

# Choosing the Best Camera for Fluorescence Microscopy

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Choosing the most suitable camera for fluorescence microscopy can appear to be a daunting task when faced with the variety of camera technologies and models on offer. The technical specifications provided may also be difficult to understand and relate to how they will affect imaging for any given application. In recent times the scientific Complementary Metal-Oxide Semiconductor cameras (sCMOS) and Electron Multiplying Charge Coupled Device (EMCCD) have been the two main camera technologies available to the life science researcher. Meanwhile the older interline Charge Coupled Device (CCD) cameras that were once widely used have now largely been displaced. sCMOS cameras are known for their large fields of view and high frame rate performance, while EMCCD-based cameras offer the greatest sensitivity, down to the single photon level. However, it should be noted that EMCCD cameras are often seen as a premium option due to their higher cost.

Since our earlier article in the December 2014 edition of **infocus**, a new generation of sCMOS

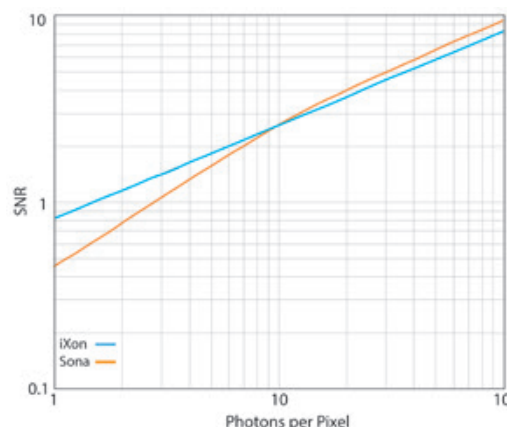


Figure 1: A comparison of the signal to noise ratio (SNR) against illumination level (photons per pixel) for back-illuminated sCMOS (Sona) and back-illuminated EMCCD (iXon).

cameras featuring back-illuminated sensors has become available, promising greater sensitivity than previous generations of sCMOS cameras based on front-illuminated sensors. The increase in sensitivity coupled with large sensor sizes and speed that sCMOS cameras are already known for make an attractive case to many fluorescence microscopy applications. But are they always more suitable? And how do they compare to EMCCD which has continued to remain the detector of choice for the most demanding low light applications?

## Key parameters to consider:

For fluorescence microscopy there are three key parameters that need to be considered:

**Sensitivity:** How sensitive is the camera? i.e. a measure of the ability to detect photons emitted from fluorophores

**Field of View:** The size of the sample area that can be seen (and resolved) in a single field of view

**Speed:** How well can the camera resolve temporal information from dynamic events?

The importance of each of these parameters will vary depending on the specific application, however, sensitivity is often the primary consideration. We will look at cameras summarised in Table 1 as examples of the different camera types available, but first, let's look at the different camera technologies.

## Competing Camera Technologies

### EMCCD

Cameras based on EMCCD sensors were introduced in 2001 and represented a significant leap forward compared to standard CCD cameras, combining ultra-sensitivity with speed. Central to an EMCCD

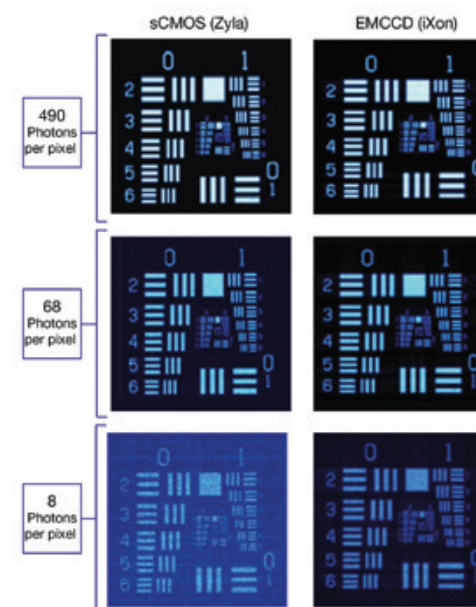


Figure 2: Comparative low light images taken with Andor Zyla front-illuminated sCMOS (1.4 electrons read noise @ 560MHz) vs Andor iXon 888 back-illuminated EMCCD (< 1e- read noise), under a range of photon intensity conditions plots. sCMOS was 2x2 binned in order to have the same effective pixel pitch (and light collection area per pixel) as the 13  $\mu\text{m}$  pixel of the EMCCD sensor. The superior sensitivity of back-illuminated EMCCD under extremely low light conditions is readily apparent.

is an on-chip amplification that multiplies even single photon events well above the read noise floor by a mechanism termed “impact ionization”. Thus, EMCCDs are capable of single photon sensitivity at high frame rates. This established EMCCD as the camera of choice in applications demanding ultra-low light measurements, such as single molecule detection and photon counting experiments. While a well optimized EMCCD can be thought of as having a “zero noise floor”, there is a downside to this technology. The EM amplification carries an additional noise source called multiplicative noise (sometimes called EM noise or Excess Noise Factor). EM noise effectively increases the shot noise (or Poisson noise) of the signal by a factor of  $\times 1.41$ . This is observed as an increase in both pixel-to-pixel and frame-to-frame signal variability. EMCCD cameras also tend to have larger pixel sizes (e.g. 13 or 16  $\mu\text{m}$ ) which are good for sensitivity at low light levels, but less suited to resolution at lower objective magnifications.

## sCMOS

sCMOS cameras were first introduced in 2010 by Andor and other camera companies as a compelling alternative solution to the aging interline CCD sensor-based cameras. sCMOS-based cameras rapidly became the technology of choice for many applications across both life and physical sciences. The popularity of sCMOS-based cameras over other camera technologies (CCD and EMCCD) is largely due to their very useful set of attributes for microscopy imaging: low noise, large sensor sizes while achieving fast frame rates. There are now many models available, typically at lower price points than EMCCD cameras, meaning that there is an sCMOS camera option for most research budgets.

There have been several refinements to the sensitivity of sCMOS sensors that have seen quantum efficiency (QE) jump from  $\sim 60$  to 82%, edging their sensitivity closer towards that of EMCCD. Until recently, sCMOS sensors have been of the front-illuminated type whereby photons must pass through the bulk silicon and the electrode structure to reach the photosensitive area of the sensor and create charge. Therefore, a portion of the light is absorbed or reflected and so reduces the efficiency of collection and conversion of photons into electrons. Back-illuminated sensors provide higher QE as obstructions to incoming light have been placed behind the photosensitive area. Back-illuminated CCD and EMCCD sensors have been available for some time, but it is only recently that sCMOS sensors suitable for microscopy have benefited from back-illuminated sensors. One advantage of this is a higher QE, for example, the new Andor Sona 4.2B-11 sCMOS camera has a QE of 95% which is comparable to that of EMCCD at the emission wavelengths of common fluorophores such as GFP.

### Sensitivity

The Signal-to-Noise Ratio (SNR) is of key importance when considering the sensitivity of any imaging sensor. This defines the ability of the camera to detect a signal and distinguish it from

the surrounding noise. The Signal to Noise ratio for a given light level at a specific wavelength can be described by the equation:

$$SNR = \frac{S}{\sqrt{S + [ND \times T^2] + NR^2}}$$

Where:

S= The signal level arriving at the detector (electrons)

ND= Dark Current (electrons/pixel/second)

NR= Read Noise (electrons)

T = Exposure (seconds)

**Note:** In the case of EMCCD cameras, the additional EM noise factor of x1.41 must be accounted for.

The approach, therefore, used by camera manufacturers is to a) maximize the light signal reaching the sensor by using a sensor with as high a Quantum Efficiency (QE) as possible and, b) seek to reduce the sources of noise within the system e.g. by cooling.

Why is sensitivity so important? At the most simple level, a more sensitive camera will be more effective at gathering light and producing a high fidelity image at low light levels that are inherent to many fluorescence microscopy applications. However, there are also many practical benefits of greater sensitivity including:

- Reduced laser illumination intensity - keep cells alive throughout study (i.e. suppress phototoxic effects) and also limit dye photobleaching
- Reduced fluorophore concentrations – help maintain accurate physiology in living specimens
- Lower exposure times - follow faster processes and get better temporal information
- Get better SNR with TIRF and confocal low light modalities – resulting in better image clarity for techniques that reject out of focus photons

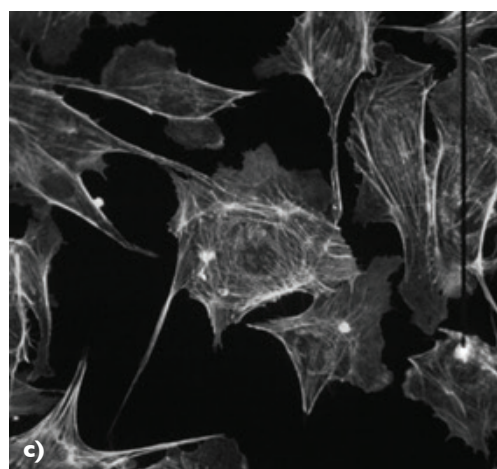
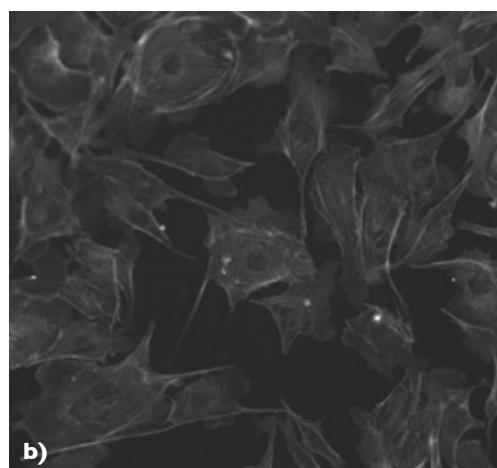
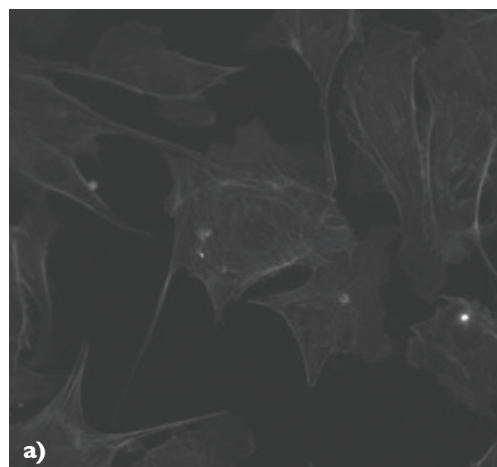


Figure 3: Comparison of a) Zyla, b) Sona, and c) iXon 888 cameras with 100ms exposure and at 60x magnification. The BPAE cells in a)-c) have a nucleus diameter of ~20 microns. The Zyla has been set with 2x2 binning to bring the smaller pixel up to a comparable effective size as the iXon and Sona. The iXon is set at an EM gain of x300. Images courtesy of Jeff Oleske, Andor Technology.

- Single Molecule Biophysics - resolve single molecules and spatial positions (Chaurasiya et al. 2018)

There are several ways cameras can be compared to evaluate their sensitivity. This can help us shortlist potential candidates for an application. However, practical comparisons of the camera under the exact conditions of the desired application are likely to be the most definitive.

Firstly, we can compare the cameras using the signal to noise equation; SNR values can typically be found on a camera's specification sheet. Moreover, the SNRs can be compared and evaluated over different levels of illumination as shown in the graph in Figure 1. Therefore, the camera which has the best SNR at the light levels required for the desired application can be determined. For example, in Figure 1 the SNRs are compared for an example of a new back-illuminated sCMOS camera (Sona) and a back-illuminated EMCCD camera (iXon) at different light levels.

The iXon EMCCD camera still provides a better SNR at light levels below ~10 photons per pixel, while Sona provides better SNR above this crossover point. It should be noted that for many fluorescence microscopy applications the image data spans the crossover point between sCMOS and EMCCD. In addition, these theoretical measurements are further complicated by the difference in pixel sizes, sensor sizes, and the conversion factor of the camera from photons to electrons.

Alternatively, a calibrated light source and target can be used to compare the images produced at the different light levels. Comparisons of sCMOS against EMCCD using this approach shown in Figure 2 that again demonstrate the superior performance of the EMCCD at low light levels against front (and back) illuminated cameras. Therefore, it is useful to compare the test pattern images of the different cameras at different light levels as this can highlight whether one camera may be better suited to a certain light level.

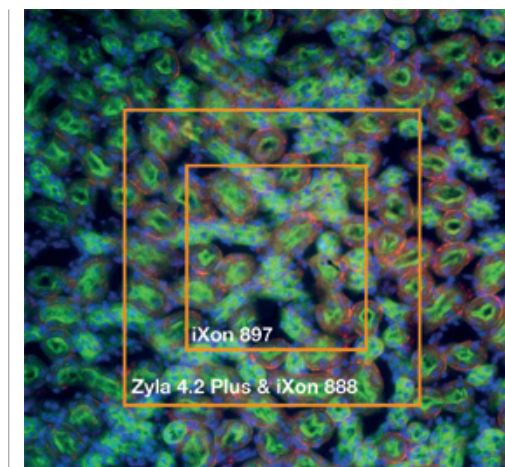


Figure 4: Comparison of the field of view of Andor's Sona 4.2B-11 (FOV 22.5 x 22.5 mm) against other camera models. Orange boxes represent relative size of the respective field of views observable using the Zyla 4.2 PLUS (13.3 x 13.3 mm) and iXon 888 (13.3 x 13.3 mm) & 897 (8.2 x 8.2 mm) camera models. Kidney cells stained with three fluorescent dyes. Glomeruli and convoluted tubules stained with Alexa Fluor 488 (green). Actin stained with red-fluorescent Alexa Fluor 568 phalloidin. Nuclei counterstained with DAPI (blue), Camera Nikon Ti2.

Another method of comparison is simply to set up the cameras for the desired application and compare the results. Indeed, this is often the most practical and effective way to compare the cameras. Consequently, in Figure 3 we compare images of the same sample from the three cameras listed in Table 1. In this example, we can clearly see that with the same acquisition settings simulating a light restricted condition, the image taken using the iXon camera gives the best contrast and clarity, largely due to the EM gain. Further, it is evident from Figure 3 that Sona also delivers a superior image to the Zyla. Moreover, from Figures 3 a), b) and c) the difference in field of view between the cameras is evident, with Sona (b) having a wider field of view of the sample than images taken using either the Zyla or the iXon cameras.

## Field of View

The camera field of view is largely dictated by the sensor size, larger sensors allowing for larger fields of view. However, the field of view must be matched with the microscope to use the available sensor area and avoid vignetting. In order to match the microscope and camera field of view, additional



magnification in the form of lens couplers is often used, as the field of view will depend on the specific objective lens used. The field of view available through modern microscopes continues to increase, with 22 mm now being common and models exceeding this now available.

Pixel size is also very important; smaller pixels will help ensure Nyquist sampling and achieve good resolution, whereas, larger pixels are advantageous for sensitivity. Therefore, a compromise on pixel size must be made to optimise the signal to noise and image resolution.

A larger on-sample field of view is of great practical use for a range of studies, as it allows more of the sample to be seen in one field of view and provides more information. Areas where this is of importance include:

- **Developmental biology** – capture whole embryos, e.g. Zebrafish
- **High Content Screening** – capture larger fields of cells, increase information content
- **Tissue Cultures** – minimize the need for image stitching, maximize throughput
- **Organoids** – unravel cell connectivity
- **Gene Editing** – screen large cell cultures for cells whose genomes have been successfully edited e.g. CRISPR constructs

Figure 4 shows an image of kidney cells taken using Andor's Sona 4.2B-II. Additionally, the respective field of views are overlaid on the image of the Zyla 4.2 PLUS and iXon 888 and 897 camera models. It is clear from Figure 4 that the field of view of the Sona camera is the largest of the models compared here. Although Zyla 4.2 PLUS and Sona 4.2B-II each have a 2048 x 2048 sensor, it is the larger 11 µm pixel size of the Sona that accounts for the difference in their respective field of views. This makes the Sona 4.2B-II the clear choice for whenever the largest field of view is required because more of the sample can be observed in one image. Zyla 4.2 PLUS and iXon 888 have the same sensor size again due to pixel size and array size differences (Table 1). The

6.5 µm of the Zyla 4.2 PLUS gives the potential for better resolution at magnification objectives e.g. x60 without the need for lens couplers to match the microscope field of view to the camera field of view.

### Speed

Biological processes can be highly dynamic making the ability to capture events at high speeds the most critical parameter. For example, studies that may require high speed imaging include:

- **Ion Signalling** - Follow fast calcium wave propagation and calcium sparks with maximum temporal dynamics e.g. in nerve cells (Voleti *et al.* 2017)
- **Cell Motility** – Speed capability is critical for following cell movement, e.g. sperm cell dynamics.
- **Intracellular transport** – Fast frame rates can be important to follow intracellular transport dynamics, including membrane dynamics or micro-tubule associated proteins (Monroy *et al.* 2018)
- **Blood flow** – perhaps one of the most temporarily challenging applications: speed is of paramount importance (Carboni *et al.* 2016)
- **Localization super-resolution** e.g. SRRF. Many raw images must be rapidly acquired for a single super-resolved output image therefore boosted speed is critical, especially if live cell super-resolution is the true goal (Gustafsson *et al.* 2016)

As with previous sCMOS models, back-illuminated sCMOS cameras like the Sona allow for imaging at high frame rates, making them well suited to applications such as those listed above. Since the available sensor sizes tend to be relatively large, Region of Interest (ROI) can often be used to provide higher frame rates at the expense of resolution, while 12-bit readout mode can be utilised to accelerate frame rate by up to 2x, albeit sacrificing the dynamic range. This is useful for imaging fast processes using low light modalities

Table 1: A summary of camera models discussed throughout this article.

Camera Model	Camera Type	Max. Quantum Efficiency (%)	Array size	Pixel Size (µm)	Field of View (mm)
Zyla 4.2 PLUS	Front-illuminated sCMOS	82	2048 x 2048	6.5	18.8
Sona 4.2B-II	Back-illuminated sCMOS	95	2048 x 2048	11	32
iXon 897	Back-illuminated EMCCD	95	512 x 512	16	11.6
iXon 888	Back-illuminated EMCCD	95	1024 x 1024	13	18.8

such as spinning disk confocal or TIRF.

It must be noted that the Zyla 4.2 PLUS with Camera Link connection will deliver higher frame rates than Sona, making the Zyla 4.2 PLUS the best option for where speed is the most important parameter. Some sCMOS camera models such as the Zyla 5.5 and Neo 5.5 have a different mode of operation called Global Shutter. Global Shutter cuts the frame rate in half but the sensor operation mode means that the whole image exposure starts and ends at the same time, eliminating spatial distortion effects. Thus, for experiments in which objects are moving rapidly e.g. for sperm motility, the Zyla 5.5 and Neo 5.5 may be the preferred choice over Rolling Shutter based cameras that use a rolling line-by-line exposure which may produce motion artefacts with fast moving objects.

EMCCD cameras still manage a high frame rate performance, especially when using crop mode, and should be considered for very low light conditions. At low light levels or short exposures times, sCMOS cameras may simply not be sensitive enough to detect the signal. In these cases, EMCCD is still the preferred option despite the on-paper disparity in frame rates.

### Conclusion

The emergence of back-illuminated sCMOS cameras is a welcome development for practitioners

in many areas of research, including fluorescence microscopy techniques. The back-illuminated sCMOS cameras add increased sensitivity to the large field of view and fast frame rate performance that front-illuminated sCMOS are well known for. Therefore, the new back-illuminated sCMOS cameras, such as the Sona, are well suited to a diverse range of applications, including high content screening and developmental biology. Moreover, they are suitable for studies that require a wide field of view and high sensitivity, so that illumination levels and fluorophore concentrations can be reduced, and exposures shortened for the most accurate measurements of cell physiology.

There are some conditions in which cameras based on the other technologies still offer advantages over the new back-illuminated sCMOS cameras. The Zyla 4.2 PLUS offers a good overall performance for general fluorescence applications at a lower price point. The Zyla 4.2 PLUS model that uses a Camera Link connection, as opposed to USB 3.0 allows for a higher data transmission rate and so provides a higher frame rate. EMCCD based cameras such as iXon remain the detectors of choice in Andor's range for the most demanding applications due to greater sensitivity even at the lowest light levels. For example, one of the main uses of EMCCD cameras in microscopy has been for single molecule biophysics experiments, and this

is unlikely to change significantly. EM amplification enables a level of sensitivity that back-illuminated sCMOS cameras still cannot reach.

This article provides an overview of the different camera technologies available and their relative strengths with regard to sensitivity, field of view and speed. The weighting of these parameters does depend on the specific needs of your research; therefore, it is good practice to evaluate a shortlist of models suitable for your microscopy system so that you can make an informed decision.

## References:

Voleti, V., Li W., Shaik M. A., Wu M., Campos C. P., Wyart C., and Hillman E. M. C., "Imaging the nervous system at different spatiotemporal scales with SCAPE microscopy," in Optics in the Life Sciences Congress, OSA Technical Digest (online) (Optical Society of America, 2017), paper BrS2B.3.

Chaurasiya K.R., Dame R.T. (2018) Single Molecule FRET Analysis of DNA Binding Proteins. In: Peterman E. (eds) Single Molecule Analysis. Methods in Molecular Biology, vol 1665. Humana Press, New York, NY

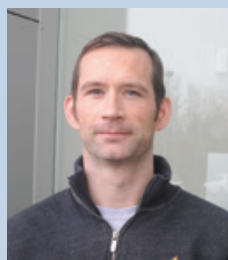
Monroy B.Y., Sawyer D. L., Ackermann B. E., Borden M. M., Tan T. C. and Ori-McKenney, K. M. Competition between microtubule-associated proteins directs motor transport. Nature Communications, volume 9, Article number: 1487 (2018)

Erik J. Carboni, Brice H. Bogner, Grant M. Bouchillon, Andrea L. Kadlak, Leslie M. Shor, Michael D. Ward, Anson W.K. Ma. Direct Tracking of Particles and Quantification of Margination in Blood Flow, Biophysical Journal, Volume 111, Issue 7, 2016, Pages 1487-1495.

Gustafsson, N.; Culley, S.; Ashdown, G.; Owen, D. M.; Pereira, P. M.; Henriques, R. Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations. Nature Communications 2016, 7, 12471

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Dr Allan Mullan is the Product Specialist for Microscopy Cameras and has worked at Andor technology for 6 years. This role brings him in touch with the latest developments in camera technology as

well as a broad range of microscopy applications. Prior to this he has worked in both the Medical Diagnostics and Pharmaceutical industries. His background in microscopy is built from his work and studies at Queen's University Belfast. He was a post-doctorate Research Fellow focused on the study of phosphate transport and polyphosphate metabolism in micro-organisms. Microscopy techniques such as widefield fluorescence, confocal, luminescence and Electron Microscopy were key parts of this work. He received his PhD in Microbiology, also while at Queen's University Belfast.

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Dr Marsh recently joined Andor Technology as a Technical Author and Copywriter. Dr Marsh maintains specifications and documentation for the Andor product

range, keeping her up to date on camera technologies and microscopy applications. She completed her PhD in Chemistry at the University of Bristol in 2018. During her PhD research in the field of aerosol science she used single particle techniques coupled with Raman spectroscopy, elastic light scattering and bright-field microscopy, to improve understanding of the impact of aerosols on human health and climate.

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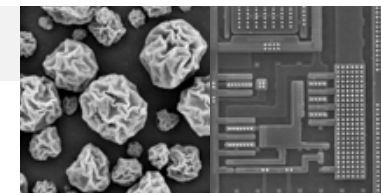
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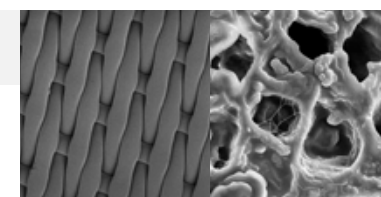
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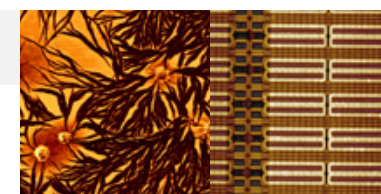
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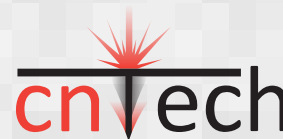
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