CCD Camera Technology: Sensors Specialized for High Content Applications

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ABSTRACT Confusion exists within the high content market segment as to which camera technology is appropriate for the assays that are commonly addressed using this cell based quantitative analysis tool. Limiting the review to the two primary camera technologies available, interline CCD and sCMOS, benefits and drawbacks are described as well as each camera's relation to assays typically addressed by high content analysis. For quantitative analysis, when users employ multiple imaging techniques (brightfield, fluorescence, confocal) flexibility in binning and sensitivity are necessary to achieve the best assay results while balancing speed of scanning hundreds to thousands of wells. Careful review of the limitations and benefits of interline CCD and sCMOS for high-throughput quantitative analysis suggests interline CCD as having the greater potential to offer reliable results through-out multiple imaging techniques.

INTRODUCTION

Understanding the differences between interline charge-coupled device (CCD) and scientific, complementary metal oxide semiconductor (sCMOS) camera technology proves to be a difficult task for many of those interested in purchasing a high content instrument. These chips, which are the detection component of a scientific camera, is only one of many variables a researcher must consider when choosing a high content instrument, but it remains a source of debate among high content users.

Specifications of a high content platform can be confusing as well causing a potential user to have difficulty distinguishing between specifications that are important and those which are not. As one of the major optical components of a high content system, the camera must be given special consideration when choosing a high content platform. The approach when evaluating camera technology for high content analysis is different than the approach taken toward technology used in traditional microscopy. High content is focused on the generation of data from many biological samples in a very short time. With a pure microscopy approach, the user is likely focused more on the image rather than the quantity of cells that make up the data. Because of these divergent approaches, technologies for these purposes should be evaluated separately as there is rarely a solution or platform that can bridge both effectively. Therefore, when evaluating camera specifications for a high content platform, reliable data is the ultimate objective when using this powerful cell based technology.

It is important to note that a decision about high content instrument quality should not be limited to the camera. The camera is just one component that works in concert with the rest of the platform with the biggest impact on assay performance coming from the preparation of the sample, not the camera.

FINDING YOUR WAY THROUGH THE MAZE OF CAMERA SPECIFICATIONS

There are a host of camera specifications a potential high content user is presented with. Each of these specifications can present confusion if the user is not clear on the value of each. In addition, certain specifications should be considered differently for the quantitative nature of high content as compared to other areas of imaging which may be more qualitatively driven.

Three primary camera features focused on when purchasing a camera for bioimaging include: sensitivity, resolution, and speed (Fig. 1). This is also true for high content. However, a fourth feature, reliability, is a critical factor in the successful execution of this cell-based screening technology. These four features all contribute to the flexible detection capabilities needed in addressing the wide variety of assays using high content platforms.

Table 1 provides an explanation of each specification and relates its importance to the high content user. Each of these features do not operate in isolation, but rather effect one another directly. That is, if a camera is optimized for speed, there is a direct effect of that optimization on resolution and sensitivity.

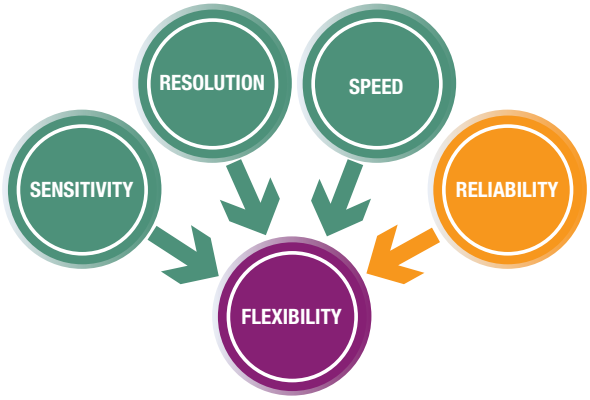

Figure 1: Camera features which effect high content platform flexibility.

CAMERA FEATURE	WHAT IS IT?	QUESTIONS REGARDING THE FEATURE'S VALUE TO YOUR HIGH CONTENT NEEDS
Flexibility	Camera's ability to reliability transition between widefield, confocal, and live cell imaging.	Is this camera capable of effectively imaging for widefield, confocal, and live cell if I decide I need all those capabilities now or in the future?
Speed	Often discussed in terms of frame rate (FR), it is the camera's ability to image fast enough to increase a user's throughput.	Can my camera image fast enough to collect the amount of information I need to make a decision quickly, or is the platform fast enough to handle the amount of plates planned to be screened?
Resolution	When discussing digital camera resolution it is often discussed in terms of pixel size, but this is the ability of a user's eye or the analysis software to distinguish between two separate points.	Will the camera be able to distinguish subcellular structures enough for the software to accurately generate the data I need?
Sensitivity	Often discussed in terms of quantum efficiency (QE), this is the sensor's ability to collect light, i.e., photons.	How can I be sure the camera on my instrument will be sensitive enough to image low levels of expression differences, e.g., two different but low intensity signals?

Table 1: Camera features and how they relate to their value for high content applications.

Therefore, a balance must be struck to find the best camera for high content imaging, and that requires the camera to be flexible enough for the operator to make adjustments as necessary based on application.

Both interline CCD and sCMOS cameras differ in their approach to addressing these four factors, but new CCD chips, like the Sony ICX694, are better able to offer the user the flexibility needed to address the variety of biological applications often seen in high content assays. sCMOS is currently marketed as being the most advanced option for high content imaging when, in fact, according to manufacturer's specifications and testing, it can lag behind new CCD chips for sensitivity and reliability. The remainder of this paper will discuss chip architecture; it's effect on speed, resolution, sensitivity, and reliability; and why CCD architecture is better optimized for high content performance as compared to sCMOS architecture.

CHIP DESIGN AND READOUT ARCHITECTURE: WHY IT MATTERS

The fundamental design of a CCD sensor consists of a series of parallel registers, a serial register, an amplifier, and an off-chip, analog-to-digital converter (Fig. 2). As the chip is exposed to light, photons rain down on the sensors, wherein each pixel begins to accumulate electrons. At the end of the exposure, the electrons are moved down the parallel registers one row at a time, into and across the serial register, and into an output node. They are then transferred to an amplifier and then to an analog-to-digital converter (ADC). As each pixel is read out, there is an inherent uncertainty in the electronic measurement, dependent upon read out speed among other factors; this uncertainty is referred to as read noise. The proportion of signal being read out to read noise is termed the signal-to-noise ratio (SNR).

Binning is the process which acts to increase the signal per pixel, without increasing read noise, thereby increasing the SNR. With binning, the user is allowed, through software, to affect the "pixel-shifting" process for increased sensitivity in the situation of low-light levels or the need for increased speed in the instance of dynamic imaging.

For example, in a 4x4 pixel array, wherein each pixel is able to hold ten electrons and there is a read noise of ten electrons, the signal-to-noise ratio is 1:1 which indicates that the signal and noise are equal, and a very poor image will result. If 2 x 2 binning is applied in this situation, the charge from two rows of pixels is shifted into the serial register. Each pixel in the serial register will then hold twenty electrons. When those electrons are shifted two pixels at a time into the summing well, the summing well/ output node will be holding forty electrons. Readout is done from the newly generated superpixel in this case, and the resultant read noise is equal to one readout event, or ten electrons. The resulting signal-to-noise ratio is 40:10 = 4:1. Although the super-pixel contains electrons from four pixels, there are fewer superpixels, thus effectively reducing the resolution.

CCD chips and sCMOS chips are designed differently, with sCMOS chips being the most efficiently designed with regard to economy of space and power. Unlike CCD sensors, sCMOS sensors do both amplification and digitization on-chip (ADC). Whereas CCDs shift pixels from the serial register into an amplifier, sCMOS has an amplifier for each pixel, and each column is read out to a column-specific amplifier and analog-to digital converter. Therefore, all of the columns in a row can be read out at once through the multiple analog-to-digital converters. This process increases speed, but the increased number of amplifiers and ADCs also causes increased variability between pixels and columns. Because CCDs readout to one amplifier and ADC, variability is much lower.

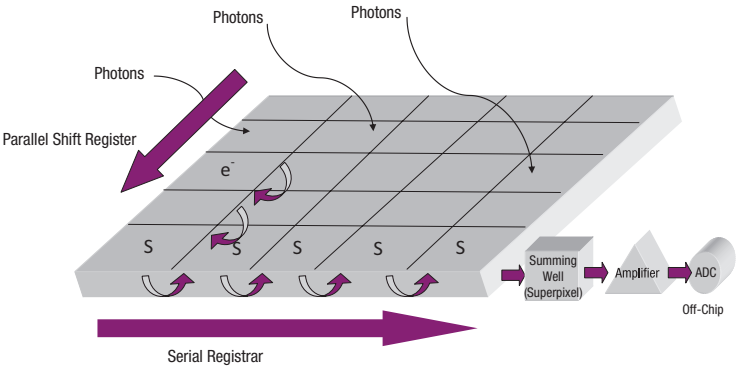

Figure 2: Readout of a CCD chip 4 x 4 pixel array. Row labelled with "S" indicates the serial register. Other pixels are part of the parallel shift register. After (summing if binning) and amplification, the signal moves off-chip and is then converted from analog to digital via the analog to digital converter (ADC).

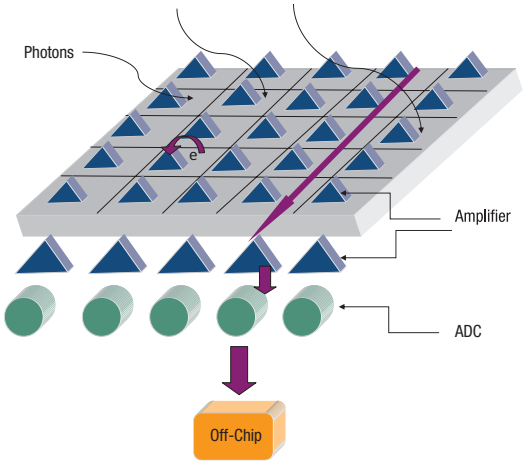

Figure 3: Readout of sCMOS chip. Each pixel has its own amplifier, and each column has its own amplifier and analog-to-digital converter (ADC). Readout is done on the chip, through multiple ADCs, thereby improving readout speed.

SHUTTERING

Shuttering refers to the imaging mode which exposes a camera sensor to light. For high content cameras, there are two shuttering modes: global and rolling. Global shuttering, the only imaging mode available for CCD cameras, exposes each row of pixels to simultaneously expose for the same amount of time and at the same time. The use of global shutter is ideal for both dynamic and fixed sample imaging. Global shuttering is also available for some sCMOS cameras, but at the cost of higher noise and a slower speed. [1]

The more dominant imaging mode for sCMOS is the rolling shutter. Rolling shutter exposes each row of pixels for the same amount of time but provides a delay between rows. Although the noise is less than using global shutter, rolling shutter is not ideal for live cell imaging due to the distortion that is generated when combined with dynamic imaging. [1], [2]

SENSITIVITY: EVERY PHOTON IS IMPORTANT

Quantum efficiency

A camera sensor's ability to collect photons to then return a signal is considered a measure of that camera's sensitivity. Quantum efficiency (QE) is the measurement detailing sensitivity, and it is expressed as a percentage of the photons reaching the sensor that are able to generate a signal. Taking into account the differences in chip architecture, Fowler et al. used a method to perform an estimation of CMOS quantum efficiency and found it to be approximately 37% [3] which is far below the average 60-70% QE [4] of a CCD camera. The release of sCMOS cameras revealed sensors with increased QE, but still below 60%, although it is expected that upcoming sCMOS cameras will incrementally improved QE over time. On the other hand, the newest bioimaging CCD cameras have a QE of nearly 75% and as high a >90% for back thinned CCD cameras. A later study on photon detection by Magnan from the Integrated Image Sensor Laboratory, showed CCD cameras without lateral overflow drains (a device preventing saturation) also to have a higher quantum efficiency than CMOS cameras. [5] Having a high quantum efficiency is especially important when a sample's signal is not very strong because very little light is being emitted to the sensor, a common challenge in high content assays. For confocal imaging, an "electronic signal may represent [between 3% and 16%] of the total fluorescence emission" [6] making camera sensitivity one of the decisive features for confocal applications.

Live cell imaging requires a highly sensitive detector in order to detect signal when light is decreased in order to reduce phototoxicity. Sensor sensitivity is so important, in fact, for live cell imaging that Brown [7] and Friguault et al. [4] only recommend CCD cameras as opposed to their sCMOS counterparts. If a camera has a low quantum efficiency, signal and thus biologically relevant information will be lost.

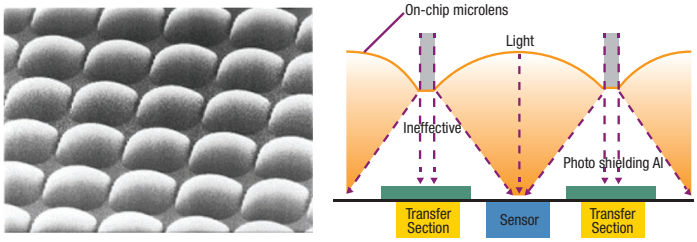

Figure 4: Microlens array (left). Light redistribution from non-photosensitive regions to photosensitive regions (right). Reproduced from King Leader, "Frequently asked questions," http://www.kl-security.com/technology/faq/. Accessed 09/20/2012.

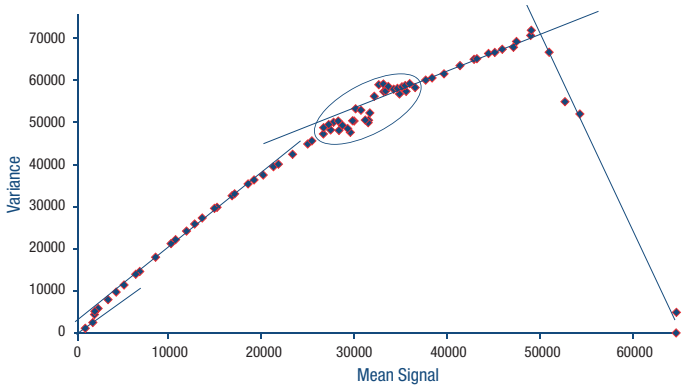

Figure 5: Mean-variance curve for sCMOS. Courtesy of Y. Sabharwal; Reproduced from Microscopy and Analysis 26 (1), January 2012, Copyright 2012 John Wiley and Sons Ltd.

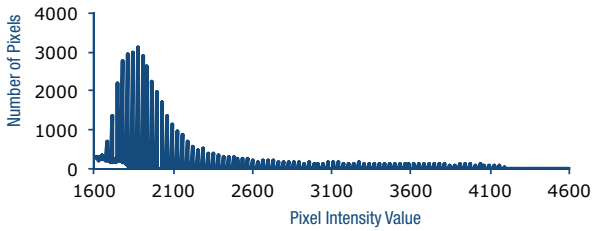

Figure 6: Intensity histogram for sCMOS. Courtesy of Y. Sabharwal; Reproduced from Microscopy and Analysis 26 (1), January 2012, Copyright 2012 John Wiley and Sons Ltd.

Dynamic range and linearity

Dynamic range refers to the maximum signal a camera can achieve (its full well capacity) divided by the sum of the camera's dark noise and read noise (see Reliability: Trusting Your Camera). In other words, both dim and bright signals are able to be quantitatively measured in a single image. Therefore, a camera's ability to perform well in low-light conditions requires a large dynamic range. There are some features a CCD sensor may employ to further enhance its sensitivity, and thus, its dynamic range assuming noise remains the same. One such feature is the already reviewed binning process. Binning of a CCD sensor can be used to boost signal by combining the electrons from multiple signals before readout. Microlens arrays (Fig. 4) are another addition to chip technology in which photons hitting non-photosensitive interline masks are collected and redistributed over the photosensitive regions of the chip. Using both these features is a powerful way to enhance the sensitivity of an interline CCD camera.

sCMOS cameras also have a method to increase dynamic range by using a different gain amplifier depending on the intensity of the pixel. Multiple gain amplifiers can have detrimental effects such as increasing the variability of the camera. Figure 5 shows the effect of multiple gains and their associated ADCs of a 6.5-µm pixel sCMOS camera on the mean-variance curve. The mean-variance curve of most scientific CCD cameras increases linearly with a slope related to the camera's gain. However, a sCMOS mean-variance graph displays two linear regions with different slopes. If biological samples with different intensities are being compared, quantitative data analysis becomes problematic due to erratic camera behavior based on the average intensity level.

Also surprising is the intensity histogram of the 6.5-µm pixel sCMOS camera (Fig. 6). During the comparison of CCD and sCMOS images, Sabharwal [8] cites another anomaly with the sCMOS camera's 16-bit images. The histogram has been mathematically stretched by inserting gaps which results in only 11-bits of data (ADC has a bit depth of 11-bits) being delivered, rather than 16-bits.

RESOLUTION AND NOISE: INCREASING SNR WITHOUT REDUCING RESOLUTION

Arguably, the signal to noise ratio (SNR) is the most weighted feature when assessing camera quality. Methods to increase signal, and the SNR, have been discussed in the previous section. For instance, binning is one method to increase sensitivity, but resolution is often reduced in the process. Reducing pixel size is often considered in order to provide for higher resolution at the expense of lower light collecting capacity and thus a low SNR. Reducing noise is another method to increase SNR. As an inherent characteristic of each camera system, noise is unpredictable with each measuring event. There are a number of noise classifications, but for simplicity we mention only read noise, dark noise, and telegraph noise.

Noise in chip technology

Read noise is the source of variability in each readout event. It is particularly apparent in low light situations, which are often common in high content screening assays. For a CCD sensor, read noise follows a Gaussian distribution across the pixels in the sensor (Fig. 7). In contrast, sCMOS read noise distributions are non-Gaussian and follow a positive-skewed distribution (Fig. 8) with 42% of the pixels having a higher than average read noise. [9],[10] Read noise from an sCMOS camera may be seen as either column to column variation (Fig. 9) across an image or a salt-and-pepper effect commonly referred to as telegraph noise.

Telegraph noise is specifically due to the unpredictable variation from pixel to pixel across a sCMOS sensor and is unique to sCMOS cameras especially in low light applications. [10] It can be removed through data post-processing, [11] but this

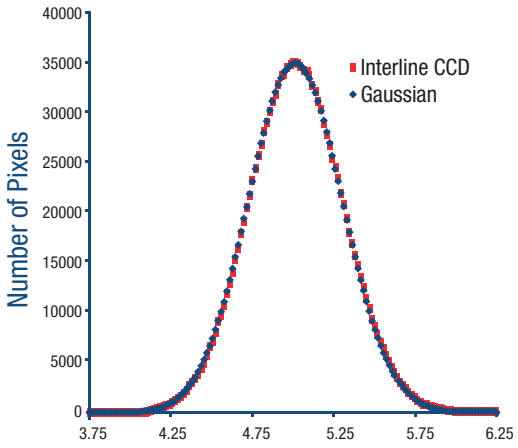

Figure 7: Gaussian distribution of read noise for CCD sensor. Courtesy of Y. Sabharwal, J. Joubert, and D. Sharma; Reproduced from Microscopy and Analysis 25, published online September 2011, Copyright 2011 John Wiley and Sons Ltd.

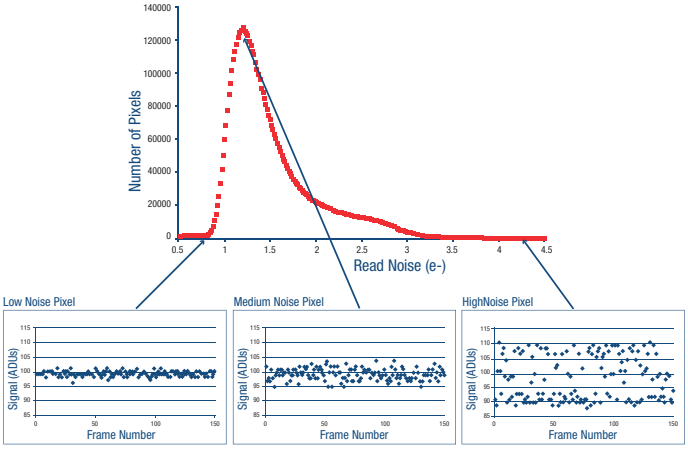

Figure 8: Non-gaussian, positively-skewed distribution of sCMOS sensor. Variability of noise among different pixels. Courtesy of Y. Sabharwal, J. Joubert, and D. Sharma; Reproduced from Microscopy and Analysis 25, published online September 2011, Copyright 2011 John Wiley and Sons Ltd.

can potentially result in an image not necessarily representing a raw image. Joubert and Sharma [11] demonstrated that averaging CMOS images to reduce read noise is less effective than averaging CCD images with Gaussian read noise due to the telegraph noise inherent in sCMOS technology. More sCMOS images needed to be averaged versus CCD images to reduce noise. Therefore, when averaging the intensities of many different cells in order to distinguish a treated from a control sample, sCMOS noise reduction will be less effective. If small differences in signal are to be measured, more samples and/ or images would be required.

Dark noise is the result of thermoexcitation, as opposed to photoexcitation. The dark noise is highly dependent on camera temperature and the rate of accumulation is referred to as dark current. Most cameras used in the bioimaging and high content areas have integrated cooling, thus reducing the dark current to where the contribution of dark current is negligible.

SPEED: IS IT WORTH IT?

Camera speed is often discussed in terms of frame rate (FR). However, speed when referring to the frame rate of the camera, has little if anything to do with speed of the high content platform and is rarely the bottleneck of "throughput". sCMOS is clearly the faster chip due to its readout process with framerates of 30 frames per second (fps) in times of continuous frame capturing, although newer cameras may allow faster continuous imaging. [1] CCD cameras are somewhat slower at 3–11 fps. [1] sCMOS cameras are able to readout in two different directions for the two halves of the chip. Therefore, frame rate is doubled, but variation is introduced. This variation is manifested

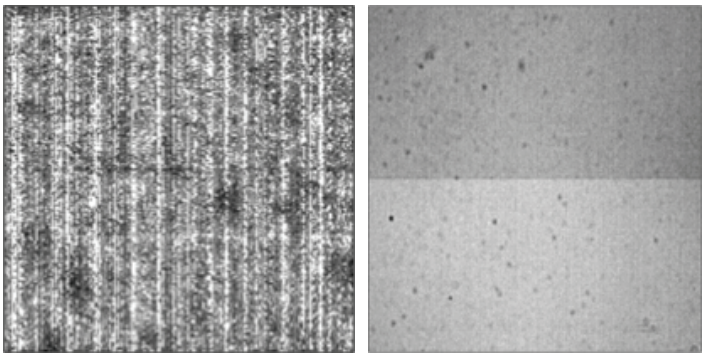

Figure 9: (Left) Column to column variation due to multiple column amplifiers of sCMOS. Reproduced with permission from Photometrics. (Right) Manifestation of bi-directional readout of sCMOS chip. Courtesy of Y. Sabharwal; Reproduced from Microscopy and Analysis 26 (1), January 2012, Copyright 2012 John Wiley and Sons Ltd.

by an image with a top and bottom half with distinctly different intensities. So although speed is something to consider when comparing camera technology, the sensitivity and dynamic range required for most applications in high content cannot be sacrificed for the sake of speed.

RELIABILITY: TRUSTING YOUR CAMERA

Interwoven throughout the previous sections were characteristics of sCMOS for which specifications can be impressive, but for certain applications, the sCMOS technology is not practical, utilized, or can be detrimental to assay performance. Performance reliability refers to the ability of the camera to capture the information from a sample with as little variability as possible in order to produce repeatable imaging and analyses across other high content imaging systems. If non-Gaussian readout noise, non-linearity, 16-bit images with only 11-bits of data, and pixel-to-pixel and column-to-column variation are considered, the ability to reliably reproduce an imaging and data analysis event is in question.

A sensor with low variability is necessary to provide the stability and low variation needed for imaging and analyzing complex bioapplications. Only statistically relevant images produced by a statistically relevant camera can provide statistically relevant data. CCD cameras provide the sensitivity, high SNR and dynamic range, and a readout process optimized for high content.

CCD DELIVERS FLEXIBILITY TO HIGH CONTENT ASSAYS

As discussed, both sCMOS chips and CCD chips have advantages. Latest generation CCD chips such as the Sony IXC684 deliver large field of view and high performance for high content screening. sCMOS, which must be differentiated from less expensive, less capable consumer CMOS chips, exhibit impressive performance for speed but lag behind in QE and resolution.

So which sensor technology is better? Remember the goal of a high content platform is to rapidly capture images which are used to derive cell level measurements to make a decision about a phenotypic change. One could easily argue that the camera, although important, is not as important as the analysis software, speed of the instrument, or the informatics database used to collate images and data. When choosing to solely review camera technology as it relates to high content platforms, CCD sensors outperform the current sCMOS technology because of the QE and higher resolution.

When users employ multiple imaging techniques (brightfield, fluorescence, confocal) flexibility in binning and sensitivity are necessary to achieve the best assay results while balancing speed of scanning hundreds to thousands of wells. Therefore, as an all around, high performance tool, the latest CCD technology delivers more value to high content assays than sCMOS does.

WRITERS NOTE

Audra Ziegenfuss is a Technical Product Manager with Thermo Fisher Scientific representing Thermo Scientific High Content Products. She has a background in cellular imaging and microscopy and has been successfully published on her collaborative imaging projects.

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