ImagEM®

EM-CCD CAMERA C9100-13, -14

EM-CCD Technical Note (Dec./2009)

HAMAMATSU

1. CCD Structures and Characteristics

There are three types of CCDs which are well known in scientific imaging. One is the interline transfer CCD (IL-CCD), a second is the Full Frame Transfer CCD (FFT-CCD) and a third is the Frame transfer CCD (FT-CCD).

1.1 Interline Transfer CCD (IL-CCD)

The structure of IL-CCD is shown in Figure 1(a). The interline CCD has vertically paired columns consisting of imaging Photodiodes (PD) and a readout register (Vertical charge transfer register (V-CCD). Electric charges generated in all the PD by incoming photons are shifted simultaneously to the adjacent V-CCD register. The V-CCD register is covered by a mask of aluminum or other opaque material to prevent photons from creating additional charges in this area during readout. Readout is accomplished by transferring each horizontal row of information in the V-CCD, line by line, up the CCD to the Horizontal serial register (H-CCD). There, charges are transferred horizontally and converted into charge voltage by AMPFDA.

The design of an IL-CCD has the advantage that the signal accumulation (exposure) and readout can be done simultaneously because PD can accumulate charges for the next frame right after the previously generated electric charges in the PD are shifted to the V-CCD. There is no possibility of image smearing in this device.

Traditionally, the design of the IL-CCD has had the disadvantage that the open ratio of the light sensitive area (fill factor) is reduced because of the presence of the masked V-CCD area. Recently this disadvantage has been dramatically improved by on-chip lenses (as shown in Figure 2) and improvement of sensor structures that allow detection of photons deeper in the PD than previous models. Overall Quantum efficiency has increased to over 70 %. New IL-CCDs like the Hamamatsu ER-150 CCD (Figure 3) used in the ORCA series of cameras, offer characteristics ideally suited to many scientific applications.

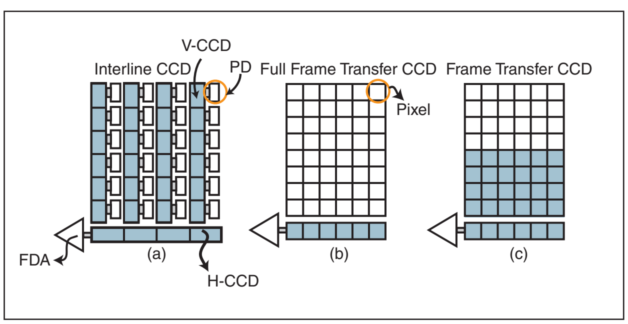


Fig. 1

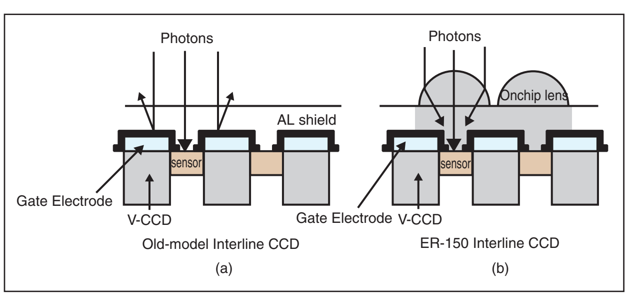


Fig. 2

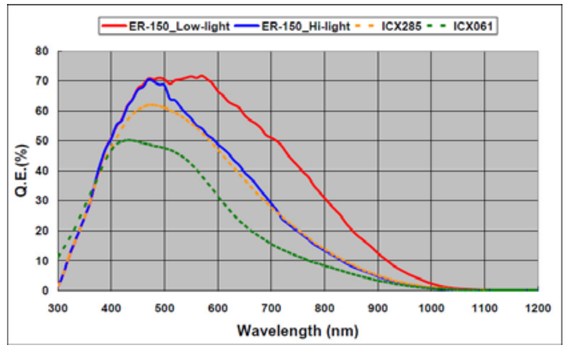


Fig. 3

1.2 Full Frame Transfer CCD (FFT-CCD) and Frame Transfer CCD (FT-CCD)

As shown in Figure 1, Frame transfer CCDs are divided into two types, Full frame transfer (FFT-CCD)(b) and Frame transfer (FT-CCD)(c).

In the case of a FFT-CCD Figure 1(b), charges generated in pixels are transferred vertically, row by row, to the horizontal serial register for readout. Unless a shutter is used during this transfer and readout, image smearing will occur. While this design offers 100 % open area ratio with full collection of incoming photons, the shutter limits the frame rate and photons falling on the shutter are lost when it is closed for readout. It is not possible to acquire signal and readout at the same time.

FT-CCDs Figure 1(c) offer both 100 % fill factor and simultaneous signal acquisition and readout. Like the FFT-CCD, the chip has no charge transfer regions in the signal acquisition area. Rather, the FT-CCD has two separate but equal regions, one with pixels exposed to the incoming photons and another region with an equal number of pixels but entirely masked to eliminate photons from being detected. As shown in Figure 1(c) one area works as the detection area and the other works as the storage area.

Accumulated charges detected in the detection area are rapidly transferred to the masked storage area, and the accumulated charges are transferred vertically, line by line, to the horizontal serial register for readout. The detection area and storage area are driven individually so the next exposure can start right after the completion of the rapid vertical charge transfer from the detection area to the storage area. This design eliminates the need for a mechanical shutter, allowing signal accumulation (exposure) and readout simultaneously like an IL-CCD.

1.3 Back-Thinned CCD

As mentioned before, in FFT-CCDs and FT-CCDs the light sensitive pixels have a charge transfer function as well. This function requires the front surface of light sensitive pixels to be covered by a semi-transparent Poly-Si electrode for the charge transfer to function, as shown in Figure 4.

Even with 100 % fill factor, the effective quantum efficiency (QE) drops into the 40 % range because the Poly-Si electrode absorbs some percentage of incoming photons depending on their wavelength. To overcome this disadvantage, Back-Thinned CCDs (BT-CCD) are becoming popular. In a BT-CCD the CCD is turned upside down and this back side of the CCD is thinned to 10 µm to 15 µm in thickness as shown in Figure 4(b). Incident photons now enter the CCD from this back thinned side, without the Poly-Si electrode in the light path. QE values of greater than 90 % can be achieved. Figure 5 compares typical QE curves of the same CCD in front- illuminated and back-illuminated versions.

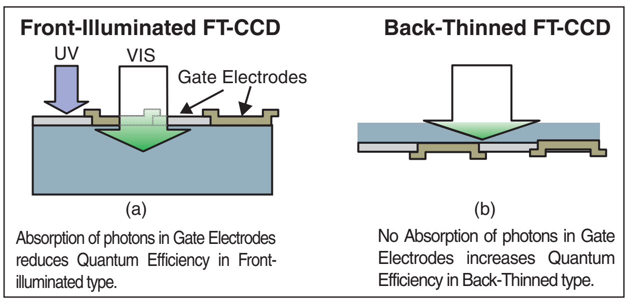


Fig. 4

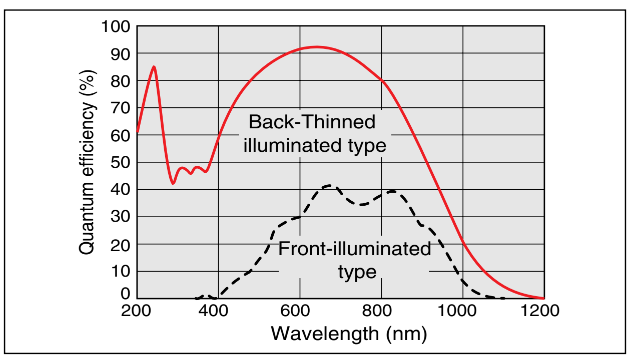


Fig. 5

2. Noise Components of CCD

As seen chapter 1, innovations in silicon based CCD technology have created many kinds of CCDs. With the Quantum efficiency of the IL-CCD reaching about 70 % and the BT design achieving more than 90 %, detection limits are nearing their theoretical limits. Signal detection in modern CCDs is often limited by how much camera noise (due to dark current and readout noise) must be overcome before the signal is apparent on the CCD. These values determine the camera performance of CCD, especially in low light applications.

2.1 Dark Current

A CCD is made from Silicon, and the dark current caused by thermal migration of electrons in silicon is a main noise factor for a CCD sensor. The dark current of a CCD depends on the temperature, and it decreases by half when the temperature drops by approximately 7 to 8 degree C. It is apparent that cooling a CCD is a very good way to reduce the dark current noise. Figure 6 shows the CCD dark current vs. Temperature.
Dark current also depends on the type of CCD. In most applications an IL-CCD can normally achieve good performance with -30 to -50 degree C cooling. In the case of an FFT-CCD or FT-CCD, most require -50 to -90 degree C cooling for low light applications. Cooling is most effective when the CCD is placed in a vacuum chamber.

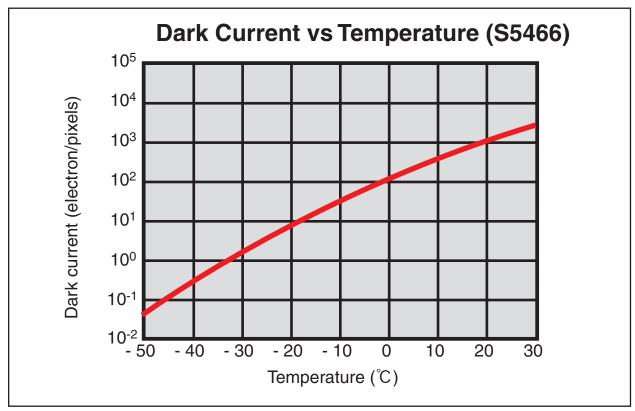


Fig. 6

Hamamatsu Photonics K.K. have developed an unique hermetic vacuum-sealed chamber with high performance cooling capability based on many years of experience with high vacuum technologies used in Photo Multiplier Tube (PMT) and Image Intensifier (I.I.) technologies (shown in Figure 7). The CCD chip and a multi-stage peltier element (Thermo electric cooler) are built into a welded metal chamber with a special window on the front to create the camera head. The chamber of the vacuum head is vacuumed and hermetically sealed to retain a high degree of vacuum (10^{-8} torr or less), to ensure great cooling performance over many years. In comparison with simpler vacuum sealed models using ordinary gaskets or o-rings, there is no need for periodic re-evacuation or maintenance. With several hundred such vacuum heads in daily use over many years, the unique Hamamatsu hermetic vacuum-sealed head reliably provides significantly better and more stable cooling performance than other designs.

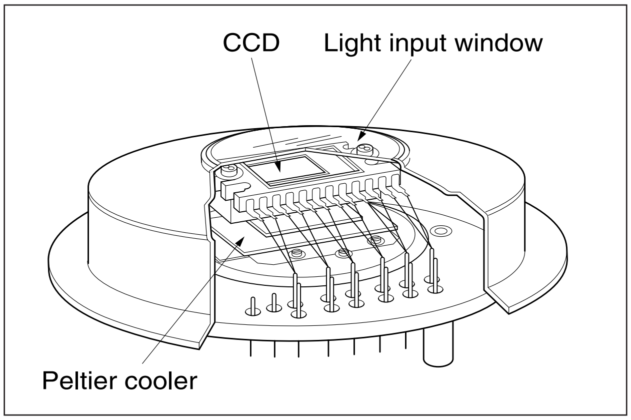


Fig. 7

2.2 Readout Noise

The largest factor influencing the detection limit of a CCD is the readout noise caused by the on-chip Floating Diffusion Amplifier (FDA) that converts accumulated charges into voltage. Accumulated charges transferred into horizontal serial register are serially transferred into the FDA pixel by pixel. Readout noise is primarily caused by the resetting of the amplifier after the accumulated charge in each pixel is converted to a voltage and the amplifier is reset for the next incoming pixel. This reset noise can be dramatically reduced by an external correlated dual sampling (CDS) circuit. Additionally readout noise depends on pixel clocking frequency and is generally lower with slower CCD clocking speeds. However, slower pixel clock speeds may limit the camera use for dynamic real-time imaging.
In summary, camera readout noise performance depends greatly on the external circuit design of the camera manufacturer and the readout speed.

As an example, there are only 3 electrons r.m.s. readout noise in cameras such as the Hamamatsu ORCA II series cooled CCD. The detection limit of such cooled CCD cameras is about 10 electrons, making it an ultra-sensitive camera.
More details of noise are explained in chapter 4 : CCD/EM-CCD noise calculations.

3. Electron Multiplier CCD (EM-CCD)

As mentioned above, CCD technological innovations are making dramatic progress. As a result of various approaches, very high sensitivity and low noise CCDs are readily available.

Despite all the advances, readout noise is still the dominant factor limiting weak signal detection. Detecting signals below the readout noise level of a camera is possible with various special methods or technologies. Detecting a lower signal than the readout noise is possible by signal integration on CCD chip. Over time the signal will accumulate and become greater than the readout noise.

Other techniques that involve signal multiplication are done with Microchannel Plates (MCP) in an Image Intensifier (I.I.) or direct electron bombardment of a CCD (EB-CCD). In these cases signal electrons are created at a photocathode and then multiplied by a high voltage in a vacuum tube before signals are readout.

A mechanism for direct multiplication of electrons on the CCD itself has been known for many years. A host of technological problems associated with this on chip multiplication process prevented the technique from being useful until just recently. In the last few years solutions to the problems have been developed and it is now becoming an effective means of ultra low light detection in biological and scientific imaging. This exciting technology has become known as an Electron Multiplier CCD (EM-CCD).

3.1 Principle of Electron Multiplication Gain in EM-CCDs

Figure 8(a) shows the structure of an EM-CCD. The basic structure is the same as a normal FT-CCD and it is shown as a back-Thinned version. Accumulated charges detected in the detection area are rapidly transferred to the storage area, and then the accumulated charge is transferred line by line to the horizontal serial register for readout, just as in a normal FT-CCD. At this point in an EM-CCD a multiplication (Charge Multiplier) register is built into the horizontal serial register. With this charge Multiplication register, signal multiplication is done by supplying a higher voltage than normal to each horizontal transfer electrode.

Figure 8(b) shows the principle of signal multiplication in the charge multiplication register. When a signal electron charge is transferred from stage to stage, the signal charge is accelerated by high electric field generated under the multiplication gate by applying a high voltage (30 to 40 V) to each multiplication electrode (multiplication gate). This high voltage is much greater than the normal horizontal transfer electrode voltage, and it generates an occasional extra electron-hole pair. This is called an impact ionization event. The probability of such an event is very small, typically about 1.0 % to 1.6 % at each stage. This is the value (g) in the formula shown below. Electrons are multiplied from stage to stage repeatedly in the gain register and high multiplication gain is achieved. Normally, there are 400 to 600 stages (N) in the gain register.
Total multiplier gain (M) can be expressed by the following formula :

$$M = (1+g)^N$$

g : probability to generate an electron-hole pair at each stage
N : total number of charge multiplication stages

Here, probability of (g) depends not only on the supply voltage of the charge multiplier register but the temperature of a sensor also has a great influence. Control and stability of both the supply voltage and the CCD temperature are very important factors when EM-CCD technology is used for quantitative measurement.

The key technical point and advantage of an EM-CCD is signal charges in the CCD are multiplied in the multiplication register before it is converted to voltage by the FDA. As mentioned before, the readout noise caused by the FDA is the limiting factor to low signal detection. Multiplication of the signal before the FDA makes the readout noise of the FDA relatively smaller as the multiplication of the original signal increases. Even at moderate gain settings, the relative readout noise becomes less than 1 electron, enabling detection of even single photon events in the signal. As readout speed increases, the readout noise increases by a square function of the frequency.

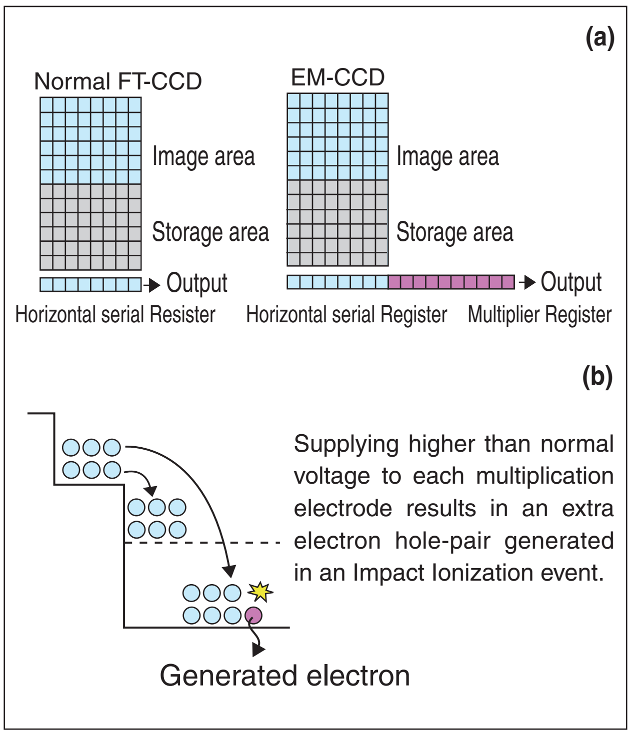


Fig. 8

To observe low intensity objects at high speeds, the EM-CCD can overcome the increase in readout noise by additional multiplication gain; again reducing the relative readout noise to less than 1 electron. This ability to use the multiplication gain register to overcome the readout noise even at high readout speeds is the chief advantage of the EM-CCD cameras for fast, scientific, low light imaging.

Figure 9 shows sequential images taken while increasing the multiplication gain factor from no multiplication gain to higher multiplication gain. With higher multiplication gain, the multiplied signal becomes larger than the readout noise and an image is visible.

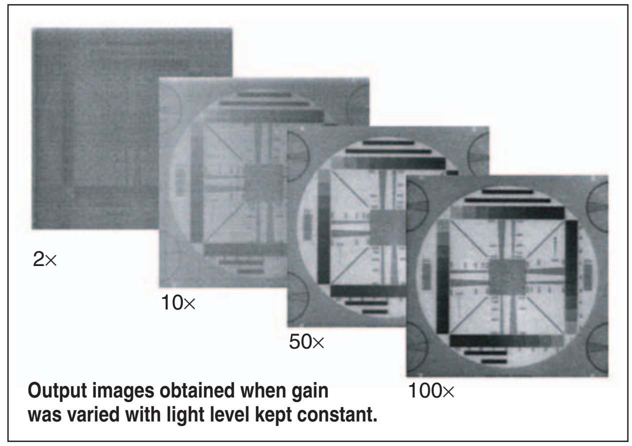


Fig. 9

3.2 EM Gain Dependence on Temperature

In the case of EM-CCD, as mentioned above, the multiplication gain factor in the multiplication register greatly depends on the temperature. It is obvious that the stabilization of temperature of a sensor becomes a very important issue. Figure 10 shows an example of an E2V CCD97 CCD and the EM gain vs. temperature.
A change of 70 degrees at the CCD changes the EM gain by about 10 times in Figure 10. In addition, as the temperature is decreased, the slope of the change increases. Temperature stability becomes increasingly important at lower cooling temperatures to maintain constant gain in an EM-CCD.

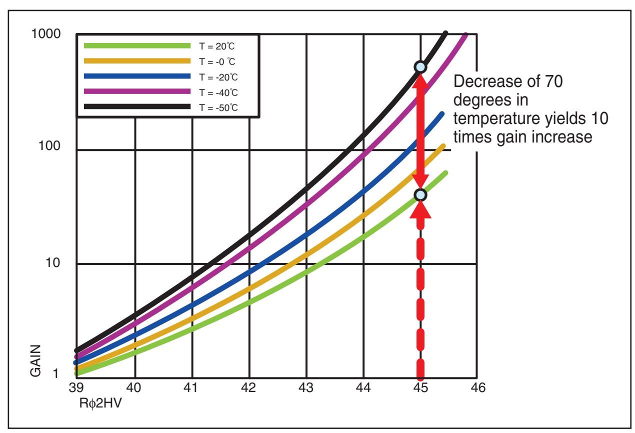


Fig. 10

3.3 Gain Ageing Characteristic

EM-CCDs have been widely used for high sensitivity applications due to the advantages of the charge multiplication register. But it was not widely known until recently that the multiplication gain tends to suffer gain ageing, a slow decrease in gain over time based on the total electric charge passed through the multiplication register. This is changing and has now been officially announced by the CCD manufacturers.

To use an EM-CCD for stable quantitative measurement over long periods, it is necessary to consider this EM gain ageing. The multiplication principle of EM-CCD is achieved by impact ionization effects of a high voltage (30 V to 40 V) applied to each multiplication electrode (multiplication gate). While the exact cause of the gain degradation is not known, it is thought that the higher than normal voltages used in the process traps accelerated electrons in the bottom of the transfer electrode. These trapped electrons may change the electric field at this point and thus create the gain ageing phenomenon. This gain ageing occurs exponentially over time and is most prominent in the early use of an EM-CCD. In order to reduce this to a minimum in actual use, every C9100 camera is factory aged for more than 100 hours and readjusted before being shipped. As a result, serious gain deterioration should not occur in C9100 series cameras. However, since this phenomenon depends on total electric charge through the multiplication register, there may be some applications where additional gain degradation can occur. Reducing gain and light intensity when the camera is not being used can help prevent this. It is wise to check the gain with a standard sample occasionally if long term standardization is required.

Tips for operating EM-CCD cameras

(1) Keep the gain adjusted to a level that offers just enough gain to overcome the readout noise. There is no increase in the Signal to Noise ratio once the readout noise becomes less than one and adding gain past this point only increases the rate of gain degradation.

(2) Reduce illumination to the detector as much as possible. Even if the gain is not increased, an increase in the signal intensity will create a larger charge in the multiplication register and increase the rate of gain degradation.

(3) Reducing the gain to the minimum setting and blocking illumination to the CCD when the camera is not being used for measurement can help maintain stable EM-CCD gain over long periods.

4. CCD and EM-CCD Noise Calculations

4.1 CCD Noise Components Calculation

There are four kinds of important noise factors that determine the S/N of a CCD.

(1) Readout noise σr

The reset noise in the electric charge - voltage conversion AMP (FDA) on the CCD is the main source. It is expressed as the dispersion σr.

(2) Noise caused by dark current $\sigma d = \sqrt{(DT)}$

This noise is the fluctuation in the dark current generated in a CCD, and it depends on cooling temperature and exposure time.

It is expressed as the square root of the product of D: Dark current (electrons/pixel/sec) and T: Exposure time (sec).

(3) Photon shot noise $\sigma s = \sqrt{(QP)}$

This noise is the fluctuation in the number of incident photons (photon shot noise). It is expressed as the square root of the product of P: Input photon and Q: Quantum efficiency.

(4) Spurious Noise σcic

Spurious noise is a charge generated by signal charge transfer process in the CCD called Clock Induced Charge (CIC).

This is a fixed value when readout clock and clock duty cycle is fixed, but it is small enough and usually it is possible to ignore it from the following calculation. From our measurement result, it is 0.01 electron/pixel/frame-readout.

Total noise N is calculated by the following expression:

$$\text{Total noise}N = \sqrt{\{(\text{Readout noise})^2 + (\text{Dark current noise})^2 + (\text{Photon shot noise})^2\}}$$
$$= \sqrt{(\sigma r^2 + \sigma d^2 + \sigma s^2)}$$
$$= \sqrt{(\sigma r^2 + DT + QP)}$$

On the other hand, Signal S is expressed by P: incident Photon and Q: Quantum efficiency as follows.

Signal S = QP

Thus, signal to noise ratio (S/N) is expressed by the following expression.

$$\text{Signal / noise} = QP / \sqrt{(\sigma r^2 + DT + QP)}$$

Figure 11 shows the change in noise (N) based on the number of incident photons under the following conditions.

Readout noise: 8 electrons
Exposure time: 100 ms
Quantum efficiency: 90 %
Dark current: 0.01 e/p/s

As shown in Figure 11, the noise characteristics can be divided into two domains. A change in the slope of the noise value occurs at 70 photons. Below this value the graph indicates that when the incident photon number is less than 70 photons the camera readout noise is the dominant factor in the noise calculation. When the incident photon number is greater than 70 photons the photon shot noise is the dominant noise factor. (In this example with the comparatively short exposure time, dark noise is too small to influence the result.) In practical terms, 70 photons define the point at which the camera noise no longer has an influence on the S/N and the number of incident photons defines the S/N.

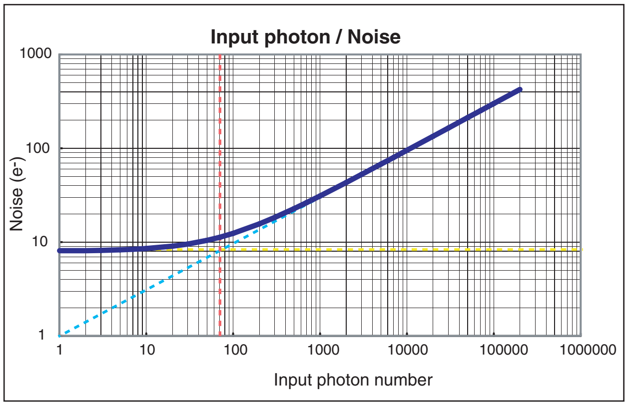


Fig. 11

4.2 EM-CCD Noise Components Calculation

In the case of EM-CCD, multiplication of the signal in the multiplication register has noise associated with that process and this will influence the S/N. Noise caused by signal multiplication is called excess noise (F) and is added to the signal. Excess noise: F is calculated by the multiplication factor M and a ratio of the dispersion of a multiplication register input signal: σin and a dispersion of the multiplication register output signal: σout.

$$F^2 = \sigma out^2 / M^2 \sigma in^2$$

It is important to note that not only are the detected signal electron charges multiplied but also any electron charge in the CCD from other sources such as dark current are multiplied with the same multiplication and noise factors.
Calculations for signal level and total noise in an EM-CCD must include multiplication noise, and are shown below.
Signal : S is expressed as the product of detected signal : QP and multiplication gain : M.

$$\text{Signal } S = QPM$$

Total noise: N is expressed as the square root of the product of the sum of signal charge :S, dark current :D, time :T, multiplication factor : M and excess Noise :F as follows.

$$\text{Total noise } N = \sqrt{\{ \sigma r^2 + F^2M^2(\sigma d^2 + \sigma cic^2 + \sigma s^2)\}}$$

In this expression, σcic is small enough to ignore from S/N calculation, total noise becomes following.

$$\text{Total Noise } N = \sqrt{\{ \sigma r^2 + F^2M^2 (DT + QP) \}}$$

S/N is calculated as shown below.

$$S/N = QPM/\sqrt{\{ \sigma r^2 + F^2M^2 (DT + QP) \}}$$
$$= QP/\sqrt{\{ \sigma r^2 /M^2 + F^2 (DT + QP) \}} \quad \text{———— (A)}$$

4.3 EM-CCD Noise Dependence on EM gain vs. Input Photons

Excess noise factor is estimated at 1.41 for calculations of multiplication register noise.
This value is included in the examples shown below.

4.3.1 EM Gain vs. S/N

Figure 12 shows how S/N is influenced by the number of the incident photons (10, 100, and 1000 (photon / pixel / frame)), and increasing multiplication gain. An exposure time of 33 ms, Quantum efficiency 90 % , and readout noise of 80 electrons are used for this example.
The graph shows S/N clearly improves with signal multiplication but there is a limit to the S/N improvement in each case. Even if higher EM gain is applied, there is a certain level at which no further improvement can be expected. When the input photon level exceeds 1000 photons/pixel in 33 ms the EM gain feature offers almost no benefit. The S/N at that point is limited only by the number of input photons (QP) and the multiplication noise (F).
This can be seen in expression (A).

Supposing the dark current is small enough, it is possible to ignore $\sigma r^2 / M^2$ as multiplication gain increases. In this case S/N is simplified as in expression below.

$$S/N = QP/\sqrt{\{F^2 (QP) \}}$$
$$= \sqrt{(QP/F^2)}$$

From this calculation S/N is constant since the incident photon number P is constant.

This example clearly shows there is no advantage to increasing gain more than required according to the numbers of incident photons. This is very important point to be considered because it effects the gain stability in the long term.

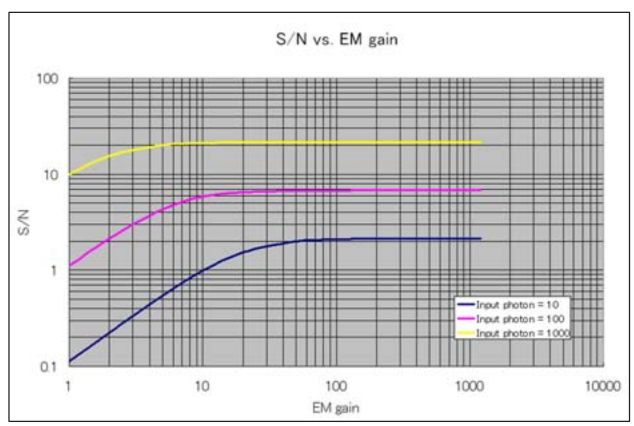


Fig. 12

4.3.2 Input Photons vs. S/N

Figure 13 shows how S/N is affected by the number of incoming photons at different gain settings. The conditions are otherwise the same as in Figure 12. In this graph, it is shown that the S/N does not improve at gain settings over 200 but does improve with the number of incoming photons. The point is that using more gain than necessary is not a benefit and will only increase the rate of gain degradation in the multiplication register. Different conditions will change the gain setting at which this happens but the lesson is the same.

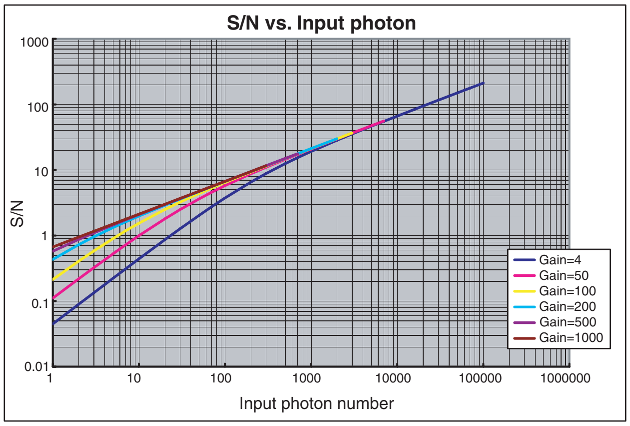


Fig. 13

Figure 14 shows how readout noise becomes relatively smaller as Gain is increased.

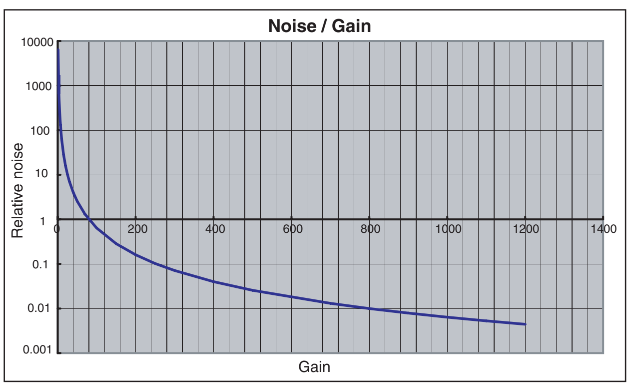


Fig. 14

4.3.3. S/N Crossover Point between Normal CCD Readout and EM-CCD Readout

Figure 15 shows how S/N changes with the number of incident photons in normal CCD readout and EM-CCD readout respectively. As shown in Figure 15, using 100 photons/pixel/frame as a reference (exposure time: 100 ms), when the incident photons are 100 photons/pixel/frame or more, normal CCD readout provides better S/N. When incident photons are less than 100 photons/pixel/frame, EM-CCD readout provides better S/N.

Notably, even with less than 100 photons/pixel/frame, normal CCD readout offers higher S/N if the EM gain is less than 50 times. Another way to look at this is that in order to improve S/N with less than 100 photon/pixel/frame conditions, it is necessary to use at least 50 times EM gain. This value is confirmed in Figure 14 by observing that the point at which the noise becomes less than one photon - at a gain of 50 times.

Since the ImagEM camera has both normal CCD and EM-CCD readout modes it is recommended to switch readout modes to maximize the S/N based on the number of incident photons.

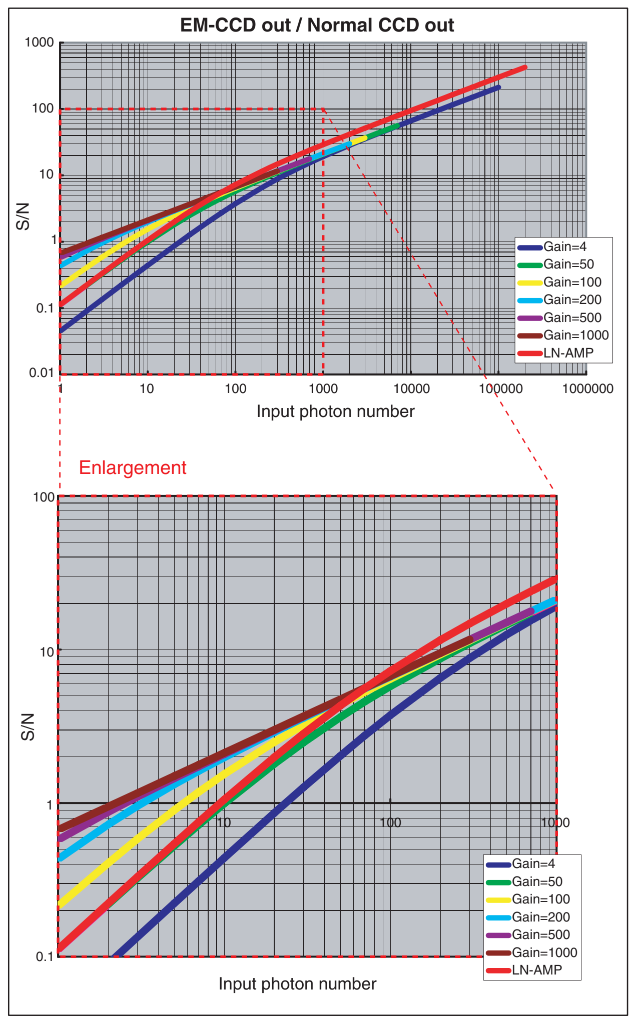


Fig. 15

5. ImagEM EM-CCD Camera Technical Note

5.1 Outline of ImagEM (C9100-13, -14) Features

Hamamatsu Photonics K.K. uses the very latest cooling and CCD camera design technologies for the ImagEM EM-CCD camera. The ImagEM camera is the result of many years of experiments and experience with deep cooling, high vacuum and low noise CCD technology. It provides optimum signal and noise characteristics for a wide range of applications. With one camera, it is now possible to capture both low and high light level images, wide dynamic range images and single photon binary images, long integration and high frame rate images and it offers a very high quantum efficiency over a broad range of wavelengths. The features below confirm that virtually any application can be addressed with this new camera.

◉ –90 ˚C cooling with Hermetic Sealed Head (C9100-13)

A newly designed hermetic sealed head includes a specially developed 4 stage peltier cooler for the CCD and the entire camera head has been designed to optimize heat radiation. With both air and water cooling capabilities built-in, temperature at the CCD can be maintained as low as –90 ˚C (–80 ˚C : C9100-14) with the water cooling operational and a water temperature of 10 ˚C or less.

◉ Temperature Stability and Gain Stability

Temperature stability is the key to gain stability in an EM-CCD camera so the ImagEM provides temperature stability to within ± 0.01 ˚C at –80 ˚C (C9100-13) or –70 ˚C (C9100-14) with water cooling.

◉ Stability of the Digitizer Offset

Stability of the digitizer offset is an important issue for reliable data in an EM-CCD camera. Fluctuation of the digitizer offset in the ImagEM camera is very well controlled; showing only a few counts even at full 16 bit digitizer resolution and maximum EM gain.

◉ Anti-reflection (AR) Coatings on Both Sides of the Vacuum Head Window

The AR coating on both sides of the window provides greater than 99 % transmission efficiency between 450 nm and 750 nm and greatly reduces reflections from both outside and inside the vacuum head (C9100-13, -14). A mask is applied on the metal components of the CCD chip to further minimize reflections from inside the vacuum chamber (C9100-13).

◉ Optimization of Dark Current

The ImagEM provides optimized driving methods for the CCD based on using the different characteristics CIC and thermal charges to minimize the contribution of both. For ease of use, these driving methods are combined with multiple clock speeds such as High scan mode for short exposure measurement, Middle or Slow scan mode for long exposure measurement.

◉ EM Gain Protection

It is important to operate the camera in ways that minimize the rate of EM gain ageing and extend the life of the camera. The ImagEM now provides user adjustable protection levels to reduce excessive EM gain ageing from unintentional events.

◉ EM Gain Readjustment

When EM gain degradation does occur, a built-in feature of the ImagEM readjusts the gain to the original values without removing the camera from the laboratory setup.

◉ Direct EM Gain Control

The ImagEM provides direct selection and setting of the EM gain in the gain register.

◉ Dual Readout Modes

The ImagEM has both an EM-CCD readout and a NORMAL CCD readout. With recent innovations, the NORMAL CCD readout offers very low noise readout and very low dark current for long integration exposures. This creates tremendous flexibility in applications. From ultra low luminescence samples to routine fluorescence microscopy, the conventional readout provides high S/N images. With the EM-CCD readout, high frame rates at low intensities are possible for live cell imaging and spinning disk confocal applications.

◉ Multiple Pixel Clock Selections

The ImagEM offers a selection of pixel clock speeds in both EM-CCD readout mode and normal CCD readout mode. According to the application, it is possible to select a clock speed that offers the best S/N. Faster clock speed offer faster frame rates and lower clock speed provides lower noise.

◉ Photon Imaging Mode (Patent Pending)

Due to the excess noise factor in the multiplication register, EM-CCD cameras have traditionally had limited use in photon counting applications. Intensified CCDs have dominated this field for many years. Using over 20 years of experience in the design and production of image intensifiers and electronic circuit technology, Hamamatsu Photonics K.K. has incorporated a unique photon counting imaging mode into the ImagEM.

◉ Real Time Image Processing

Traditionally, background correction and sensor non-uniformity corrections in images required separate software operations and processing time in a computer. The ImagEM is equipped with on-board digital signal processing functions that offer real time image processing that replaces the software and computer processing. When implemented, highly corrected images emerge directly from the camera at full frame rates. A special recursive filter function is also included to dramatically reduce the effects of excess noise when the EM-CCD operation is selected. This real time filter provides full frame rate images of averaged images; creating exceptional quality EM-CCD images.

◉ External trigger / Synchronous Readout Trigger (Patent Pending)

When an EM-CCD camera is combined with a real-time confocal microscope, it is very important to synchronize the camera readout with the rotation of the disk in the case of spinning disk type or with the galvanometer in case of a mirror scanning type. Vertical smear or a non-uniform intensity (banding) appears in images unless the same number of scan points are created in every point in the image. To overcome this, the ImagEM is designed with a specially developed synchronous readout trigger that assures even intensity in every image.

◉ Programmable Trigger Signal Output

To simplify the control and synchronization of peripheral devices, the ImagEM is equipped with a versatile programmable trigger signal output. It is possible to freely control delay time, pulse width, and polarity of the trigger signal output with external commands.

◉ Multiple Heads Capability

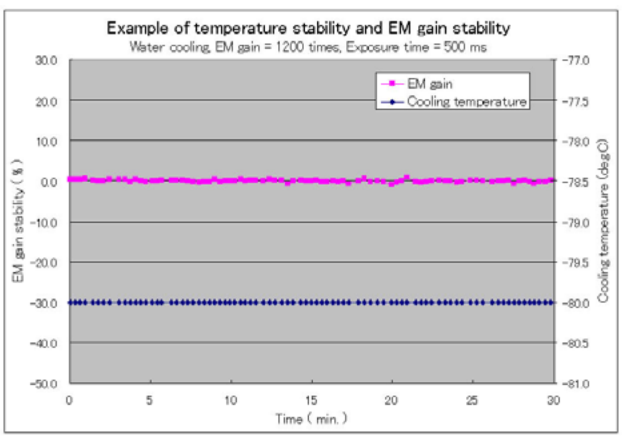
Scientific imaging often requires the simultaneous acquisition of data from multiple cameras. Until now this has been difficult but a new feature of the ImagEM is the ability to synchronously drive two or more cameras. Synchronization is accurate to within one pixel clock operation. To take full advantage of this feature over a wide range of applications, each camera is able to operate synchronously even with individual exposure settings and individual EM gain settings. With this option, multiple wavelength imaging and multiple polarization angle imaging is simply and reliably done with high precision.

5.1.1 Temperature Stability and Gain Stability

The ImagEM provides dramatically reduced dark current with new –90 ˚C (C9100-13) or –80 ˚C (C9100-14) cooling capacity. A newly designed hermetic sealed vacuum head with a 4 stage peltier element and a highly efficient heat radiation structure means great cooling using only air or even lower cooling using water; all in the same camera head and with no modifications. Considering the importance of cooling to the gain and especially the gain stability in EM-CCD cameras, the ImagEM provides temperature stability of ± 0.01 ˚C (typical), ± 0.05 ˚C (maximum) at –80 ˚C (C9100-13) or –70 ˚C (C9100-14) with water cooling. If the room temperature is stable, ± 0.03 ˚C (typical: C9100-13) or ± 0.05 ˚C (typical: C9100-14) stability is possible under air cooled operation. As a result, gain stability is kept better than ± 1% for both cooling modes (See Figure 16 and Figure 17).

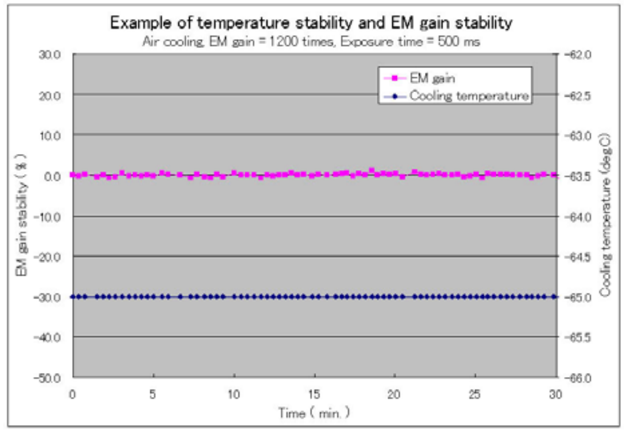
To compensate for the difference in gain values when switching between the water cooled operating temperature of –80 ˚C (C9100-13) or –70 ˚C (C9100-14) and the air cooled operation at –65 ˚C (C9100-13) or –55 ˚C (C9100-14), each camera has a built-in gain correction table. This table ensures a constant gain factor regardless of the cooling mode used.

Due to the superb cooling of the ImagEM, the dark current of the CCD has been reduced by 100 times. When used with a circulating water chiller, the CCD temperature is maintained and regulated at –80 ˚C (C9100-13) or –70 ˚C (C9100-14) with a water temperature of 20 ˚C . When using the fan assisted air cooling feature, the temperature is maintained and regulated at –65 ˚C (C9100-13) or –55 ˚C (C9100-14) in air temperatures up to 30 ˚C. Combining this very low dark current with the slow scan readout from the normal CCD mode, the ImagEM is able to capture images over a wide range of applications including those that require very long integration times.



- Example of C9100-13 water cooled
  -80 ˚C (Water temperature : +20 ˚C)
  Temperature stability ± 0.01 ˚C(typical)
  EM gain stability ± 1 % (typical)

Fig. 16



- Example of C9100-13 forced-air cooled
  -65 ˚C (Room temperature :Stable at +20 ˚C)
  Temperature stability ± 0.03 ˚C(typical)
  EM gain stability ± 1 % (typical)

Fig. 17

5.1.2 Stability of the Digitizer Offset

The stability in the digitizer offset of an EM-CCD is important since the digitizer offset is the baseline that is often subtracted from the data for quantitative analysis.

As previously mentioned, the cooling temperature and the stability of the cooling temperature are known to play a major role in the gain and gain stability of EM-CCD cameras. As the cooling temperature is lowered to enhance the gain characteristics, other noise factors in the CCD like clock induced charge (see 5.1.4) and thermal charge (see 5.1.4) become more obvious. Since these charges occur in the CCD and are multiplied by the gain register, they have the potential to create instability in the offset as well.

Considering the importance of this digitizer offset stability, evaluating the fluctuation or lack of it is an important tool for quantitative users. Using a Hamamatsu C9100-13 with water cooling (Temp -80 ˚C), exposure time of 30.5 ms, and gain of 1200×, with the scan mode is set to high (11 MHz readout) and the CCD in the dark,100 exposures were acquired to create the table below.

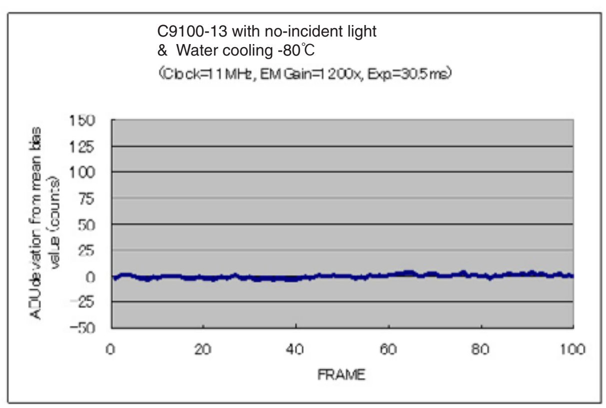


Fig. 18

5.1.3 Anti-Reflection (AR) Coatings on Both Sides of the Vacuum Head Window

C9100 series cameras feature a single window on the front of the hermetic vacuum-sealed head. This single window is designed to reduce the loss of light at a certain level due to reflections on the glass. The improvement to the ImagEM is the addition of AR coating on the both inside and outside of the window. This two sided AR coating helps improve light transmission through the window and minimize reflections and stray light caused by reflections from metal components within the vacuum head as well.

The AR coating provides greater than 99% transmission efficiency between 450 nm and 750 nm and over 90% transmission efficiency between 400 nm and 850 nm (See Figure 19).

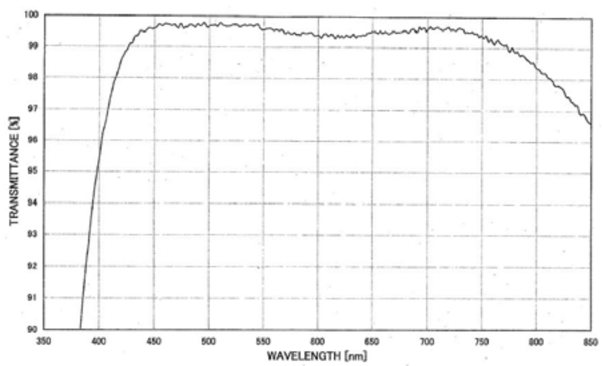
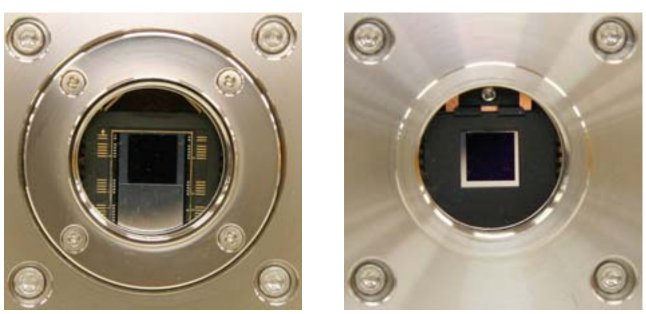


Fig. 19

The new two sided AR coating offers new levels of protection from ghost images and stray light for improved S/N in the ImagEM images.



EM-CCD without AR and mask

EM-CCD with AR and mask (C9100-13 only)

Fig. 20

5.1.4 Optimization of Dark Current

In a normal cooled CCD camera, the dark charge (dark current) is assumed to be just the thermal charge and clock induced charge (CIC) is ignored since it is less than 1 electron and so small in comparison to the dominant readout noise. With an EM-CCD camera the CIC can no longer be ignored since the gain features of the EM-CCD reduce the effective readout noise to less than 1 electron. In this case both the thermal charge and the CIC have to be considered and combined to create the actual dark charge (dark current).

Clock Induced Charge (CIC)

The images below illustrate the contribution of CIC in an EM-CCD under different methods of driving the vertical transfer on the chip. One drive method will optimize the CIC contribution and a second drive method will optimize the contribution of thermal charge. Since CIC is constant and thermal charge is time dependent, CIC will dominate the dark charge in images taken at short exposures and thermal charge will dominate the dark charge in images taken at longer exposures.

To illustrate the contribution of CIC to dark charge these images (See Figure 21) were taken by C9100-13 with the temperature of the CCD regulated to -65 ˚C, a gain of 1200×, and a 30 ms exposure time with no illumination. (A region of 100 × 100 pixels was selected and enlarged). The standard drive method is shown on the left and the optimized drive method is shown on the right.

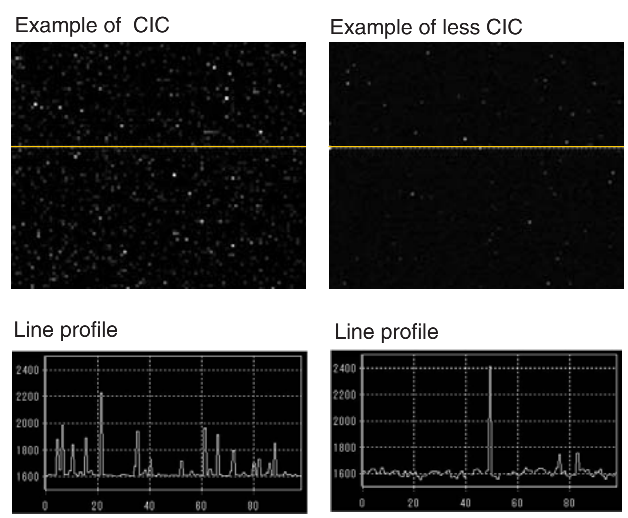


Fig. 21

Thermal Charge (TC)

To illustrate the contribution of thermal charge (TC) to dark charge, these images (Figure 22) were taken by C9100-13 with the temperature of the CCD regulated to -65 ˚C, a gain of 1200×, and a 10 minute exposure time with no illumination. (A region of 100 × 100 pixel was selected and enlarged) The standard drive method is shown on the left and the optimized drive method is shown on the right.

Due to the extremely low dark charge in the ImagEM, an exposure time of 10 minutes was necessary to acquire enough dark charge to be visible. In the image on the left it is possible to see thermal noise patterns of circles and stripes plus some bright white spots caused by cosmic rays. In the image on the right, using the dark charge optimized drive method, only the cosmic rays can be seen.

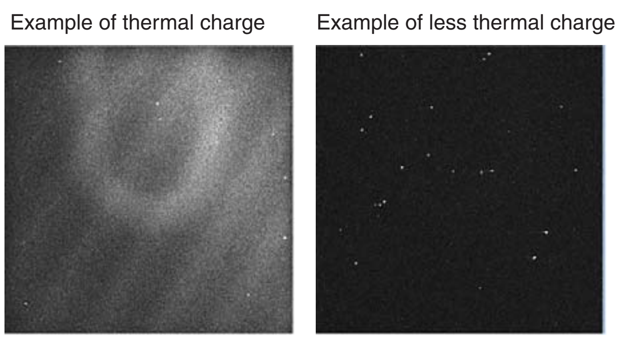


Fig. 22

Total Dark Charge

Combining CIC and thermal charge produces the total dark charge in the CCD. These charges are summed in quadrature and can be plotted on a log/log scale to see the combined effect over time
(See Figure 23) .

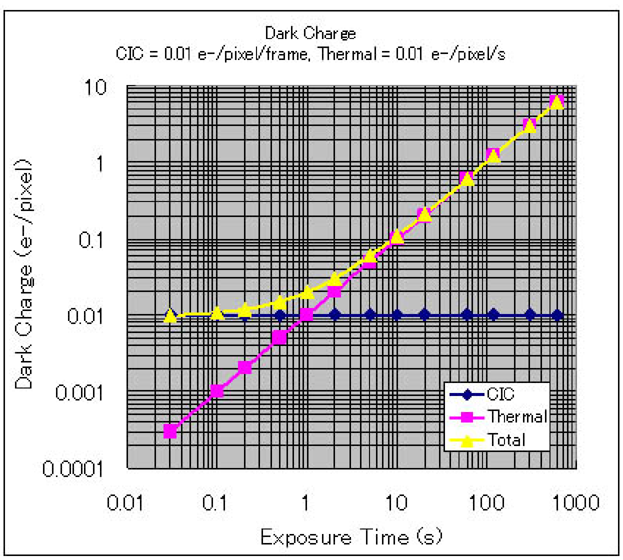


Fig. 23

Optimizing Drive Methods

While it is apparent that both CIC and thermal charges can be optimized by driving the vertical transfer with different methods, it also means that it is not possible to optimize them both at the same time. It is however possible to optimize them independently depending on the exposure conditions.
The ImagEM changes the optimal drive setting based on the scan mode to create the lowest possible dark current in each of the three scan modes (11 MHz, 2.75 MHz, and 0.69 MHz) (See Figure 24).

ImagEM Dark charge optimization			
Scan mode	11 MHz	2.75 MHz	0.69 MHz
Optimized for:	CIC	TC	TC

Fig. 24

In addition to the three possible scan modes, there are two cooling methods, water and air cooling. Combining these features with the information from the chart above (Figure24) leads to the conclusion that due to the different dark charge characteristics of the two cooling methods, it is possible to further optimize the dark charge control by selecting scan speeds based on exposure time. The following chart (Figure25) shows the recommended combinations if other experimental factors allow the settings.

ImagEM Scan mode optimization				
Cooling / Exposure	Air < 1 s	Air > 1 s	Water < 10 s	Water > 10 s
Optimal scan mode	11 MHz	2.75 MHz or 0.69 MHz	11 MHz	2.75 MHz or 0.69 MHz

Fig. 25

5.1.5 EM Gain Protection

EM-CCD devices are known to exhibit a loss of gain over time called gain ageing. This deterioration in the gain factor can be overcome by adjusting the voltage applied to the stages in the gain register but there is a limit to how much voltage can be applied before destroying the device. It is important to operate the camera in ways that minimize the rate of gain ageing and extend the life of the camera.

Since the rate of gain ageing depends on the incident light levels and gain settings combined, it is possible to minimize both most of the time. Occasionally mistakes are made, like changing lenses or objectives with the gain on or having illumination on the CCD for long periods without knowing it. To prevent excessive gain ageing from such unintentional events, the ImagEM provides two levels of protection.

Level 1 is the EM gain warning. This warning is an audible alarm or software warning that excessive output conditions have occurred which may damage the detector. This warning can be set to one of three levels or disabled completely by the operator.

Level 2 is the EM gain protection mode. This feature will switch the camera to protection mode, stopping the charge transfer through the gain register. The degree of protection is based on a combination of the critical signal level and the number of frames the operator selects in the gain warning dialog box. Like the warning feature it may also be disabled by the operator.

5.1.6 EM Gain Readjustment

Over time, all EM-CCD cameras exhibit gain ageing, also called gain degradation. Fortunately, the gain can be readjusted by raising the voltage in the gain multiplication register, but only within limits. Reducing the rate of gain ageing by careful use of the detector is helpful and the ImagEM includes effective EM gain protection (see 5.1.5) but at some point it will be necessary to readjust the gain to the original values.

The EM gain readjustment for the ImagEM is simple and the software, labeled "EMGREAD.exe", can be found in the DCAM modules. For example, on the HCImage disk included with the camera, look in "HCImage\Drivers\DCAM\DCAMAPI\Tools\EMGREAD.exe" to find the software.

5.1.7 Direct EM Gain Control

Previously, EM gain controls divided the actual EM gain range into 256 steps. Using a chart in the camera manual, it was possible to estimate the actual EM gain of the camera. For instance, if the gain 100 was selected from the EM gain control in the software window, the actual gain was between 4× and 1200× for the ImagEM and the chart in the manual would indicate this to be equivalent to 40× gain in the multiplication register.

Direct EM gain control is now possible using any software that supports the Hamamatsu DCAM application. Setting the software control to 100 means the gain register will provide 100× multiplication(See Figure26). Like all EM-CCD devices gain ageing occurs over time and this value will change slightly but it can easily be checked and readjusted to the nominal value as described before.

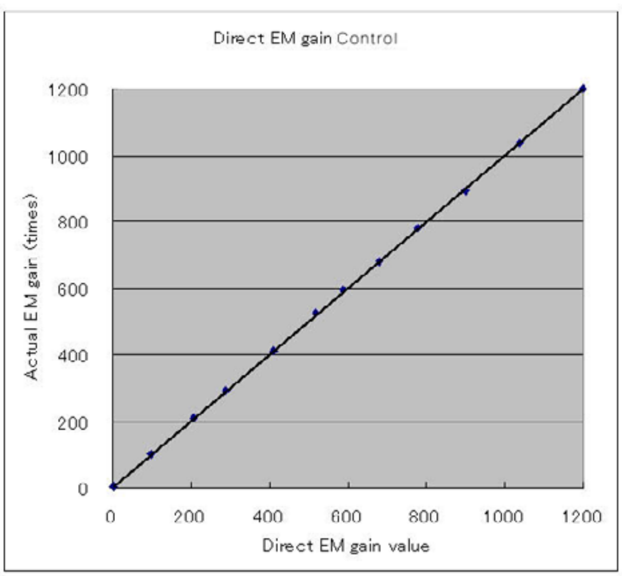


Fig. 26

5.1.8 Dual Readout Modes

The ImagEM offers two readout modes. The traditional electron multiplication mode has usually been used for rapid imaging of live cells in low light situations. The addition of a normal CCD amplifier creates the NORMAL-CCD readout mode and extends the capabilities of the camera into additional areas of imaging where long term integration with low noise readout is required.
Figure 27 is a diagram of the CCD with both readout modes shown.
Accumulated charges are transferred from the image storage area of the CCD into the Register Elements as part of the readout process. When the camera is operated in the EM-CCD mode, the accumulated charges would normally be transferred to the right in the diagram and then through the multiplication register to the EM-AMP. In the case of NORMAL-CCD readout, the accumulated charges are transferred to the left in the diagram, directly into the CCD-AMP from the register elements. This reduces readout noise and creates high S/N values in the image.

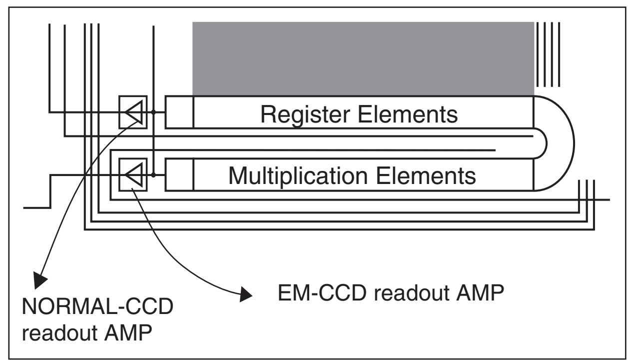


Fig. 27

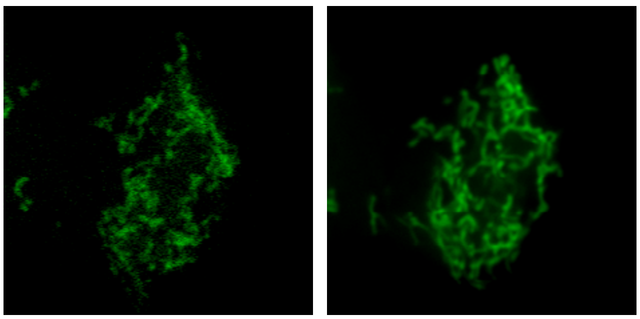
It is easy to select the readout mode in ImagEM according to the experimental conditions. EM-CCD readout works well for dynamic and real time image acquisition in low light and NORMAL-CCD readout works well for imaging normally done with a cooled CCD camera, even for ultra-low light luminescence imaging. The S/N cross-over point of these two modes (EM-CCD readout mode at 11 MHz clock speed and NORMAL-CCD readout mode at 2.75 Hz clock speed) is where the incident photon number is around 100 photons/pixel/frame. (See Figure 15)

5.1.9 Image Reversal Function

Since the ImagEM includes both NORMAL-CCD readout and EM-CCD readout the images from these two methods would normally be mirror images of each other due to the directional readout of each mode. To simplify the data handling by the end user, an automatic image reversal function is applied to the EM-CCD mode and the images from the two modes will be created with the same orientation.

Comparison of readout modes
(Images of labeled intracellular proteins)

EM-CCD readout mode	NORMAL-CCD readout mode
High sensitivity live imaging	**High precision imaging**
Exposure time : 30 ms	Exposure time : 3 s



Images of a fluorescently labeled HeLa cell.
·Camera : C9100-13
·Objective lens 100× ·Yokogawa CSU22
·Excitation 488 nm laser (3 mW) ·ND 10 %T

Fig. 28

5.1.10 Multiple Pixel Clock Selections

The ImagEM is equipped with multiple pixel clock speeds in both the EM-CCD readout mode and the NORMAL-CCD readout mode. Since the pixel clock speed changes the readout noise characteristics of the camera, it is possible to select a pixel clock speed that best suits your S/N requirements in either mode.
Typical readout noise figures at various pixel clock speeds are listed in Figure 29.
Readout noise shown below is the figure at minimum EM gain (4 times).

Type number		C9100-13	C9100-14
EM-CCD readout (4×)	11 MHz	25 electrons	10 electrons
	2.75 MHz	20 electrons	8 electrons
	0.69 MHz	8 electrons	3 electrons
	(Pixel clock)	(Readout noise)	

Fig. 29

Using the 0.69 MHz pixel clock speed, it is possible to readout through the EM-AMP register with approximately 8 electrons readout noise (C9100-13). In this case, the necessary EM gain to bring the relative readout noise to less than 1 electron becomes very small, meaning that even a small EM gain setting provides high S/N. This can be a very sensitive imaging method. It is important to note that due to the structure of the FT-CCD, the minimum exposure time is limited by the time required to readout the accumulated charges in the storage area.
Figure 30 shows the EM gain setting at which the noise becomes less than 1 electron (C9100-13).
Figure 31 shows the minimum exposure time related to different pixel

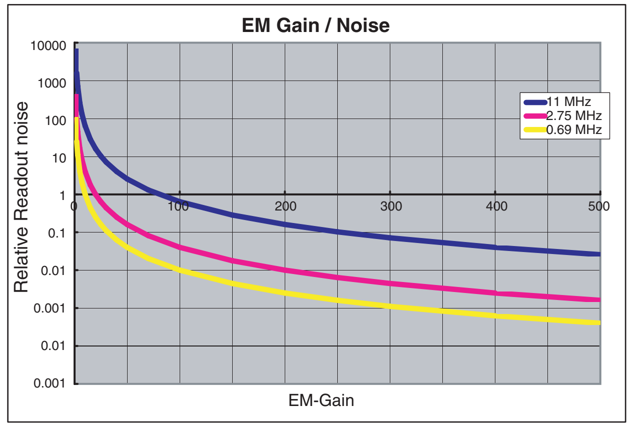


Fig. 30

Type number		C9100-13	C9100-14
EM-CCD readout (4×)	11 MHz	30.5 ms (31.9 frames/s)	103 ms (9.5 frames/s)
	2.75 MHz	122 ms (8.0 frames/s)	413 ms (2.4 frames/s)
	0.69 MHz	488 ms (2.0 frames/s)	1652 ms (0.6 frames/s)
	(Pixel clock)	(Minimum exposure time)	

Fig. 31

Figure 32 and Figure 33 show the relationship of different pixel clock speeds to the readout noise, minimum exposure time and frame rate in the NORMAL-CCD readout mode.

Type number		C9100-13	C9100-14
NORMAL-CCD -readout	2.75 MHz	17 electrons	19 electrons
	0.69 MHz	8 electrons	10 electrons
	(Pixel clock)	(Readout noise)	

Fig. 32

Type number		C9100-13	C9100-14
NORMAL-CCD -readout	2.75 MHz	8.0 frames/s (122 ms)	2.4 frames/s (413 ms)
	0.69 MHz	2.0 frames/s (488 ms)	0.6 frames/s (1652 ms)
	(Pixel clock)	(Minimum exposure time)	

Fig. 33

As shown in Figure 32, the readout noise using the 0.69 MHz pixel clock equals the low noise performance of a conventional cooled CCD camera, suitable for applications such as luminescence imaging where long exposures and slow frame rates are normal.

5.1.11 Photon Imaging Mode (Patent Pending)

Since there is no way to eliminate the excess noise factor from the electron multiplication process in an EM-CCD, it has always limited the image quality at very low light levels. Because of their smaller excess noise factors Image intensifiers have dominated applications where the signal level is in the single photon range.

The ImagEM has a special Photon Imaging mode to overcome this limitation. For many years, Hamamatsu Photonics K.K. has been making photon counting cameras with special image intensifiers. Based on this experience, unique circuits for driving the electron multiplication process have been designed and included in the ImagEM to enable high quality images in ultra low light .

Figure 34 clearly shows the benefits of the special Photon Imaging mode. Using a standard test target, the image on the left, taken using the EM-CCD readout mode, is almost unrecognizable as a test target but the image on the right, taken with the Photon Imaging mode, can be clearly seen to be a test target.

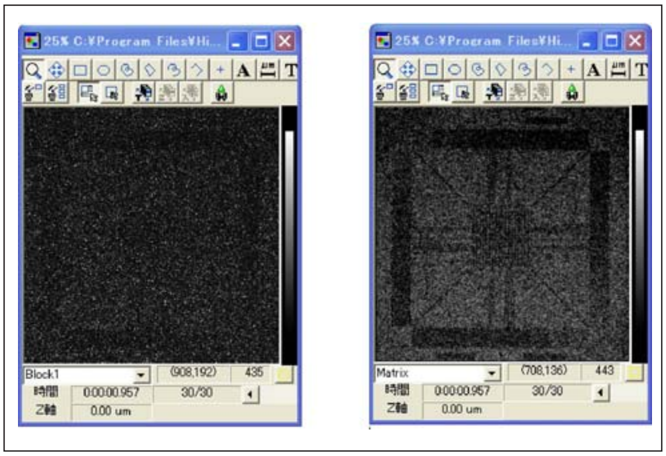


Fig. 34

Figure 35 shows a similar test using a dot chart. As shown in the image on the right, it is possible to detect each dot clearly with the photon imaging mode. The photon imaging mode offers new opportunities in microtiter plate readers, DNA chip readers and other applications where the signal of interest is composed of isolated spots of low intensity.

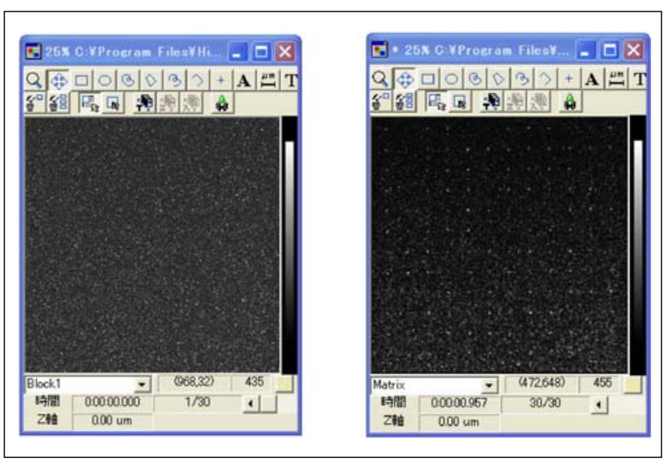


Fig. 35

Features of the photon imaging mode

- Must be used in the EM-CCD readout mode of operation, has no effect in NORMAL-CCD readout mode of operation.
- Most useful for signal levels at which maximum EM gain has no apparent signal or very little signal.
- Increases signal intensity 5× in mode 1, 13× in mode 2 and 21× in mode 3.
- Quantitative linear signal output in each mode means quantification is possible if mode is constant.
- Improved spatial resolution at very low light levels.

5.1.12 Real-time Image Processing Features

The design of ImagEM incorporates a fundamentally different way of thinking about how to produce the highest quality images from a digital camera. Rather than rely on software and computer processing after the camera output, some of the most common image processing functions are included in the camera itself. Selecting these functions provides real time image improvement and increased S/N. Otherwise, the ImagEM outputs raw data that is preferred for quantitative analysis.

Function 1 : Real time Background Subtraction

The ImagEM normally does not need dark current subtraction due to the superb cooling but in very long exposures in the NORMAL-CCD readout mode, it may still offer some benefit to subtract dark current. In brightfield images, it is often beneficial to subtract backgrounds to eliminate spots and other image artifacts. In both cases, it is usually best to add a constant value (offset) to the result to avoid zero values in a fluorescence image or to create a similar intensity to the normal background in brightfield images. The value of this offset can be freely adjusted to suit the requirements.

ImagEM does all this and will store 4 different reference images (1 reference image in C9100-14) for this operation. Images with different binning, subarray and exposure settings can all be stored and used as reference images.

Function 2 : Shading Correction

It is common in microscopy to find uneven illumination in images. Correcting this unevenness improves the results and often reveals additional details in the image. The ImagEM can calculate an intensity difference map for every pixel, and apply this map to the original image to remove the difference; correcting the shading of the microscope or other illumination system.

Function 3 : Recursive Filter

A Recursive filter is used to significantly improve S/N in images by averaging noise in both the signal and the camera. In EM-CCD cameras it is especially effective because of the low number of signal photons and the excess noise factor in the multiplication process. Real time images are produced by averaging the values in the same pixel over several images at the selected pixel clock rate. Each output image is the result of an average of some number of previous images. For static or slowly changing images this is not a problem. For dynamic images, image smearing will result.

Having these real time image processing features built into the ImagEM is an important and effective way to speed up image acquisition. Producing processed images in the camera relieves the computer CPU of many very time intensive operations, especially when high frame rate sequential images are required. When acquiring 4D and 5D images, the number of peripheral devices and image handling operations can cause frame dropping due to CPU stress. Entire experiments may become meaningless if frames are lost and the real time image processing functions in the ImagEM can help prevent this.

Calculation of recursive filter (e.g. N=4)

Vout(n) = Vout(n-1) + (Vin(n) - Vout(n-1)) / 4

Vout(n) : Present output image
Vout(n-1) : Output image of 1 previous frame
Vin(n) : Present input image

Function 4 : Frame Averaging

Frame averaging is a processing of multiple frames that result in the output of a simple average of the last current frame and a number of previous frames that equal the desired frame number. During the averaging time, the last previously averaged image is used as the output until a new average is completed. At that point the output is refreshed and the next average is started. This form of temporal averaging provides significant increases in the S/N since both detector noise and signal are averaged.

Calculation of frame averaging (e.g. N=4)

Vout(n) = (Vin(n) + Vin(n-1) + Vin(n-2) + Vin(n-3)) / 4

Vout(n) : Present output image
Vin(n) : Present input image
Vin(n-1) : Input image of one previous frame
Vin(n-2) : Input image of second previous frame
Vin(n-3) : Input image of third previous frame

Function 5 : Spot Noise Reducer

This image processing function operates on random spots of intensity by comparing incoming images and eliminating signals that meet the criteria for noise in one image but not in others. Small bright signals are compared to previous frames to see if they occur in more than one frame. If they occur in only a single frame the value is replaced the value of the comparison frame. If similar value occurs in two frames then the original value is kept in both.

This processing eliminates noise elements like cosmic rays to increase the signal to noise ratio and overall quality in images.

Caution should be exercised using spot noise reducer if the signals of interest have properties similar to random noise events.

5.1.13 External Trigger / Synchronous Readout Trigger (Patent Pending)

The ImagEM has various external trigger modes for synchronization with peripheral devices. In particular, The ImagEM has both an External trigger IN where an external device is master and the ImagEM is the slave and an External trigger out where the ImagEM is the master controlling external devices. A unique and important external trigger mode called Synchronous readout trigger, is designed to be used for the synchronization of the ImagEM with a confocal microscope. This mode eliminates the vertical smearing or banding noise that comes from a mismatch between camera readout and the confocal disk rotation speed.

External Edge Trigger

Using External Edge trigger, the camera starts an exposure at the (positive or negative) edge of an external trigger signal. The exposure time is defined by the application software. The camera ignores any additional trigger input until the exposure is completed and frame readout is done completely, even if another trigger signal is received during the exposure.
Using this mode means the frame rate will be slower than the internal trigger mode because the minimum frame rate is the sum of the exposure time plus frame readout time. Figure 36 shows the timing chart for the external edge trigger using the positive edge. The minimum permissible exposure time setting is 10 µs, so the frame rate with 10 µs exposure time setting is close to the frame rate in the internal trigger mode.

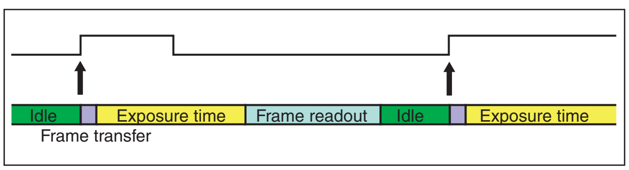


Fig.36 External Edge Trigger In (Positive Edge)

Level Trigger

Using external Level trigger the exposure time is fully controlled by the trigger pulse. If the trigger polarity is selected for a positive trigger, the camera starts an exposure at the positive edge of external trigger, and maintains the exposure time during the positive period. The camera stops the exposure and begins the frame readout at the negative edge of the trigger.
In this external level trigger mode the frame rate is slower than the internal trigger mode because the minimum frame rate is the sum of the exposure time plus frame readout time. Figure 37 shows the timing chart for the external level trigger using the positive edge. The minimum exposure time setting is 10 µs, so the frame rate with 10 µs exposure time setting is close to the frame rate in the internal trigger mode.

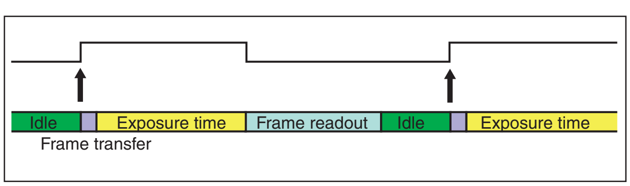


Fig.37 External Level Trigger In (Positive Level)

Start Trigger

This trigger mode is used for fast frame acquisition. A single trigger input from either the positive or negative edge can be used to start the exposure. The camera can simultaneously expose and readout in this mode and it operates in the internal trigger mode. No additional trigger signals are needed for continuous imaging at high frame rates.

Synchronous Readout Trigger (Patent Pending)

Synchronous readout trigger is a special external trigger mode which is designed to be used for synchronizing the ImagEM with a confocal microscope. There are two ways to do this.

(1) Synchronous Readout mode 1

In this mode, the external trigger in is used and the trigger interval defines the exposure time. When the trigger in signal is synchronized with the spin cycle timing of the disk in a confocal microscope, the camera readout cycle will synchronize properly even when the spinning disk speed is not constant since the trigger interval depends on the disk speed. Without proper synchronization, images will show uneven illumination (called banding noise). Figure 38 shows the timing chart for this mode.
An added feature of this mode is that it is possible to get almost the same frame rate as the internal trigger mode because the exposure and frame readout can be done simultaneously.

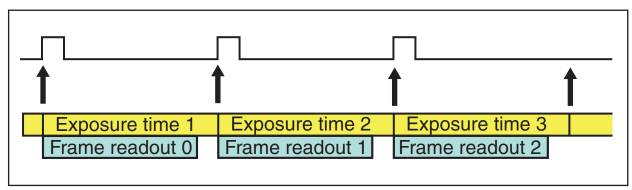


Fig.38 Synchronous Readout Trigger mode 1

(2) Synchronous Readout mode 2

In this mode, the external trigger in is used and the number of trigger pulses defines the exposure time. Some spinning disk confocal microscopes use pulses from pinholes in the disk for timing. Setting the trigger in signal in the ImagEM to start exposure on one pulse from the spinning disk and finish exposure after some number of pulses will synchronize the exposure and readout with the spin cycle timing of the disk in a confocal microscope. The camera readout cycle will synchronize properly even when the spinning disk speed is not constant since the trigger interval depends on the number of disk pulses. Without proper synchronization, images will show uneven illumination (called banding noise).
As an example, if one confocal scanning disk rotation is completed by 4 pulses, the camera should be set for one exposure time to equal 4 pulses from application software.
The camera starts the exposure on the first pulse, and finishes the exposure time when 4th pulse is detected.
Figure 39 shows the timing chart in this mode with 4 pulses for one exposure cycle.
An added feature of this mode is that it is possible to get almost same frame rate as the internal trigger mode because the exposure and frame readout can be done simultaneously.

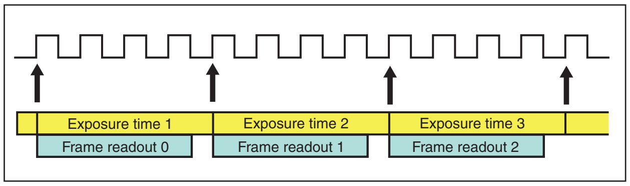


Fig.39 Synchronous Readout Trigger mode 2

5.1.14 Frame Rate

The frame rate of the ImagEM depends on both the scan speed (readout speed) and the synchronization mode, even with a uniform exposure time.

The table below shows (Figure 40, 41) the fastest possible frame rate using the fast scan mode (11 MHz) in each trigger mode.

● C9100-13

Trigger mode	Fastest frame rate 11 MHz mode		
	1×1 binning (exp. time 30.5 ms)	2×2 binning (exp. time 15.6 ms)	4×4 binning (exp. time 8.2 ms)
Internal trigger mode Start trigger mode Synchronous readout trigger mode	Approx. 32 Hz	Approx. 61 Hz	Approx.112 Hz
Edge trigger mode Level trigger mode	Approx. 16 Hz	Approx. 31 Hz	Approx. 56 Hz

Fig. 40

● C9100-14

Trigger mode	Fastest frame rate 11 MHz mode		
	1×1 binning (exp. time 103 ms)	2×2 binning (exp. time 52.8 ms)	4×4 binning (exp. time 27.5 ms)
Internal trigger mode Start trigger mode Synchronous readout trigger mode	Approx. 9.5 Hz	Approx. 18.4 Hz	Approx. 34.2 Hz
Edge trigger mode Level trigger mode	Approx. 4.8 Hz	Approx. 9.2 Hz	Approx. 17.1 Hz

Fig. 41

5.1.15 Programmable Synchronization Output

The Programmable Synchronization output is an important feature of the ImagEM.
For biomedical imaging, timing control of peripheral devices such as excitation light sources, filter changers, mechanical shutters, stage and focusing controls is essential. Triggering these peripherals with accurate timing directly from the camera can improve the speed of image acquisition for high speed applications. The ImagEM offers freely programmable synchronization output, delay time, pulse width, and trigger signal polarity.

5.1.16 Multiple Heads Control Feature

Scientific imaging often requires the simultaneous acquisition of data from multiple cameras. Until now this has been difficult but a new feature of the ImagEM is the ability to synchronously drive two or more cameras. Synchronization is accurate to within one pixel clock operation. To take full advantage of this feature over a wide range of applications, each camera is able to operate synchronously even with individual exposure settings and individual EM gain settings. With this option, multiple wavelength imaging and multiple polarization angle imaging is simply and reliably done with high precision.
For details of multiple camera control, please request information from our sales office.

5.2 Calculation of Photons from Output Gray Levels

Although the EM-CCD has electron multiplication gain, if the multiplication factor is known, incident photon numbers can be estimated from the output image, similar to a normal cooled CCD camera. The following formula will allow a close approximation of the photon numbers if the light is a single wavelength and a large number of pixels are used. The difference between an EM-CCD and a regular CCD is the multiplication noise factor from the EM gain register and this leads to a small uncertainty in any of the calculations of exact photon numbers.

Number of photons

$$= (\text{Out put intensity} - \text{Dark intensity}) \times \text{Conversion Factor} / (\text{Analog gain} \times \text{EM gain} \times \text{Q.E.} / 100)$$

Number of photons (photon) : Number of incoming photons
Output intensity (count) : Output signal intensity with illumination
Dark intensity (count) : Output signal intensity without illumination
Conversion Factor (electron / count) : Characteristic value for the camera (Listed in manual)
Analog gain (times) : Analog gain set on the camera
EM gain (times) : EM gain set on the camera
Q.E. (%) : Conversion efficiency of photons to electrons (Depend on wavelength)

Refer to the specific example below in case of C9100-13.
An image is acquired by the ImagEM when the wavelength of incoming light is 700 nm. The pixel output intensity is 12 000 counts and dark intensity (digitizer offset) is 2000 counts. The camera controls are set to: analog gain is 1×, EM gain is 1200× and exposure time is 30.5 ms. According to the calculation below, the incident photon number at a certain pixel is approximately 54 photons at 30.5 ms exposure time.

Number of photons

$$= (\text{Output intensity} - \text{Dark intensity}) \times \text{Conversion Factor} / (\text{Analog gain} \times \text{EM gain} \times \text{Q.E.} / 100)$$

$$= (12\,000 - 2000) \times 5.8 / (1 \times 1200 \times 90 / 100)$$

$$\fallingdotseq 54 \text{ (photons)}$$

5.3 Specifications

			ImagEM Enhanced	ImagEM 1K
Type number			C9100-13	C9100-14
Camera head type			Hermetic vacuum-sealed air/water-cooled head ①	
Window			Anti-reflection (AR) coatings on both sides, single window	
AR mask			yes	No ②
Imaging device			Electron Multiplier Back-Thinned Frame Transfer CCD	
Effective no. of pixels			512 (H) × 512 (V)	1024 (H) × 1024 (V)
Cell size			16 µm (H) × 16 µm (V)	13 µm (H) × 13 µm (V)
Effective area			8.19 mm (H) × 8.19 mm (V)	13.3 mm (H) × 13.3 mm (V)
Pixel clock rate	EM-CCD readout		11 MHz, 2.75 MHz, 0.69 MHz	
	NORMAL CCD readout		2.75 MHz, 0.69 MHz	
EM (electron multiplication) gain (typ.) ③			1x or 4x to 1200 x	1x or 10x to 1200 x
Ultra low light detection			Photon Imaging mode (1, 2, 3)	
Fastest readout speed			31.9 frames/s to 405 frames/s	9.5 frames/s to 231 frames/s
			417 frames/s (Binning option)	242 frames/s (Binning option)
Readout noise (r.m.s.) (typ.)	EM-CCD readout	EM gain 4x (C9100-13) 10x (C9100-14)	25 electrons (at 11 MHz)	10 electrons (at 11 MHz)
			20 electrons (at 2.75 MHz)	8 electrons (at 2.75 MHz)
			8 electrons (at 0.69 MHz)	3 electrons (at 0.69 MHz)
		EM gain 1200x	1 electron max. (at 11 MHz)	
			1 electron max. (at 2.75 MHz)	
			1 electron max. (at 0.69 MHz)	
	NORMAL CCD readout		17 electrons (at 2.75 MHz)	19 electrons (at 2.75 MHz)
			8 electrons (at 0.69 MHz)	10 electrons (at 0.69 MHz)
Full well capacity (typ.) ④			370 000 electrons (Max. 800 000 electrons)	400 000 electrons (Max. 730 000 electrons)
Analog gain			1/2 times to 5 times	
Cooling method / temperature ⑤	Forced-air cooled		-65 °C stabilized (0 °C to +30 °C)	-55 °C stabilized (0 °C to +30 °C)
			-75 °C (Room temperature : Stable at +20 °C)	-65 °C (Room temperature : Stable at +20 °C)
	Water cooled ⑥		-80 °C stabilized (Water temperature : +20 °C)	-70 °C stabilized (Water temperature : +20 °C)
			-90 °C (Water temperature : lower than +10 °C)	-80 °C (Water temperature : lower than +10 °C)
Temperature stability	Forced-air cooled		±0.03 °C (typ.) (Room temperature : Stable at +20 °C) (-65 °C stabilized)	±0.05 °C (typ.) (Room temperature : Stable at +20 °C) (-55 °C stabilized)
	Water cooled		±0.01 °C (typ.) (Water temperature : +20 °C [Operated with circulating water cooler]) (-80 °C stabilized)	±0.01 °C (typ.) (Water temperature : +20 °C [Operated with circulating water cooler]) (-70 °C stabilized)
Dark current ⑦ (typ.)	Forced-air cooled		0.01 electron/pixel/s (-65 °C)	0.01 electron/pixel/s (-55 °C)
	Water cooled		0.001 electron/pixel/s (-80 °C)	0.001 electron/pixel/s (-70 °C)
Exposure time ⑧	Internal sync mode		30.5 ms or more	103.3 ms or more
	External trigger mode		10 µs or more	10 µs or more
A/D converter			16 bit	
Output signal / External control			CameraLink	
Sub-array			Every 16 lines (horizontal, vertical) size, position can be set (refer to the table on the right)	
Binning			2×2, 4×4 (8×8, 16×16) ⑨	
External trigger mode ⑩			Edge trigger, Level trigger, Start trigger, Synchronous readout trigger	
Trigger output ⑩			Exposure timing output, Programmable timing output (Delay and pulse length are variable)	
Image processing features (real-time)			Background subtraction, Shading correction, Recursive filter, Frame averaging, Spot noise reducer ⑪	
EM gain protection			EM warning mode, EM protection mode	
EM gain readjustment			Available	
Lens mount			C-mount	
Power requirements			AC 100 V to 240 V, 50 Hz / 60 Hz	
Power consumption			Approx. 140 V·A	
Ambient storage temperature			-10 °C to + 50 °C	
Ambient operating temperature			0 °C to + 40 °C	
Performance guaranteed temperature			0 °C to + 30 °C	
Ambient operating/storage humidity			70 % max. (with no condensation)	

● Fastest Readout Speed (Internal synchronization mode, Unit : frame/s typ.)

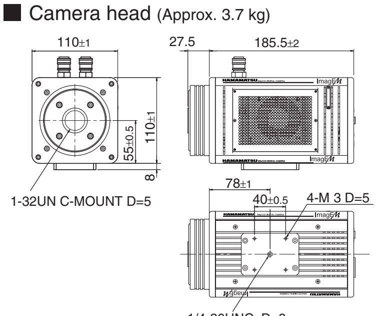
C9100-13						
Binning	Effective vertical width (Sub-array)					
	512	256	128	64	32	16
1 x 1	31.9	59.6	105	170	245	315
2 x 2	60.9	107	172	248	318	370
4 x 4	112	178	254	323	373	405
8 x 8 ⑨	177	252	320	369	401	417 ⑫
16 x 16 ⑨	248	313	360	389	405	413

C9100-14							
Binning	Effective vertical width (Sub-array)						
	1024	512	256	128	64	32	16
1 x 1	9.5	18.4	34.3	60.4	97.7	141	182
2 x 2	18.4	34.2	60.4	97.6	141	182	212
4 x 4	34.2	60.3	97.5	141	181	211	231
8 x 8 ⑨	60.2	97.2	140	180	210	229	240
16 x 16 ⑨	96.6	139	178	207	226	236	242

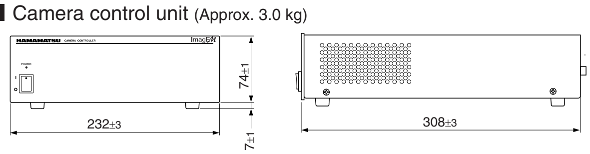
① The hermetic sealed head maintains a high degree of vacuum 10^{-8} Torr, without re-evacuation.
② AR mask is not placed because the proportion of CCD area to the window is large therefore reflection is quite small.
③ Even with electron multiplier gain maximum, dark signal is kept low level at low light imaging.
④ Linearity is not assured when full well capaicty is over 370 000 electrons (C9100-13) or 400 000 electrons (C9100-14), because of CCD performance.
⑤ The cooling temperature may not reach to this temperature depends on the operation condition.
⑥ Water volume 1.2 liter/min.
⑦ Typical thermal charge value (not guaranteed).
⑧ Image smearing may appear when the exposure time is short.
⑨ 8 × 8 and 16 × 16 binning are available on special order. Please consult with our sales office.
⑩ C-MOS 3.3 V with reversible polarity.
⑪ Recursive filter, frame averaging, spot noise reducer cannot be used simultaneously.
⑫ Fastest readout speed is at 8x8 binning, sub-array16.

5.3.1 Dimensional Outlines (Unit : mm)

■ Camera head (Approx. 3.7 kg)



■ Camera control unit (Approx. 3.0 kg)



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