Fast, Multi-modal Nanoscopy with SRRF-Stream (Super-Resolution Radial Fluctuations)

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ABSTRACT

Super-resolution radial fluctuations (SRRF) is a synthesis of temporal fluctuation analysis and localization microscopy [7]. One of the key differences between SRRF and other super-resolution methods is its applicability to live-cell dynamics because it functions across a very wide range of fluorophore densities and excitation powers.

SRRF is applied to data from imaging modes which include widefield, TIRF and confocal, where short frame bursts (e.g. 50 frames) can be processed to deliver spatial resolution similar to or better than structured illumination microscopy (SIM). With sparse data e.g. direct STORM, SRRF provides resolution similar to Gaussian fitting localization methods. Thus SRRF could provide a route to super-resolution without the need for specialized optical hardware, exotic probes or very high power densities.

We present a fast GPU-based algorithm, "SRRF-Stream" and apply it to imagery from our new multi-modal imaging platform, Dragonfly. SRRF-Stream runs on an NVidia GPU, and streams images direct from camera to GPU for real time processing. We present preliminary data from EMCCD and sCMOS cameras, and various fluorophores including fluorescent proteins and organic dyes.

BACKGROUND

Method	Reported Resolution nm	Photon Increase	Acquisition Time sec	Imaging Mode	Power Density W cm^{-2}
STED	XY = 20	100	60	Confocal	10^4 – 10^9
	XYZ = 30	1,000	1,000		
STORM	XY = 20	100	> 20	Widefield	10^3 - 10^4
	Z = 20	15,000	1,500		
SIM	XY = 100	10	0.1 - 1	Widefield	10 - 10^2
	Z = 370	20	10		
SOFI / SRRF	XY = ~60	~10	0.1 - 1	Confocal, Widefield TIRF	1 - 10^2
	Z = 100	20	10		

Table 1 shows the relative performance for the primary super-resolution methods vs SRRF. SRRF can exceed the lateral resolution limit of SIM, and has the added advantage of trading spatial and temporal resolution. STORM and STED can provide the highest resolutions at the cost of very high power densities and long acquisition times [1].

Super- resolution, exceeding the diffraction limit has been achieved by four primary methods [1-6]: See Table 1. Stochastic fluctuation analysis (SFA), such as SOFI [6] and SRRF, depends 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.

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

THE SRRF ALGORITHM

The SRRF algorithm [7] was developed by the Henriques group at UCL. It analyses radial and temporal fluorescence intensity fluctuations in an image sequence. The number of images per sequence can be varied to trade spatial and temporal resolution. This is especially important for live cell studies where phototoxicity is a concern. Typically, 10 times the light dose is required to double resolution.

SRRF-STREAM IMPLEMENTATION

Images are acquired in rapid sequence (e.g. 100 fps) at a given focal plane and wavelength. As they are acquired SRRF-Stream streams the images to the GPU where the images are spatially processed and buffered. At the sequence end, temporal processing is performed and the result is delivered to the imaging pipeline.

In the first step, each image is super-sampled to increase grid size. The intensity gradient is computed at each pixel and interpolated with a Catmull-ROM B-Spline algorithm to populate the sub-pixel gradient map. Each sub-pixel is weighted according to its local gradient convergence (radiality) and further weighted by the pixel intensity in the raw image – high radiality associates fluctuations at or close to the sub-pixel [8].

SRRF-STREAM IMPLEMENTATION

Finally, temporal processing is applied: accumulation, maximum projection and autocorrelation methods can be used. The resultant image pixel size is typically reduced by a factor of between 4 and 8 and the image is typically 2k x 2k or 4k x 4k pixels.

Using the Andor GPU Express Platform we implemented a CUDA version of the SRRF algorithm and then integrated it with image streaming from Andor iXon EMCCD and Zyla sCMOS cameras.

Numerous images from the SRRF-Stream algorithm were compared pixel by pixel to those from the NanoJ implementation and found to be within errors expected from integer arithmetic rounding. See Results Table 2 for performance figures of the algorithm in its current form.

RESULTS

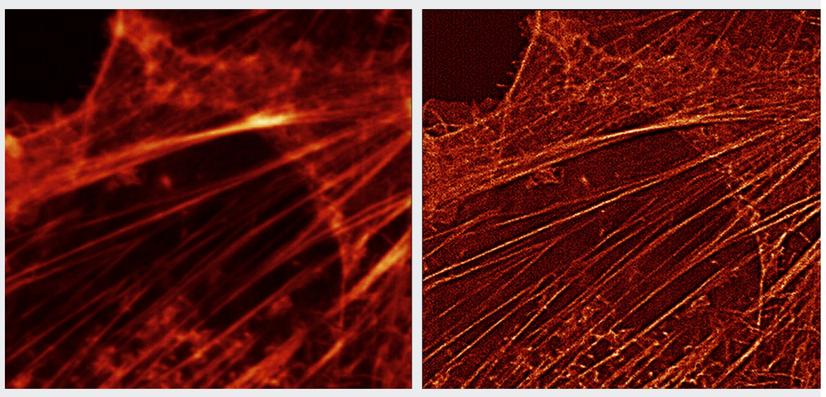

Figure 1 Shows a Dragonfly confocal data set from a fixed specimen labelled with Alexa488 Phalloidin . On the left the frame averaged result of 100 sCMOS frames is compared to the SRRF-Stream result. Resolution and contrast enhancement is apparent (for more information see Figure 4). We found that fixed pattern noise in sCMOS sensors was apparent as a very low intensity mesh-like background, which can be substantially eliminated by denoising. In EMCCD data this background was not apparent, however.

NVidia GPU	Data size uint16	SRRF-Stream ms	NanoJ GPU ms	Speed up X
K5000	512 x 512 x 100	478	10,618	22.2
	1024 x 1024 x 100	1,878	42,319	22.5
	2048 x 2048 x 100	7,527	177,214	23.5
M4000	512 x 512 x 100	284	5,828	20.5
	1024 x 1024 x 100	1,115	23,802	21.4
	2048 x 2048 x 100	4,422	93,881	21.2

Table 2 Comparing SRRF-Stream with the NanoJ (ImageJ plugin) released open source by the Henriques lab. NanoJ SRRF runs on a GPU if available, but is not fully optimized, yet is 12-15 times faster than the CPU execution (Xeon 3.5 GHz 4 Core). These tests are for pixel zoom 4X, i.e. output contains 16 times more pixels. Andor's SRRF-Stream has been optimized for not only execution, but also for data flow to and from the GPU, enabling integration with camera acquisition. Andor plans to make SRRF-Stream available for our cameras running under Micromanager in June and Fusion by the end of 2017.

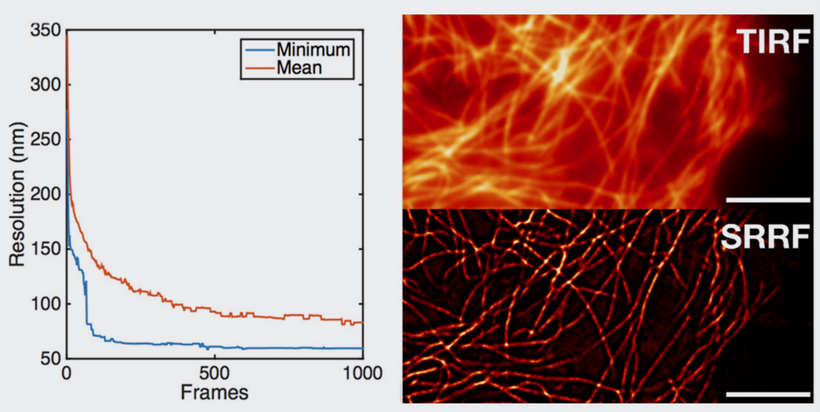

Figure 2 The evolution of FRC (Fourier Ring Correlation) resolution with number of frames in the SRRF-Stream time series, computed from an EMCCD TIRF live cell dataset. Mean shows the average from all ROI's analyzed by FRC, while Minimum shows the best resolution of the ROI group.

RESULTS (CONTD.)

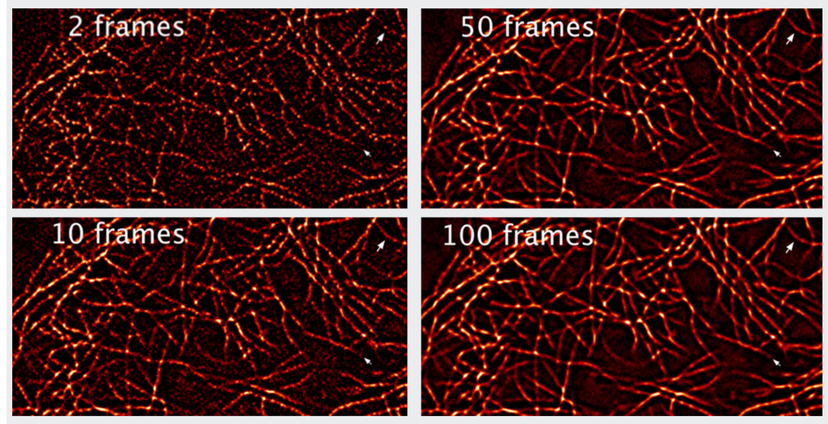

Figure 3 The images show SRRF-Stream results after processing the indicated number of frames. Comparing to Figure 2, SRRF-Stream resolution reaches 200 nm with <10 frames, ~150 nm with 50 frames and ~130 nm with 100 frames. These translate to temporal resolutions of 0.1, 0.5 and 1.0 seconds, which is adequate for many live cell studies. For structural studies with fixed specimens and drift correction, resolutions of <80 nm can be achieved.

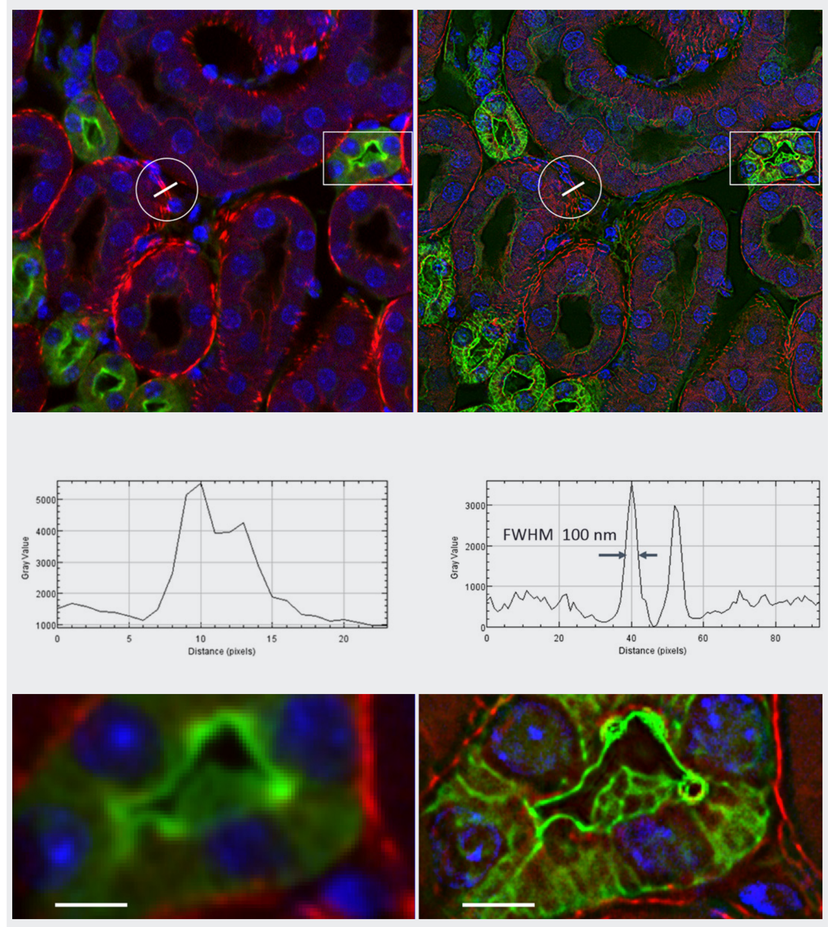

Figure 4 Confocal data from a triple labelled kidney section acquired with Dragonfly 40 micron pinhole, Planapo 60X/1.4 oil objective. Top: 512x512 raw data is contrasted with 2048x2048 SRRF-Stream image processed from 100 frames. Middle: line profiles of highlighted features (like scaling), showing 100 nm FWHM from striated features in kidney vasculature. Bottom: Zoomed highlighted regions illustrating resolution boost: scale bar 2 µm.

CONCLUSION

SRRF-Stream offers great potential for fast multi-modal super-resolution microscopy. Further detailed evaluation is needed to establish the boundaries of performance and application.

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