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## SRRF-Stream - Releasing The Potential of Real-Time Super-Resolution Microscopy

SRRF-Stream super-resolution processing is carried 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 visualising 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 visualised hours to days after the acquisition.

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**ANDOR**  
an Oxford Instruments company

**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 **Orion Life** and **Orion Ultra EMCCD** camera functionality. SRRF-Stream presents a powerful super-resolution approach that is also **widely accessible**, being applicable to **most existing modern fluorescence microscope** and fully compatible with **conventional fluorophores** such as fluorescent proteins. That is to say, with SRRF-Stream there is no requirement to use specialised photo-switchable fluorophores as is typically required for localisation super-resolution approaches. The resolving power of SRRF-Stream is excellent, yielding a resolution improvement from **2- to 6-fold (50-150nm final resolution)** for most datasets.

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SRRF-Stream is also highly applicable to **live cell microscopy** utilising 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 **10<sup>3</sup> times** lower excitation power than that typically used in localisation based super-resolution approaches. Furthermore, super-resolution image rates in **excess of 30 fps** can be achieved and output in real time, meaning even rapid physiological processes can be followed with fast 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 intracellular resolution that can readily discern **sub-organellar** structure.

**Key features of SRRF-Stream:**

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

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