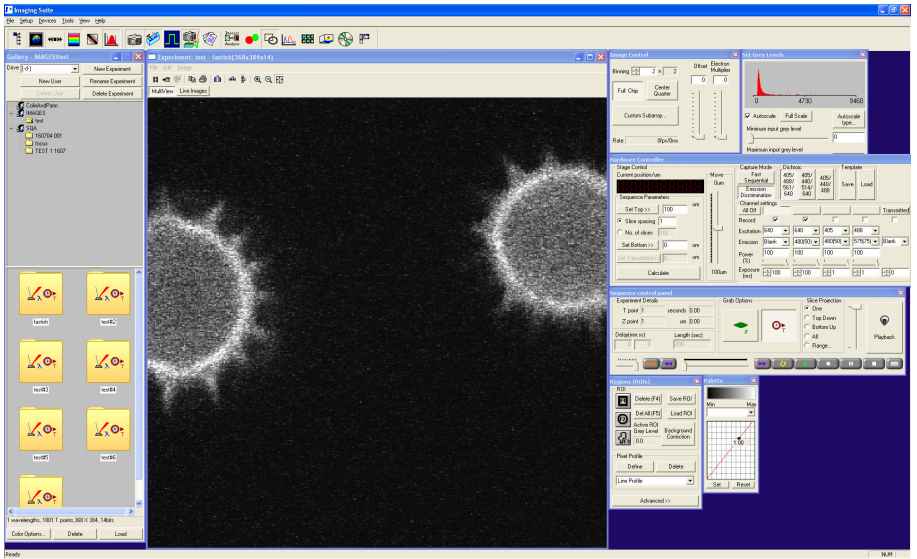
9. Click **Live** on the Sequence Control panel.
10. On the confocal head, push the ocular control fully in.  
You should now see an image appear in the Image Window. If the Image Window is all white, reduce the camera exposure time on the Image Control panel, and/or reduce the brightness of the brightfield illumination. If the Image Window is black or very dim, then increase the camera exposure time on the Image Control panel, and/or increase the brightness of the brightfield illumination. If you still can not see an image in the display, check that there is no obstruction in the optical path, for example an epifluorescence cube.
11. Check that the Hardware Controller is displayed.  
If not, click  on the toolbar.
12. Turn the key on the confocal head fully to the right.
13. On the Hardware Controller, select one channel button.
14. Turn the Brightfield illumination off.  
Laser light should be visible at the microscope sample.



**WARNING**

*Do not stare at this light, and do not attempt to use the microscope eyepieces.*

A fluorescence image should now be displayed in the Image Window of the UltraVIEW ERS software, similar to Figure 13. If not, make small adjustments to the microscope focus, increase the laser power and increase the camera exposure time. Once you have this image you are ready to record an experiment.



**Figure 13** Fluorescence image displayed in Image Window

## ***Optical Set-up***

---

1. Place the sample on the microscope stage (applying oil or water to the objective/sample, if appropriate).
2. Bring the sample into focus using transmitted light or wide field fluorescence.
3. Set the microscope light path 100% to the camera port.  
This directs the image to the scanner.
4. For a purely confocal image, ensure the microscope illumination is switched off.

**NOTE:** You may combine microscope illumination with the scanner illumination to produce a combined confocal and non-confocal image.

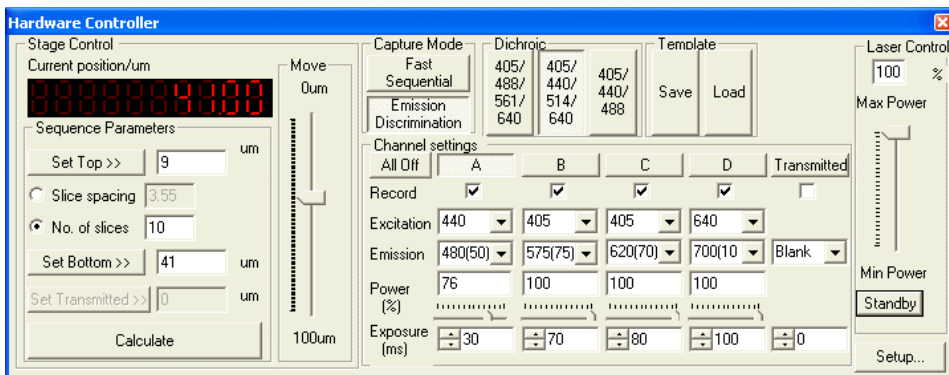
5. Set the scanning head light path to the eyepiece by pulling out the scanning head optical path control knob.
6. Ensure that there are no filters or dichroic mirrors in the microscope optical path, as these would affect the operation of the scanner.
7. Check that all the laser connections are properly made.
8. Set the illumination power to minimum.
9. Switch the laser on and open the laser shutter.
10. Turn on the scanner with the key switch.
11. Use the shutter icon on the filter panel to open the scanner interlock shutter.
12. Observing the image at the confocal scanner eyepiece, adjust the microscope focus until an image is visible.
13. Adjust the confocal scanner eyepiece focus as required to produce a sharp image, increasing the illumination power as necessary.  
You should now be able to see a single plane of the sample, and be able to alter the plane by changing the microscope focus, or from the Z axis control if applicable.
14. Adjust the illumination power as required to provide a clear image without bleaching the sample.

15. Once the image at the eyepiece is satisfactory, push the scanning head optical path control knob fully in to direct the image to the camera.  
The system is now ready for use.

## Hardware Control

### Laser control

The laser is controlled from the Hardware Controller.



- Before image capture, select the **Dichroic** by clicking the appropriate button. The dichroic buttons vary between different versions of UltraVIEW ERS:

Version	Dichroic
ERS with laser engine	<ul style="list-style-type: none"> <li>• 405/488/561/640</li> <li>• 405/440/514/640</li> <li>• 405/440/488</li> </ul>
ERS with gas lasers	<ul style="list-style-type: none"> <li>• 488/568/647</li> <li>• 647</li> <li>• 488</li> </ul>

- Lasers are switched on by clicking the appropriate button (**488nm, 568nm, 647nm**). The channel button (A,B,C, or D) is depressed to indicate which line is active. Only one laser line can be activated at any time. Having activated the laser, the amount of laser light reaching the sample is regulated by the **Power** slider.

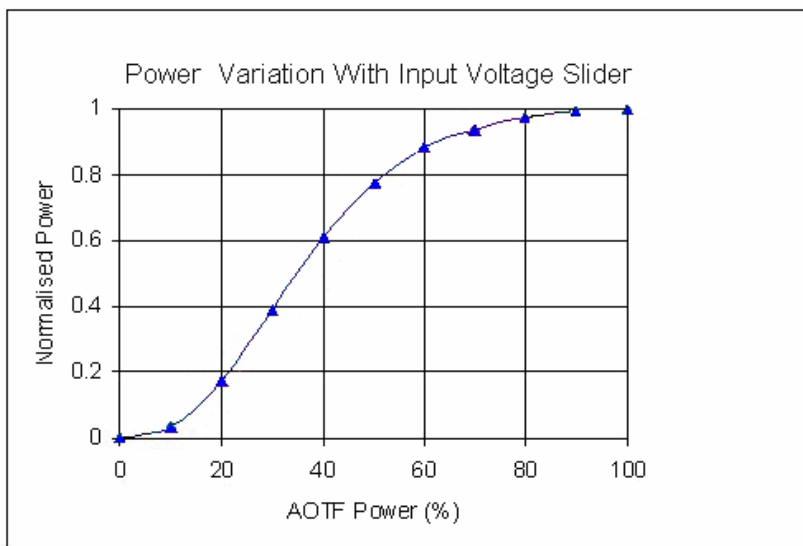
**NOTE:** Increasing the amount of excitation light increases the signal, but may bleach the sample at a faster rate.

**NOTE:** The **Laser Control** section controls the Argon Ion laser which is used for the 488 nm and 514 nm laser lines.

### ***AOTF transfer characteristics***

The laser output power from the AOTF does not vary proportionally with the input power control voltage. Typical values are shown in Figure 14.

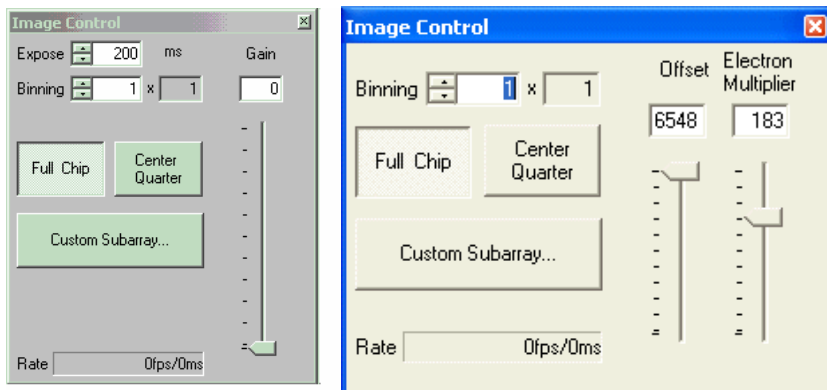
**NOTE:** Only the Ar ion and Kr laser lines use AOTF.



**Figure 14 AOTF Power Variation**

## Camera control

The Image Control dialog displayed will depend on which type of camera is installed on your system. Figure 15 shows the dialogs displayed for the Orca camera and the EMCCD (Electron Multiplier Charge Coupled Device). The **Electron Multiplier** slider on the EMCCD is similar to the **Gain** slider on the Orca, but rather than just making the signal bigger (and increasing the background noise as well), the Electron Multiplier increases the sensitivity of the CCD, increasing the wanted signal without increasing the noise as much; so the signal-to-noise ratio is improved.



**Figure 15 Image Control for the Orca camera (left dialog) and EMCCD (right dialog)**

Increasing the exposure time increases the brightness of the captured image, but reduces the capture rate (given at the bottom of the dialog in fps).

Pixels can be combined using Binning. Increasing pixel binning increases the brightness of the image (and at low exposure times increases the capture ratio). Binning the pixels, however, reduces the lateral resolution of the image.

If the sample only fills a small area of the screen, this region can be imaged by only using a small area of the CCD chip:

- Click **Center Quarter** to narrow the view to the central portion of the chip.
- User-defined subarrays can be defined by clicking **Custom Subarray**.
- Right-click the mouse to re-size the subarray.

**NOTE:** Both binning and subarray reduce the size of the image files.



## ***Z axis control***

You use the **Stage Control** section on the Hardware Controller to change the Z plane view of the sample. The **Move** slider controls the Z-position between 0 and 100  $\mu\text{m}$ . The **Current position/ $\mu\text{m}$**  is shown at the top left of the dialog.

Before image capture:

1. With the camera in Live mode, move the slider until the view is just above the sample.
2. Click **Set Top**.
3. Move the slider to view through the sample until the view is just below the sample.
4. Click **Set Bottom**.
5. Select either **Slice spacing** or **Number of Slices**, and then enter the required value in the adjacent field.

A smaller Z-slice (0.1-0.3  $\mu\text{m}$ ) is optimal for high-quality Z-reconstructions, but for thick or rapidly moving samples, a smaller Z-spacing results in longer data capture times.

6. Click **Calculate**.

The spacing and number of slices in the stack will be set.

The **Move** slider will now cover the required Z movement.

## ***Delay Capture***

A delay between images (or stacks) may be incorporated into the image capture protocol. The **Delay** period can be entered in the Sequence Control panel in minutes and seconds.

**NOTE:** In order to facilitate statistical analysis, the delay time is calculated from the start of one stack to the start of the next stack. Therefore if the delay time entered is less than the time taken to capture the stack, no delay between stacks will be observed.

## Data Capture

---

There are three modes of capture in UltraVIEW ERS: single images, single stacks and multiple timepoints/stacks. Image capture may be performed with either one or more wavelengths.

Image acquisition control is performed by the Hardware Controller and the Sequence Control panel.

### Single images


Single images in one or more wavelengths may be captured:

1. On the Sequence Control panel, ensure that the Z button displays one slice



only, , and that the time button displays an equals sign, .




2. Select the appropriate wavelength(s) on the Hardware Controller.
3. On the Sequence Control panel, click .

### Single Z stacks

1. To capture a single Z stack, first set the top and bottom of your Z-stack as described in *Z axis control* on page 57.
2. Set the **Slice spacing** as required.
3. Check the appropriate wavelengths in the Channel settings section.


4. Click the Z button to display multiple Z stacks, .



5. Ensure that the time button indicates the equals sign, .






6. Click .



Clicking  before the end of the capture will abort the series.

## Multiple time points

1. Set the Z position (if one slice is required) or for x,y,z,t (4D) imaging, set the Z controls as described in *Z axis control* on page 57.
2. Select the required wavelengths on the Hardware Controller.

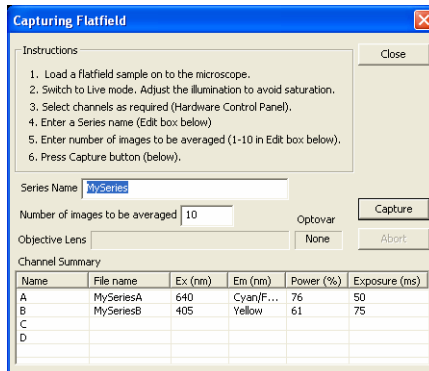


3. In the Sequence Control panel, click the time button,  and for 4D imaging, activate the Z button.
4. Click  to start the experiment.  
Clicking  will immediately stop the capture for an x,y,t series.

**NOTE:** In a 4D series, clicking  once will stop the experiment at the end of the stack. Clicking  twice in succession will stop the experiment immediately. To avoid having incomplete image stacks, data from the stack in progress will not be recorded.

## Flat field correction

The flat field correction compensates for uneven light illumination across the sample. There is a set of instructions that describe how to collect flat field images in the Capturing Flatfield dialog.



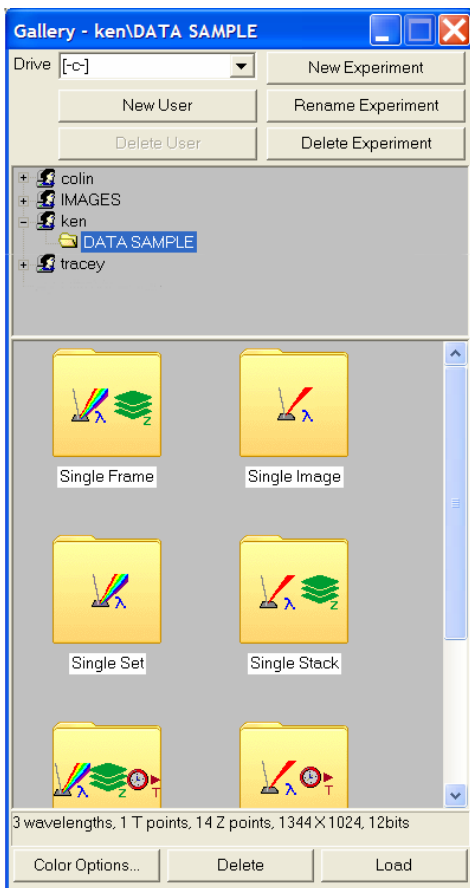
When you press **Capture**, a number of background flat field images with the excitation light switched off are collected and averaged. Next, the flat field image with the excitation light on is collected and averaged. Finally, the background image is subtracted from the flat field image to give the corrected flat field image. For a description how to apply background and flat field corrections to your images see *Background subtraction and flat field correction* on page 68.

## ***Routine Tasks***

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### ***Managing users, series and experiments***

Data is managed in the Gallery Window.



Experiments are organized by users and data series (sequences). Experiments may consist of a number of different series. Each series can be a single or multiple wavelength, and consist of single or multiple Z-steps or timepoints. The icon on the series folder indicates the type of experiment.

- Click **New User** to add a user to the list.
- Before a new experiment is started, click **New Experiment**.
- To rename a series folder, right-click on the folder and select **Rename**. The name is highlighted blue and can be edited.
- Load a series into the image window by double clicking on the folder.

OR

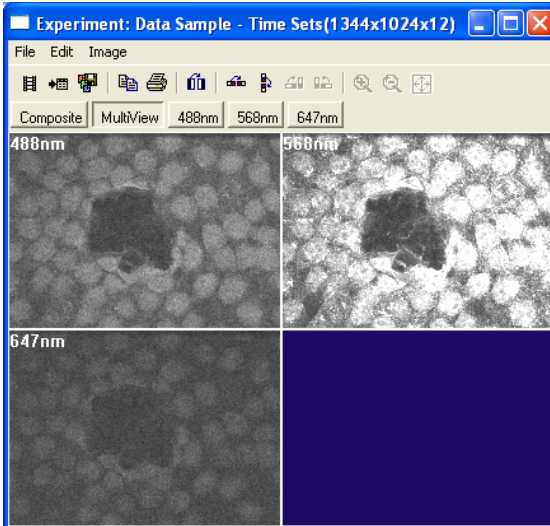
Highlight the series of interest and click **Load** at the bottom of the Gallery window.

- To delete an experiment, highlight the experiment in the top half of window and click **Delete Experiment**.
- To delete a series, click **Delete Series** at the bottom of Gallery window.


## ***Series Playback***

To view the image:

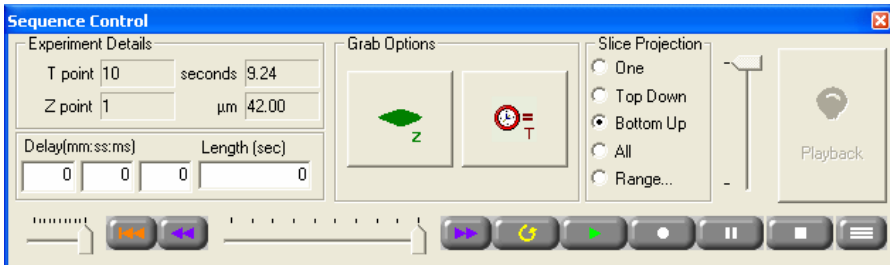
1. Open the Image Window.



The image observed can be changed by clicking **MultiView** to view all channels, **Composite** for a composite view of the channels, or an individual channel. The properties of the composite image are defined in the Processing

and Analysis Options dialog, opened by clicking  on the main toolbar.

2. Open the Sequence Control Panel.



The Sequence Control Panel controls resemble those of a video cassette recorder and function in the same way. Sequences can be played through time, or, with Z series, through a single stack by clicking the stack button,

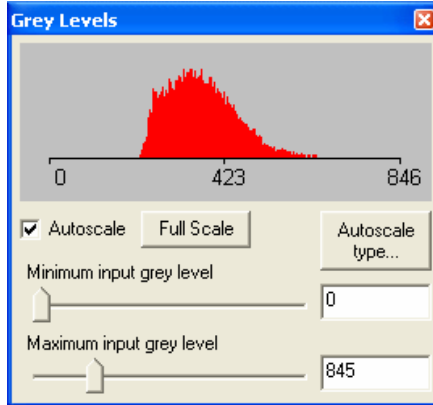


The arrow pointers (for Z and time) can be moved to view a particular slice or timepoint.

Image stacks can also be visualized as Z projections. Selecting **All** results in a maximum intensity projection of all slices in the stack viewed, while **Top Down** builds a projection from the top of the stack and **Bottom Up** from the bottom. **Range** enables you to define a projection of slices, centered on the slice indicated by the Z pointer.

## Rescaling grey levels

UltraVIEW ERS saves images as 12-bit grey images. Often the image of the sample does not use all 4096 grey levels. In order to facilitate visualization of the images, the grey levels viewed in the Image Window can be altered using the Grey Levels dialog.



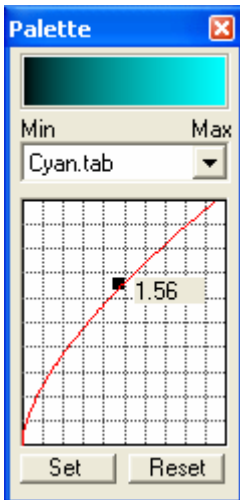
- Selecting **Autoscale** rescales the grey values in the image according to the brightness of the image displayed.
- To change the grey levels manually, clear the **Autoscale** check box and move the sliders to optimize the image.

Increasing the **Minimum input** reduces the background grey levels while reducing the **Maximum input** makes objects appear brighter.

**NOTE:** Adjusting the grey level settings of the image does not alter the raw image data.

## ***Pseudocolour (look up tables)***

The color of the images may be changed from greyscale to pseudocolour using the Palette dialog.



1. Select a color from the drop-down list.
2. Click **Set** to apply the color to the selected image.

Image contrast adjustments using gamma correction factors enable utilization of the entire range of pixels in the image.

- Click and drag the black square on the red graph line using your mouse. The shape of the line changes. Both the displayed value of gamma, and the image is updated.

**NOTE:** Clicking **Reset** only resets gamma.

When the value of gamma is one, the output signal is directly proportional to exposure time, or the intensity of light falling on the sensor. A double logarithmic plot of exposure time or intensity versus output current will give a straight line.

If gamma is less than 1, the dark features in the image become brighter. However, overall image contrast is reduced between the very bright and midtone grayscale values.

When gamma is greater than 1, the bright features become darker and overall contrast is increased.



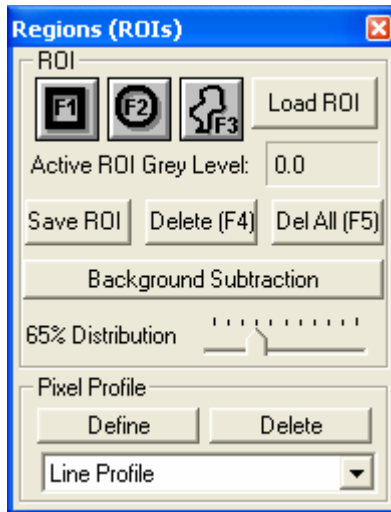
## Data Analysis

Following image capture there are a number of options available regarding image processing.

**NOTE:** You must select a Region of Interest before selecting an analysis type from the Set up analysis tab of the Processing and Analysis Options dialog.

### Regions of Interest (ROIs)

Regions of Interest (ROIs) may be drawn around areas in the image before you can extract information from areas of a cell. The region can be rectangular, circular or freehand. This is done using the Regions (ROIs) dialog:

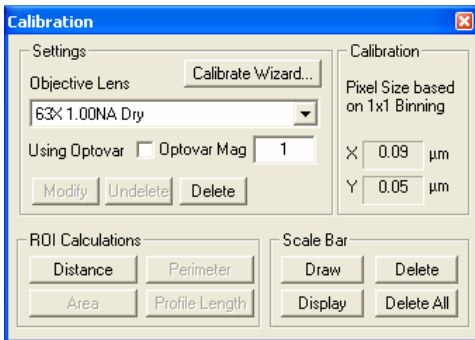


- Background fluorescence may be subtracted by drawing an ROI on a region of the image that contains no sample, adjusting the % **Distribution** scale and then clicking **Background Subtraction**.

**NOTE:** ROIs are also used to calibrate the system with different objectives. A graticule is required for this process. Calibration is made easier if the default size of the ROI is increased. To change the default size of ROIs, use the System Options dialog which is displayed when you select **Options** from the Tools menu.

## Calibration

The Calibration dialog is used to calibrate your images and perform simple measurements.




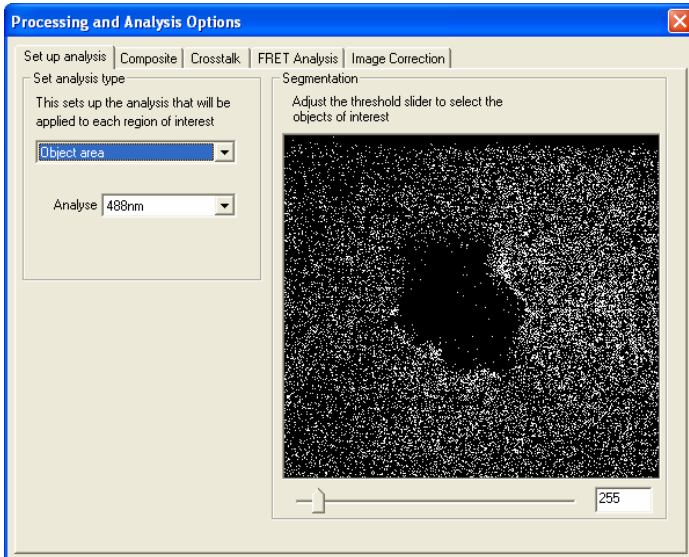
If you have a calibrated slide you can generate a calibrated scale on the Image Window. The calibrated scale allows you to determine the size of an object on your image in microns ( $\mu\text{m}$ ) based on its size in pixels. A calibration wizard leads you through the calibration routine.



The ROI Calculations section allows you to perform simple spatial measurements on your images such as determining the distance between two points, and calculating the area of an ROI. The Scale Bar section allows you to draw scale bars on your images that display absolute distances in microns.

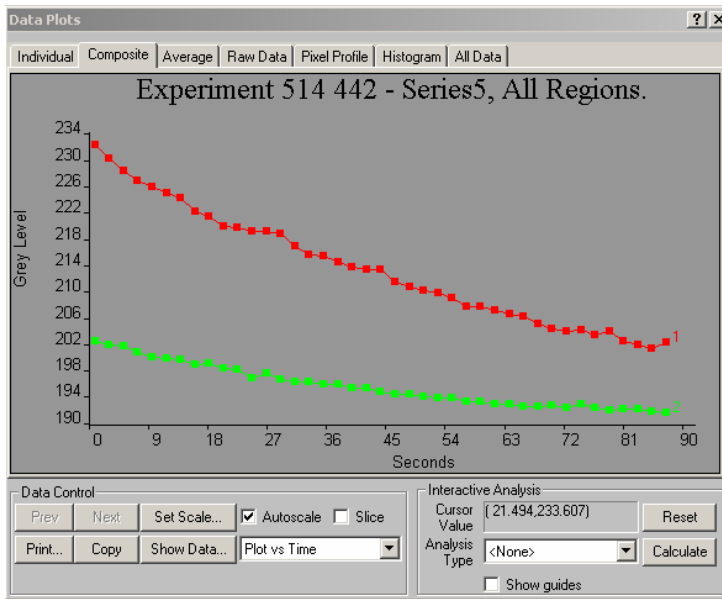
## Graphing

Graphs may be generated of a sequence of images. To define the type of graph you want to generate:

1. Open the Processing and Analysis Options dialog by clicking  on the toolbar.



2. Select the channel from the **Analyse** drop-down list.
3. Open the Data Plots dialog by clicking  on the toolbar.
4. To generate a graph, play through the sequence first by clicking  on the Sequence Control panel.  
A graph is generated on the Data Plots dialog.




**NOTE:** The tabs at the top of the dialog offer different ways to display the data.

- Raw data is displayed by clicking **Show Data**.  
From the resulting dialog, data can be exported as text files.

### ***Background subtraction and flat field correction***

To apply a background subtraction or flat field correction to each of your data channels:

- Open the Processing and Analysis Options dialog by clicking  on the toolbar, and then select the **Image Correction** tab.
- In the Channel Assignment for Correction table, choose **Yes** from the **Flatfield Correction** or **Bkgnd Subtraction** drop-down lists as required.  
If you select Flatfield Correction, Bkgnd Subtraction is automatically selected.  
If you wish to subtract the background using a Region of Interest (ROI), select the **Use ROI Background** check box.
- Click **Save**.

## ***FRET (Fluorescence Resonance Energy Transfer)***

In the UltraVIEW software, FRET is calculated by sensitized emission according to the following simplified formula devised by Wouters et al. (Trends Cell Biol. 11:203-211 (2001)). In the formula, the first letter in each two letter term is the excitation wavelength that gives maximal excitation of the Donor (D) or Acceptor (A). The second letter refers to the emission filter used to measure maximal emission of the Donor (D) or Acceptor (A):

$$E_A = (DA-DD.a-AA.b)/AA$$

Where  $E_A$  is the sensitized emission (FRET) of the acceptor

DD is the fluorescence measured in the donor channel when illuminated with donor excitation ("donor channel").

DA is fluorescence measured in the acceptor channel when illuminated with donor wavelength ("FRET" channel).

AA is fluorescence measured in the acceptor channel when illuminated with acceptor excitation ("acceptor channel").

a is the Donor correction factor for donor emission bleedthrough into the FRET channel. This is a ratio of donor detection efficiencies through the donor and acceptor emission filters. It is calculated by taking a sample containing donor fluorophore only, exciting at a single wavelength and dividing the background-corrected intensities detected through the acceptor filter by the donor filter (i.e.  $a=DA/DD$ ).

b is the Acceptor correction factor for acceptor cross-excitation by donor illumination. It is calculated by taking a sample containing acceptor fluorophore only, exciting using the donor (DA) and acceptor (AA) wavelengths and detecting through the acceptor emission filter. b is the ratio of acceptor fluorescence emission through the FRET and acceptor channels (i.e.  $b=DA/AA$ ).

In this simplified formula, acceptor emission bleedthrough into the donor channel and donor cross-excitation by acceptor illumination are neglected as normally they do not significantly affect the result.

**To acquire FRET images:**

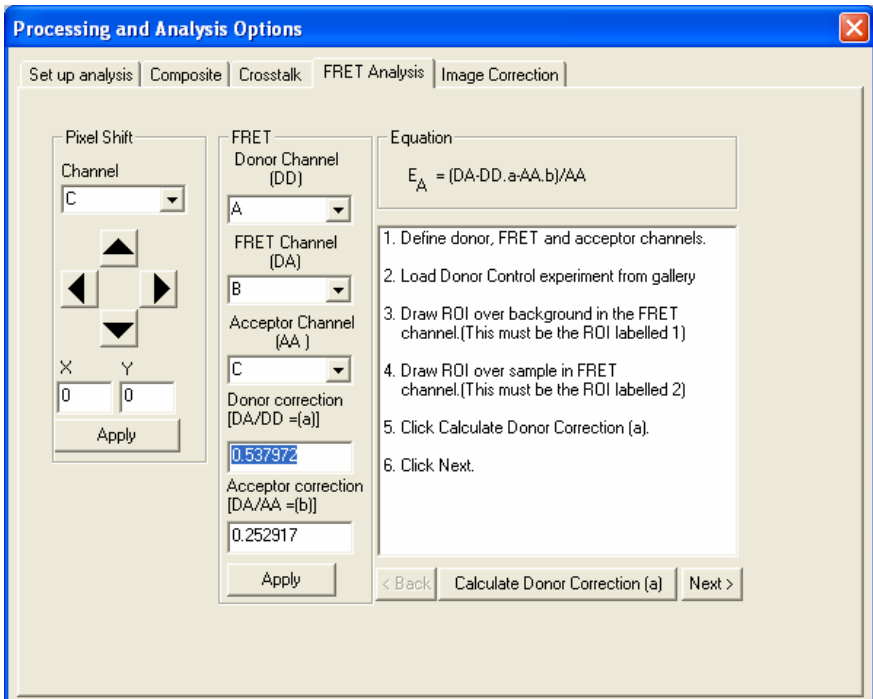
1. In the Hardware Controller dialog, select **Emission Discrimination**.
2. In the Hardware Controller dialog, select the appropriate **Dichroic** button.
3. Mount the FRET sample on the microscope.
4. In the Hardware Controller dialog, set up the Donor (DD) channel by selecting the appropriate **Excitation** wavelength and **Emission** filter.
5. In the Hardware Controller dialog, set up the FRET (DA) channel and Acceptor (AA) channel according to the FRET pair under study.  
Ensure that the camera exposure time and the laser power are equal for the Donor Channel (DD) and FRET Channel (DA).  
To reduce the variance in the data set, ensure that fluorescence grey levels in the Acceptor channel are brighter than in the FRET channel. Use the Regions dialog to compare the grey levels in the two channels.
6. Acquire data on the Donor Channel (DD), Acceptor Channel (AA) and FRET Channel (DA) for the FRET sample.
7. Mount the control sample that contains only the donor fluorophore onto the microscope.
8. Acquire data on the Donor Channel (DD), Acceptor Channel (AA) and FRET Channel (DA) for the donor control sample.
9. Mount the control sample that contains only the acceptor onto the microscope.
10. Acquire data on the Donor Channel (DD), Acceptor Channel (AA) and FRET Channel (DA) for the acceptor control sample.

**NOTE:** You must use the same system settings (laser power, camera exposure time, binning and gain) when acquiring data for the two control samples and FRET sample.

**To analyze FRET images:**

1. Open the Processing and Analysis Options dialog, and then click on the FRET Analysis tab.
2. In the FRET section, select the **Donor Channel**, **FRET Channel** and **Acceptor Channel** from the drop-down lists.

All of the steps that are necessary to perform a FRET Analysis are described in the FRET Analysis wizard on the right side of the page.



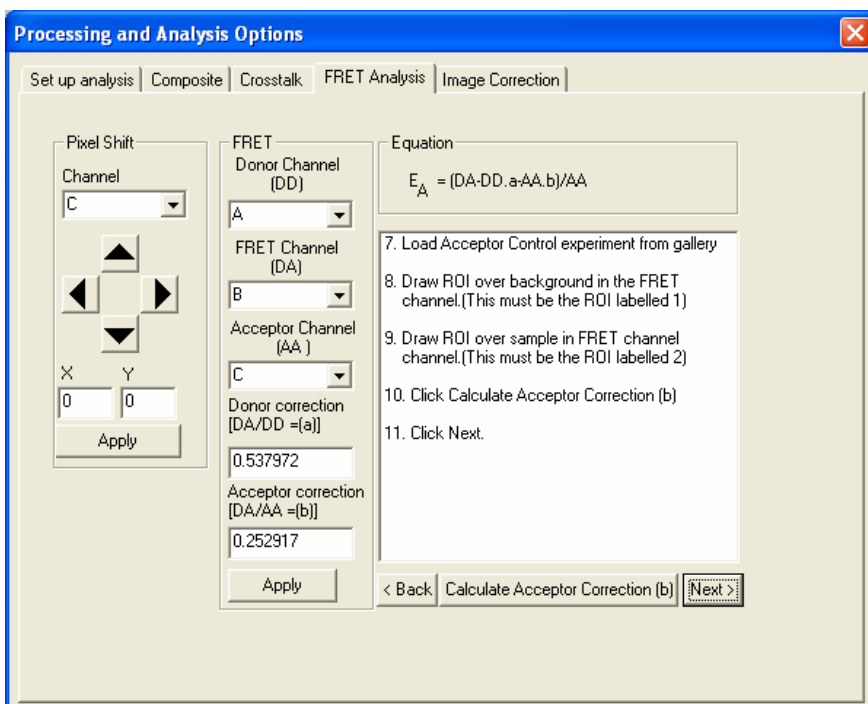
3. Load the image of the control sample that contains only the donor fluorophore.  
The first step in the analysis is to calculate the Donor correction a.
4. Draw a Region Of Interest (ROI) on the background of the image from the FRET Channel.  
This background area must not contain any sample.
5. Draw an ROI on the image from the FRET Channel that covers a representative area of sample.

6. Click **Calculate Donor Correction (a)**.

The calculated value is displayed in the Donor correction field. Typically this value will be between 0 and 1.

7. Click **Next**.

The Acceptor page of the wizard is displayed. The second step in the analysis is to calculate the Acceptor correction b.



8. Load the image of the control sample that contains only the acceptor.

9. Draw a Region Of Interest (ROI) on the background of the image from the FRET Channel.

This background area must not contain any sample.

10. Draw an ROI on the image from the FRET Channel that covers a representative area of sample.

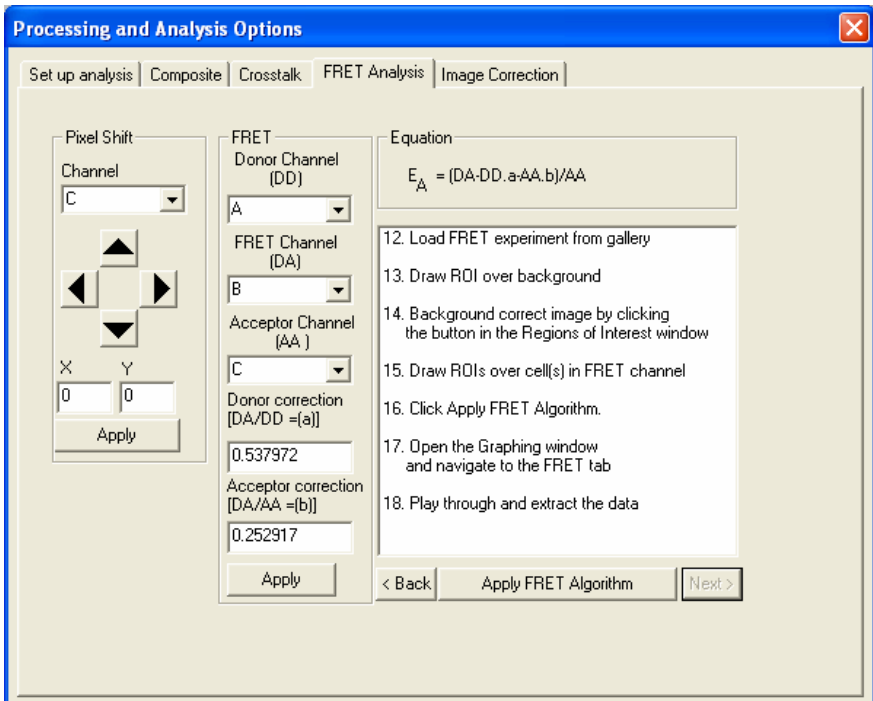
11. Click **Calculate Acceptor Correction (b)**.

The calculated value is displayed in the Acceptor correction field. Typically this value will be between 0 and 1.



12. Click **Next**.

The FRET page of the wizard is displayed. The third step in the analysis is to calculate the FRET in the experimental sample.

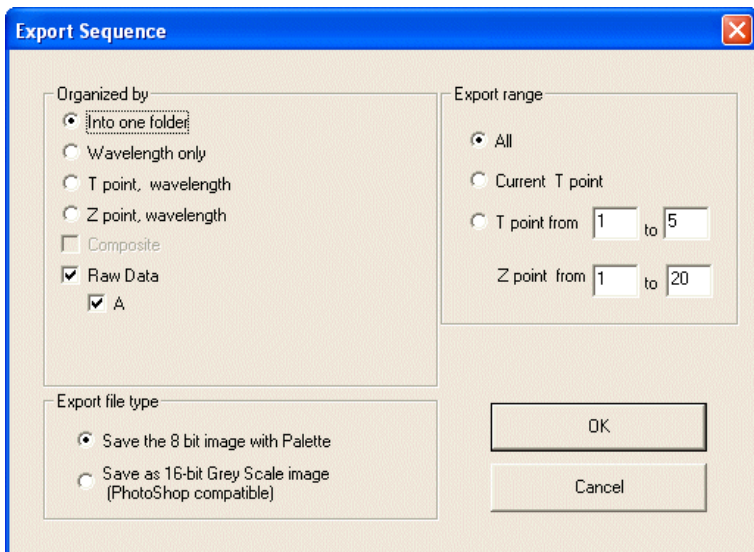


13. Load the image of the FRET sample.
14. Draw a Region Of Interest (ROI) on an area of the image from the FRET Channel that contains no sample. Perform a background correction on the image.
15. Draw as many ROIs as appropriate on the image from the FRET Channel that cover a representative area of sample.
16. Click **Apply FRET Algorithm**.

The resulting FRET image can be viewed by clicking the **FRET Image** button in the Image Window. FRET data can be viewed in the Data Plots dialog.

## Image and Movie Export

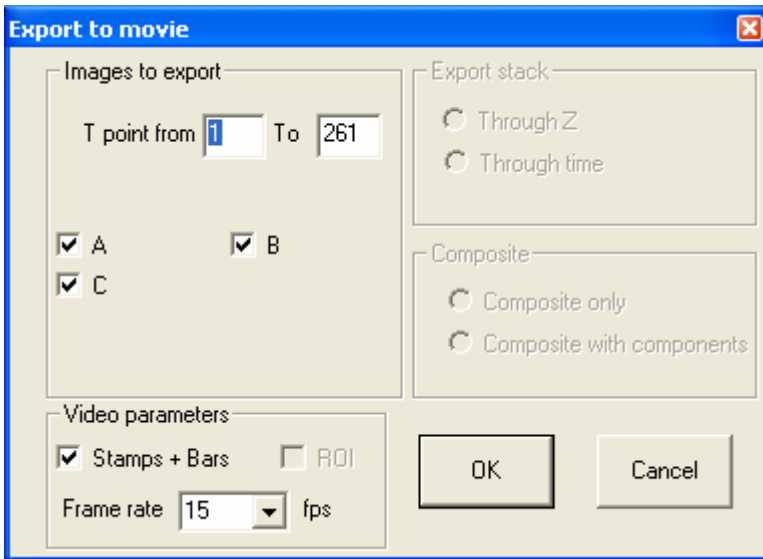
- Export images by selecting **Export sequence** from the File menu.



**NOTE:** Sequences can be exported as 8-bit tiff files or 16-bit Photoshop compatible files. For 8-bit tiffs, the grey levels for each channel must be set individually prior to export.

**NOTE:** Select **Composite** to export the results of the composite view. If you select **Raw Data** you can choose to export either the 8-bit image as displayed on the screen with the Palette (Save the 8 bit image with Palette), or the raw 16-bit Grey Scale image (Save as 16-bit Grey Scale image).

- Export movies by selecting **Export sequence as movie** from the File menu. You can choose which frames/Z-positions are exported.



## ***Crosstalk Correction***

Under certain circumstances, dyes with long wavelength excitation maxima may also be excited by shorter wavelength excitation lines, causing crosstalk of the signal and the appearance of some signal in the lower wavelength channel.

While crosstalk can be minimized by using dyes with well-spaced excitation maxima, crosstalk can also be minimized by ensuring that the lower wavelength dyes give stronger signals than the higher wavelength dyes.

Nevertheless, if there is crosstalk in the image, this can be corrected using the Crosstalk tab on the Processing and Analysis Options dialog.

**NOTE:** If the pixel values in crosstalk corrected regions fall below the background level then the image has been over-corrected.

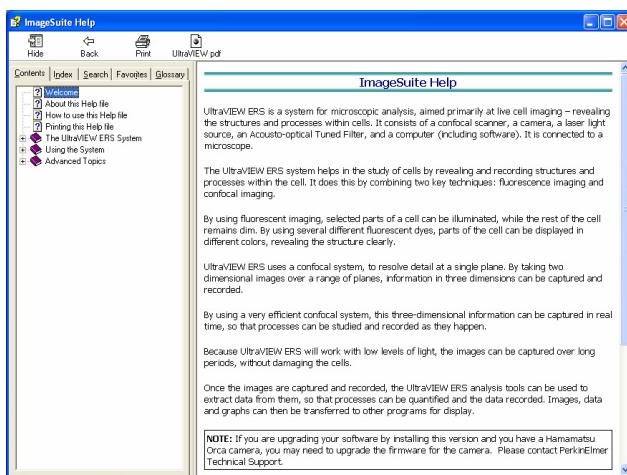
## Help System

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The Help system provides more detailed information on using the UltraVIEW ERS system.

This is a HTML based help system that uses a tripane window:

- At the top of the window is a toolbar for common tasks, such as printing and going back to the previous page.
- On the right is the main window that displays the help topics using a format described below.
- On the left is a tabbed window that gives you four different ways to select which topic you want to view, as discussed below.




### Main Window

The layout of the Help has been designed to aid navigation to the exact information you need without having to read additional information that may not be necessary to the query you have.



Some of the Help is written as a series of answers to questions that you may ask. Each question is in the form of Dynamic HTML. That is, clicking on the blue underlined text displays the answer that particular question. In this way, only the Help that you really need is displayed. To remove the additional text simply click on the blue underlined text again. However, if you are not sure about the question you are asking or you wish to display all the Help on a particular page, click **Show All** to display all the hidden text on the page. Click **Hide All** to remove it again.

## ***Tabbed Window***

### ***Contents***

On the **Contents** tab the topics are arranged by subject in books .

1. Click on one of the books to open the book and display an overview of the topics in the book.

The book opens () and topics () are displayed.

2. To display a topic, click on the topic icon ()

The topic is displayed.

### ***Index***

The **Index** tab enables you to search the index of the help for a word.

- Type the word you want to look up in the text box and choose **Display**, or click on the topic in the alphabetical list.

The topic you have chosen is displayed.

### ***Search***

The **Search** tab enables you to search the whole of the help for a word, phrase, or set of words.

#### **Defining search terms**

This help file can use boolean, wildcard and nested expressions to narrow down the search. For instance, the AND, OR, NOT, and NEAR operators enable you to precisely define your search by creating a relationship between search terms. The following table shows how you can use each of these operators. If no operator is specified, AND is used. For example, the query "spacing border printing" is equivalent to "spacing AND border AND printing."

To search for	Use	For example	Will give
More than one term in the same topic.	AND	baseline AND correction	Topics that contain both the words "baseline" and "correction".
Either term in a topic.	OR	spectrum OR spectra	Topics containing either the word "spectrum" or the word "spectra" or both.
The first term without the second term.	NOT	peak NOT table	Topics containing the word "peak" but not the word "table".
Both terms in the same topic, close together.	NEAR	third NEAR derivative	Topics containing the word "third" within eight words of the word "derivative".

**NOTE:** The |, &, and ! characters don't work as boolean operators (you must use OR, AND, and NOT).

### To perform a Search:

1. Select the search criteria you require.
  - Search previous results** enables you to narrow down your search if the previous query gave too many results.
  - Match similar words** will look for variations of a word like searching for "add" will also look for "adds" and "added".
  - Search titles only** will look for the selected words only in the topic titles.
2. Type the words or phrase you want to search for in the text box and click **List Topics**.
 

The topics containing the word or phrase are displayed, including a **Rank** to help you choose the topic you want to view first.
3. Select a topic from the list and click **Display**.
 

The topic you have chosen is displayed in the main window.

## ***Favorites***

- The **Favorites** tab enables you to mark a topic as one you want to be able to get back to quickly.

### **To set up a favorite:**

- With the topic displayed, switch to the Favorite tab and click **Add**.  
The topic name is added to the list.

### **To go to a favorite topic:**

- Select the topic from the list and click **Display**.  
The topic is displayed in the main window.

**NOTE:** To remove a topic from the list, select the topic and click **Remove**.

## ***Glossary***

The **Glossary** tab contains some common terms and their definitions.

- Select the term from the list in the top pane.  
The definition is shown in the bottom pane.

## ***Printing the Help File***

- If you wish to print the currently displayed Help page, click **Print** on the Toolbar at the top of the Help dialog.  
If a book is currently selected in the left-hand tabbed window, clicking **Print** will give you the option to print the current page or all the pages in the selected book.

The entire help file is also provided as a pdf file which can be opened from the Toolbar within the Help File.



- Click **UltraVIEW' pdf** to view the pdf file.

**NOTE:** You must have Adobe Reader installed to be able to open the pdf file.

## ***Output***

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### ***Output to printers and slide makers***

Output to any printer or slide maker supported by Windows is possible. If you have purchased a printer, refer to your Windows manual for instructions on installing software for the printer, including networked printers not physically attached to the workstation.

Each of the different forms of data presentation in the software has either a **Print** button or command on the File menu. After the desired result is displayed on the screen, selecting this option will bring up the standard print dialog box, allowing you to select the printer to use, and other print properties such as paper orientation.

### ***Output to other programs***

In the same manner, each data presentation format has a **Copy** button or menu command, which will copy the data to the Windows clipboard. This can include images, graphs, numerical data, etc.

Once the data is copied, you can paste it into another application, such as a spreadsheet or image processing software.

Numerical data from the **Show Data** button on the intensity plot window can be pasted into spreadsheet programs in columnar format ready for further numerical processing.

### ***Output to disk files***

Data may be output to disk files for transfer to colleagues across a network, by e-mail or on removable disk.

The graphical output windows have a **Save As** menu command that enables the displayed image to be saved as a TIFF (a common format on both Macintosh and IBM compatible computers) or a compressed JPEG (JPG) file.

Textual output can be saved into an industry standard ASCII format file, and the character separator between columns of data can be chosen to be either tabs, spaces or commas. This allows the data to be read into most statistical and graphical packages for custom processing.



## ***Saving Disk Space by Archiving Experiments***

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There are a number of different techniques by which digitized image data may be compressed to make the best use of storage media such as magneto-optical disks, or streaming tape. So called 'lossy' compression methods, such as the JPEG standard, actually reduce the amount of information in the file as part of the compression process, so when the image is de-compressed it is not quite the same. For photographic images this technique can be quite acceptable, but for quantitative data such as that generated with the system, a 'lossless' technique is needed.

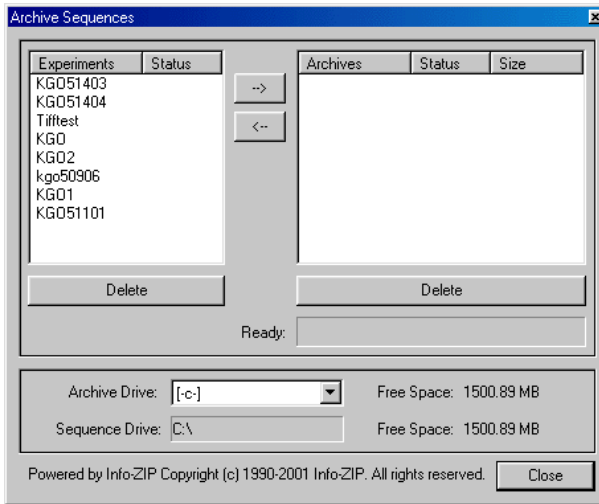
Lossless compression techniques such as the Lempel-Ziv-Huffman (LZW) encoding scheme compress data so that the exact same image is restored on de-compression. The technique itself is quite involved, but generally involves eliminating sequences of repeated pixels. Some images compress better than others, for example an image with large regions of black will compress very well, whereas a very noisy, bright image would not be so compressible. With the more advanced techniques, as used by the products described here, ratios of between 3 - 15 times compression are possible.

**NOTE:** Very large files (greater than 1 GB) may not extract properly and should not be archived in this way.

To perform archiving, it is necessary to use the Archive Manager:

1. Select **Archive Manager** from the Tools menu.

This displays your experiments in two lists. On the left are all your current experiments that have not yet been archived, while on the right are the archived experiments.



2. Check that the **Archive Drive** is the one that you want.
3. Select an experiment to archive by clicking on it in the left-hand list so that it is highlighted.
4. Click -->.  
This starts the compression and transfer of the experiment into the archive region. It will then remove it from the current area.

**NOTE:** When you want to retrieve an archived experiment, select it in the right list, and then click <--. The experiment will be decompressed as it is transferred.

**NOTE:** Archive Manager can also be used to Delete an unwanted experiment from the current or archived regions.

# **Maintenance and** **Troubleshooting**

## ***Hardware Maintenance***

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The system does not require regular maintenance or calibration other than occasional checking and cleaning.

### ***Definitions***

**RESPONSIBLE BODY:** Individual or group responsible for the use and maintenance of the equipment and for ensuring that the **OPERATORS** are adequately trained.

### ***System checks***

The system should be checked from time to time, and in any case when it has been moved or out of use for a long period. For further information, see *Periodic checks* on page 15.

### ***System cleaning***

#### ***Casings***

Remove power cord before cleaning.

The casing of each unit may be cleaned using a soft lint-free cloth, moistened if required with a little water. Do not use abrasive or solvent based cleaning materials. Do not use copious amounts of water, or allow water to enter the casing.

#### ***Scanning head***

The unit requires no maintenance, other than protection from mechanical shock, dust and dirt. If the eyepiece lens becomes dirty, clean it carefully using suitable lens cleaning materials. Always protect open ports using the caps provided. This is particularly important for the FC connector and the optical fiber tip. Whenever these are disconnected, they **MUST** be covered by their protective caps.

## ***Troubleshooting***

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Please follow this troubleshooting guide if you are having problems with your confocal imaging system. This gives remedies for the most common problems you might experience. If this does not help, contact your local PerkinElmer Technical Support Representative.

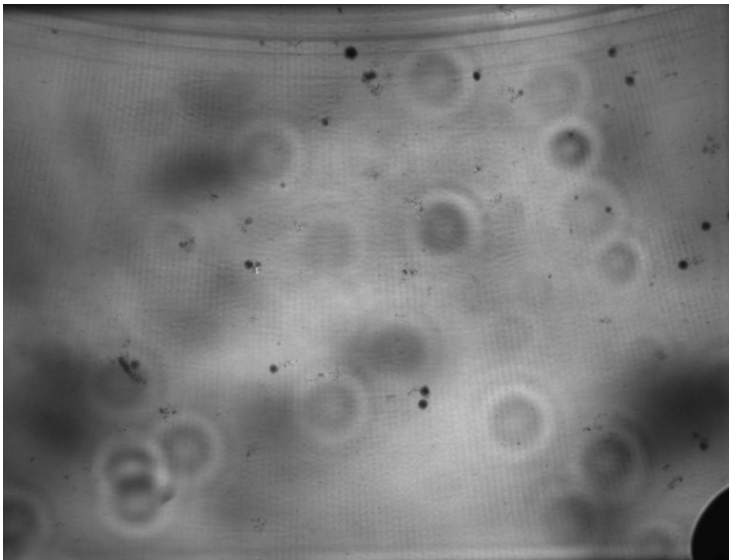
### ***Scanner head***

If you cannot get a confocal image through the UltraVIEW ERS scanner's eye piece, check the Brightfield image using transmitted light. Also check that the UltraVIEW ERS scanner's red LED (to the right side of the key switch) is on. Then check against the following table.

<b>Problem</b>	<b>Probable Cause(s)</b>	<b>Remedy</b>
No Brightfield image	Scanner's Eye piece/Camera exchange knob is set for Camera side (knob is pushed into the scanner body)	Pull out the knob
	Light path of microscope is not set for camera port	Select 100% camera port
Good Brightfield image, but no confocal image: LED is on	Laser is off	Switch it on
	Laser's shutter is closed	Open the shutter
	Optical fiber is disconnected	Reconnect the fiber
	Optical fiber is mis-aligned	Please contact your local PerkinElmer Technical Support Representative
	Optical fiber is dirty	
	Microscope's fluorescent filter set is in the light path	Select open light path
	System is completely out of focus	Focus either with transmitted light or fluorescent light

<b>Problem</b>	<b>Probable Cause(s)</b>	<b>Remedy</b>
	Specimen is very dark	Use the pollen grain slide
No confocal image: LED is off	Scanner's key switch is off	Turn it to the laser on position
	Scanner's interlock connector is not properly seated	Check the connector seating

### ***Image problems***



**Figure 16 Concentric patterns and black dots in an image**

If you see many small concentric patterns and/or black dots as shown in the image above, there may be stains and/or particles on the optical parts in the light path. To find out what part is affected, check against the following table.

<b>Problem</b>	<b>Probable Cause</b>	<b>Remedy</b>
Image of contamination rotates when scanner is rotated against the microscope, after loosening the adapter screw	The contamination is on the specimen, the objectives, or inside the microscope	Make sure there are no unnecessary optical parts in the light path, and check and clean in turn the specimen, the outside surface of the objective, the inside surface of the objective (after removing it from the nosepiece), and the camera selector mirror in the microscope (see the microscope manual)
Image of contamination does not rotate when scanner is rotated, but rotates when camera is rotated against the scanner after loosening the screws of the camera C-mount adapter	The contamination is affecting an internal component of the scanner head, either the filters or another surface	We strongly advise that you do not open any part of the scanner head as this should only be done in class 1000 clean room conditions. Please contact your local PerkinElmer Technical Support Representative
Image of contamination does not rotate when camera is rotated	The contamination is affecting the camera	Remove the camera and C-mount from the scanner. Unscrew the C-mount from the camera and clean the camera's front window carefully with a lint-free cloth. If the problem persists, DO NOT attempt to open the camera head. Contact your local PerkinElmer Technical Support Representative

## Camera

<b>Problem</b>	<b>Probable Cause</b>	<b>Remedy</b>
Live image completely black	Grey level mapping set too low	Set grey level or autoscale using grey level map
	Light path on the microscope not set correctly	Make sure the light is directed to the camera port
Live image completely white	Grey level mapping set too high	Set grey level or autoscale using grey level map
	Camera saturated by too much light	Decrease the illumination or decrease exposure time
Live image has low contrast and is mid-grey	Grey level mapping range set too narrow	Set grey level or autoscale using grey level map
	Camera saturated by too much light	Decrease the illumination or decrease exposure time
No live image	No signal from the camera	Check cable connections
Regions of Interest are not covering the correct part of the image	Applying a camera subarray after ROIs have been set	If you need to use camera subarrays and ROIs, then always set the subarray first
Camera not working correctly	The camera firmware requires updating	Please contact your local PerkinElmer Technical Support Representative



# **Appendices**

## ***Appendix 1 - Filters and Dichroics***

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In UltraVIEW ERS the laser excitation beam (405 nm, 440 nm, 488 nm, 514 nm, 561 nm, or 640 nm) passes through a 45° mirror, into the microscope and then onto the sample. The excitation light from the sample passes back along the same path, reflects on the dichroic mirror, and passes through an emission filter before reaching the camera.

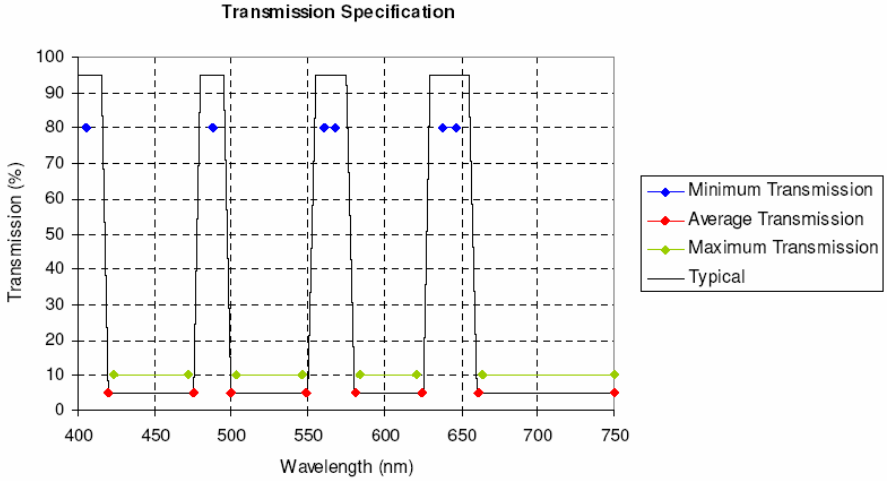
All the wavelengths cannot be covered with one dichroic mirror and emission filter set, therefore each UltraVIEW version is provided with a choice of three sets:

<b>Version</b>	<b>Dichroic</b>
ERS with laser engine	<ul style="list-style-type: none"><li>• 405/488/561/640</li><li>• 405/440/514/640</li><li>• 405/440/488</li></ul>
ERS with gas lasers	<ul style="list-style-type: none"><li>• 488/568/647</li><li>• 647</li><li>• 488</li></ul>

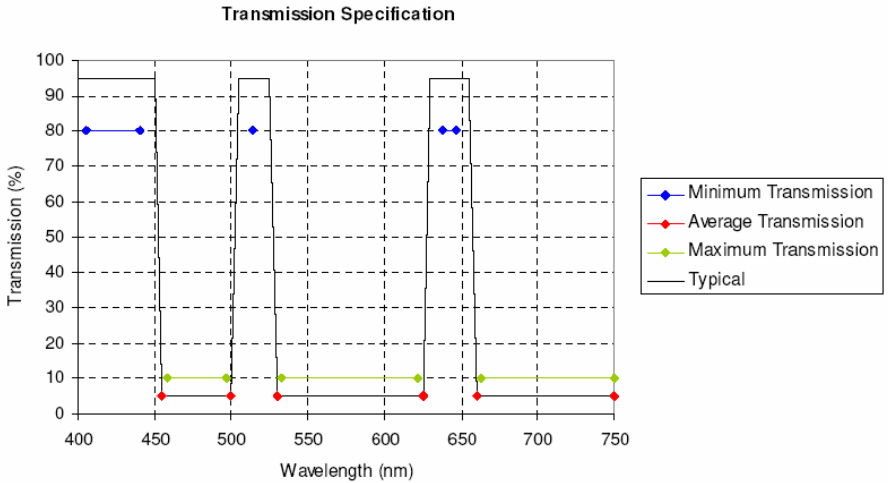
The figures below show the spectral characteristics of the dichroic mirrors and emission filters.

## Dichroic Mirrors

L7205021 **405-488-561-568-638-647**

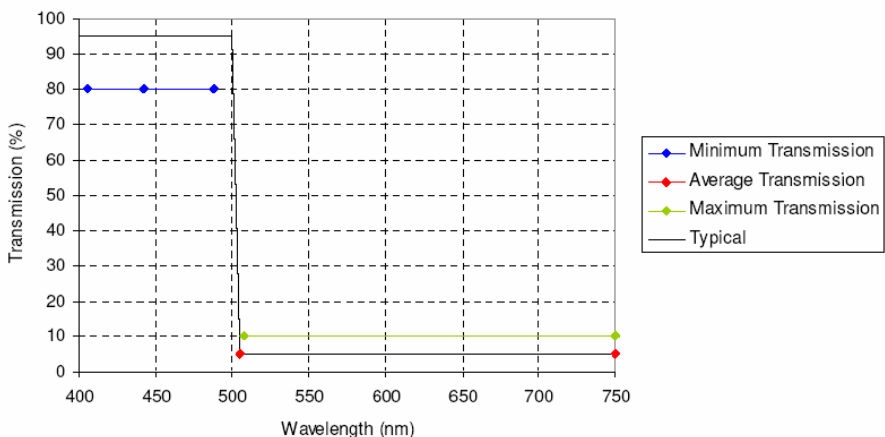


L7205022 **405-440-514-638-647**



L7205023 **405-440-488**

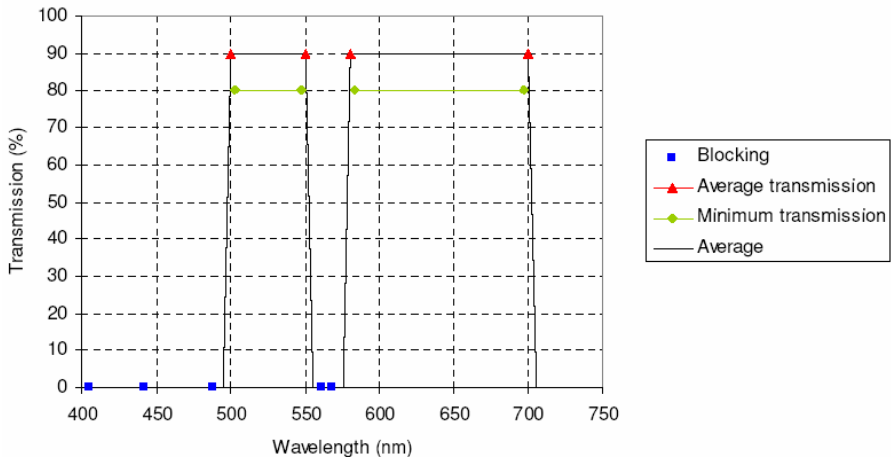
Transmission Specification



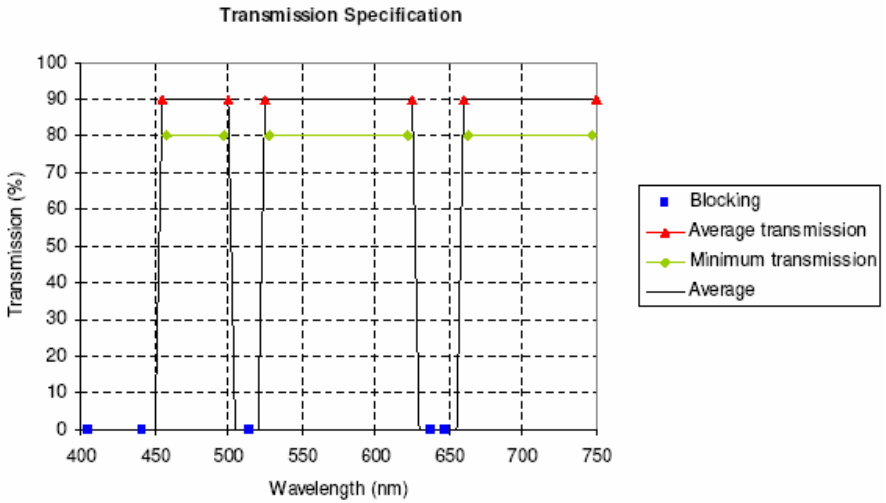
**Emission Barrier Filters (Confocal Head)**

L7205001 **EM525/50-640/120H**

Transmission Specification

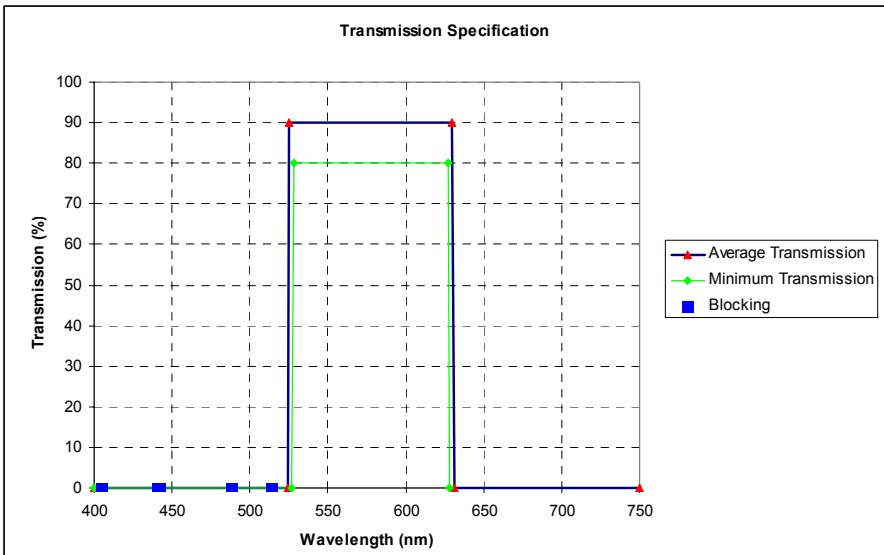


L7205002 **EM477/45-575/100-705/90H**

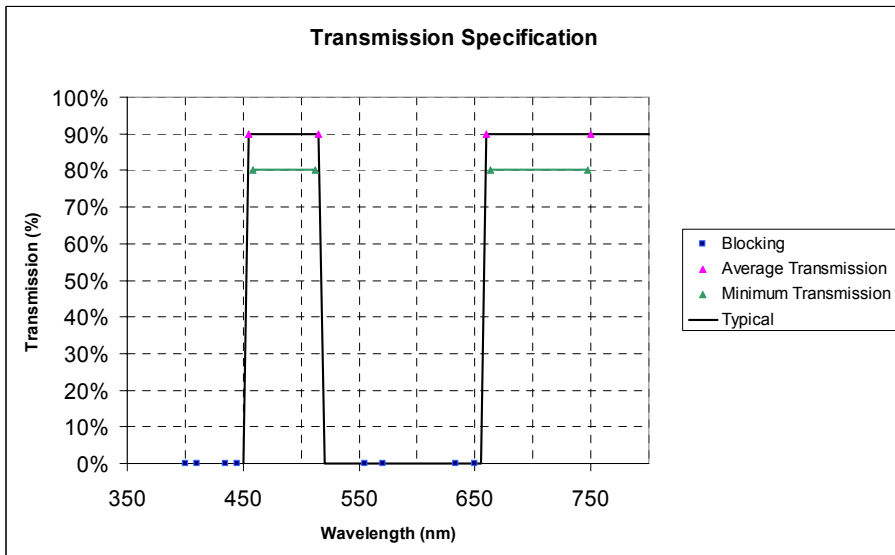


**Emission Discrimination Filters**

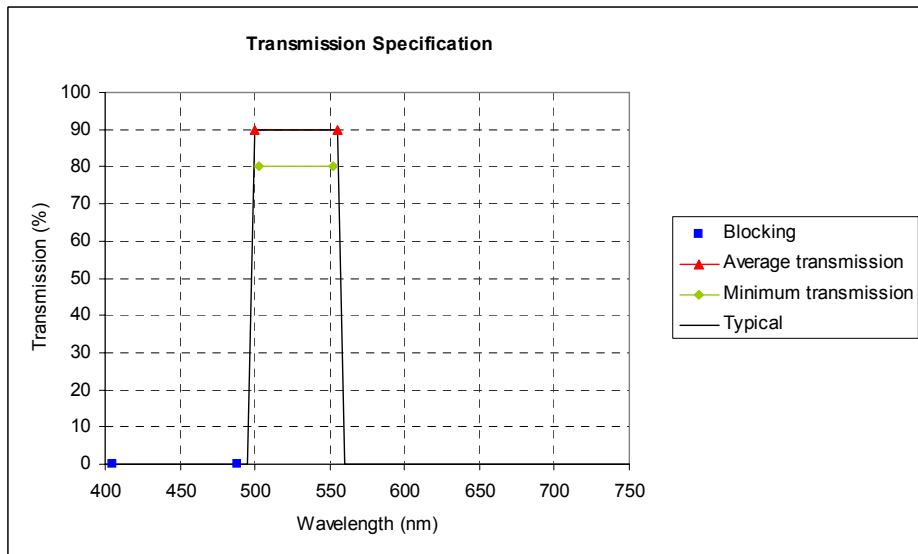
L7205003 **EM578/105W (Yellow)**



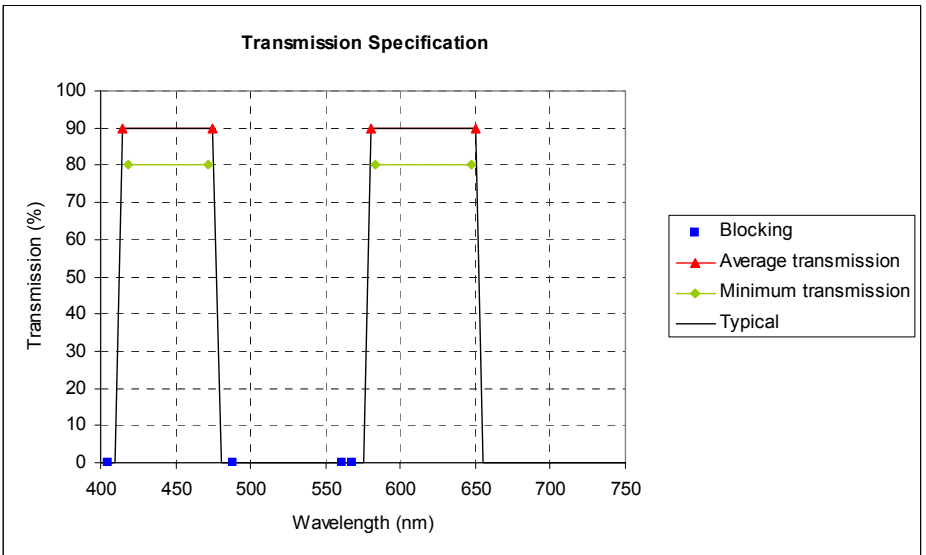
L7205004 **EM485/60-705/90W (Cyan/Far Red)**



L7205005 **EM527/55W (Green)**



L7205006 EM445/60-615/70W (Blue/Red)







Products from other manufacturers may also form a part of your PerkinElmer system. These other manufacturers are directly responsible for the collection and processing of their own waste products under the terms of the WEEE Directive. Please contact these manufacturers directly before discarding any of their products.

Consult the PerkinElmer web site (above) for manufacturer's names and web addresses.

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