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Revisiting Gen I- and Gen II-sCMOS Cameras for Live-Cell Fluorescence Imaging

- Global Shutter vs Rolling Shutter -

1. Introduction

The sCMOS (Scientific CMOS) camera contains multiple transistors within a single pixel and the number of these transistors determines the camera function and performance. Generation I (Gen I) sCMOS cameras employ a 5-transistor structure to achieve global shutter operation (described later on). Generation II (Gen II) sCMOS cameras, on the other hand, use a 4-transistor structure only offering rolling shutter operation (described later) yet delivering high sensitivity due to high quantum efficiency (QE) on the camera light-receiving surface and low readout noise (see Table 1 and Figure 1). It is becoming recognized that these features make the Gen II-sCMOS cameras the best performing camera for live-cell fluorescence imaging.

But some of us may lately be uneasy after hearing the unfamiliar term “rolling shutter” and viewing greatly distorted images of a rotating fan that were captured in rolling shutter mode. Figure 2 shows graphs of sCMOS camera pixel row positions and exposure timings. In global shutter mode, exposure of all pixel rows starts simultaneously as shown in Figure 2 (a). In rolling shutter mode, on the other hand, the start of exposure time is slightly delayed along the pixel row positions from the center to the outer side as shown in Figure 2 (b).

So recently, some reports are pointing out “negative points” of the rolling shutter such as the (1) spatial distortion and (2) time difference occurring due to the difference in exposure timing.

However, in actual live-cell fluorescence imaging, exactly what effect does the rolling shutter have on an image? We investigated these so-called “negative points” of the rolling shutter by way of simulation and measurement of actual samples.

Our research clearly revealed that the rolling shutter has negligible actual adverse effects on live-cell fluorescence imaging even when imaging the flagellar movement of sea urchin sperm and calcium changes in myocardial cells which exhibit the fastest movement and changes among samples usually observed by live-cell fluorescence imaging, and furthermore, using flashed illumination and global exposure timing reproduces exactly the image dynamics of global shutter, but with reduced sample bleaching and improved SNR.

Moreover, the Gen II-sCMOS camera features of high quantum efficiency and low readout noise demonstrate that the rolling shutter provides live-cell fluorescence imaging with excellent signal-to-noise image quality.

		Gen I	Gen II
Number of Transistors		5	4
Operable Shutter Mode		Global (or Rolling)	Rolling
Maximum Frame Rate	Global	50	-
	Rolling	100	100
Quantum Efficiency (550 nm) [%]		57	72
Readout Noise [e]	Median	2.3 (global)	1.2
	Average	3.0 (global)	1.6
	RMS	3.6 (global)	2.0

Table 1: Comparison of Gen I and Gen II

The Gen I-sCMOS camera has a 5-transistor structure within each pixel to achieve global shutter operation. The Gen II-sCMOS camera has a simple 4-transistor design that allows making the light-receiving area larger to enhance the quantum efficiency. The readout noise in global shutter operation is higher than that in rolling shutter operation.

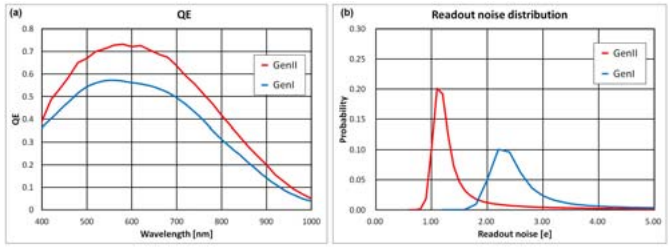

Figure 1: Quantum efficiency (QE) curve and readout noise distribution. For Gen I-sCMOS, the readout noise is shown in global shutter mode.

As can be seen from Figure 1 (a), the Gen II with its 4T design exhibits higher QE over the entire wavelength range. In Figure 1 (b), the readout noise distribution for Gen II was obtained from actual measurements. The readout noise distribution curve for Gen I was created by changing the noise distribution for Gen II so that the median value for Gen I readout noise comes to the above listed value of 2.3.

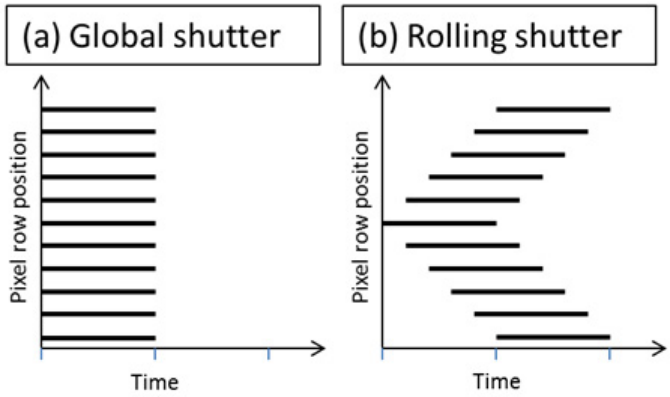

Figure 2: Exposure timing by pixel row position

In the global shutter (a) shown on the left, exposure of all pixel rows starts simultaneously. In the rolling shutter (b) shown on the right, the exposure start time is slightly delayed along the pixel row positions from the center to the outer side.

2. Distortion simulation

What kind of imaging conditions will create a “distorted image” in rolling shutter mode?

We know that usually the faster the camera target moves or the shorter the camera exposure time, the more likely that distortion will occur. Using a rotating fan as a sample target, we tried simulating the camera operation to clarify under what conditions the fan image becomes drastically distorted.

Simulation results revealed that the image becomes distorted in rolling shutter mode under the following conditions:

(1) Fan rotation speed is 10 Hz (rotates 10 times per second)

(2) Gen I (global shutter): Exposure time of 1 ms
Gen II (rolling shutter): Exposure time of 1 ms

Figure 3 shows simulation results under these conditions. As can be seen, the shape of the fan blades has become distorted in rolling shutter mode.

Now once again consider the fact that the fan rotates 36 degrees (one tenth of a revolution) per one image frame (10 ms). When observing cells and microorganisms under a microscope, there are virtually no cases in which the target moves at a speed where it travels one tenth of a revolution on the screen in a period of 10 ms (under standard measurement conditions of 30 fps with an EM-CCD, this is a speed at which the target moves three tenths of a revolution in one frame). This might be hard to understand in terms of rotations, so let’s try calculating the moving speed of a fan blade tip in order to convert it to linear movement. If we let the fan blade length be 1000 pixels which is roughly half of the screen (2000 pixels × 2000 pixels), we then obtain 1000 pixels*sin(36 deg) ≈ 600 pixels for a 10 ms period. In other words, this is equivalent to a speed that travels approximately one-third the distance from the top edge to bottom edge of the screen of 2000 pixels (moving speed from one edge of the screen to the other at 30 fps). So, as you might expect, there are no cases where live cells move at such high speeds.

This means that significant distortion such as seen in Figure 2 (b) will not occur in live-cell fluorescence imaging.

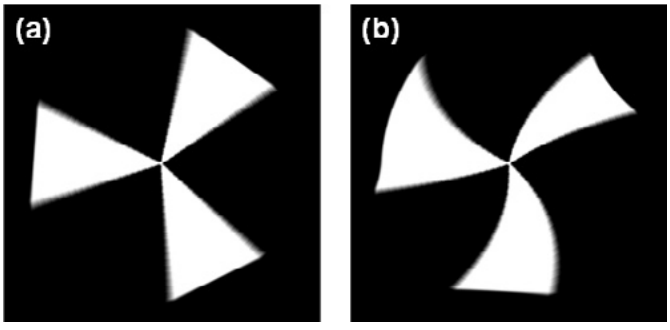

Figure 3: Images (simulation) captured from a fan rotating at 10Hz

Figure 3 (a) shows an image captured by Gen I in global shutter mode at a frame rate of 50 fps and an exposure time of 1 ms. No distortion is observed. Figure 3 (b) shows an image captured by Gen II in rolling shutter mode at a frame rate of 100 fps and an exposure time of 1 ms. The shape of the fan blades is distorted.

3. Image with noise simulation

In live-cell fluorescence imaging, the other most important elements are camera sensitivity and noise characteristics.

As already mentioned, compared to the Gen I-sCMOS camera, the Gen II-sCMOS camera has higher quantum efficiency and lower readout noise (see Figure 1). To find out how these features have effects on imaging of a dark moving sample unique to live-cell fluorescence imaging, we made simulations (see Figure 4) using a camera simulation engine we developed in-house.

We used the flagellum of sea urchin sperm that have extremely quick movement. Converting this flagellum movement into fan rotation gives an estimated rotation speed equivalent to 0.68 Hz (optical magnification of 40 times).

Assume that a fan rotating at 0.68 Hz emits two photons per pixel in a 10 ms period. Figure 4 shows results from a simulation of rotating fan images. The images (a) to (c) were captured by Gen I-sCMOS (global shutter) and the images (d) to (g) by Gen II-sCMOS (rolling shutter) when the frame rate was varied in a range from 100 fps (exposure time of 10 ms) to 10 fps (exposure time of 100 ms). A shorter exposure time is desirable when observing moving objects, but when the light level is very low such as in fluorescence observation, the number of photons incident on the camera during the short exposure time is small and so the image signal-to-noise ratio becomes poor as seen in (a) and (d). This means that extending the exposure time is unavoidable in order to improve the image signal-to-noise ratio. But this also causes image blurring to occur as in (a)→(b)→(c) and in (d)→(e)→(f)→(g). At an exposure time of 100 ms in (c) and (g), blurring is definitely occurring, so in this case an exposure time of about 50 ms as in (b) and (f) is preferred. At this exposure time, the Gen II-sCMOS gives an image with a better signal-to-noise ratio as seen in (f).

Moreover, the images (d) to (g) captured by Gen II-sCMOS (rolling shutter) have none of the distortion such as seen in Figure 3. This is because the fan motion is slow and exposure time is long compared to the conditions in Figure 3. Furthermore, extending the exposure time has the effect of suppressing distortion.

As shown in this example, extending the exposure time is unavoidable to some extent since the light level in live-cell fluorescence imaging is low, and in most cases the signal-to-noise ratio and blurring are more serious problems than image distortion. All of this shows that with its high quantum efficiency and low readout noise, the Gen II-sCMOS can provide images with minimal blurring and a good signal-to-noise ratio.

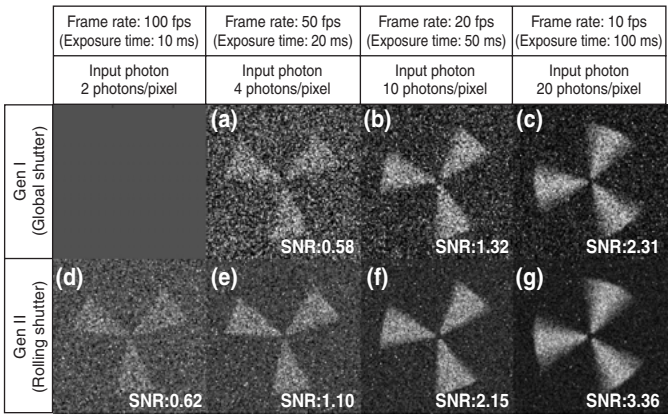

Figure 4: Rotating fan simulation images with camera sensitivity and noise taken into account

The images (a) to (c) in the upper row were captured by Gen I-sCMOS (global shutter), and the images (d) to (g) in the lower row by Gen II-sCMOS (rolling shutter). The Gen I-sCMOS camera is unable to capture images at a frame rate of 100 fps. The SNR stands for the signal-to-noise ratio (SNR = average of fan area / standard deviation of fan area).

4. Biological sample observation

Fluorescence observation using sea urchin sperm and C. elegans as samples is shown below.

Sea urchin sperm

Observation was made under the following conditions:

Gen I-sCMOS: Global shutter, exposure time 10 ms
Gen II-sCMOS: Rolling shutter, exposure time 10 ms
Fluorescence dye: Calcein-AM
Excitation wavelength: 488 nm
Fluorescence wavelength: 510 nm
Optical magnification: ×100
Imaging was performed using a beam splitting optical system to divide the light equally into 2 cameras.

Figure 5 shows images of sea urchin sperm captured with each camera.

Comparing both images shows no obvious distortion such as occurred in Figure 3.

Moreover, the Gen II-sCMOS camera has a better image signal-to-noise ratio as seen in (b).

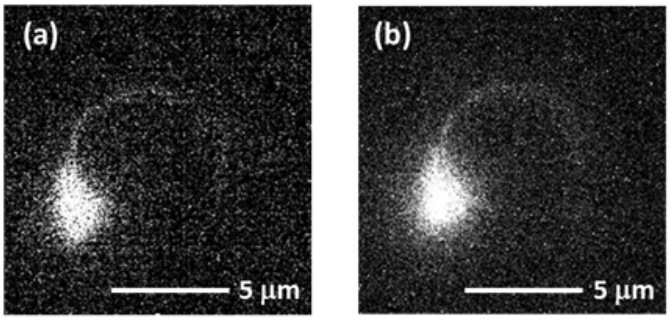

Figure 5: Fluorescence images of sea urchin sperm

The image (a) was captured by Gen I-sCMOS (global shutter) and the image (b) by Gen II-sCMOS (rolling shutter). In both images, there is absolutely no distortion such as seen in Figure 3 (b), and the image quality is clearly better with the Gen II-sCMOS

C. elegans

Observation was made under the following conditions:

Gen I-sCMOS: Global shutter, frame rate 10 fps, exposure time 10 ms
Gen II-sCMOS: Rolling shutter, frame rate 10 fps, exposure time 10 ms
Fluorescence dye: DiI (incubated in the DiI-containing medium)
Excitation wavelength: 561 nm
Fluorescence wavelength: 600 nm
Optical magnification: ×20
Simultaneous imaging was performed using a beam splitting optical system.

Figure 6 shows C. elegans images acquired with each camera.

Timestamp T for each image is as follows: T=0 ms for (a) and (d), T=100 ms for (b) and (e), T=200 ms for (c) and (f).

In this case, there is no problem with the image signal-to-noise ratio because the fluorescence intensity is high, so we shall focus on whether there is any image distortion.

The images (a) to (c) acquired by global shutter and the images (d) to (f) acquired by rolling shutter are nearly the same and so the rolling shutter has absolutely no distortion problems.

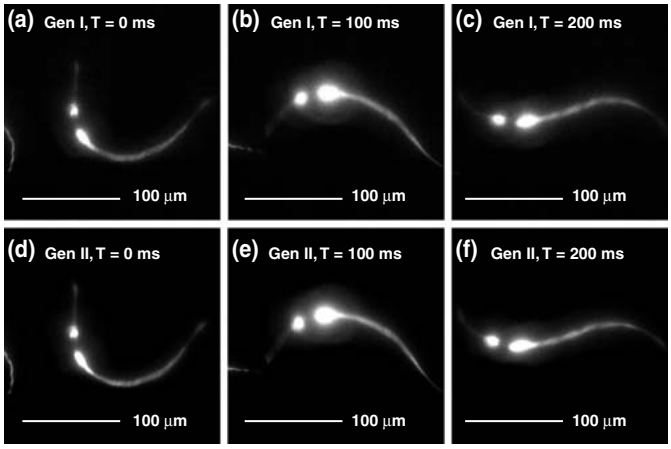

Figure 6: Fluorescence images of C. elegans

In Figure 6, (a) to (c) show images captured by Gen I-sCMOS (global shutter) and (d) to (f) show images captured by Gen II-sCMOS (rolling shutter). Timestamp T for each image is as follows: T=0 ms for (a) and (d), T=100 ms for (b) and (e), T=200 ms for (c) and (f). Comparing these images by timestamp shows that they provide the same results.

5. Fluorescence Intensity measurement over elapsed time

Calcium imaging in cardiomyocyte cells

To demonstrate that the rolling shutter time difference exerts negligible effects on measurements of fluorescence intensity changes over time, we performed calcium imaging in cardiomyocyte cells. In this section, we used an EM-CCD camera, which is normally used in this kind of application, as a global shutter camera for the measurement.

Measurement was performed under the following conditions:

EM-CCD: Global shutter, 2×2 binning, frame rate 200 fps, exposure time 5 ms

Gen II-sCMOS: Rolling shutter, 4×4 binning, frame rate 200 fps, exposure time 5 ms

Fluorescent dye: Fluo8 AM

Excitation wavelength: 488 nm

Fluorescence wavelength: 510 nm

Optical system magnification: ×10

Simultaneous imaging was performed using a beam splitting optical system.

The results are shown in Figure 7. In the figure, (a) is an image captured in global shutter mode (EM-CCD) and (b) is an image captured in rolling shutter mode (Gen II-sCMOS). Fluorescence intensity changes over time are measured for three cells in each image and plotted in (c) to (e). The time response of each cell is well matched, and the images clearly show there are no adverse effects from the time difference, which has been a concern.

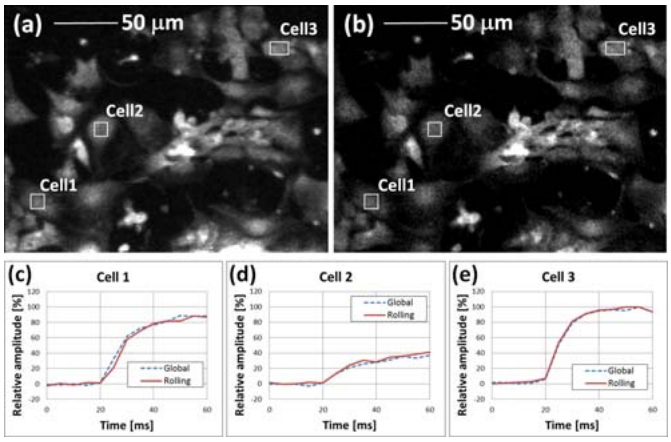

Figure 7: Calcium imaging in myocardial cells

In Figure 7, (a) shows an image captured by EM-CCD (global shutter) and (b) shows an image captured by Gen II-sCMOS (rolling shutter). Graphs (c) to (e) show the response time of three cells 1 to 3 in each image. Their time responses show a good match between global shutter and rolling shutter modes.

6. Global Exposure Timing mode

There are a few cases where short (e.g. less than 1/ frame rate) exposure times are required.

Our Gen II-sCMOS has a Global Exposure Timing mode that synchronizes the exposure with external illumination. In this mode, sample bleaching is greatly reduced compared to Gen I-sCMOS in global shutter, and simultaneously provides the same “freeze motion” characteristics as global shutter, but with the much higher SNR performance of rolling shutter

If you would like to know more about the Global Exposure Timing, please contact us.

7. Conclusion

All of the above conclusively proves that drastic image distortion in rolling shutter mode occurs only when capturing images of high-speed motion that is unlikely to occur for cells and microorganisms usually observed by live-cell fluorescence imaging. Under realistic conditions, there is virtually no distortion in images obtained in rolling shutter mode and problems are more likely to occur from the image signal-to-noise ratio and blurring. Also it was clearly demonstrated that the Gen II-sCMOS with its high quantum efficiency and low noise delivers images with minimal blurring and a high signal-to-noise ratio.

Moreover, measurements of fluorescence intensity changes over time showed negligible difference between global shutter and rolling shutter modes.

In those special cases where very short exposure times are appropriate, the Global Exposure Timing mode of the Gen-II sCMOS camera is the preferred imaging method.

Overall, there is no doubt that the Gen II-sCMOS is an excellent camera for fluorescence microscope imaging, including live cells.

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