

USER MANUAL





High Performance EMCCD & CCD Cameras for Life Sciences

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http://www.photometrics.com/support

#### Normal Wear Item Disclaimer

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#### **Vacuum Integrity Lifetime Warranty**

Photometrics warrants the vacuum integrity of all our products for a period of up to sixty (60) months from the date of shipment. We warrant that the detector head will maintain the factory-set operating temperature without the requirement for customer pumping. Should the detector experience a Vacuum Integrity failure at anytime within sixty (60) months from the date of delivery all parts and labor needed to restore the vacuum integrity will be covered by us. Responsibility for shipping charges is as described above under our Basic Limited Two (2) Year Warranty.



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#### Owner's Manual and Troubleshooting

You should read the owner's manual thoroughly before operating this product. In the unlikely event that you should encounter difficulty operating this product, the owner's manual should be consulted before contacting the Photometrics technical support staff or authorized service representative for assistance. If you have consulted the owner's manual and the problem still persists, please contact the Photometrics technical support staff or our authorized service representative. See Item 12 in the following section of this warranty ("Your Responsibility") for more information.

#### Your Responsibility

The above Limited Warranties are subject to the following terms and conditions:

You must retain your bill of sale (invoice) and present it upon request for service and repairs or provide other proof of purchase satisfactory to Photometrics.

You must notify the Photometrics factory service center within thirty (30) days after you have taken delivery of a product or part that you believe to be defective. With the exception of customers who claim a "technical issue" with the operation of the product or part, all invoices must be paid in full in accordance with the terms of sale. Failure to pay invoices when due may result in the interruption and/or cancellation of your two (2) year limited warranty and/or any other warranty, expressed or implied.

All warranty service must be made by the Photometrics factory or, at our option, an authorized service center. Before products or parts can be returned for service you must contact the Photometrics factory and receive a return authorization number (RMA). Products or parts returned for service without a return authorization evidenced by an RMA will be sent back freight collect.

These warranties are effective only if purchased from the Photometrics factory or one of our authorized manufacturer's representatives or distributors.

Unless specified in the original purchase agreement, Photometrics is not responsible for installation, setup, or disassembly at the customer's location.



Warranties extend only to defects in materials or workmanship as limited above and do not extend to any product or part which has:

- been lost or discarded by you;
- been damaged as a result of misuse, improper installation, faulty or inadequate maintenance, or failure to follow instructions furnished by us;
- had serial numbers removed, altered, defaced, or rendered illegible;
- · been subjected to improper or unauthorized repair; or
- been damaged due to fire, flood, radiation, or other "acts of God" or other contingencies beyond the control of Photometrics.

After the warranty period has expired, you may contact the Photometrics factory or a Photometrics-authorized representative for repair information and/or extended warranty plans.

Physically damaged units or units that have been modified are not acceptable for repair in or out of warranty and will be returned as received.

All warranties implied by state law or non-U.S. laws, including the implied warranties of merchantability and fitness for a particular purpose, are expressly limited to the duration of the limited warranties set forth above. With the exception of any warranties implied by state law or non-U.S. laws, as hereby limited, the forgoing warranty is exclusive and in lieu of all other warranties, guarantees, agreements, and similar obligations of manufacturer or seller with respect to the repair or replacement of any parts. In no event shall Photometrics' liability exceed the cost of the repair or replacement of the defective product or part.

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# **Table of Contents**

1.	Introdu	iction	1
2.	Installation		2
	2.1	Installing Filters into the DualView Lambda Filter Cube	2
	2.2	Installing Dichroic Mirrors into the DualView Lambda Filter Cube	3
	2.3	Inserting the DualView Lambda Filter Cube into the Photometrics DualView Lambda	3
	2.4	Auxiliary Component Mounts	5
	2.5	Installing the Photometrics DualView Lambda in the Light Path	5
3.	Opera	tion	6
	3.1	Adjusting and Locking the Aperture	7
	3.2	Adjusting the Position of the Images.	7
	3.3	Focusing the Camera on the Aperture	8
	3.4	Centering Images on the Camera	9
	3.5	Image Optimization (Trim Adjustment)	11
	3.6	Single Channel (Non-Split) Mode	. 12
4.	Technic	cal Summary	. 13



#### IMPORTANT INFORMATION

## Please Read Before Installing and Operating Your Photometrics DualView Lambda

For maximum reliability Photometrics recommends using the equipment within certain guidelines. If in any doubt, then please contact our technical support team:

Phone: +1 800.874.9789

Email: support@photometrics.com

Online: <a href="http://www.photometrics.com/support">http://www.photometrics.com/support</a>

Note: Please do not adjust any control before reading this manual.

The Photometrics DualView Lambda is supplied pre-aligned and centered. Please refer to this manual before making any adjustments.

When changing the DualView Lambda filter cube, care should be taken to not touch the optical surfaces. If any lenses, filters or mirrors are marked, they should be wiped with a clean lens tissue.



### Chapter 1.

1

#### Introduction

In fluorescence imaging applications, it is often useful to acquire simultaneous images at two emission wavelengths. Traditionally such applications have been restricted by the speed of an electronic filter changer, or by the cost and complexity of adding a second camera to a system. In many instances the region of interest (ROI) does not require the full resolution of the camera, so the ideal solution would be to simultaneously image at two different wavelengths on the same camera chip. In conjunction with a research microscope and a suitable CCD or EMCCD camera, the Photometrics DualView Lambda allows the researcher to do exactly this.

The Photometrics DualView Lambda is supplied with a rectangular aperture to define a ROI and includes controls to allow the two images to be positioned accurately and conveniently within the camera frame. Images can be acquired using any imaging software and processed either manually off-line or using an appropriate analysis tool. The instrument is usually configured to attach to the c-mount output port of a research microscope, with a c-mount CCD or EMCCD camera fitted to its output. The design allows for a connection to a variety of alternative devices. Please consult with the Photometrics support team before using in any other configuration.

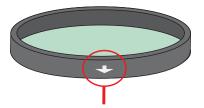
### Chapter 2.

### Installation

Before using the Photometrics DualView Lambda image splitter, install the appropriate set of filters and mirror into the DualView Lambda filter cube. Instructions for these procedures are in sections 2.1 and 2.2. Always take extreme care when changing or adjusting the filter and mirror sets to avoid damage or soiling. If you purchased your Photometrics DualView Lambda with a full filter set pre-installed, go to section 2.4. as the following may not be pertenant.

# 2.1 Installing Filters into the DualView Lambda Filter Cube

The DualView Lambda filter cube has spaces for two 25mm filters. These are held in place by locking rings which can be removed using the tool provided. To fit a filter, remove the locking ring, then adjust the filter orientation so the arrow points into the DualView Lambda filter cube and towards the light path. Then simply replace and tighten the locking ring.



The arrow should align with the filter cube, toward the light path.



Turn the locking ring counter-clockwise to remove it.



Carefully place the filter inside the recess.

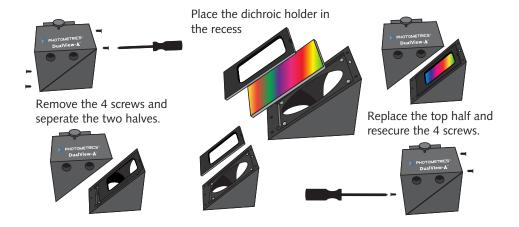


Replace the locking ring and rotate it clockwise to secure.

In most applications, the dichroic beamsplitter will have longpass characteristics. The longer wavelength of the two filters should be located in the straight through filter position, and the shorter wavelength filter in the second position. (Please refer to the technical summary in section 4 for details of the light path.)

# 2.2 Installing Dichroic Mirrors into the DualView Lambda Filter Cube

To fit the dichroic mirror into the DualView Lambda filter cube, remove each of the four screws that secure the two halves together. Then, gently pull the two halves apart. There is one steel locating rod in each half of the cube to ensure the correct alignment when it is reassembled. Once the two halves of the cube are seperated, place the dichroic mirror (active side down) into the rectangular recess. Then simply reassemble the cube and tighten the screws.



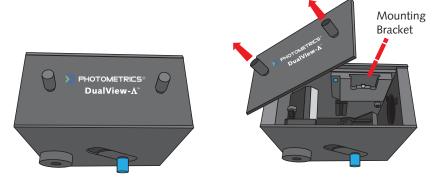
Photometrics strongly recommends using Chroma Ultraflat 2mm thick dichroic mirrors, designated as "UF2". This is essential to obtain proper registration between the two paths.

# 2.3 Inserting the DualView Lambda Filter Cube into the Photometrics DualView Lambda

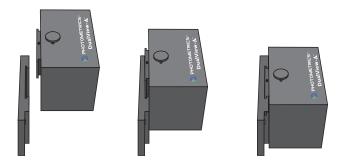
The DualView Lambda filter cube is designed to be easily accessible in order to facilitate quick and easy changes of filter sets.

Access the filter cube mount by removing the access panel. Use the two handles to pull gently and firmly. The filter cube mount will now be visible.

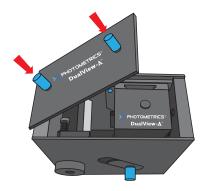
The mount attached to the rear of the DualView Lambda filter cube is designed to lock in place with the bracket on the internal wall of the Photometrics DualView Lambda. The small handle on top of the cube should now be facing out. Once the DualView Lambda filter cube is mounted successfully, replace the access panel using the two handles.

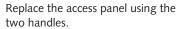


Remove the access panel using the two handles.



Mount the filter cube onto the bracket on the interior of the Photometrics DualView Lambda.

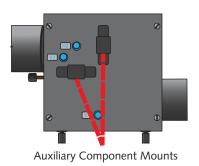






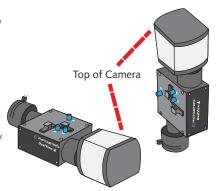
# 2.4 Auxiliary Component Mounts

To control the relative intensity of the two pathways, Photometrics recommends using neutral density filtering (optional) in the brighter pathway using the auxilliary component mounts. This is required only when one image is disproportionately brighter than the other. This auxilliary position can also be used for corrector lenses to reduce chromatic abberation and ensure both pathways are focused on the camera sensor.



# 2.5 Installing the Photometrics DualView Lambda in the Light Path

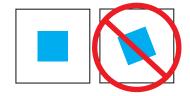
Before installing the Photometrics DualView Lambda, it is important to first setup the microscope, camera and software to give a clear image of an object of less than half the size the camera frame. Ideally this should be a real sample with the appropriate optical properties for the installed filter set. Failing this, a standard bright field image can be used, but this may lead to arbitrary intensity differences between the spectrally resolved images.



First, the CCD or EMCCD camera should be mounted on the microscope C-mount output and the port adjusted to give the sharpest possible image. Once a clear image can be seen, the camera should be switched off and removed from the microscope.

Finally, mount the Photometrics DualView Lambda to the microscope and align the diaphragm with the output of the microscope. Secure the camera on the output port of the Photometrics DualView Lambda, aligning the top of the camera so it lies parallel with the top of the Photometrics DualView Lambda. If fitting onto a vertical mount, the tops should also be aligned in the same direction.

A 1mm nylon spacer is included with each unit. This may be required on the camera output mount, particularly when mounting a shutter in addition to the Photometrics DualView Lambda. Having secured the camera to the output port of the Photometrics DualView Lambda, it



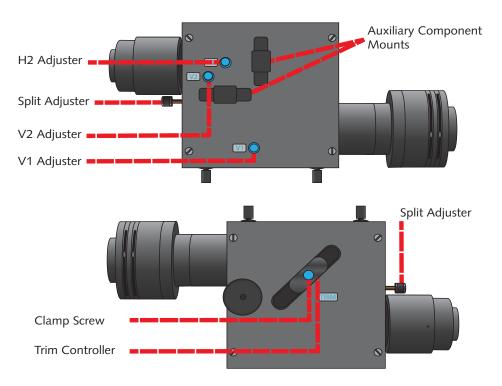
is possible to adjust the image to see a sharp picture of the aperture edges with the sample in focus. The image should line up with the edges of the aperture and should not have any rotation or slant in either direction.

The Photometrics DualView Lambda may already be aligned with two super imposed images. When it is first installed, a single central image is seen on the screen. If this is the case, they may be separated with the Split Adjuster control.

# Chapter 3.

# **Operation**

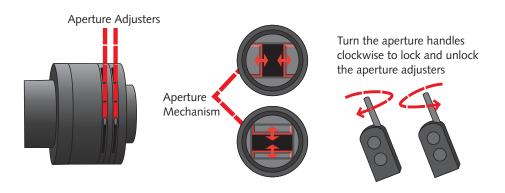
The Photometrics DualView Lambda uses a single control for adjusting image separation and allows the use of varying sample sizes. There are additional controls available for refining the ROI and centering the image.



# 3.1 Adjusting and Locking the Aperture

An adjustable rectangular aperture is provided with the Photometrics DualView Lambda. This enables the ability to determine the ROI, both vertically and horizontally. Once the ROI is defined, the aperture can be locked in place using the aperture adjusters. (See diagram below.)

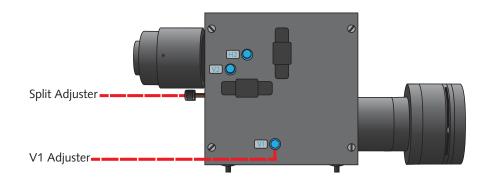
To define the ROI, adjust each of the two levers on the input port of the Photometrics DualView Lambda. To lock the aperture in place, tighten the levers by rotating them clockwise. To loosen them, rotate in the counter-clockwise direction.

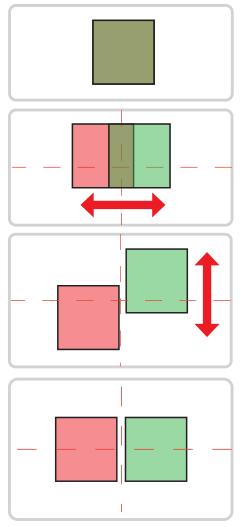


# 3.2 Adjusting the Position of the Images

Once the camera is setup, Photometrics recommends becoming familiar with the operation of the alignment adjustments. Please note that the diagram's overleaf indicates the system is using an appropriately aligned and centered rectangular diaphragm. An opposite image, similar to that shown, will be observed when the Photometrics DualView Lambda is first used, with the two images being displayed superimposed.

When the camera is mounted correctly, the split adjuster and the aperture control are the only controls that will be frequently adjusted. The remaining adjusters on the body of the Photometrics DualView Lambda should remain untouched unless the filter set has become unaligned.





Here we see the two superimposed images. Although your image may be monochromatic, colors have been used here to define the two images.

Turning the Split control counter-clockwise will seperate the two images along the horizontal axis. Only small adjustments are required when using the horizontal image seperation adjustment.

If the left and right images are at different vertical heights, adjust them using the V1 and V2 adjusters. Turning V1 will alter the position of the shorter wavelength image, and V2 will alter the position of the longer wavelength image.

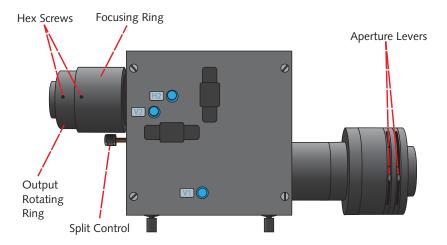
Recording can begin when the spectrally resolved images are side by side on the camera chip. When conducting experiments, set the aperture to mask the region of interest tightly so the two images are located as closely as possible on the camera chip.

# 3.3 Focusing the Camera on the Aperture

If the Photometrics DualView Lambda aperture is not in sharp focus, adjust the fine focus on the camera as follows:

- 1. Set an aperture size of less than half the width of the camera frame.
- 2. Slacken the two hex screws that retain the output rotating ring to allow the camera to rotate.
- 3. Separate the two images slightly with the Split control.

- 4. Slightly loosen the hex screws on the focusing ring. Adjust the focus while ensuring the Photometrics DualView Lambda is secure. Hold the camera until the apertures edges are in sharp focus.
- Lock the focusing ring by tightening the hex screws. Ensure the camera top is aligned with the top of the Photometrics DualView Lambda.
   Then, lock the output rotating ring.
- 6. Tighten the two hex screws that lock the camera's rotating ring.



# Centering Images on the Camera

When initially installed or if the filter block goes out of alignment, the Photometrics DualView Lambda images can show visible aberrations. Or, in extreme cases only, a single image may be observable for any setting of the separation control. In these situations, reconfigure the filter set by following this procedure:

1. Define a small area using the Photometrics DualView Lambda aperture and center the shorter wavelength image horizontally using the Split control.

10

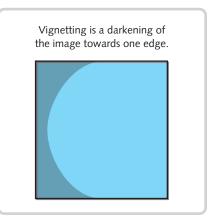
- 2. Center the same image vertically using the vertical offset adjustment marked V1 on the Photometrics DualView Lambda body.
- 3. The short wavelength is now correctly positioned.
- 4. To center the long wavelength pathway, superimpose the longer wavelength image on the shorter wavelength image that is centered. Do this using the V2 and H2 adjusters.
- 5. Center the image horizontally by making smaller adjustments of H2. Once the image is exactly superimposed on the long wavelength image in the center of the field of view, the vertical position of the image can be adjusted.
- 6. Adjust the vertical position with V2.
- 7. Recheck the horizontal alignment of the image and repeat steps 5 and 6 if needed.

Note: If the images are superimposed off-center, image aberrations may result. Ensure the short wavelength image is correctly centered before positioning the long wavelength image.

# 3.5 Image Optimization (Trim Adjustment)

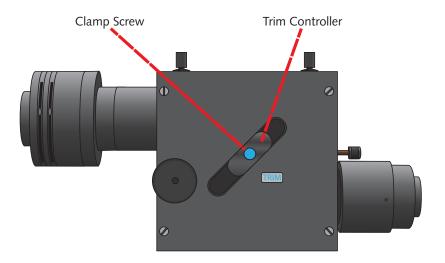
Vignetting is when an image appears darker on one edge. This effect can be easily corrected by adjusting the trim control.

To adjust the trim control, loosen the clamp screw on the underside of the Photometrics DualView Lambda by a quarter of a turn. This frees the slider which controls the trim control. Gently move the slider until the vignetting is eliminated. If the slider is moved too far, the vignetting will become apparent on the other image, and will require adjustments in the



opposite direction until both images are the same intensity. It is important to remember to re-tighten the clamp screw once the adjustment is complete.

If the Photometrics DualView Lambda is configured into single channel (non-split mode, refer to section 3.6) and trim adjustment is required to compensate for vignetting being observed, it is likely the slider will need to be at the extreme of its range of movement to either end.





# 3.6 Single Channel (Non-Split) Mode

When dual splitting is not required, the Photometrics DualView Lambda unit can be used in bypass mode, allowing the unit to remain *in situ* on the microscope while using the full camera chip to generate one image.

There are several ways this can be achieved:

#### A: Removing the filter cube

Suitable if no emission filters are required in the light path

- 1. Remove the dichroic mirror cube (see section 2.3)
- 2. Center the image using the Split control
- 3. Open the aperture
- 4. Adjust the Trim control to remove vignetting (see section 3.5)

#### B: Blocking the longer wavelength path

Suitable if only the shortest wavelength channel is required

- 1. Leave the dichroic mirror cube in place and block the long wavelength path using the shutter plate provided. Most dichroic mirrors have long-pass characteristics, in which case the longest wavelength will be the transmitted channel. (See section 4 for help differentiating the channels).
- 2. Center the image using the Split control
- 3. Open the aperture
- 4. Adjust the Trim control to remove vignetting

#### C: Blocking the shorter wavelength path

Suitable if only the longest wavelength channel is required

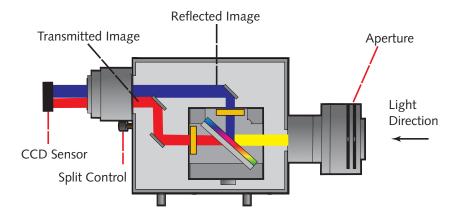
Follow the steps for option B, but block the short wavelength path

(reflected channel) with the shutter plate.

### Chapter 4.

# **Technical Summary**

The Photometrics DualView Lambda uses a conventional dichroic mirror to seperate light into two different spectral bands. This wavelength selection is usually augmented by the use of bandpass filters as shown below.



The design is loosely based on technology described by Kinosita et al. (J.Cell Biol. (1991) 115, 67-73), but includes several proprietary features, most notably:

A rotating mirror cradle to give precise symmetrical control of the degree of separation while maintaining identical path lengths.

A half-size fully silvered mirror at the output port for recombining the images.

Fine adjustment controls for camera focus and vertical alignment.

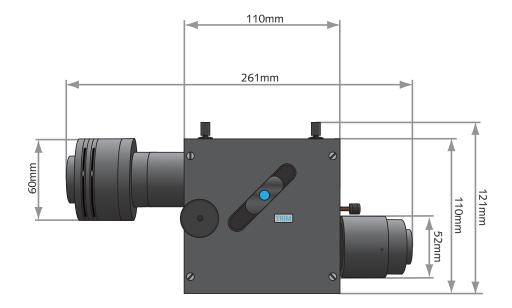
Optional ROI definition using adjustable rectangular aperture.

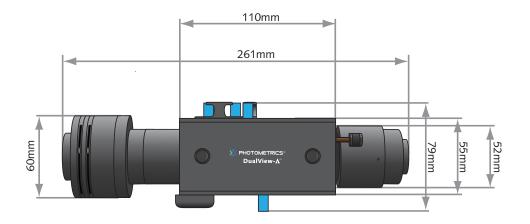
The system is supplied with high grade AR coated achromatic doublet lenses and dielectrically coated mirrors for maximum throughput.

**Dimensions excluding couplings:** H55, W110, D110 (mm) **Dimensions including couplings:** H55, W261, D110 (mm)

Approximate weight: 1.26 kg

Power consumption: 0





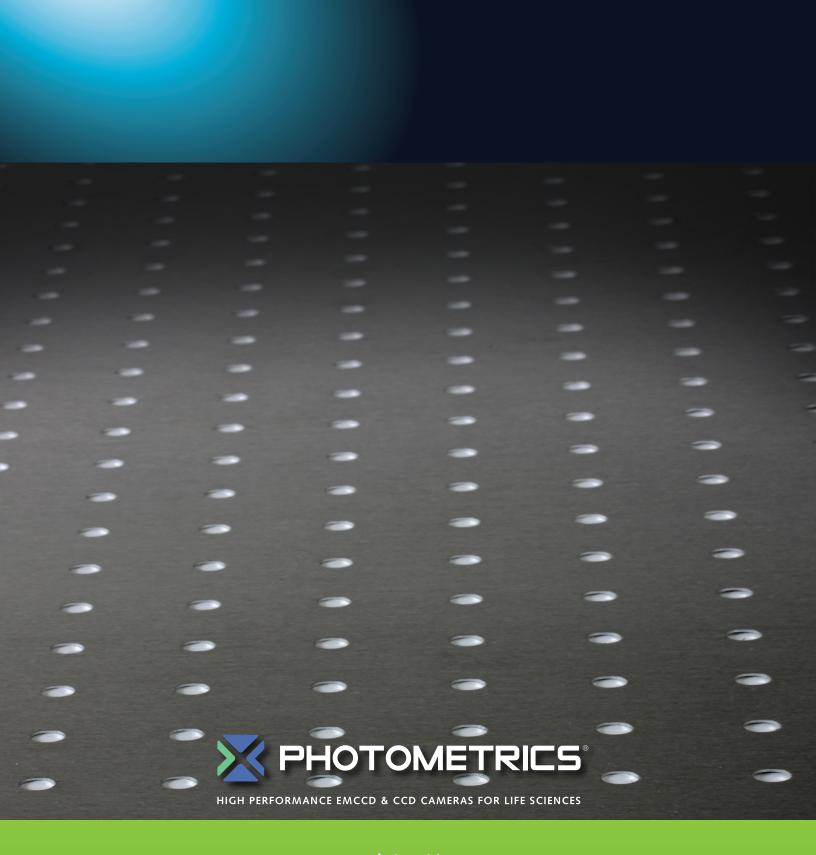




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