

# Technical Note

## ‘SRRF-Stream’: Real-Time Super-Resolution in a Camera

### Introduction

Andor ‘SRRF-Stream’ is a *Real-Time Super-Resolution Microscopy* module that is offered as an extension of iXon Life & Ultra EMCCD and now Sona back-illuminated sCMOS\*. 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 super-resolution processing is carried out at a rate up to *30x faster* than the ImageJ implementation of SRRF, ‘*NanoJ-SRRF*’. Furthermore, SRRF-Stream processing is carried out *in parallel* with data acquisition, as opposed to being restricted to post-processing, resulting in a significant *workflow advantage*. SRRF-Stream images can readily be viewed via the ‘Live Mode’ of the acquisition software. With this capacity, SRRF-Stream allows researchers to directly optimize imaging settings while directly visualizing the super-resolution images; an extremely desirable trait when compared to other super-resolution methods based on data post-processing, where the final super-resolution images are only visualized hours to days after the acquisition.

SRRF-Stream is also highly applicable to *live cell microscopy*, utilizing fluorescence excitation powers in the mW/cm<sup>2</sup> to W/cm<sup>2</sup> 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.

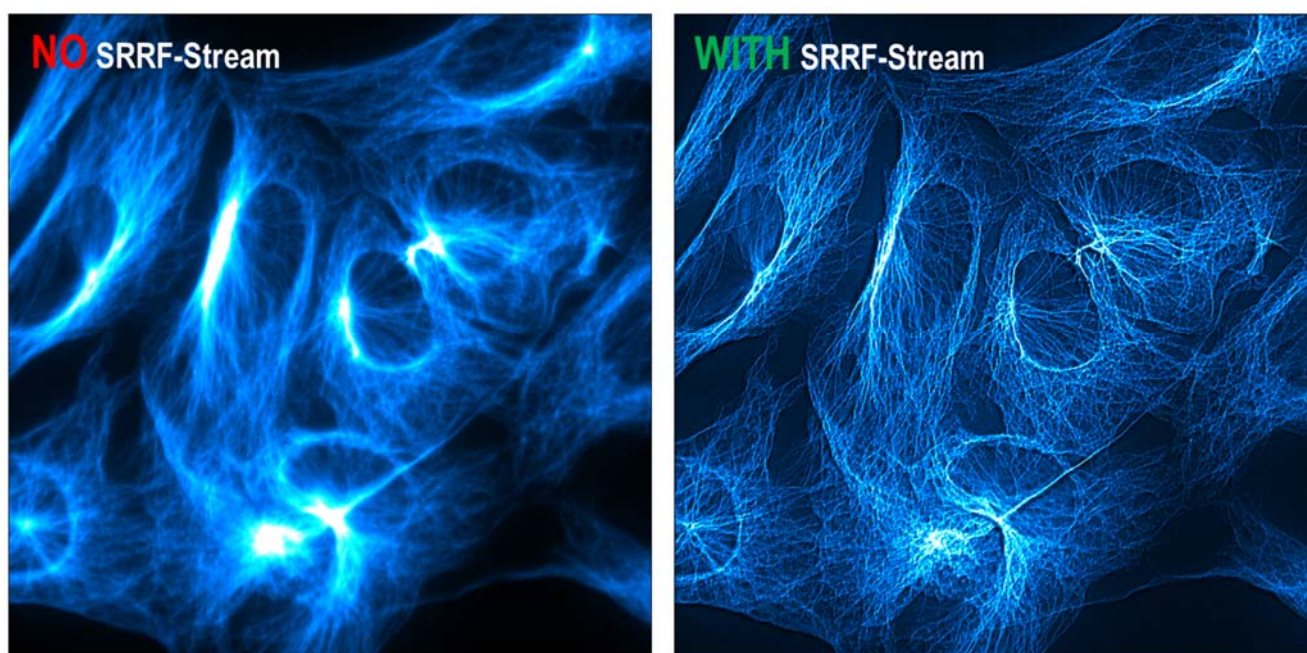
### Key features of SRRF-Stream:

- ✓ **Real Time** – enhanced workflow, avoids post-processing.
- ✓ **Super-Resolution** - 2 to 6-fold improvement (50-150 nm final resolution).
- ✓ **Low Excitation Intensities (mW-W/cm<sup>2</sup>)** – prolonged live cell observations and accurate physiology.
- ✓ **Conventional Fluorophores, e.g. GFP** – simple labelling, no photo-switching required.
- ✓ **Live Cell Dynamics** – full FOV images every 1-2 sec. Smaller ROI sizes can readily achieve super-resolution at > 10 Hz.
- ✓ **Cost-Effective** – convert conventional fluorescence microscopes to super-resolution microscopes: widefield, TIRF, confocal.

\*Sona back-illuminated sCMOS cameras supported in SRRF-Stream+ versions onwards.

## The SRRF Algorithm

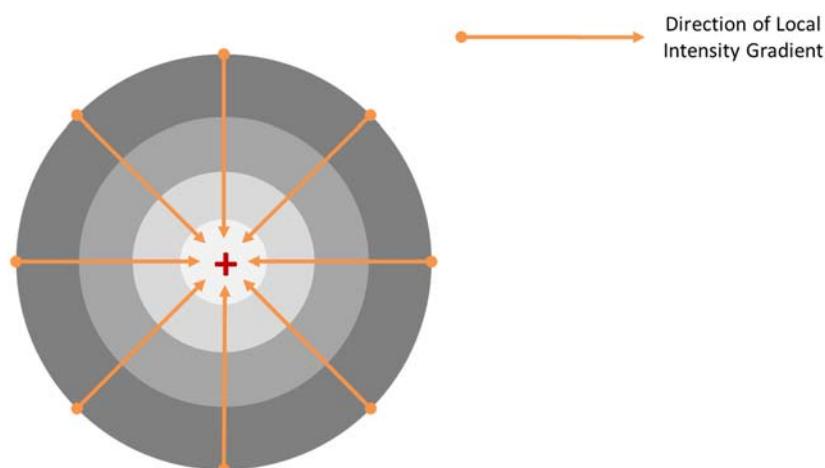
Super-resolution radial fluctuations (SRRF – pronounced ‘Surf’) is a synthesis of temporal fluctuation analysis and localization microscopy. One of the key differences between SRRF and other super-resolution methods is its applicability to image live-cell dynamics because it functions across a very wide range of fluorophore densities and excitation powers. The SRRF algorithm was developed by the Henriques group at UCL [1]. It analyses radial and temporal fluorescence intensity fluctuations in an image sequence. The number of ‘input’ images per sequence can be varied to trade spatial and temporal resolution. This can be especially important for live cell studies where phototoxicity must be minimized and, on occasion, faster physiological processes must be followed.



**Figure 1 – SRRF-Stream readily generates large field of view super-resolution images at fast frame rates.** Microtubule structure in fluorescently labelled BPAE cells (Fluocells, ThermoFisher), comparing a widefield image with and without use of SRRF-Stream. The full 1024 x 1024 pixel field of view of the iXon Ultra 888 camera was used. A x63 high-NA objective was used, with further 2x magnification and 560nm illumination. 100 raw ‘input’ images were recorded for every resultant super-resolution image, resulting in a super-resolution image rate of 0.5 Hz. For a fair comparison without SRRF-Stream, 100 standard widefield images were recorded and then averaged. For SRRF-Stream, a 4x radiality magnification was used, yielding a 4096x4096 pixel super-resolution image.

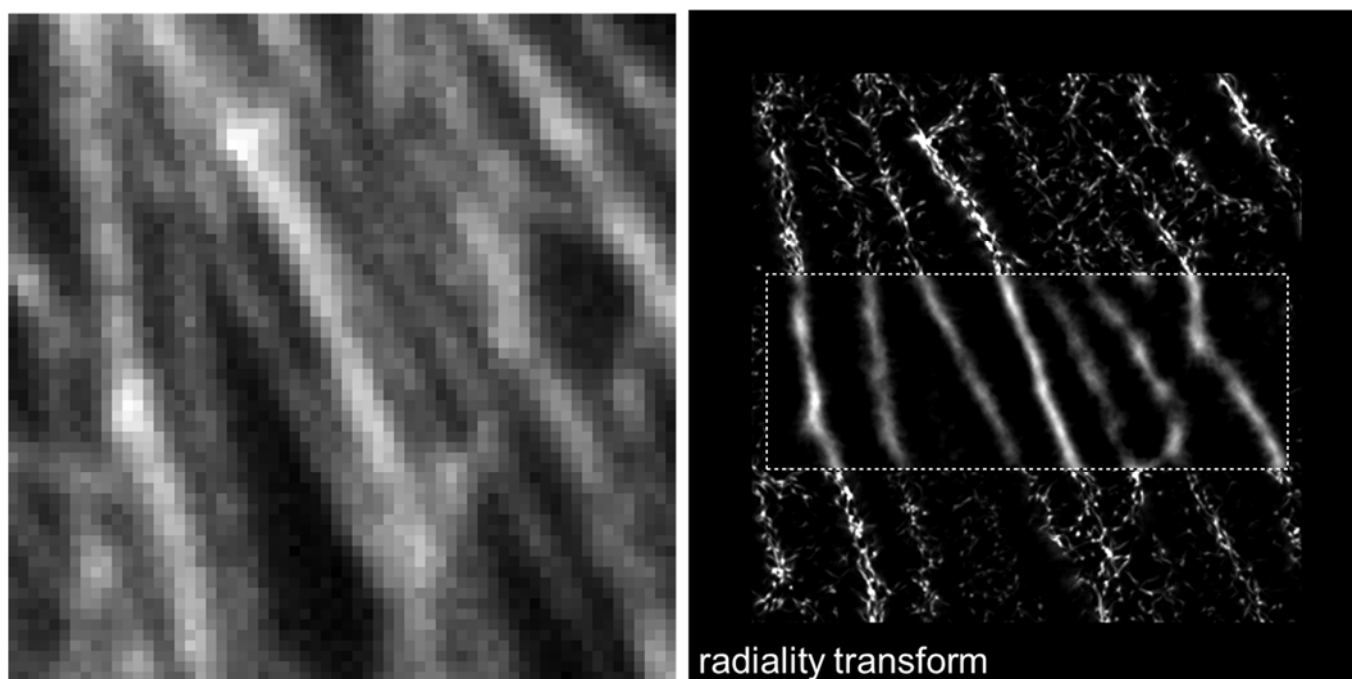
SRRF uses the concept of Radiality Fields [3] as the first important step towards achieving super-resolution. This approach essentially analyses intensity gradients across a user-defined set of radials, intersecting the centre point of analysis. It analyses such radial gradients across every point in the image, usually at an interpolated user-defined resolution that is greater than the native pixel resolution of the acquiring camera. This results in a highly complex field of data that contains rich information on local intensity gradients. This in turn provides detailed information on the accurate location of fluorophores within the individual image.

However, the SRRF process requires that multiple images (typically 50-100) are acquired and input in order to derive a single super resolved output image. Such Stochastic Fluctuation Analysis (SFA) methods, including both SRRF and SOFI [2], depend on the analysis of (short) image time series, with relatively low exposures and fast sampling. The value assigned to an output pixel depends on the original brightness and the correlation of fluorescence fluctuations in the pixel. Background tends to be poorly correlated and so significant gains in contrast can also be won.



**Figure 2** – Diagram representing a simplified case of a single fluorophore and direction of local radial intensity gradients. The fluorescent marker is at the centre of the intensity gradient field, aka. the point of maximum radial symmetry.

In SOFI, improved resolution can be derived from computing higher order cumulants. In SRRF, resolution gains are achieved from continuous interpolation of the radially field. SFA methods handle high fluorophore densities, making SRRF applicable to widefield, confocal and TIRF images with relatively low excitation intensities.



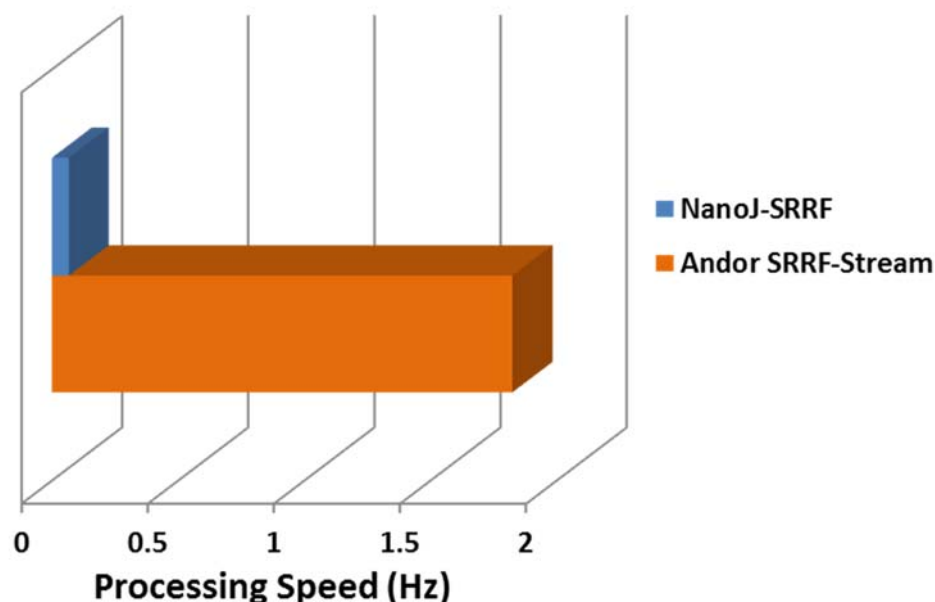
**Figure 3** – Stages of the SRRF algorithm, represented in images. LHS shows a single widefield raw image of a dense data set, acquired with only 40 mW/cm<sup>2</sup>. The RHS shows a radiality transform of that image. The inset shows the result of Stochastic Fluctuation Analysis on 100 radiality transform images, generated accordingly from 100 raw widefield images. Thus, the inset represents the quality of the final SRRF output super-resolution image.



SRRF is readily applied to data from imaging modes which include widefield, TIRF and confocal, where short frame bursts (e.g. 50-100 frames) can be processed to deliver spatial resolution similar to or better than structured illumination microscopy (SIM) [4], typically between 50-150 nm. When SRRF-Stream is used with sparse datasets e.g. STORM type data [5], SRRF provides resolution similar to Gaussian fitting localization methods, offering down to less than 50 nm resolution. Thus, SRRF provides a highly flexible and adaptable route to super-resolution without the need for specialized optical hardware or exotic probes, and utilizing relatively low power densities.

## The Andor 'SRRF-Stream' Implementation

Working in close collaboration with the Henriques Lab, Quantitative Imaging and Nanobiophysics Group, UCL, Andor have markedly accelerated the execution of the SRRF algorithm via an implementation called 'SRRF-Stream'. SRRF-Stream has been enhanced to run optimally on new iXon EMCCD cameras. Andor are expert in advanced GPU processing optimization techniques, employed in this instance to execute the SRRF algorithm up to 30x faster than the existing ImageJ-based post processing implementation of SRRF ('NanoJ-SRRF'), using the same GPU card. This significant acceleration enables workflow enhancement, by further allowing data acquisition and SRRF processing to operate in parallel. That is to say, as soon as the first input image is transferred to the GPU card, the processing begins; SRRF-Stream does not wait until the full image sequence has been recorded before beginning the processing. Since processing is significantly faster than the camera can acquire data (typically 6 - 9x faster than even Andor's 'overclocked' iXon EMCCDs), SRRF-Stream now accomplishes *real time* super-resolution, with large field of view super-resolution images. An EMCCD full field of view super-resolved image can be readily generated at a rate of approximately 1 fps, easily pushing up to > 10 fps through use of smaller standard or Image Crop ROI



sizes.

**Figure 4** - This graph compares the rate of processing of blocks of 100 raw input images (1024 x 1024 pixels), to yield resultant SRRF super-resolution images of 4096 x 4096 pixels. SRRF-Stream is compared to NanoJ-SRRF, the processing occurring on the same Nvidia GTX 1070 GPU card. The SRRF-Stream acceleration subsequently allows data acquisition and processing to happen in parallel, yielding a further workflow improvement over NanoJ-SRRF.

## Measuring fast live cell processes with SRRF-Stream

Some live cell processes, such as mitosis, can be followed adequately using time-lapse imaging and the camera can readily be operated in full pixel resolution coupled with optimal settings for achieving best super-resolution, whilst maintaining necessary frame rate. Even when combining multi-color and z-stack time-lapse super-resolution imaging, relatively large fields of view can be maintained.

However, some physiological processes may require multiple SRRF-output frames per second. Since with SRRF-Stream the frame rate is limited by the camera, then making sacrifices to field of view and/or the number of input frames per output SRRF image, will yield a proportional increased in frame rate.

Furthermore, using the specialized iXon feature called 'Crop Mode', in conjunction with Region of Interest, yields significant frame rate increases. Note that Crop Mode essentially 'fools' the sensor into thinking it is smaller than it actually is, and thus it ignores the need to readout and dump charge from the pixels that surround the specified ROI. As such, Crop Mode must be used in conjunction with a simple accessory called 'OptoMask' in order to optically shield regions outside of the defined ROI from photons which would otherwise distort the image.

The table below shows SRRF-Stream super-resolution frame rates achievable for the iXon 888 model for different combinations of ROI size (Standard ROI and Crop Mode ROI) and input frames per super-resolved output image. For example, a 128x128 ROI of the iXon 888, used in Crop Mode, and inputting 50 frames per output image (thus sacrificing some degree of resolving power) would output 14 fps, more than adequate for following most live cell dynamic processes. Note also that if a 4x magnification is used in SRRF-Stream (as is typical), a 128x128 ROI becomes a 512x512 super-resolution image.

|                   | Standard ROI Mode                       |   |  |
|-------------------|---|---|--|
| iXon 888 ROI Size | Camera Frame rate - Standard ROI (fps)  | SRRF-Stream Frame rate - 100 input frames (fps) | SRRF-Stream Frame rate - 50 input frames (fps) |
| 512 x 512         | 50                                      | 0.5   | 1.0  |
| 256 x 256         | 95                                      | 1.0   | 1.9  |
| 128 x 128         | 171                                     | 1.7   | 3.4  |
|                   | Crop Mode ROI Mode                      |   |  |
|                   | Camera Frame rate - Crop Mode ROI (fps) | SRRF-Stream Frame rate - 100 input frames (fps) | SRRF-Stream Frame rate - 50 input frames (fps) |
| 512 x 512         | 78                                      | 0.8   | 1.6  |
| 256 x 256         | 251                                     | 2.5   | 5.0  |
| 128 x 128         | 697                                     | 7   | 14   |

**Table 1** - SRRF-Stream super-resolution frame rates achievable for the iXon 888 model for different combinations of ROI size (Standard ROI and Crop Mode ROI) and input frames per super-resolved output image.

## Optimal imaging criteria for SRRF-Stream

SRRF yields better results if the initial images are acquired at or close to Nyquist conditions, irrespective of whether using widefield, confocal or TIRF modalities. For relatively large pixel cameras such as iXon 897 or iXon 888 models, this would ideally involve additional magnification, available either from a tube lens in the microscope or from a C-mount coupler with built-in magnification. The table below shows some example combinations of Objective Mag and Coupler Mag, resulting in varying degrees of oversampling of the resolution limit. Ideal oversampling is 2.3 or greater, but note that conditions down to 1.5 can still adequately be used to yield good results with the SRRF algorithm.

|          | Region of Interest | Pixel Size (um) | Objective Mag (x) | NA   | Coupler Mag (x) | Resolution Limit on Sensor - Rayleigh (um) | NYQUIST Sampling (Res limit ÷ pixel size) |
|----------|--------------------|-----------------|-------------------|------|-----------------|--|---|
| iXon 897 | 512 x 512          | 16              | 100               | 1.49 | 1               | 24.56                                      | 1.5                                       |
|          | 512 x 512          | 16              | 100               | 1.49 | 1.5             | 36.85                                      | 2.3                                       |
|          | 512 x 512          | 16              | 60                | 1.4  | 1.5             | 23.53                                      | 1.5                                       |
|          | 512 x 512          | 16              | 60                | 1.4  | 2               | 31.37                                      | 2.0                                       |
|          |                    |                 |                   |      |                 |  |   |
| iXon 888 | 1024 x 1024        | 13              | 100               | 1.49 | 1               | 24.56                                      | 1.9                                       |
|          | 1024 x 1024        | 13              | 100               | 1.49 | 1.5             | 36.85                                      | 2.8                                       |
|          | 1024 x 1024        | 13              | 60                | 1.4  | 1.5             | 23.53                                      | 1.8                                       |
|          | 1024 x 1024        | 13              | 60                | 1.4  | 2               | 31.37                                      | 2.4                                       |

Table 2 - Example combinations of Objective Mag and Coupler Mag, resulting in varying degrees of oversampling of the resolution limit.

## SRRF-Stream Applications

With its ability to smash through the classical diffraction limit, and furthermore, to accomplish this in real time, with non-complex sample labelling, conventional equipment and with low intensity illumination, SRRF-Stream paves the way to unlock previously unseen cellular structure and behaviour, at unprecedented spatio-temporal resolution in a low-photodamage friendly manner.

What observational capabilities does SRRF-Stream unlock?

- ✓ Elucidation of protein structure analysis at a sub-organelle level.
- ✓ Tracking of single molecules inside cells.
- ✓ Using this tracking to gain insight into individual molecular machinery underpinning cellular physiology.
- ✓ With this new information, update models of cell function.

Example applications of SRRF-Stream:

- ✓ Membrane fusion involving individual SNARE protein machinery.
- ✓ Dynamic changes of and within synaptic vesicles.
- ✓ Dendritic spines reformation due to synaptic plasticity and learning.
- ✓ Signal transduction processes and cell-to-cell communication and differentiation.
- ✓ DNA structure fluctuations including expression and inhibition.
- ✓ Intracellular skeleton reassembly – actin fibres meshwork changes

## What is needed to access SRRF-Stream?

SRRF-Stream is optimized to operate on iXon Life and iXon Ultra EMCCD cameras (both '897' and '888' sensor models), both on NEW cameras and as an UPGRADE pathway to existing cameras.

**NEW iXon cameras** - iXon Life and iXon Ultra that are ordered to ship with SRRF-Stream are specifically modified to be compatible with and to work optimally with SRRF-Stream. When ordering a new camera, SRRF-Stream should be ordered also, effectively as an optional accessory (see the iXon Life and iXon Ultra specification sheets for ordering details). Andor will then ensure each camera is configured and optimized to operate with SRRF-Stream before shipping, alongside the SRRF-Stream installer and license. Ordering a NEW iXon camera with SRRF-Stream is the best way to ensure absolute optimal image quality of SRRF-Stream processed images.

**Upgrade route** – It is possible to upgrade your current iXon camera, provided you currently own an iXon Ultra 888, iXon Ultra 897, iXon Life 888 or iXon Life 897 model. For further details on SRRF-Stream Upgrades, a separate Technical Note has been written entitled [\*\*"SRRF-Stream Upgrade: Boost your Current iXon EMCCD with Super-Resolution Capability"\*\*](#).

Sona back-illuminated cameras are also now compatible with SRRF-Stream+. Either order with a new camera, or order the "SRRF-upgrade" for your existing Sona camera to unlock super-resolution capabilities. To upgrade Sona sCMOS cameras you do not need to return the camera to the factory.

### To access SRRF-Stream capability, the following is required:

- ✓ A **SRRF-Stream compatible** iXon Life or iXon Ultra EMCCD OR Sona back-illuminated sCMOS
- ✓ SRRF-Stream license and installer (enables SRRF-Stream in camera – specific to camera serial number).
- ✓ A CUDA-compatible NVidia GPU card\*.
- ✓ MicroManager (64-bit) software or Andor SDK2/3.
- ✓ 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.*

*Note that Andor have also made available a SRRF-Stream PC workstation that comes pre-installed with a recommended and tested GPU card, alongside SRRF-Stream enabled MicroManager (linked to the serial number of your iXon camera) and Andor SDK2 with SRRF-Stream. See Appendix for further details and workstation configuration.*

## SRRF-Stream in MicroManager

To make SRRF-Stream widely accessible, it has been fully integrated into MicroManager (Open Imaging) open source microscopy software platform. This integration is implemented via a simple installer that will ship as part of SRRF-Stream.

The  $\mu$ Manager user simply enables SRRF-Stream and (optionally) sets the required SRRF parameters via the 'Device Property Browser'. The SRRF-Stream output is then shown in the  $\mu$ Manager image viewer as per any other image acquisition. Acquisition protocols are then set-up as per normal, for example time-series, multi-channel and Z-stack, except now the images will be super-resolution 'output' images.

## Features of SRRF-Stream in MicroManager

- ✓ Outputs real time super-resolution images in Live Mode.
- ✓ Multi-dimensional - Integrated with Multi-channel, Z-stack and Time-series acquisitions.
- ✓ SRRF-Stream parameters readily adjustable alongside other camera parameters through 'Device Property Browser'.
- ✓ Simple Installer.

|  |             |
|--|-------------|
| Andor-ReadMode                               | Image       |
| Andor-ReadoutMode                            | 30.000 MHz  |
| Andor-ReadoutTime                            | 46.6116     |
| Andor-Region of Interest                     | Full Image  |
| Andor-SRRF Status                            | Ready       |
| Andor-SRRF   Enable                          | Enabled     |
| Andor-SRRF   Interpolation Type              | Catmull-Rom |
| Andor-SRRF   Number of Frames per Time point | 100         |
| Andor-SRRF   Radiality Magnification         | 4           |
| Andor-SRRF   Ring Radius                     | 0.5         |
| Andor-SRRF   Temporal Analysis Type          | Mean        |
| Andor-Shutter (External)                     | IMP         |
| Andor-Shutter (Internal)                     | Mean        |
| Andor-Shutter Closing Time                   | 27          |
| Andor-Shutter Opening Time                   | 27          |
| Andor-SpuriousNoiseFilter                    | None        |

**Figure 5** – The 'Device Property Browser' of MicroManager, through which SRRF-Stream parameters are configured.

## SRRF-Stream in Andor SDK2/3

When ordered, SRRF-Stream also comes with an installer for use with Andor SDK2/3. Note, SRRF-Stream is not natively part of SDK2/3 by default, it is incremental to it. SRRF-Stream consists of a simple interface with functions for:

1. **Initialisation** with input data dimensions and data type on a per-camera basis:
  - Input data dimensions include width and height of acquired frames, and number of time frames required to produce a SRRF image.
  - Data types allowed include unsigned 16-bit integer and floating point.
  - Dual camera support is provided via an input SDK2/3 camera handle.
2. **Setting of SRRF parameters** - again on a per camera basis.
  - To include radiality magnification, ring radius, temporal analysis type, and interpolation type.
  - Optionally default settings may be used.
3. **Copying and processing** of acquired frames:
  - Buffers containing acquired frame data may be passed into the SRRF-Stream library directly from GPU memory for processing: Useful for CUDA library or GPU-Express library users.
  - Alternatively, buffers containing acquired frame data may be passed directly into the SRRF-Stream library from CPU memory: In this case SRRF-Stream manages all required copies to/from GPU.



#### 4. Retrieval of intermediate (and final) outputs:

- At any point during an acquisition the currently held SRRF output image may be retrieved from the SRRF-Stream library.

#### 5. Finalisation for cleaning up of all resources.

## SRRF-Stream Settings

The default settings of SRRF-Stream in MicroManager generally yield fantastic resolution improvements. However, the user is entirely open to experimentation using the available SRRF-Stream settings:

**Interpolation Type:** Catmull-Rom is the default interpolation type and tends to yield superior resolution. You would only need the alternative 'B-Spline' type if for some reason you were challenged by processing speed, but since even with mid-range GPU cards SRRF-Stream processes at a much faster rate than the iXon can acquire data, it should not be necessary.

**Number of Frames per Time Point:** Generally, 100 input frames yield superb resolution improvements. However, if you wish to follow dynamic events and are limited by the frame rate of the camera, then you can of course opt to use fewer input frames. Even using as few as 20-30 frames can still yield resolution improvements, although in practise a minimum of 50 frames should be considered a 'safe' lower limit.

**Radiality Magnification:** Radiality is calculated on a magnified pixel grid; the radiality magnification setting determines how many magnified pixels each original image pixel is split into. For example, a radiality magnification of 5 will split each image pixel into 5x5 magnified pixels. Generally increasing the radiality magnification will improve resolving power, but it is not a linear relationship, rather a case of marginal further gain at higher magnifications. The cost of increasing magnification is increased computational time. The default setting of 4 tends to yield very good resolutions, however feel free to experiment with this factor, especially since SRRF-Stream is so efficient at data processing. Note that if the original camera resolution is 1024x1024 and a radiality magnification of 4 is applied, the SRRF-Stream output image will be 4096x4096 pixels.

**Ring Radius:** To calculate the radiality at each co-ordinate in the image, SRRF needs to calculate the intensity gradients in a ring surrounding each co-ordinate. The ring radius setting determines the radius of this ring. For low density data sets, setting this radius to be low will increase resolution of the image; however, setting the radius too low in noisy datasets will reduce precision and may introduce patterning into the resulting image. It is worth experimenting with this setting on different sample types if you feel you would like to try improving the image further, but the default value of 0.5 is a good general setting and will often yield best results.

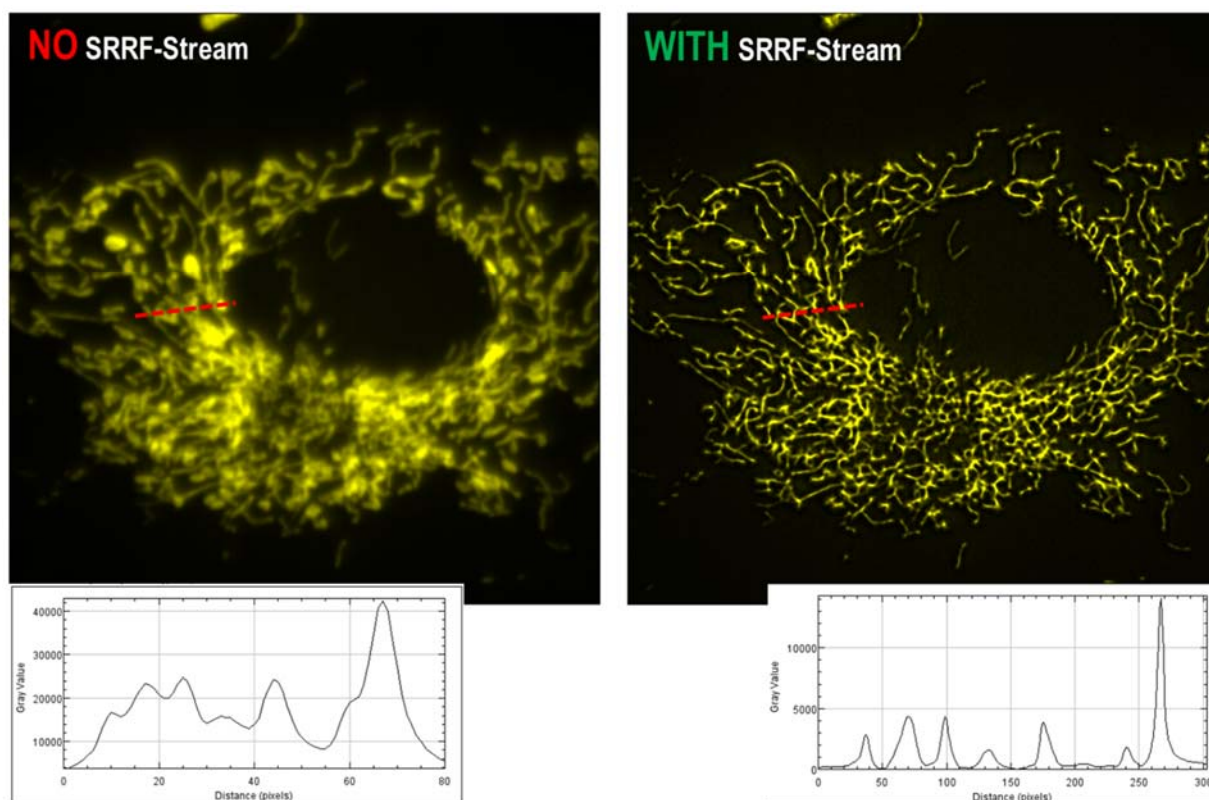
**Temporal Analysis Type:** Temporal Analysis determines the method by which the final image is combined following the radiality transform calculation.

The default 'Mean' setting displays the final image as an Average Intensity Projection of the radiality transforms of each individual frame in the original image. It is often a good starting point for analysis, especially for blinking data sets where fluorophores overlap, or for data sets acquired where fluorophores display limited intensity fluctuations such as in conventional TIRF or widefield microscopy.

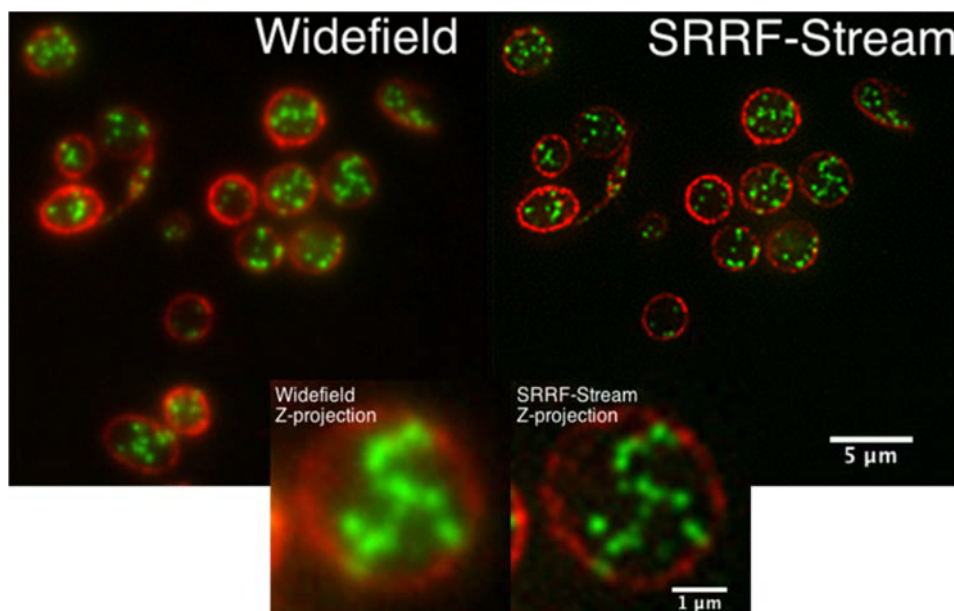
'MIP' displays the final image as a Maximum Intensity Projection of the radiality transforms of each individual frame in the original image. This option works most successfully for sparse data sets i.e. classical PALM/STORM experiments.

## SRRF-Stream Resolution Enhancements

The following image comparisons, all of which have been acquired using Andor SRRF-Stream, demonstrate the impressive resolution improvements that can readily be obtained.

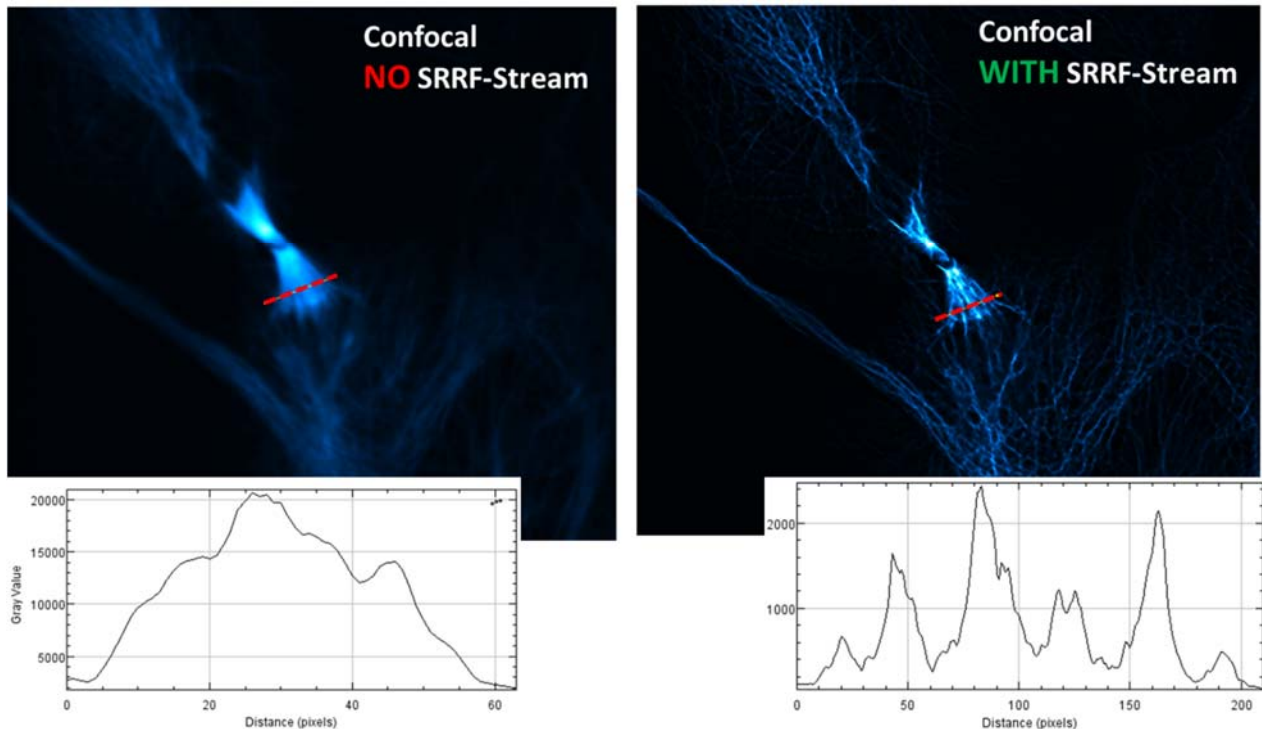


**Figure 5** - Image comparison of a fluorescently labelled BPAE cell, recorded with a widefield fluorescence microscope and a SRRF-Stream enabled iXon Life 888 EMCCD camera. A x63 objective was used, with further 2x magnification and 560nm illumination. 100 raw 'input' images were recorded for every resultant super-resolution image, resulting in a super-resolution image rate of 0.5 Hz. For a fair comparison without SRRF-Stream, 100 standard widefield images were recorded and then averaged. While the original image was of a larger field of cells, a zoomed ROI of one cell is shown here in order to more easily display a line intensity profile comparison through a small region. The improvement in resolving power is readily apparent.



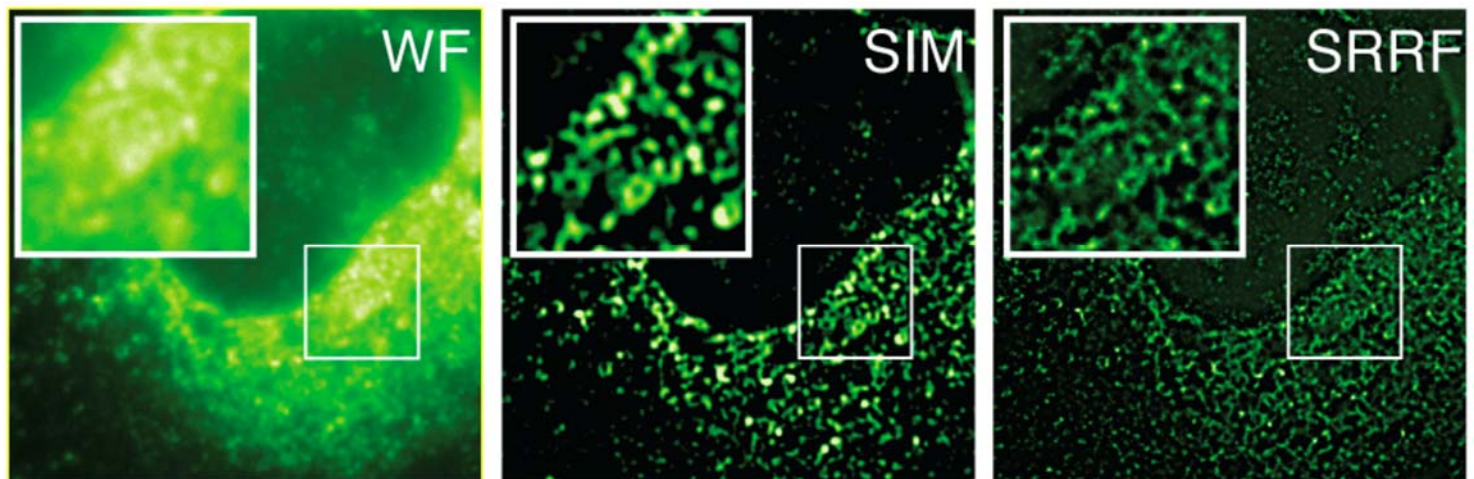
**Figure 6** - Comparative standard-widefield and SRRF-Stream-widefield images of blood platelets, red membrane, green internal granules. Sample courtesy of Cutler laboratory at UCL.



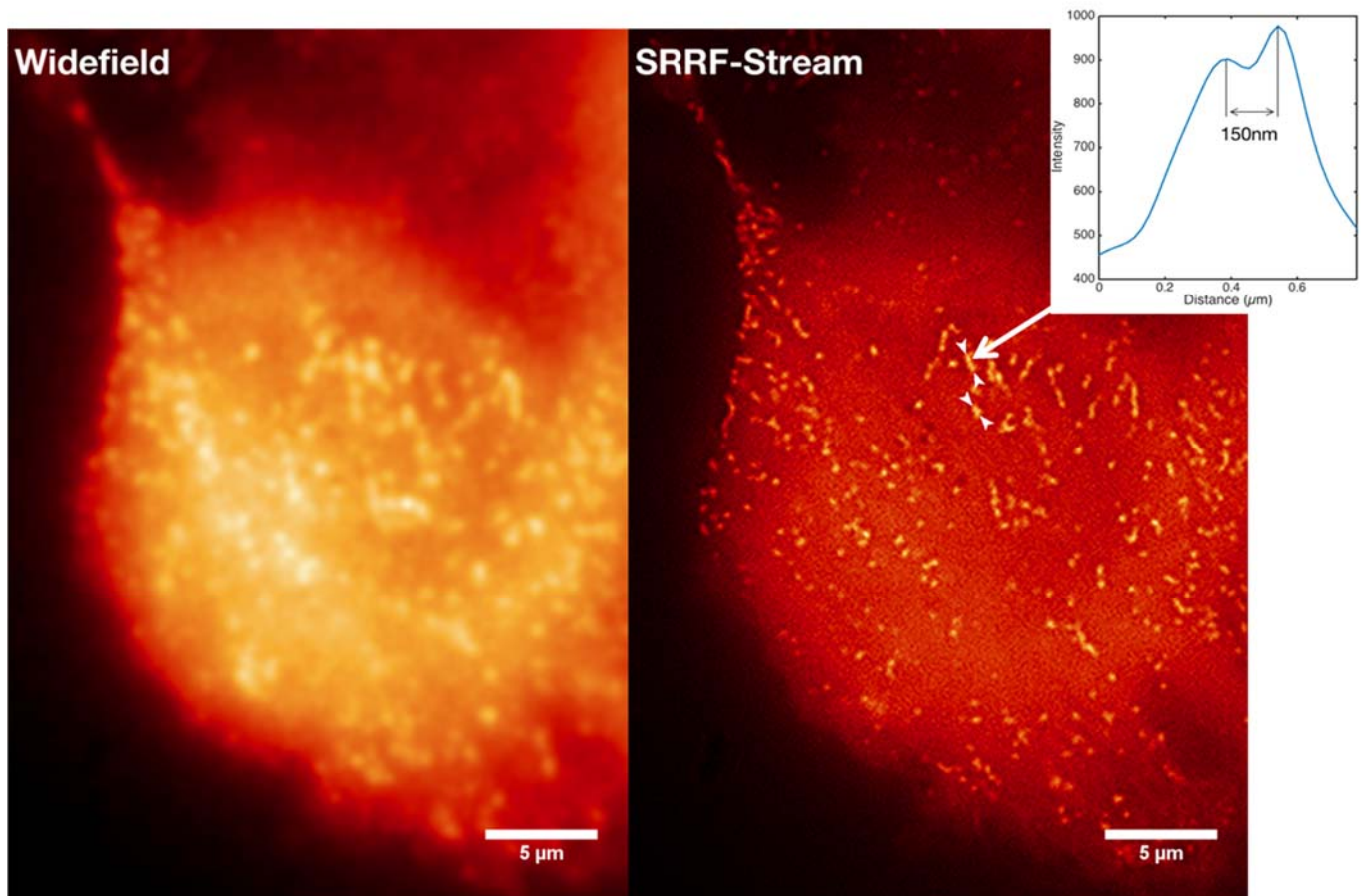


**Figure 7** - Comparison of a fluorescently labelled U2OS cell line\* recorded with an Andor Dragonfly confocal spinning disk fluorescence microscope and a SRRF-Stream enabled iXon Life 888 EMCCD camera. A x63 objective was used, with further 2x magnification and 488nm illumination. An unprecedented improvement in resolving power can be observed in the level of detail in the mitotic spindle. This is further evidenced in the comparative line intensity profile drawn through this region.

\*U2OS cell line was fixed, stained with anti-alpha-tubulin primary antibody (green, AF488) and phalloidin (red, rhodamine) to visualize F-actin, DAPI staining to visualize nuclei. Samples prepared by Klebanovych A., Laboratory of Biology of Cytoskeleton, IMG of the AS CR, v.v.i.



**Figure 8** - HCV infected cells stained with anti-NS5A. Comparison of Widefield (WF), Structured Illumination Microscopy (SIM) [4] and SRRF images (SRRF of the widefield image). The images are of the same field of cells, recorded on the same microscope, using identical objective and optical path. The only difference being that SIM was recorded using an sCMOS detector with 6.5  $\mu\text{m}$  pixels whereas the Widefield and resultant SRRF was recorded using an iXon EMCCD detector with 16  $\mu\text{m}$  pixels. The superior resolving power of SRRF is evident, indicative that SRRF is achieving a greater than 2-fold improvement over the classical diffraction limit. SIM is theoretically limited to a 2-fold reduction of the classical diffraction limit. Sample courtesy of the Grove lab at UCL.



**Figure 9** - Comparative images of Clathrin coated pits of live HeLa cells, labelled with mCherry, recorded on a widefield microscope at 2 FPS. 100 raw 'input' images were recorded for every resultant super-resolution image, resulting in a super-resolution image rate of 2 FPS. A line intensity profile is shown through a small region of the SRRF-Stream image, indicating resolution of structures that are 150nm apart. Sample preparation by Caron Jacobs (Ricardo Henriques and Mark Marsh labs at UCL)

## References

1. Gustafsson N., Culley S., et al (2016) Nat. Comms.
2. Dertinger T., et al (2009) PNAS
3. Parthasarathy R. (2012) Nat. Meth.
4. Gustafsson M.G.L. (1999) J. Microsc.
5. Bates M.J., et al (2008) Science

Update 02 Nov 2020

SRRF-Stream has been enhanced further, providing all the same benefits, but with improved image quality. Read about the latest version "SRRF-Stream+" in the technical note.

Andor Sona back-illuminated sCMOS cameras are also supported in the new SRRF-Stream+ making this technique available to an even wider range of applications.



## Appendix: SRRF-Stream Workstation

Andor's SRRF-Stream PC Workstation has been configured and tested by Andor to provide optimal price/performance for SRRF-Stream with iXon Ultra and iXon Life cameras ('888' and '897' models). It comes pre-installed with a recent build of MicroManager (64-bit) and an up to date version of Andor SDK2 (64-bit) and includes the recommended CUDA-compatible NVidia GPU card, capable of delivering real time super-resolution performance when combined with Andor's highly efficient SRRF-Stream implementation.

Alternatively, you could readily opt to use the PC and GPU card specification of Andor's SRRF-Stream Workstation as a guide to configuring and ordering your own PC workstation through independent channels, if that is preferred. However, please note that such a self-build PC configuration would not be supported by Andor.

### Detailed specification of the SRRF-Stream PC Workstation (Andor part code: MSC-13069):

**Note that specifications will be updated to the latest equivalent models. This will be confirmed at time of ordering.**

- Dell Precision Tower 5810 XCTO Base
- Intel Xeon Processor E5-1630 v3 (Four Core HT, 10MB Cache, 3.7GHz Turbo)
- Dell Precision Tower 5810 685W TPM Chassis, v2, BW
- 16GB (4x4GB) 2400MHz DDR4 RDIMM ECC
- No Media Card Reader
- 500GB 7200 rpm Hard Drive
- 2x Additional 500GB 2.5" Serial-ATA (7,200 RPM) Hard Drive
- Integrated Intel AHCI chipset SATA controller (6 x 6.0Gb/s) - SW RAID 0/1/5/10
- 512GB Adata DP900 SSD 2.5 inch SATA 6Gb/s, 1M Hrs MTBF {CS}
- Heatsink for single CPU T5810
- 8x Slimline DVD+/-RW Drive
- Chassis Intrusion Switch
- 1 NVIDIA Quadro M4000 8GB (4 DP) (1 DP to SL-DVI adapter)
- Internal Speaker
- Dell Optical Mouse MS116 Black
- Dell Multimedia Keyboard - UK (QWERTY) - Black
- C4 SATA / SSD 2.5 Inch, 3-4 Hard Drives (Integrated Controller)
  
- Dell Precision Resource DVD
- Windows 10 Pro 64bit English, Dutch, French, German, Italian
- Windows 10 Pro OS Recovery 64bit - DVD
- McAfee Security Center 30 day trial, Digital Delivery

### Optional Monitor

- Dell UltraSharp U3417W - 34.14" Curved LED (Andor part code: FUS-MNTR-34W)
- or
- Dell UltraSharp UP3017 - 30" with PremierColor (Andor part code: FUS-MNTR-30)