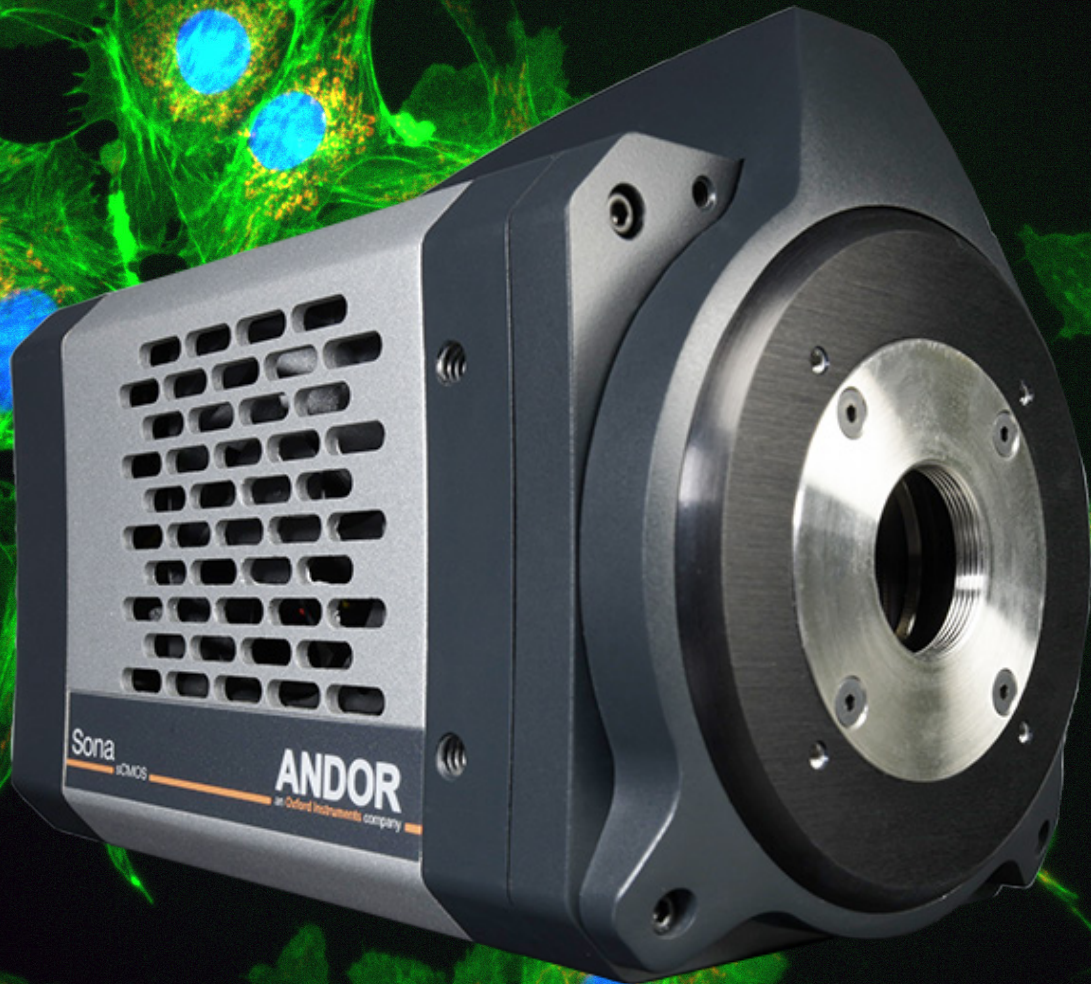




ANDOR
an Oxford Instruments company



**Purchasing a Back-illuminated
sCMOS for Microscopy?**

**Seven Reasons To Choose
Sona**

Technical Note

Purchasing a Back-illuminated sCMOS for Microscopy: 7 Reasons to Choose Andor Sona

Introduction

The new Sona microscope sCMOS camera platform has been meticulously designed from the ground up to optimize performance, extracting the absolute best out of sensors that have been selected for integration. Back-illuminated sCMOS sensors are growing in popularity, primarily due to the best-in-class Quantum Efficiency (QE) performance that they offer. It therefore makes sense to combine these sensors with best-in-class performance across all other key parameters. For those who have made the decision to purchase a back-illuminated sCMOS camera, here we offer 7 key reasons that the Andor Sona models should be your back-illuminated sCMOS of choice.

1- The Most Sensitive Back-illuminated sCMOS Available

Sona 4.2B-11 and Sona 2.0B-11 back-illuminated sCMOS models each feature **95% Quantum Efficiency (QE)** with **market-leading vacuum cooling to -45 °C**.

The darkcurrent of GPixel back-illuminated sCMOS sensors is relatively high, compared to that of BAE/Fairchild Imaging sCMOS sensors that are utilized in Zyla and Neo sCMOS cameras. This places additional emphasis on the need to deep cool the sensor in order to suppress the noise floor, i.e. minimizing the camera detection limit. Due to the unique vacuum design, Sona thermoelectrically cools to -25 °C using only the internal fan for heat dissipation. Furthermore, Sona can utilize liquid assisted cooling to push down to a hugely competitive -45 °C!

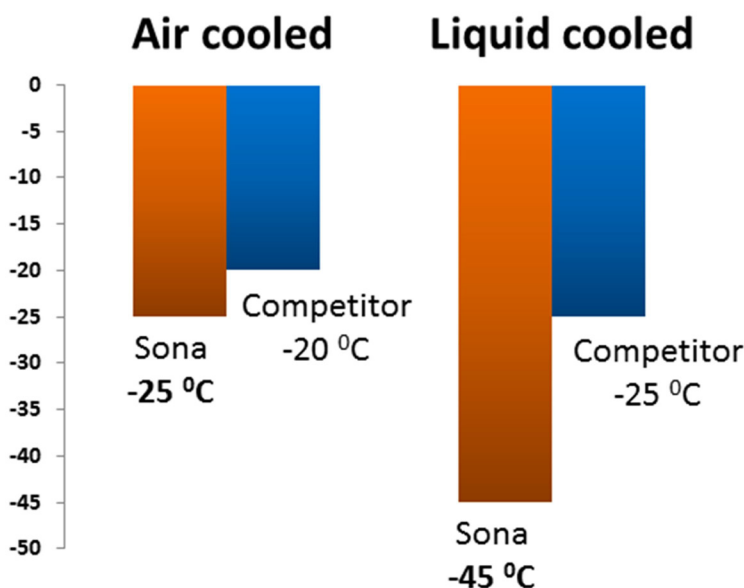


FIGURE 1: Air (fan) cooled and liquid cooled performance of Sona back-illuminated sCMOS models, versus the nearest competitive back-illuminated sCMOS camera type.

Back-illuminated sensors are valued specifically for their enhanced sensitivity – it makes sense to choose the most sensitive camera adaption of this high-end technology.

Having the most sensitive Back-illuminated sCMOS camera carries a host of practical advantages within fluorescence microscopy:

- ✓ **Reduced laser illumination intensity** - keep cells alive throughout study (i.e. suppressing phototoxic effects) and also limit dye photobleaching
- ✓ **Reduced fluorophore concentrations** - maintaining accurate physiology in living specimens
- ✓ **Lower exposure times** - follow faster processes
- ✓ **Better SNR with TIRF and confocal low light modalities** - better image clarity with techniques that reject out of focus photons.

2 – Largest Field of View Available: Sona 4.2B-11

The Sona 4.2B-11 model offers the largest field of view solution, compared with competitive back-illuminated sensors that also use the same GPixel GS400 BSI sensor type.

The Sona 4.2B-11 is native F-mount and can be compared against “Competitor A” below, a camera using the same sensor but cropped down to 1608 x 1608 pixel format. By cropping the sensor down, this camera can avoid sensor glow issues that affect the edges of this sensor. However, Sona 4.2B-11 uses a unique **Anti-Glow Technology** approach that enables the full native 2048 x 2048 of the array to be harnessed. Figure 2 shows the 62% larger field of view advantage offered by Sona 4.2B-11.

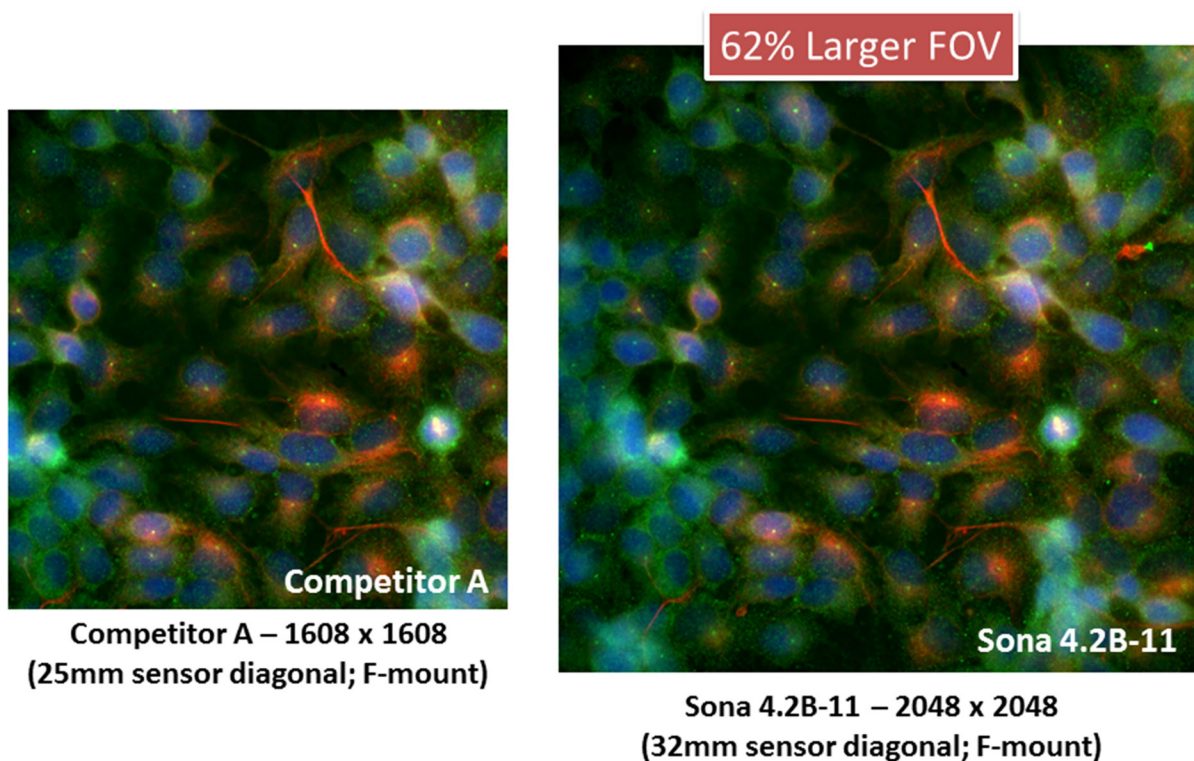


FIGURE 2: “F-mount competitive solutions” – Field of View comparison between Sona 4.2B-11 and a competitor F-mount camera, utilizing the same GS400B back-illuminated sCMOS sensor but restricted to 1608 x 1608 max resolution. Captured using a Nikon Ti2 with 60x objective and integrated 1.5x tube lens. The Sona 4.2B-11 has 62% more active pixels and offers a compelling field of view solution.

Maximizing on-sample field of view with Back-illuminated sCMOS cameras is vital for a range of studies, including:

- ✓ **Developmental biology** – capture whole embryos, e.g. Zebrafish
- ✓ **High Content Screening** – capture larger fields of cells, increase information content.
- ✓ **Tissue Cultures** – minimize stitching, maximize throughput.
- ✓ **Organoids** – unravelling cell connectivities.
- ✓ **Gene Editing** – screening large cell cultures for cells whose genomes have been successfully edited.

For further detail on Andor’s unique approach to optimizing sCMOS Field of View, see Andor’s Technical Note entitled: “Optimizing Field of View and Resolution in Microscopy: Matching sCMOS Camera to Objective Lenses”.

3 – Enhanced Mounting Flexibility: One camera, Multiple ports

(a) Sona 2.0B-11

The Sona 2.0B-11 is native C-mount and is **adaptable to various microscope C-mount port diameters, up to 22mm**. The 1400 x 1400 full array size of this model is suited to modern 22mm C-mount ports and maximizes the field of view available through this common mount type.

However, pre-configured, centrally positioned ROIs are available, directly relating to various smaller microscope port sizes:

ROI Size	C-Mount Port Diameter	Example Microscopes
1400 x 1400	22 mm	Nikon Ti2, Olympus IX83/73
1220 x 1220	19 mm	Leica DMI8
1157 x 1157	18 mm	Various research microscopes

TABLE 1: Pre-configured ROIs of the C-Mount Sona 2.0B-11 model, shown alongside the corresponding microscope Port Diameter / Field Number for which they are optimized.

Alternatively, smaller ports can be used with the full 1400 x 1400 array size by utilizing the **Andor Magnifying Coupler Unit**. This is a complimentary coupler that can readily connect to the port, expanding the image available from the microscope onto the larger sensor area. A 2x coupler also has the benefit of achieving Nyquist resolution utilizing a 60x objective, which in turn further optimizes the on-sample field of view.

(b) Sona 4.2B-11

The Sona 4.2B-11 offers ultimate flexibility to all ports. The full array is 32mm, which when coupled with the Nikon Ti2 F-mount and in-built 1.5x tube lens offers an unparalleled on-sample field of view and superb uniformity.

Alternatively, the **Andor Magnifying Coupler Unit** can be used with a large variety of modern research fluorescence microscopes and corresponding ports, providing additional 2x magnification onto the large sensor area of the Sona 4.2B-11. Since we are 2x magnifying the image onto a 32mm diameter sensor area, then the Magnifying Coupler Unit can be attached to any port that offers an image output of 16mm or greater. This describes the vast majority of available ports.

Finally, while the Sona 4.2B-11 is native F-mount, the user can readily convert the camera to either C-mount or T-mount, simply by selecting these additional accessories at time of order.

Note that the Andor Magnifying Coupler Unit is T-mount at the camera attachment end, requiring that the camera is converted to T-mount before use with this item. This has the advantage that the coupling will be absolutely light tight - light leak being sometimes otherwise associated with F-mount side port usage.



FIGURE 3: *The Andor Magnifying Coupler Unit (orderable with Sona models)*

4. Superior Quality & Longevity – UltraVac™

Why vacuum technology? As well as affording superior minimization of the noise floor, the **performance longevity** benefits of Andor's vacuum sensor enclosure should not be overlooked.

REASON 1: Sensor Protection - Unless protected, back-illuminated silicon sensors are susceptible to attack from moisture, hydrocarbons and other gas contaminants, resulting in gradual performance decline, **including QE decline**. Ultravac™, Andor's *proprietary* approach to vacuum enclosures, with minimized out-gassing, offers the ultimate level of sensor protection.

REASON 2: No re-backfilling of sensor enclosure required - Cameras that do not use vacuum enclosures instead use a method called back-filling, whereby the sensor enclosure contains a positive pressure of dry gas, separated from the external atmosphere by only O-ring seals. Over time, moisture and gas from the atmosphere will ingress into the sensor enclosure and compromise the system, resulting in loss of cooling capability and often moisture appearing on the sensor. The cameras then have to be sent back to the factory for repair, re-backfilling and resealing, often outside of the warranty period. The UltraVac™ uses a **hermetic vacuum seal**, completely preventing any gas ingress from the outside environment. The vast majority of cameras hold their cooling performance **indefinitely**.

Sona is the **ONLY** back-illuminated sCMOS camera on the market that benefits from a vacuum sensor enclosure. Vacuum sensor technology is one of Andor's core technology strengths, and we have a fantastic track record of vacuum integrity and associated camera longevity, stretching back more than 25 years.

5- Higher Quantitative Accuracy

Sona 4.2B and Sona 2.0B each offer **Extended Dynamic Range** functionality, supported by a 16-bit data range. Harnessing an innovative ‘dual amplifier’ sensor architecture, we can access the **maximum pixel well depth AND the lowest noise simultaneously**, ensuring that we can quantify extremely weak and relatively bright signal regions in one snap. This functionality is useful for accurately visualizing and quantifying many challenging samples that have both weak and bright regions, such as neurons.

To achieve best in class quantification accuracy, Andor have implemented enhanced on-head intelligence to deliver **linearity of > 99.7%**.

Why do we need superb linearity? Many applications require accurate quantitative information rather than simply structural detail; any measurement where intensity correlates to quantity or concentration will benefit from superior linearity. This can refer to physiological parameters such as calcium, pH, cAMP etc with single and dual wavelength dyes. Related to this is FRET analysis, which is used to measure concentration (by chelation e.g. calmodulin) as well as distance or co-localization at the nanometer scale. Gene expression analysis with fusion proteins also requires superb quantitative accuracy, where again intensity is directly correlated to concentration. Localization Super-Resolution Microscopy can also be impacted by poor linearity because the Gaussian fit could become skewed.

6- Fast Speed Mode

Sona 4.2B and Sona 2.0B offer fast frame rate capability, rendering them ideal for following dynamic cell processes such as ion signalling, cell motility and blood flow, while avoiding image smear. Region of Interest (ROI) and 12-bit readout mode can be utilised to considerably boost frame rates further.

12-bit mode for 2x speed boost! Sona 4.2B and Sona 2.0B are each architected to offer both 16-bit and 12-bit modes. 12-bit is selected specifically to accelerate frame rate by 2x, while sacrificing wide dynamic range, useful for imaging fast processes using low light modalities such as spinning disk confocal or TIRF.

	Max Frame Rate (fps)	
ROI size	Sona 4.2B-11	Competitor B
2048 x 2048	48	24
1608 x 1608	60.5	30

Table 1: Not all commercial cameras utilizing the GS400 sensor offer this boosted speed capability. Maximum frame rates of Sona 4.2B-11 are shown here versus a competitive camera using the GS400 sensor that does not offer this 12-bit fast speed mode.

Maximizing speed with Back-illuminated sCMOS cameras is extremely useful for a range of studies, including:

- ✓ **Ion Signalling** - Follow fast calcium wave propagation and calcium sparks with maximum temporal dynamics, with further accelerations possible through use of ROIs. For elongated smooth muscle cells, rectangular ROIs can be used (up to the full width of the sensor) without imposing a speed compromise!
- ✓ **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 dynamic, including membrane dynamics.
- ✓ **Blood flow** – perhaps one of the most temporarily challenging applications: speed boost is critical!
- ✓ **Localization super-resolution** – Back-illuminated sCMOS is increasingly popular for localization super-resolution, as the higher QE yields higher SNR and therefore better localization accuracies. However, many raw images have to be rapidly acquired for a single super-resolved output image: boosted speed is critical, especially if live cell super-resolution is the true goal.

7- Enhanced Flexibility: Adapt to multiple microscopy experiments and set-ups

The Sona platform has been designed to meet the needs and challenges of modern research, which often requires that the camera has inherent flexibility to be adapted across multiple set-ups and experimental configurations. The following areas of flexibility are inherent to the Sona platform:

- ✓ **Air and liquid Cooling** – not only can the Sona be used in liquid cooled operation to maximize sensitivity in extreme low light conditions, use of liquid cooling rather than fan assisted cooling can also be beneficial in experiments that are particularly vibration sensitive, such as electrophysiology experiments or combined optical/AFM set-ups.
- ✓ **16 and 12-bit modes** – The 16-bit mode is the High Dynamic Range mode, ideal for imaging samples that have both weak and bright signal regions. The 12-bit Fast Speed mode can be utilised to double the available frame rate from any selected ROI size, superb for adapting to low light experiments that require excellent temporal resolution, such as ion signalling or blood flow imaging.
- ✓ **Adapt to multiple ports or objectives** - As covered in more detail in point 3, both Sona 4.2B-11 and Sona 2.0-11 models can be readily used across a range of microscopes with varying port sizes and coupling attachments (e.g. F, C and T-mount). This makes the camera easily transferable across multiple experimental set-ups. Furthermore, the **Andor Magnifying Coupler Unit** can be utilized to adapt the 11 μm pixel sensor to lower magnification objectives, thus maximizing the on-sample field of view.
- ✓ **Flexible pixel binning** – The Sona models feature on-camera flexible pixel binning, user definable to 1 pixel granularity. Greater binning flexibility can be useful for some applications where resolution can be sacrificed in favour of enhanced photon collection area per pixel - e.g. extremely lower light bioluminescence experiments.
- ✓ **Timestamp** – The Sona platform can generate a timestamp for each image that is accurate to 25 nanoseconds. Accurate timestamps can be important where precise knowledge of frame time impacts temporal dynamic analysis. This is especially important for fast events, where computer and interface latencies need to be considered. Areas include signalling cascades, vesicle trafficking, lipid dynamics, synaptic re-modelling, action potential studies using opto-genetics and opto-physiology. Timestamps can also be useful for FRAP Analysis, facilitating the estimation of diffusion rates.