Technical Note

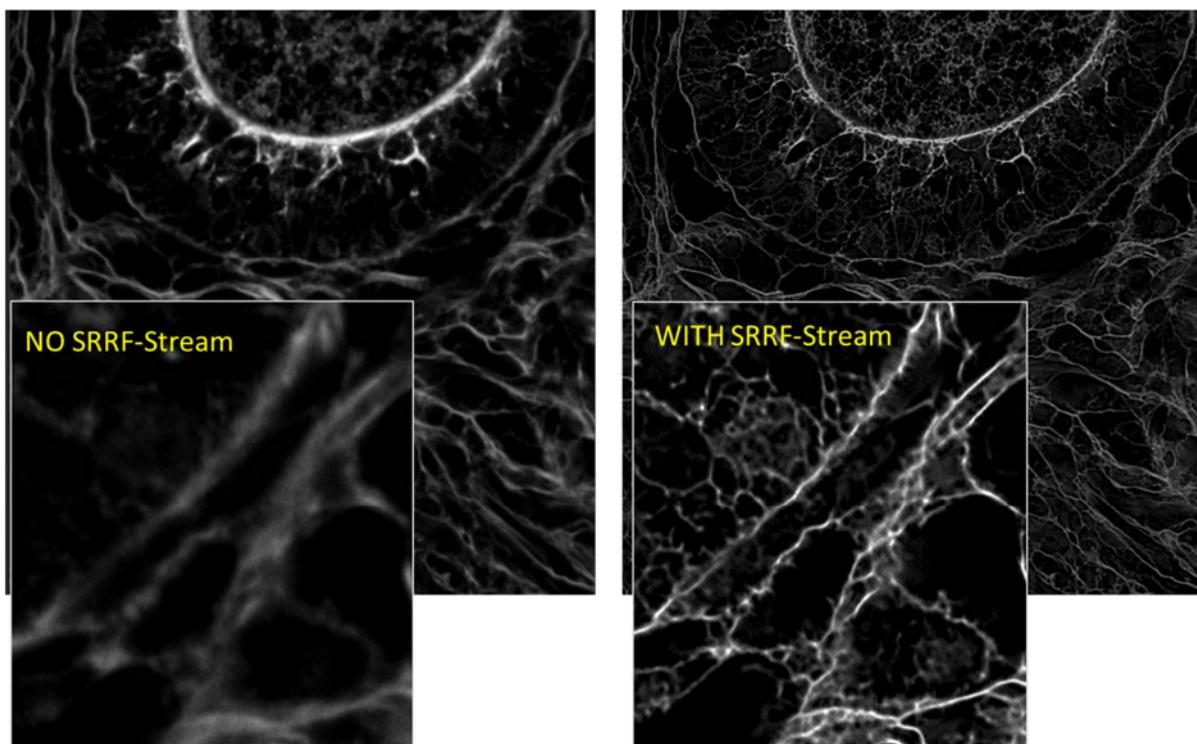## SRRF-Stream Upgrade: Boost your Current iXon EMCCD with Super-Resolution Capability

What is SRRF-Stream?

Andor 'SRRF-Stream' is a Real-Time Super-Resolution Microscopy module that is offered as an extension of iXon Life and iXon Ultra EMCCD camera functionality. SRRF-Stream presents a powerful super-resolution approach that is also widely accessible, being (a) applicable to most existing modern fluorescence microscopes, and (b) compatible with conventional fluorophores, such as fluorescent proteins. That is to say, with SRRF-Stream there is no requirement to use specialized photo-switchable fluorophores as is typically required for localisation super-resolution approaches. The resolving power of SRRF-Stream is excellent, yielding a resolution improvement between 2- and 6-fold (50-150 nm final resolution) for most datasets.

SRRF-Stream is also highly applicable to live cell microscopy, utilizing fluorescence excitation powers in the mW/cm^2 to W/cm^2 range. Thus, it is therefore possible to achieve high-performance super-resolution microscopy with $\geq 10^6$ times lower excitation power than that typically used in localization based super-resolution approaches. Furthermore, super-resolution image rates in excess of 10 fps can be achieved, and output in real time, meaning even rapid physiological processes can be followed with high temporal resolution. Large field of view super-resolution images can also be achieved in real time, meaning large fields of cells can be viewed, yet with an intracellular resolution that can readily discern sub-organelle structure.

> For further detailed information on SRRF-Stream, please download the Technical Note entitled:
>
> **"SRRF-Stream: Real Time Super-Resolution in a Camera"**

NO SRRF-Stream

WITH SRRF-Stream

Can I Upgrade my Current iXon EMCCD for SRRF-Stream functionality?

Yes, provided you currently own an **iXon Ultra 888**, **iXon Ultra 897**, **iXon Life 888** or **iXon Life 897** model, Andor can now upgrade your camera to unlock SRRF-Stream super-resolution microscopy capability. This represents an extraordinarily cost-effective means to access powerful, real time super-resolution technology from your current conventional microscope and camera, potentially obviating the need to invest in a fully dedicated super-resolution system.

What is the upgrade process?

1. Contact your local Andor sales representative and tell them you wish to upgrade your existing eligible iXon model. If you do not know who this is, simply request a SRRF-Stream quote via the Andor web page and we will contact you!
2. Your sales representative will request the serial number of your existing iXon. We will use this to confirm upgrade eligibility.
3. Your sales engineer will also advise you as to a recommended performance specification of PC and CUDA-compatible NVidia GPU card for SRRF-Stream. Alternatively, you can purchase a SRRF-Stream workstation direct from Andor as part of your order.
4. Place an order using product code 'SRRF-UPG-IXON', upon which we will ship the SRRF-Steam upgrade package (including installer and license) to you, specific to your iXon serial number. Note that if you own more than one iXon camera, the version of SRRF-Stream that we will provide will only operate on the iXon camera you have specified that you want to upgrade; it will not work on other iXon cameras.
5. *IMPORTANT: If you own an iXon Ultra 888 or iXon Life 888 that has not previously been optimized for SRRF-Stream at time of order, then you <u>must</u> also place an order for SRRF-Stream Camera Optimization (product code: 'SRRF-OPTIM-IXON'). The core reason for this is explained in greater detail later in this technical note.*

SRRF-Stream Upgrade Checklist:

- ✓ An existing iXon Ultra or iXon Life; 888 or 897 model
- ✓ SRRF-Stream Upgrade License and Installer ('SRRF-UPG-IXON') - upgrades iXon to SRRF-Stream compatible
- ✓ *If you own an iXon 888 model:* SRRF-Stream Optimization (SRRF-OPTIM-IXON)
- ✓ A PC with CUDA-compatible NVidia GPU card*; or Andor's **SRRF-Stream Workstation**
- ✓ MicroManager software (64-bit, latest nightly latest build or version 1.4.22) or alternatively Andor SDK2 (64-bit, version 2.97.30005 onwards). Note, these softwares come pre-installed on Andor's SRRF-Stream Workstation if ordered.
- ✓ A fluorescence microscope with widefield, TIRF or spinning disk confocal modality.

* The Nvidia GPU card should have Compute Capability v3.0 or above and 4GB or greater on-board GPU RAM. Note that Andor have done extensive testing using the 'mid-range' GTX 1070 and found that, with SRRF-Stream, it is more than adequate to process data much faster than the rate of iXon data acquisition.

Why do we need to 'SRRF-Stream Optimize' the iXon 888 models?

Andor are being very careful to ensure we provide the best possible image quality in our SRRF-Stream enabled cameras. We are being particularly careful when it comes to the iXon 888 models, because the image sensor may exhibit some low level (sub-read noise) structure that can be effectively pulled out and made visible by the SRRF algorithm. This effect can be particularly apparent under very low light conditions. It is less visible in imaging conditions under which the image is dominated by photon shot noise from signal and background. It is important to understand that this is not structure that appears under standard imaging, it has only become an issue when used in conjunction with SRRF functionality.   Figure 2 (a) provides a view as to how this structure may manifest in some 888 cameras in low light conditions, apparent as occasional low level column structure in the image. In some cases, you may see several of these columns across the width of the full image area.

Andor have developed an additional process that we use to optimise our new iXon cameras for optimal image quality in SRRF-Stream, which we apply when the camera is ordered with SRRF-Stream functionality. This optimization process MUST also be applied when upgrading existing iXon 888 models in order to REDUCE* the column structure effect. For this, the camera will be shipped back to Andor's manufacturing base in Belfast, Northern Ireland. Note, a standard return shipping charge would also apply.

*While the SRRF-Stream optimization process will reduce the column effect on upgraded cameras, Andor cannot guarantee the extent to which the effect will remain, even after optimization. The only way to guarantee absolutely optimal image quality is to order a new iXon Life 888 or iXon Ultra 888 with SRRF-Stream. We would simply not ship a new camera for SRRF-Stream usage unless it passes a specific quantitative test threshold that relates to this column effect.

Recommended acquisition settings to further optimize iXon 888 SRRF-Stream image quality:

It has been observed that the possible SRRF-Stream column effect on iXon Ultra and iXon Life 888 models may be further significantly reduced by following these acquisition parameter guidelines:

- Do not use EM Gain greater than x500. This is sufficient multiplication gain to ensure read noise is negligible. Higher EM gain values can bring out the column effect in very low light images.
- Use of faster Vertical Clock Speeds, settings readily accessible through the Device Property Browser of MicroManager or through SDK2.
  Two alternative recommendations for this:
  (a) **Simplest approach to column effect reduction** - 1.1 μs/row Vertical Clock Speed combined with 'Normal' Vertical Clock Voltage.
  (b) **Most effective approach to vertical column reduction, but slightly more complicated** - 0.6 μs/row Vertical Clock Speed combined with +1, +2, +3 or +4 Vertical Clock Voltage. Whilst this absolute fastest vertical shift speed is recommended to minimize possible SRRF-Stream column effects with iXon 888 models, it also yields a significantly distorted image when used with 'Normal' Clock Voltage. The recommended approach is to step through the available Vertical Clock Voltage settings until image quality has been restored. This is then the recommended Vertical Clock Voltage setting for that specific camera under the fastest Vertical Clock Speed setting, and this combination can then be used for all SRRF-Stream acquisitions. It does not need to be investigated again.
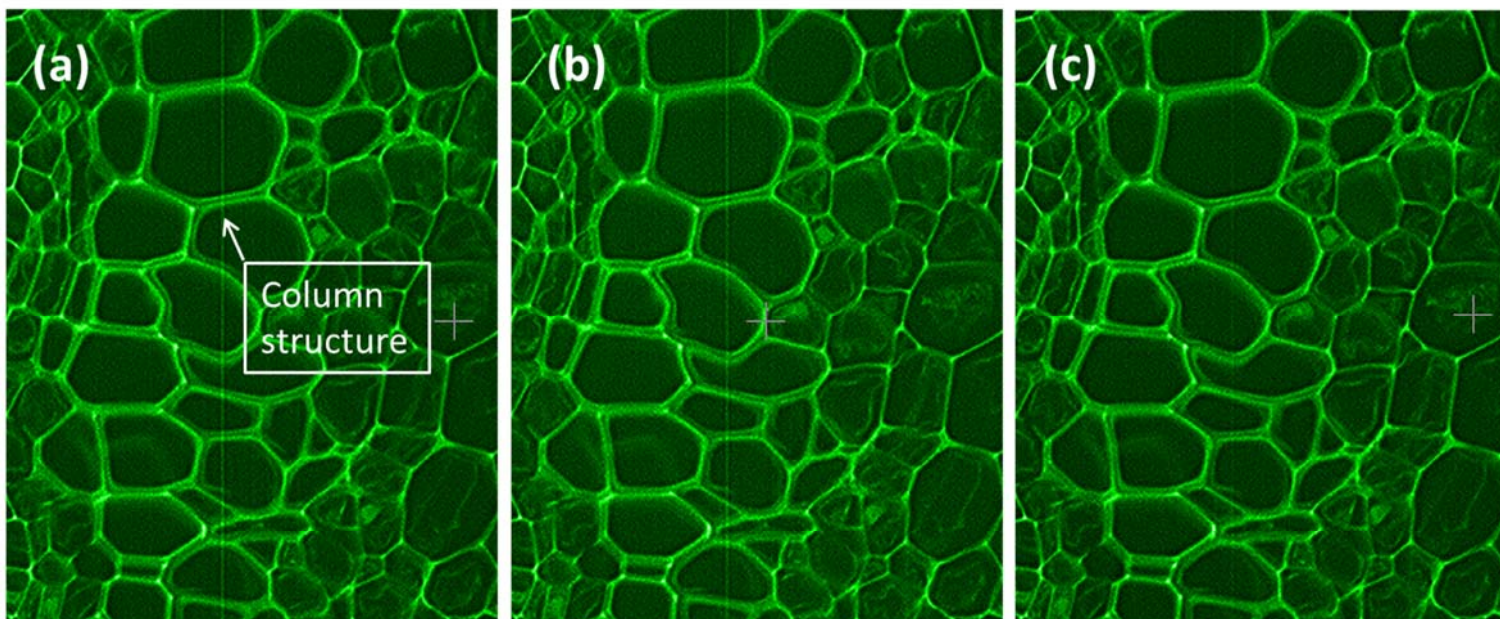
Andor-Trigger	Software
Andor-VerticalClockVoltage	Normal
Andor-VerticalSpeed	4.33
Core-AutoFocus	0.60
Core-AutoShutter	1.13
Core-Camera	2.20
Core-ChannelGroup	4.33
Core-Focus	

Figure 1 – Section of Micromanager Device Property Browser showing Vertical Clock Speed selection and Vertical Clock Voltage selection.

Figure 2 – Column structure apparent in a zoomed in section of a low light SRRF-Stream image, captured with iXon Ultra 888. Use of a x500 EM Gain setting and a faster vertical shift speed of 1.1 µs/row has been used to reduce the effect. *Note that while use of these parameters will reduce the appearance of such structures, it cannot be guaranteed to eliminate them in a SRRF-Stream upgraded camera, depending on the relative severity of the column structure.*