

# A One-of-a-kind Solution for Dual Wavelength Imaging

With the ORCA-D2, you can now capture dual wavelength images simultaneously, at wavelength ranges of your choice, and with a wide field of view – a unique combination that simplifies the challenges of conventional FRET and ratio imaging techniques. The ORCA-D2 contains two CCD devices. Interchangeable optical blocks are used to set the wavelength ranges for each CCD. During image capture, the camera automatically corrects focus, alignment and color shifting to produce high-quality images.

Other benefits of the ORCA-D2 include high sensitivity and low noise, courtesy of Hamamatsu's advanced CCD cooling technology; wide dynamic range; standard IEEE1394b interface; and Hamamatsu's proprietary vacuum-sealed chamber technology, which promotes long-term maintenance-free operation with even the most demanding applications.

### APPLICATIONS

- Ratio imaging
- Single and dual wavelength fluorescence microscopy, FRET
- Blue to NIR fluorescence applications
- Colocalization and FISH applications
- Dual wavelength TIRF microscopy, real-time confocal microscopy
- Combined transmission and fluorescence imaging
- Multi-focal point imaging microscopy



# **FEATURES**

# Simultaneous capture of wide-field dual wavelength images

# 🔴 Dual wavelength imaging

The unique camera design makes it possible to capture simultaneous dual wavelength images with a full field of view, even when the intensities differ significantly. Such images are difficult to obtain with conventional FRET or ratio imaging techniques.



▲ Internal structure of camera head

## Selectable wavelengths

By changing optical blocks, you can select your wavelengths of interest. Hamamatsu offers three types of optical blocks for different wavelength ranges.





# Application example 1 Ca<sup>2+</sup> measurement using YellowCameleon 3.6 (optical block: DM 510 nm)

The following images are an example of ratio imaging. Separated CFP and YFP (FRET) are measured with dual CCD devices. This sequence observed the YellowCameleon 3.6 (Ca<sup>2+</sup> sensor based on CFP-YFP FRET) expressed Ins-1 cell response with a depolarizing stimulus.

#### CFP-channel (CCD1)

- YFP-channel (CCD2)
- Temporal change of brightness



Samples courtesy of Hideo Mogami, Ph.D. Dept. of Physiology, Hamamatsu University School of Medicine Dept. of Environmental Biology, Okazaki Inst. for Integrative Bioscience Sample: Ins-1 cell (insulin-producing cell) ORCA-D2 optical block: A11400-03 (DM 510 nm, Em1 483 nm/32 nm, Em2 542 nm/27 nm) Microscope: Olympus IX71 Objective lens: Olympus LUCPIanFLN 60x, NA 0.70

# Easy setup

### Auto-correction of focus and alignment

During dual wavelength image acquisition, the camera will automatically correct image alignment, color mismatch, etc. Hardware and special software work together to adjust focus\* and alignment to your experimental setup. Calibration results are saved in the software, eliminating the need to readjust for the same setup.

\*Please note that auto-correction may not be possible in some experimental setups.



### Focus adjustment

Images with different focal plane can be acquired at the same time through the use of the camera's special software and adjustable CCD device position.

#### Pollen with a 10 µm diameter

The following images are examples of using different focal plane imaging at the same time. CCD1



High-sensitivity imaging from visible to near-infrared

### Dual light mode

Two modes (high light, low light) are available for different situations. Low light mode provides high sensitivity over a wide spectral range from visible to near-infrared.

#### 🔴 Max. quantum efficiency over 70 %

The ORCA-D2 offers high sensitivity from visible to near-infrared with its ER-150 interline CCD. In low light mode, sensitivity at 700 nm is nearly twice that of the ICX285\* device used in most other scientific CCD cameras.

\* ICX285 is a conventional high-sensitivity CCD device which is used for scientific instrumentation.



# Application example 2

### Membrane Potential Measurment using Mermaid (optical block : DM 550 nm)

Spontaneous oscillation of membrane potential in mouse cardiomyocyte expressing Mermaid. Intensity changes of dual fluorescence from Mermaid were measured simultaneously at 50 frames/s, using ORCA-D2. Green and Red channel of camera were used for mUKG and mKO respectively.





Spectral response (This sample is typical of the CCD characteristics, not guaranteed.)



## SYSTEM CONFIGURATION



#### SPECIFICATIONS

Type number			C11254-10B (ORCA-D2)		
Camera head ty	ре		Hermetic vacuum-sealed head		
Imaging device			ER-150 progressive scan interline CCD		
Effective numbe	r of pixels		1280 (H) $ imes$ 960 (V) $ imes$ 2CCD chip		
Cell size			6.45 $\mu$ m (H) $ imes$ 6.45 $\mu$ m (V)		
Effective area			8.26 mm (H) × 6.19 mm (V)		
Pixel clock rate			20.00 MHz/pixel		
Readout speed	1 × 1		11.2 frames/s		
	Binning	2 X 2	20.2 frames/s		
		$4 \times 4$	33.6 frames/s		
		8×8	50.5 frames/s		
Readout noise (r.m.s.) typ.			8 electrons		
Full well capacity	1 × 1		18000 electrons		
typ.	Binning	2×2	36000 electrons		
Dynamic range typ. ①			2250 : 1 typ. (1 × 1)		
Cooling method			Peltier device + Forced-air cooled		
Cooling tempera	ature		- 10 °C (Ambient temperature: + 20 °C)		
Dark current			0.01 electrons/pixel/s (- 10 °C)		
A/D converter			12 bit		
Exposure time			117 μs to 60 s		
Sub-array			Yes		
Contrast intensit	ication		Analog gain (10 times max.), offset		
External trigger	mode		Edge trigger, Level trigger,		
			Synchronous readout trigger, Start trigger		
Trigger output			Programmable timing output,		
			Trigger ready output		
Lens mount			2/3-inch bayonet mount (flange back focus 48 mm)		
Interface			IEEE1394b-2002		
External control			IIDC 1394-Based Digital Camera		
			Specification Ver.1.32		
Power requirem	ents		AC 100 V to AC 240 V, 50 Hz / 60 Hz		
Power consump	tion		Approx. 70 V·A		
Ambient storage	temperat	ure	- 10 °C to + 50 °C		
Ambient operati	ng temper	ature	+ 10 °C to + 35 °C		
Ambient storage	/ operating	humidity	70 % max. (no condensation)		

#### Optical blocks

Type number	_	Annlingtions		
	Dichroic	Em1	Em2	Applications
A11400-01	None	0 %	100 %	1CCD camera
A11400-02	Half	50 %	50 %	Beam splitter
A11400-03	510 nm	483 nm / 32 nm	542 nm / 27 nm	CFP / YFP
A11400-04	550 nm	520 nm / 35 nm	593 nm / 40 nm	FITC / TRITC
A11400-05	630 nm	593 nm / 40 nm	692 nm / 40 nm	Су3 / Су5
A11400-08	570 nm	520 nm / 35 nm	641 nm / 75 nm	GFP/mCherry

A11400-01 is included with camera. Other optical blocks are optional

#### DIMENSIONAL OUTLINES

(Unit: mm)

#### Camera head (Approx. 3.0 kg)



#### Camera control unit (Approx. 3.5 kg)



Homepage Address http://www.hamamatsu.com

Calculated from the ratio of the full well capacity and the readout noise

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