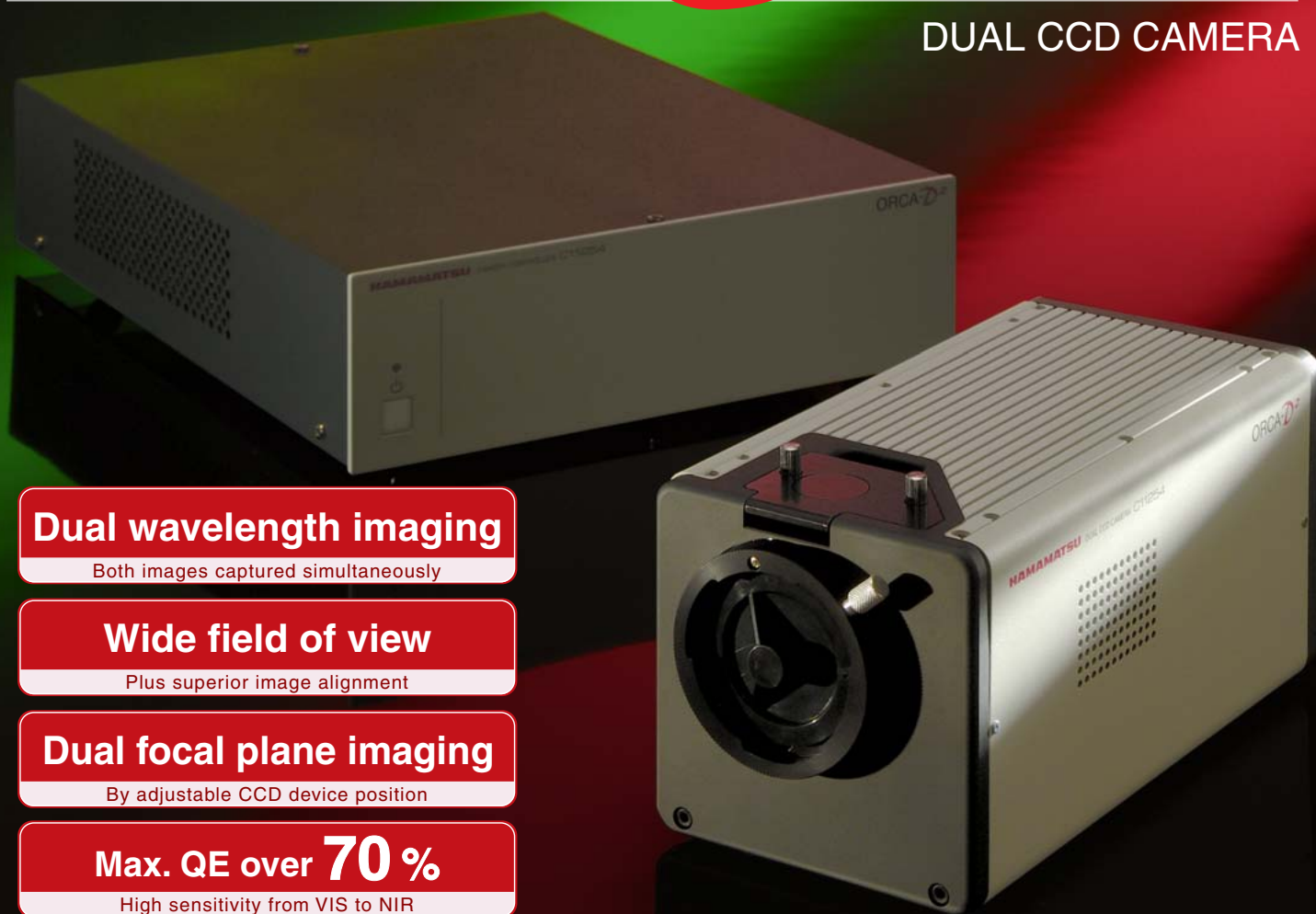


ORCA-D[®]2



DUAL CCD CAMERA



Dual wavelength imaging

Both images captured simultaneously

Wide field of view

Plus superior image alignment

Dual focal plane imaging

By adjustable CCD device position

Max. QE over 70%

High sensitivity from VIS to NIR

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

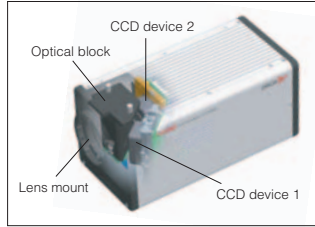
HAMAMATSU

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.



Comparison with conventional methods

ORCA-D2

- ✓ Simultaneous capture at dual wavelengths
- ✓ Selectable wavelengths
- ✓ Auto-correction of color or image shifting
- ✓ Wide field of view

W-view optics

- ✓ Simultaneous capture at dual wavelengths
- ✓ Selectable wavelengths
- ✗ Steps required to correct color or image
- ✗ Reduced field of view

3CCD color camera

- ✓ Simultaneous capture at dual wavelengths
- ✗ No selection of wavelengths
- ✗ No correction of color or image shifting
- ✓ Wide field of view

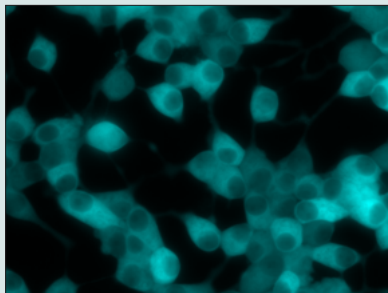
Filter wheel

- ✗ No simultaneous capture at dual wavelengths
- ✓ Selectable wavelengths
- ✗ No correction of color or image shifting
- ✓ Wide field of view

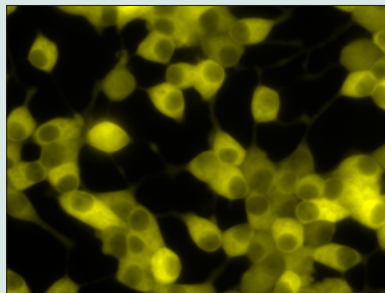
Application example 1 Ca^{2+} measurement using YellowCameleon 3.6 (optical block: DM 510 nm)

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^{2+} sensor based on CFP-YFP FRET) expressed Ins-1 cell response with a depolarizing stimulus.

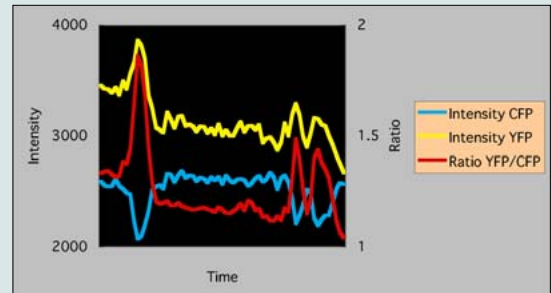
● CFP-channel (CCD1)



● 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 LUCPlanFLN 60x, NA 0.70

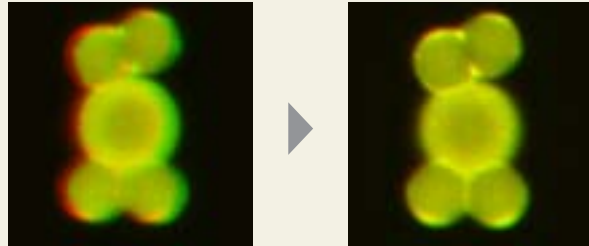
Easy setup

● 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.

Example of correction of focus and alignment



▲ Before

▲ After

Sample: Multi fluorescent beads (6.0 μm to 7.9 μm)

ORCA-D2 optical block: A11400-04 (DM 550 nm, Em1 520 nm/35 nm, Em2 593 nm/40 nm)

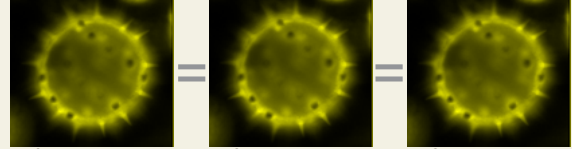
● Focus adjustment

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

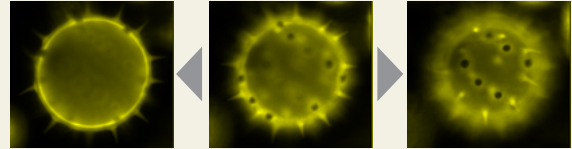


▲ $\pm 0 \mu\text{m}$

▲ $\pm 0 \mu\text{m}$

▲ $\pm 0 \mu\text{m}$

● CCD2



▲ +4 μm

▲ $\pm 0 \mu\text{m}$

▲ -4 μm

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.

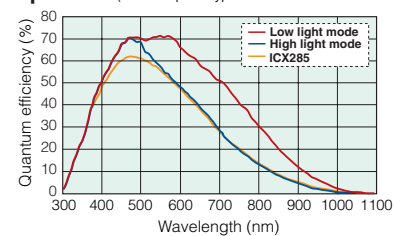
● Max. quantum efficiency over 70 %

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.

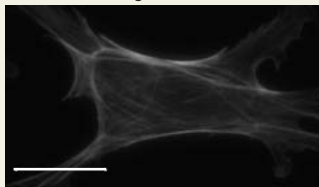
* ICX285 is a conventional high-sensitivity CCD device which is used for scientific instrumentation.

Spectral response

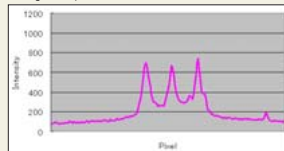
(This sample is typical of the CCD characteristics, not guaranteed.)



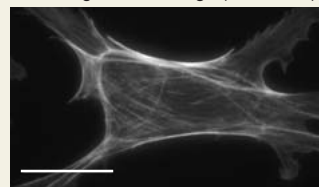
■ ICX285* image



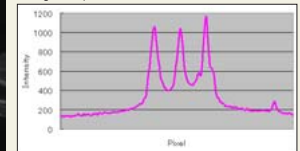
▼ Brightness profile of area marked with white horizontal line



■ Low light mode image (ORCA-D2)



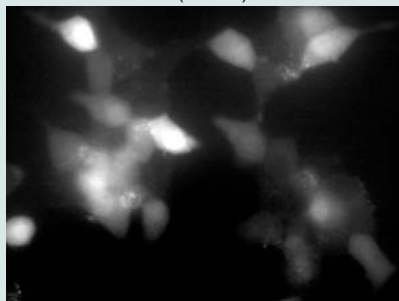
▼ Brightness profile of area marked with white horizontal line



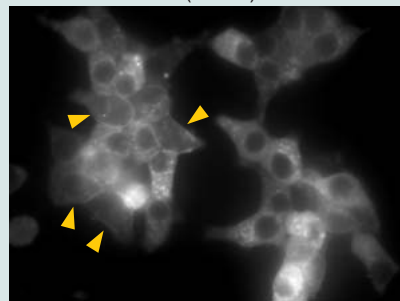
Application example 2 Ca^{2+} and PKC dual imaging using Fluo-4 and DsRed (optical block: DM 550 nm)

The images below show fluorescence signal from Fluo-4 AM loaded cells expressing PKC-DsRed when excited by 480 nm light. Observable in this time sequence is the translocation of PKC-DsRed to the cell membrane concurrent with free Ca^{2+} elevation in response to a depolarizing stimulus.

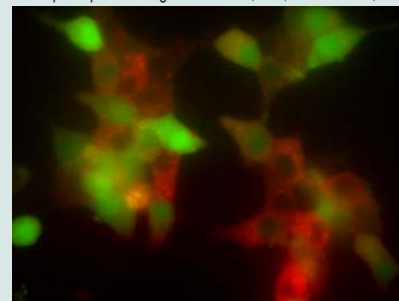
● Fluo-4-channel (CCD1)



● DsRed-channel (CCD2)



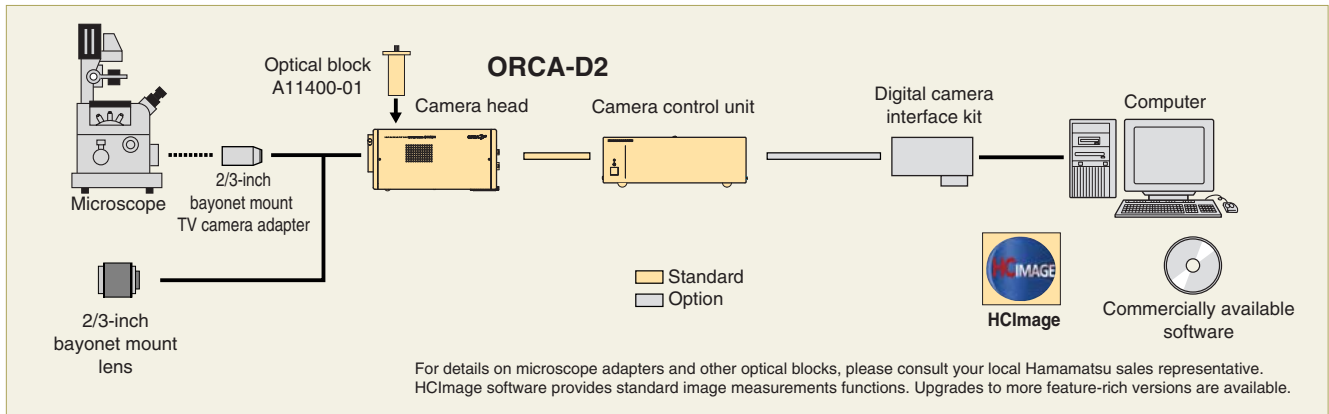
● Superimposed images of Fluo-4 (CCD1) and DsRed (CCD2)



Images 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: A11400-04 (DM 550 nm, Em1 520 nm/35 nm, Em2 593 nm/40 nm)
Microscope: Olympus IX71
Objective lens: Olympus UPlanAPO 60x / 1.20 W

SYSTEM CONFIGURATION



SPECIFICATIONS

| | | | |
|--------------------------------------|---|-----------------|---------------|
| Type number | C11254-10B (ORCA-D2) | | |
| Camera head type | Hermetic vacuum-sealed head | | |
| Imaging device | ER-150 progressive scan interline CCD | | |
| Effective number of pixels | 1280 (H) × 960 (V) × 2CCD chip | | |
| Cell size | 6.45 μm (H) × 6.45 μm (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 × 2 | 20.2 frames/s |
| | | 4 × 4 | 33.6 frames/s |
| | | 8 × 8 | 50.5 frames/s |
| Readout noise (r.m.s.) typ. | 8 electrons | | |
| Full well capacity typ. | 1 × 1 | 18000 electrons | |
| | Binning 2 × 2 | 36000 electrons | |
| Dynamic range typ. ① | 2250 : 1 typ. (1 × 1) | | |
| Cooling method | Peltier device + Forced-air cooled | | |
| Cooling temperature | - 10 °C (Ambient temperature: + 20 °C) | | |
| Dark current | 0.1 electrons/pixel/s (- 10 °C) | | |
| A/D converter | 12 bit | | |
| Exposure time | 117 μs to 60 s | | |
| Sub-array | Yes | | |
| Contrast intensification | 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 requirements | AC 100 V to AC 240 V, 50 Hz / 60 Hz | | |
| Power consumption | Approx. 70 V·A | | |
| Ambient storage temperature | - 10 °C to + 50 °C | | |
| Ambient operating temperature | + 10 °C to + 35 °C | | |
| Ambient storage / operating humidity | 70 % max. (no condensation) | | |

① Calculated from the ratio of the full well capacity and the readout noise

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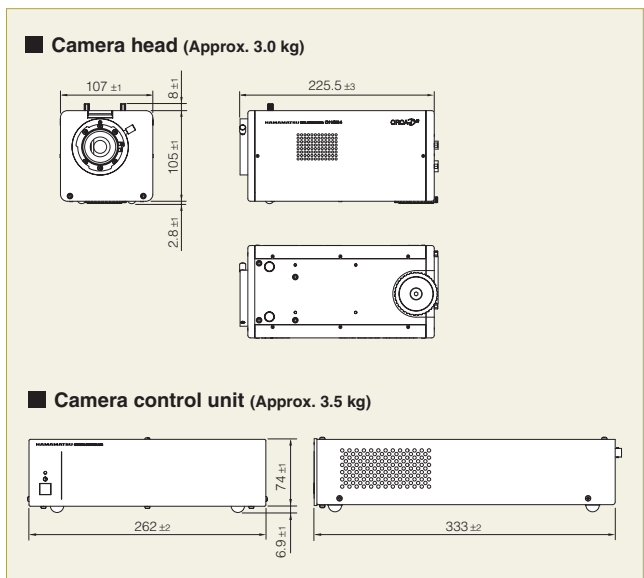
● Optical blocks

| Type number | Optical block | | | Applications |
|-------------|---------------|----------------|----------------|---------------|
| | Dichroic | Em1 | Em2 | |
| 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 | Cy3 / Cy5 |
| 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)



Homepage Address <http://www.hamamatsu.com>

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