VisiSRRF – A universal and affordable superresolution technique for all fluorescence microscope modalitie

In this 21st century microscopists developed amazing techniques to break down the resolution limits of conventional optical microscopy (~200nm) achieving resolutions in the tens of nanometers. Many of these techniques require specialized equipment and/or unique fluorophores and are often not compatible with life cell imaging or suffer from low temporal resolution.

VisiView App

VisiSRRF Super Resolution Algorithm

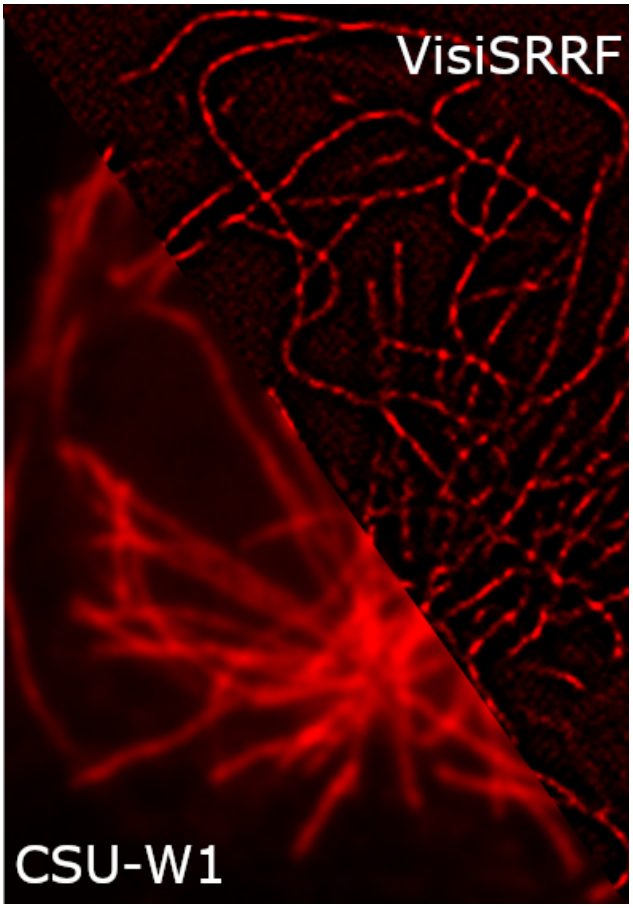

Figure 1: labelled Mikrotubuli (samples are a courtesy of Prof. Leane Lehmann, JMU Würzburg). Comparison of SRRF result (100x 15ms exp, middle) and long exposed raw data (1500ms exp, right) at equal laser power. Image shows one cell and only a very small part of the FOV. Acquired with Teledyne Prime 95B sCMOS

Superresolution techniques

VisiSRRF on the other hand can be applied to every already established fluorescence microscope and can improve the resolution from any fluorescent image without the need for specially designed fluorophores. SRRF identifies radial fluctuations in a temporal series of images and recalculates the origin of fluorophores (Gustafsson et al. 2016, Culley et al. 2018). The resolution which can be achieved with SRRF is around 70nm.

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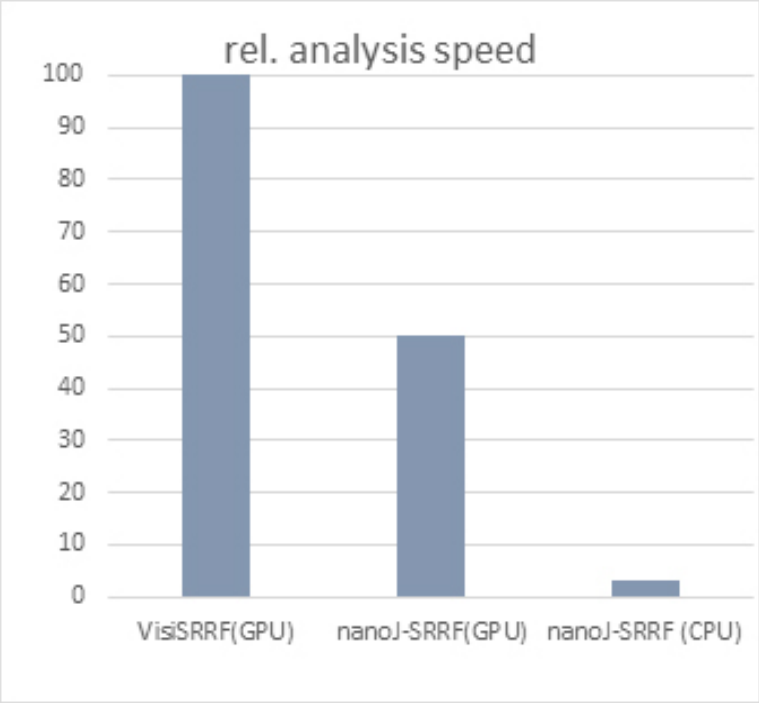
VisiView SRRF?

VisiView can apply VisiSRRF in post-processing as well as during image acquisition. The latter – called SRRF acquisition mode - is simply done by marking a check-box in the main acquisition dialog. Thanks to our ViRTEx Realtime Experiment controller images are acquired at maximum speed and cells are exposed to light only as much as necessary. VisiSRRF analysis is based on nanoJ-SRRF algorithm (by Ricardo Henriques and co.), but it is faster and more convenient to use as it is an integral part of VisiView.



Why is the SRRF algorithm suited for live cell imaging while other SR algorithms are not?

1. It does not require sparsely distributed molecules or fluorophores, of course densly stained, and thick samples will impinge on radiality measures.
2. It works with any conventional fluorophore, although temporal fluctuation and thus resolution might be higher with blinking fluorophores.
3. It only requires very low signal to noise images and hence very low excitation light power(2 × 10−4 kW cm−2 to 2 × 10−1 kW cm−2) which allows long-term multiple imaging.
4. Low signal to noise images are acquired quickly at low exposure times (~10ms). This is very important as about 100 images are necessary to achieve optimal frame number/resolution ratio. Hence the dynamic of living cells is at least partially still represented.

Applying SRRF to widefield and to spinning disk data and: how does SRRF compare to 3D deconvolution?

ASRRF can improve the resolution of all kind of imaging modalities, but the relative improvement is larger for widefield data than for confocal data.

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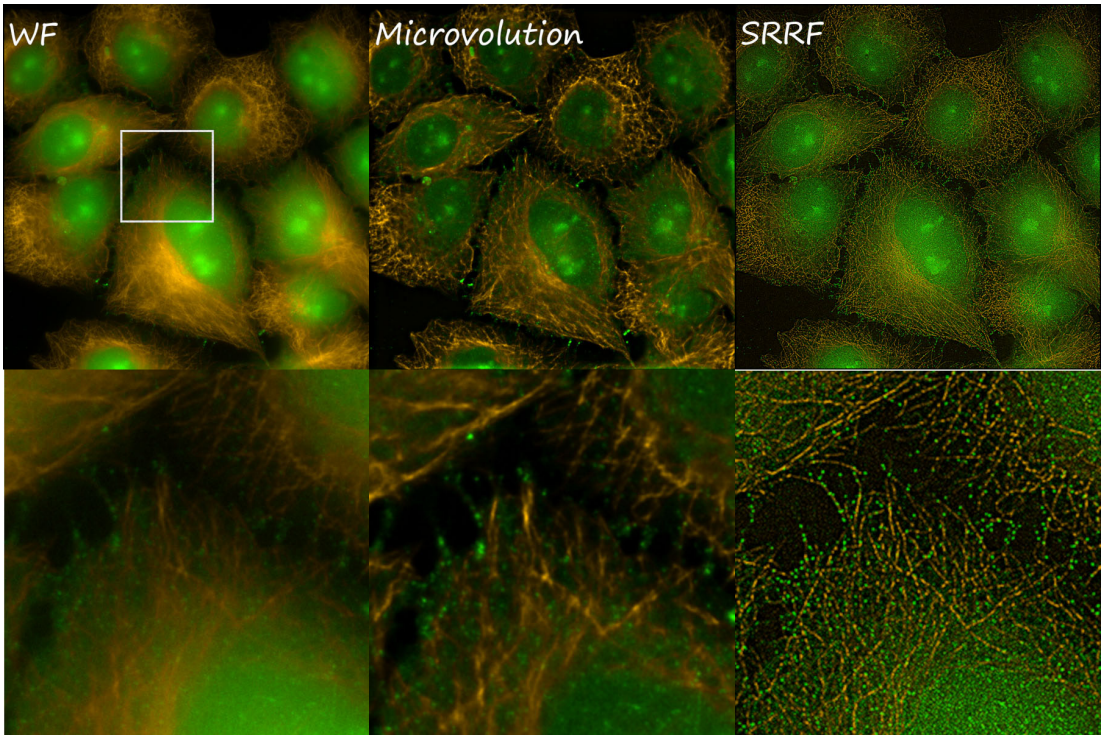

Figure 2:Comparison of SRRF and Microvolution algorithms based on widefield (WF) source data. Images show xxx cells with microtubules stained by AF594 (orange) and viral DNA stained by AF488 (green). Samples are a courtesy of Prof. Dr. M. Schelhaas (Institute of Cellular Virology, Münster, Germany)

Applying SRRF to widefield and to spinning disk data and: how does SRRF compare to 3D deconvolution?

3D deconvolution always requires 3D Data and deconvolves images based on a point spread function (PSF) acquired from your instrument. Alternatively it can estimate the PSF based on the imaging parameters (theoretical PSF). Both methods require additional and tedious steps before deconvolution can be applied. In contrast to that SRRF only requires the 2D Data and can be applied without knowledge of the PSF.
3D deconvolution is useful for deblurring images, increasing contrast and improving resolution. However, the direct comparison of SRRF and 3D deconvolution applied to the same structures shows that SRRF achieves a better resolution and more precisely relocates the origin of the fluorophores to present the circular structure of the viral DNA as well as the tubular structure of the microtubules.