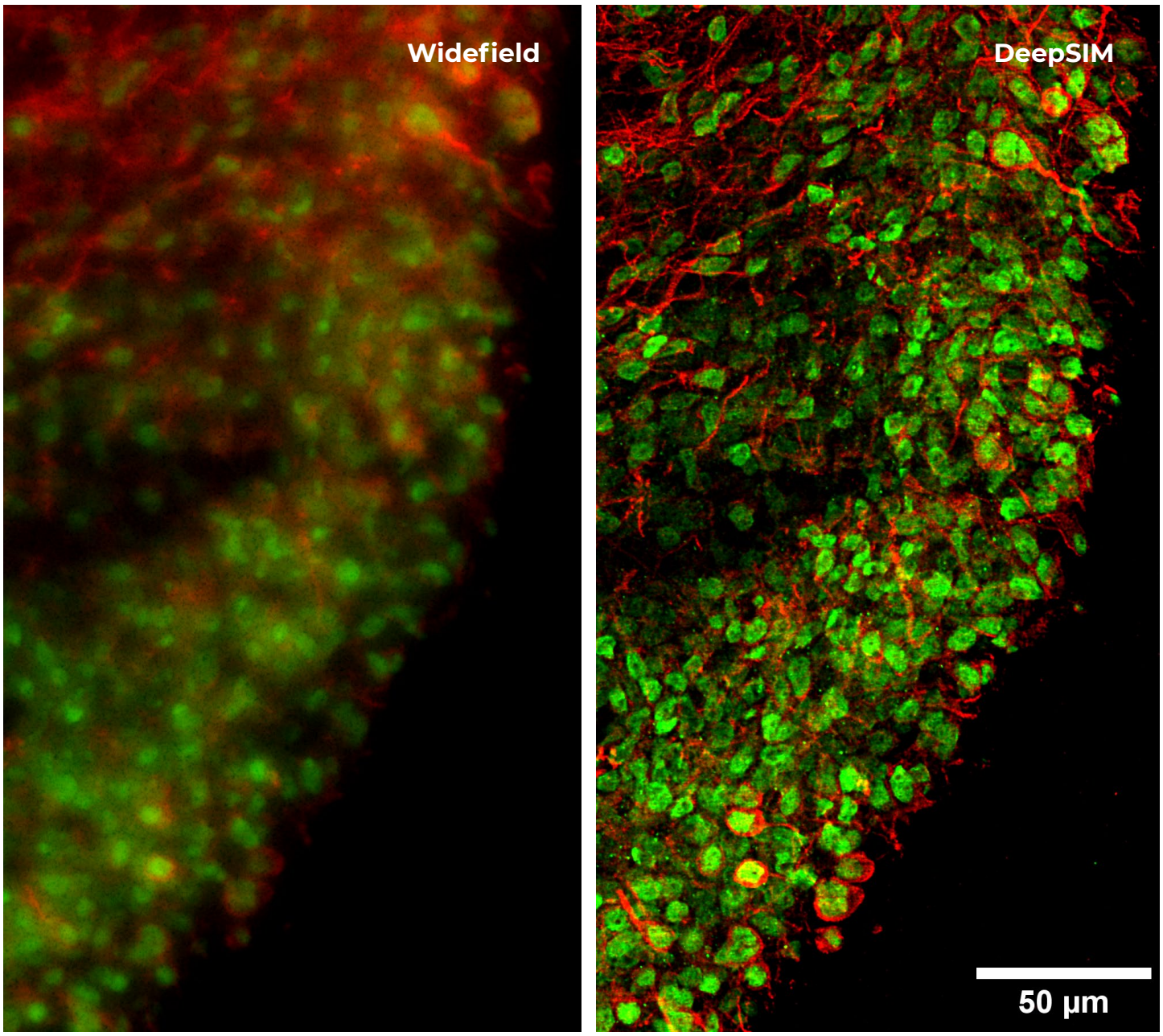


INTRODUCING THE DEEPSIM SUPER-RESOLUTION SYSTEM, A SEAMLESS EVOLUTION OF YOUR MICROSCOPY

Technical Note

Introduction

A fundamental goal of microscopy is to observe and analyze biological processes and structures at the subcellular scale. However, the size of the smallest structures that can be observed is set by the diffraction limit of light, meaning that details smaller than ~200 nm cannot be resolved. Overcoming this barrier is still an active subject of investigation in modern super-resolution microscopy but often, due to their cost and complexity, super-resolution setups are not common tools in all research laboratories. **At CrestOptics, we believe that super-resolved microscopy data should be accessible to all scientists to progress their research. This is the reason why we have developed DeepSIM, a super-resolution module based on a lattice multi-spot structured illumination.**

The DeepSIM module is compatible with upright and inverted microscopes with a camera port. Thanks to its high accessibility and extreme robustness, DeepSIM offers the possibility to every laboratory to integrate super-resolution data acquisition as part of the daily imaging pipeline. **The DeepSIM super resolution module can be integrated either as stand-alone instrument (Fig. 1) or in combination with X-Light V3 spinning disk setup ensuring a seamless evolution from widefield to confocal and from confocal to super-resolution.**

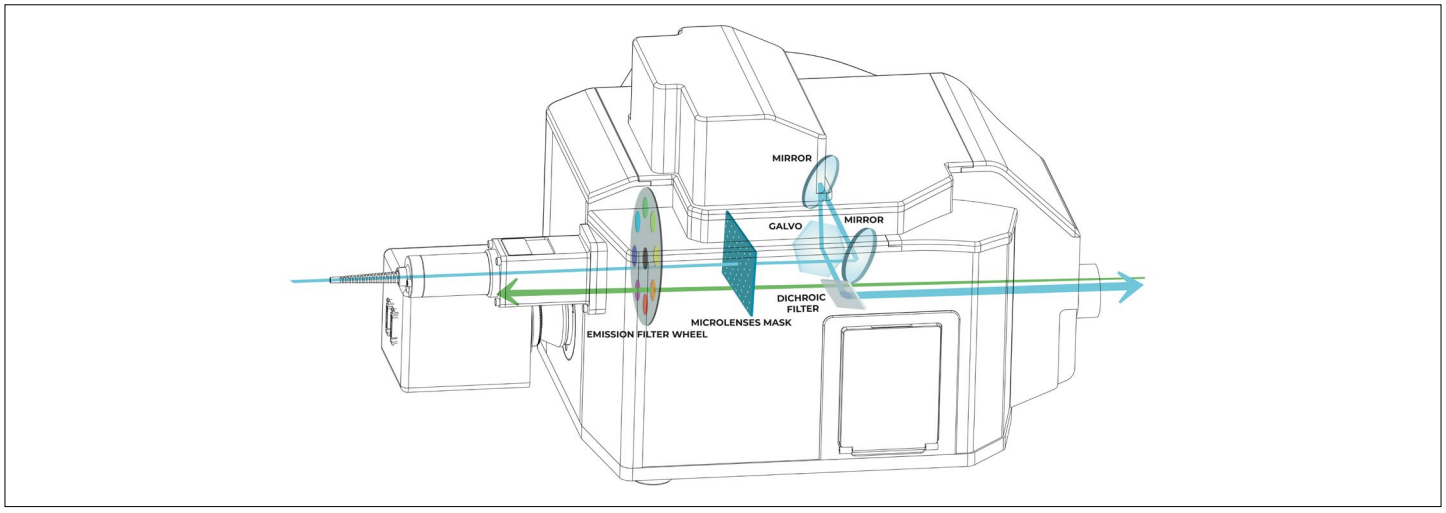


Figure 1: Schematic diagram of the DeepSIM stand-alone. Laser light (cyan line) enters via a multi-mode fiber and passes through the micro-lens array; light is focused through the pinholes of the mask and the lattice pattern is projected to the sample via a motorized galvo. Emission light (green line) coming from the sample goes through a dichroic mirror and emission filters wheel; the selected emission frequency is detected by the camera. For the DeepSIM X-Light architecture in combination with the X-Light V3 spinning disk refers to the technical note "Live cell imaging put at ease".

Due to light dosage and required sample preparation techniques, several techniques commonly used for super-resolution are not suitable for imaging live cells. **DeepSIM can capture raw images over 100 Hz allowing to follow living phenomena in combination with a great degree of optical sectioning and low photo-toxicity**. **This enables DeepSIM to generate super-resolved images deep into fixed and live biological samples, all while using conventional fluorescent dyes and molecules.** This opens the possibility of observing sub-diffraction limited processes and structures within cells and thick tissues that may previously not have been possible to visualize before in the same sample preparation with conventional microscopy.

DeepSIM working principle: the optics

Traditional SIM was invented by Mats Gustafsson ***(Ref. 1)*** and relies on a known periodic illumination patterned made of lines (grid) which are applied to the unknown sample, and by the interference between the grid and the sample new additional spatial information are revealed. With CrestOptics DeepSIM, restrictions of the Gustafsson SIM, such as the limit of going deep into the sample due to poor signal contrast and the unsuitability to follow fast live processes due to the slow scanning, have been overcome. **In the DeepSIM, the light is structured by a mask made of a lattice of spots diffraction limited (i.e., multi-spot lattice SIM) generated by micro lenses.** Through the mask, the light pattern is projected on the sample, and by shifting the mask of a precise angle with a galvo mirror, the sample is entirely scan and multiple raw images are acquired. Raw images are collected and combined by an algorithm to computationally reconstruct a super-resolved image, achieving double spatial resolution that the optical system is capable of **(Fig. 2).**

Since flexibility is a must at CrestOptics, in the DeepSIM system three multi-spot lattice patterns (i.e., three different micro-lens arrays) are available to satisfy a wide range of applications. The three different acquisition solutions related to the lattice design will be explored in the following "Biological applications" paragraph.

In summary, the DeepSIM structured illumination is generated by a micro-lens arrays enabling optimal light throughput, uniform light intensity and optimal out of focus light rejection. Compared to other SIM approaches based on stripes patterns, **DeepSIM ensures high contrast for in-depth acquisition, a great speed increases in imaging acquisition and robust elaboration, eventually ensuring an optimal optical sectioning.**

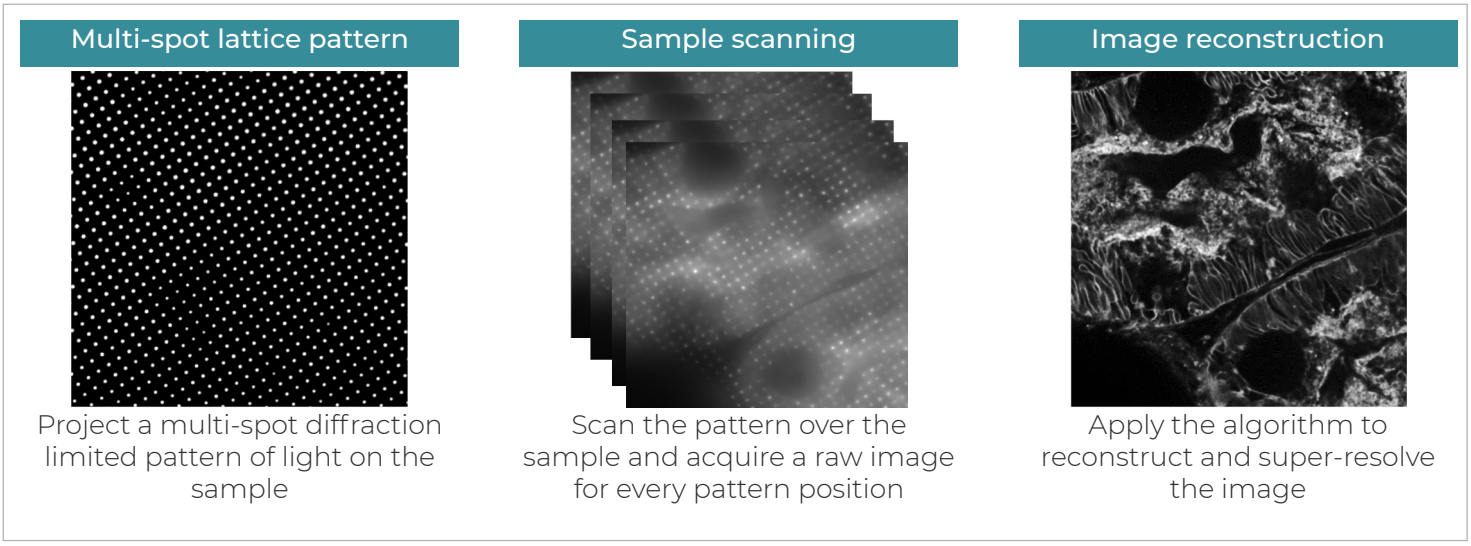


Figure 2: Overview of acquisition steps and data processing with DeepSIM in order to generate a super-resolved image.

DeepSIM working principle: the computation

The final step to get a super-resolved image is to apply a reconstruction algorithm on the acquired raw images. The computation is based on the point spread function (PSF) of the optical system, similarly to what deconvolution process does. Due to the small PSF and good optical sectioning, DeepSIM leads to an overall 2x increase in lateral and axial resolution. Simplifying, the algorithm extracts in focus biological information subtracting out of focus signal contribution. **The DeepSIM approach is a modified version of the joint Richardson-Lucy (jRL) deconvolution and applied to multi-spot SIM enables an improvement in resolution by a factor 2 respect to widefield *(Refs 2;3; Fig 3)*.**

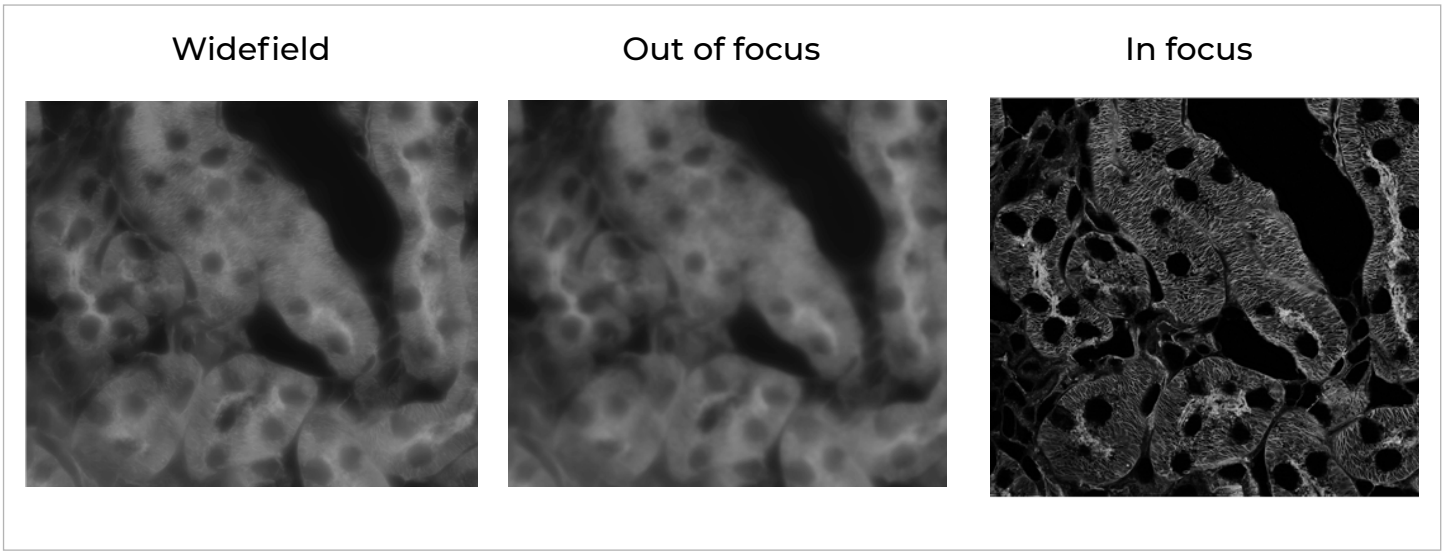


Figure 3: Image examples of a widefield data formed by in focus and out of focus information, out of focus signal calculated by our model and in focus product outcome obtained after computation.

Like the traditional Richardson-Lucy deconvolution method, image reconstruction is achieved by a Maximum Likelihood Estimation (MLE), a statistical approach which, starting from raw images, leads to the output super-resolved image through an iterative method tending to mathematically minimize statistical parameters. In computational mathematics, an iterative method is a mathematical procedure that uses an initial value to generate a sequence of improving approximate solutions for a class of problems, in which the n-th approximation is derived from the previous ones **(Fig. 4)**. In fact, the number of iterations usually required is about 25 iterations and is automatically set. Anyway, the operator can modify this value via software with an “Advanced mode” option available. To note, a highly parallelized CUDA code is used for fast elaboration. **Overall, the DeepSIM computational approach is a special solution which differs from the image reconstruction from the Gustafsson SIM.**

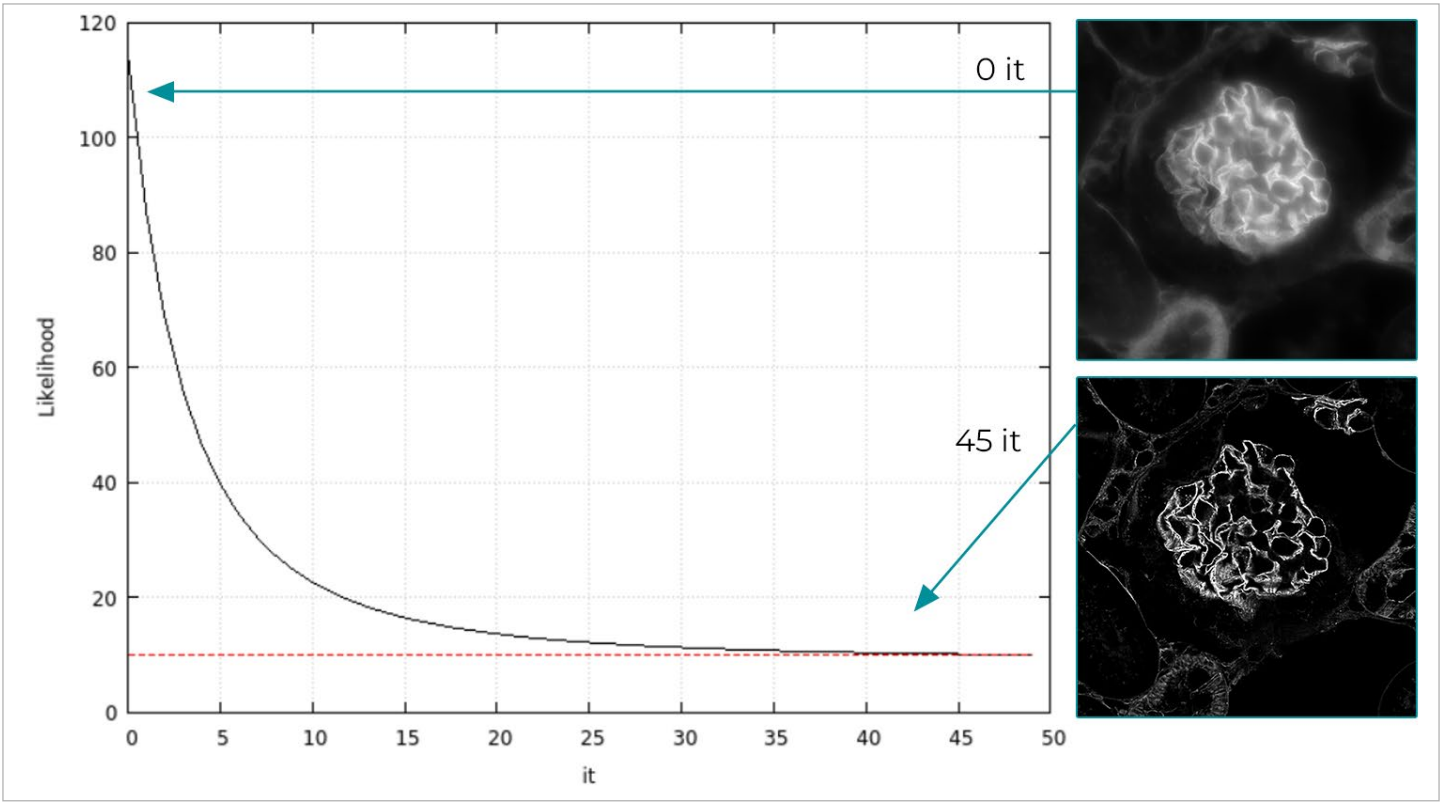
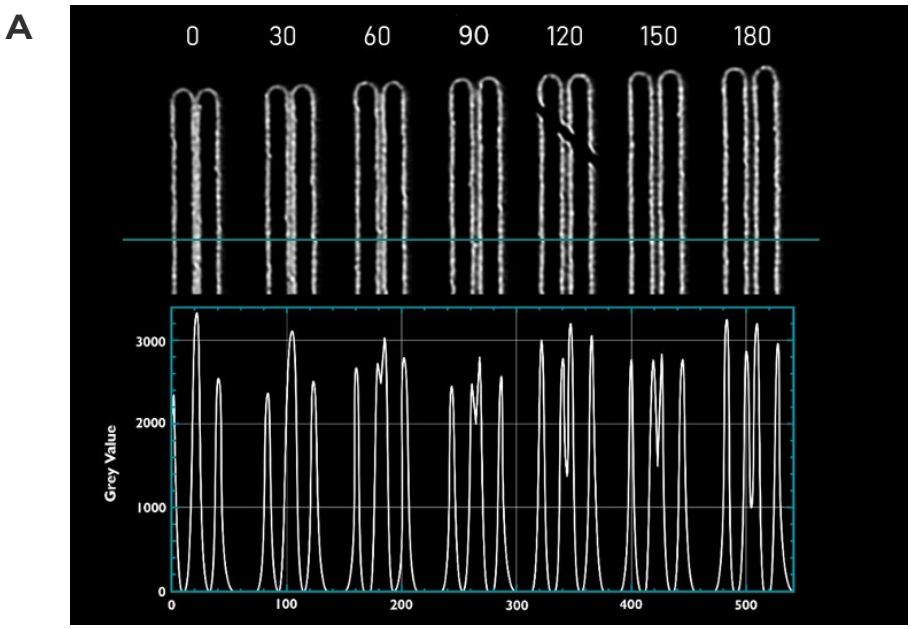
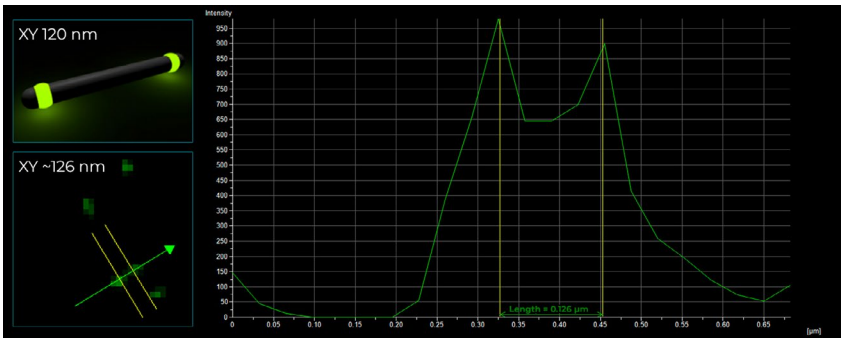
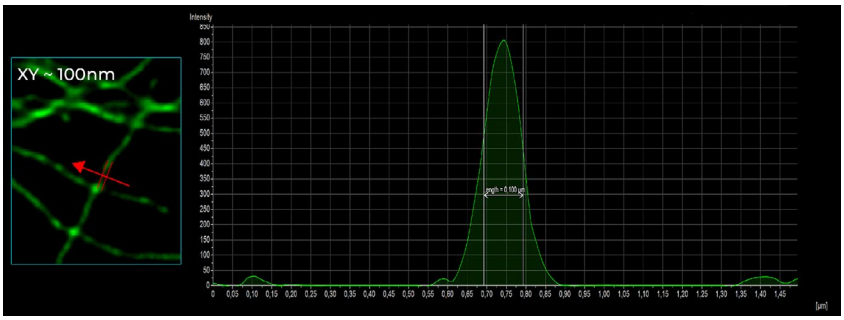


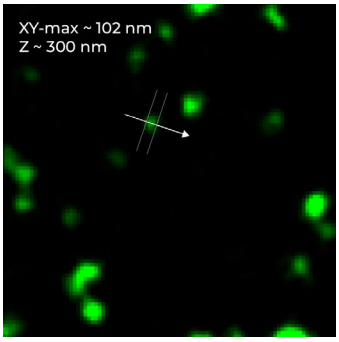
Figure 4: The jRL algorithm requires a mathematical model of the image formation process and precise knowledge of the illumination structure to iteratively combine a multitude of single raw data files into the final super-resolved image. It maximises the likelihood (Y axis) of an estimated super-resolved image to be the source of the raw images when imaged in presence of Poisson noise (photon shot noise) and the Gaussian noise (camera read out noise). According to Bayes's theorem, the high-resolution SIM image maximizes the probability of an estimate X being the origin of the acquired data. Each step of the iteration provides an update of the estimate X that is a closer approximation of the original acquired data.

The DeepSIM approach, a combo between how the sample is illuminated and how computationally super-resolved data are obtained, allows an improvement of the image optical sectioning leading to a resolution enhancement in all 3D dimensions (XY-lateral resolution: ~100 nm; Z-axial resolution: ~300 nm). Resolution measurements (i.e., full width at half maximum (FWHM) value) in XYZ directions have been done with different tools: Argolight SIM-slide, Gattaquant SIM-Nanorules, biological structures such as cellular cytoskeleton and TermoFisher Tetraspeck Beads. See **Fig. 5** for technical details.









FWHM	Bead size
XY-min	80 nm +/- 3.3 nm
XY-max	102 nm +/- 3.3 nm
Z	290 nm +/- 11 nm

Figure 5: FWHM measurements. A) Argolight SIM-slide pattern where bars are progressively separated. The distances resolved are indicated in the figure and values are given in nanometers. B) SIM-Nanorules of 120 nm. DeepSIM measures 126 nm in XY plane. C) Tubulin (cytoskeleton) in microglia cell: 100 nm are measured in XY plane. D) Beads of 100 nm: 102 nm resolved in XY plane, and 290 nm resolved in Z direction. All measurements have been performed at 510 nm emission wavelength with a 100X oil 1.45 NA Plan Apochormat objective.

Deep SIM reconstruction algorithm preserves linearity. The behaviour of the in-focus information has been tested by a linear increase in the exposure time and the measured increase in signal intensity (i.e., the in-focus information obtained with the same reconstruction parameters, such as number of interactions) is linear with the exposure time used to get the raw images (e.g., doubling the exposure time, a double number of counts in the final image is observed; **Fig. 6**)

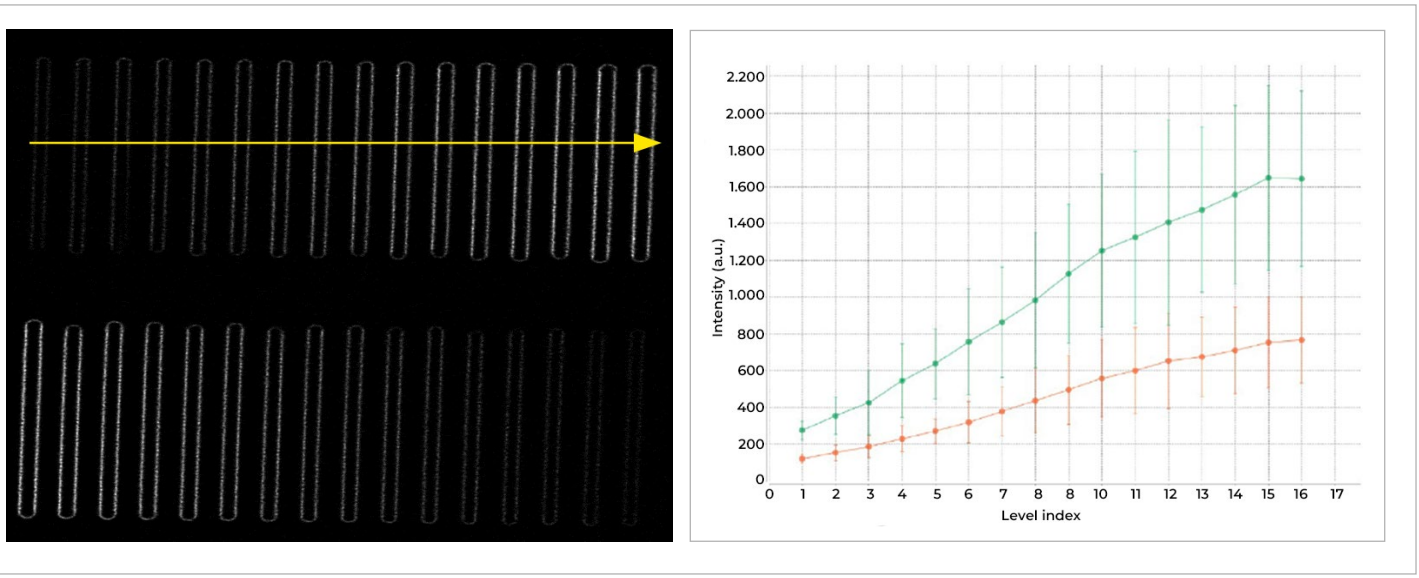


Figure 6: Intensity measurements along the 16 stripes (Argolight SIM-slide pattern: bars with progressive intensity). By doubling the exposure time (green line: 500 ms; orange line: 250 ms) a double number of counts (Y axis, Intensity) is obtained in the final image.

DeepSIM: biological applications

Super-resolution microscopy allows the visualization of subcellular organization with unprecedented detail, but also confront biologists with the challenge of selecting the best-suited approach for their peculiar research questions. Super-resolution commercial solutions are currently limited to a small fraction of possible applications due to slow acquisition rate, and an inadequate depth penetration compromise the whole imaging outcome. Moreover, super-resolution approaches requiring photoconvertible/photo-switchable fluorophores lead to a dedicated sample preparation. In this regard, **the DeepSIM represents a reliable, easy-to-use, and affordable solution to enhance the imaging resolution over the traditional 200 nm light diffraction-limit and get super-resolved data on conventionally prepared samples even from complex, heterogeneous specimens. To satisfy a wide range of applications, the DeepSIM system is equipped with three structured illumination masks which generate specific 2D-lattice multi-spot patterns by means of optimized micro-lens arrays (Table 1)**. The three masks share the same micro-lens diameter and differ in the spacing between spots. Lateral and axial resolution is conserved among the three DeepSIM masks.

More specifically, the ***High Throughput*** mask is suitable for cell monolayers and thin tissues up to 50 µm thickness. With just 17 raw images required for each frame, this imaging option provides a temporal resolution over 10 fps to track live-cell dynamics at a cellular and sub-cellular level with low photon dosage and reduced phototoxicity. Because of light scattering caused by the inhomogeneity of tissues, achieving super-resolved images is even more

difficult within thick specimens where light penetration is limited. To approach biological structures of increasing complexity, the DeepSIM is provided with a ***Standard*** and ***Deep imaging*** masks. With a higher micro-lens spacing compared to the High Throughput, **Standard** and ***Deep imaging*** masks enable optimal out of focus light rejection and higher contrast for in-depth acquisitions over 100 µm depth. **This expands the applicability of the DeepSIM to any conventionally prepared samples suitable for confocal microscopy, including 3D cell models and cleared tissues over 100 µm thickness (Fig. 7)**. The compatibility of the DeepSIM with low magnification (20X objectives) allows to benefit from all the advantages of dry lens, like the absence of immersion solution or a greater working distance, without compromising the quality of the image. To note, additional benefit of the DeepSIM system is the multi-line laser compatibility in the visible spectrum and in NIR up to 750 nm, allowing for a wide range of fluorophore options.

"The different DeepSIM configurations increase the plasticity of every existing microscope system and gives researchers more flexibility in applications, considering the DeepSIM as a powerful platform for super-resolution imaging with standard sample preparation and, potentially, all fluorescent dyes and proteins"

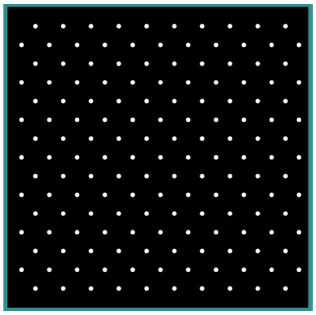
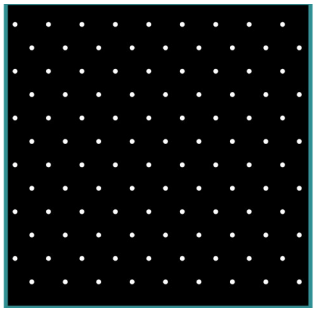
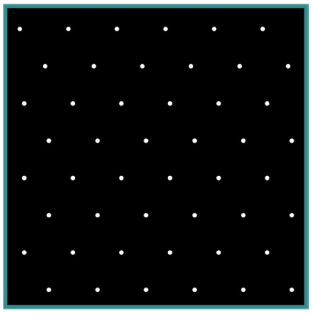
Mask definition	High Throughput	Standard	Deep imaging
N° of raw images	17	37	65
Schematizations of the multi-spot lattice patterns			
Suggested applications	Live cell imaging, cell monolayer/ tissue (up to ~ 50 µm)	Live imaging, thin samples, thick samples (up to ~80 µm)	Dense and homogeneous structures, deep imaging (>80 µm)

Table 1. DeepSIM set of three different micro-lens masks available for different acquisition modalities. Numbers of raw images required for each mask are specified.

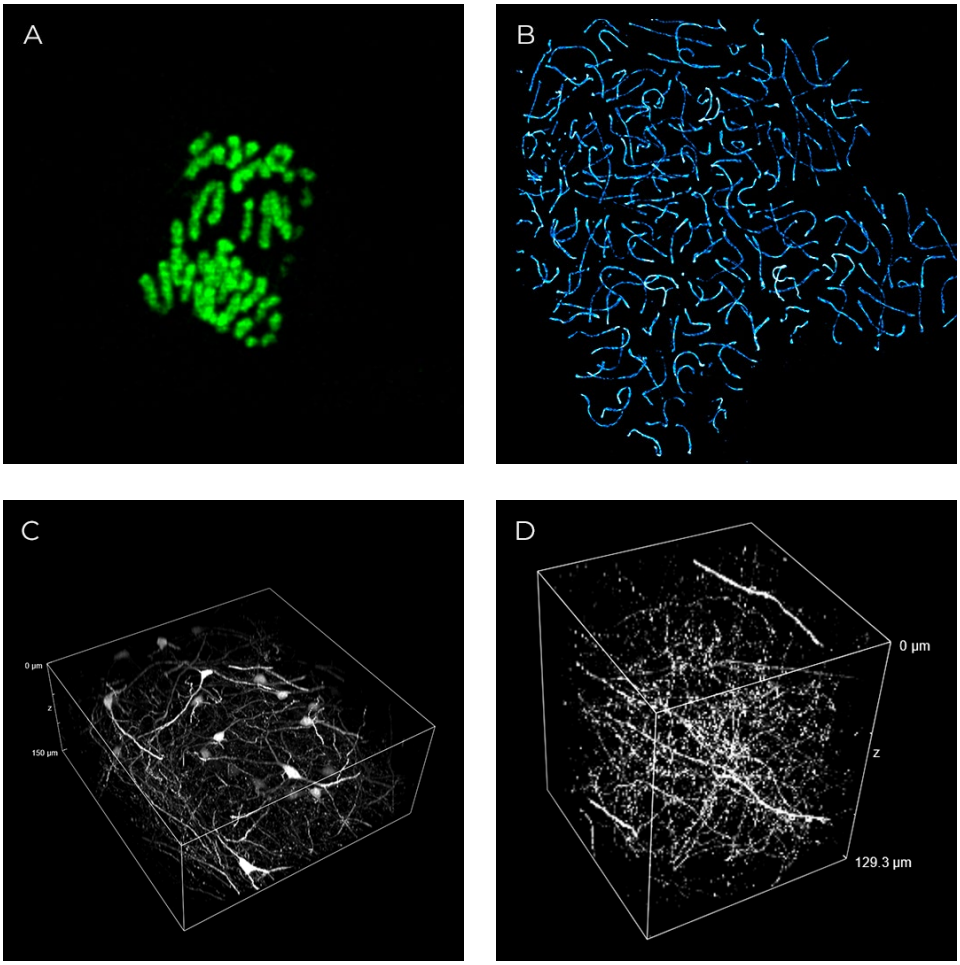


Figure 7: A) 12 hours time-lapse imaging of dividing HeLa cells stained with SPY 505-DNA probe (Spirochrome), 60X oil. Images were obtained with "High Throughput" DeepSIM mask (Link video). B) Chromosome spread showing Synaptonemal complex protein 3 (SYCP3), 100X oil, "High-Throughput" mask was used. C) Cleared mouse brain section (150 µm acquisition thickness) with GFP-expressing neurons, 20X dry, "Standard" mask was used. D) Mouse brain section (130 µm acquisition thickness) with GFP-expressing neurons. 60X oil. Images were obtained with "Deep imaging" mask.

Refer to our latest **DeepSIM application notes** to appreciate the strengths of the CrestOptics DeepSIM for different biological samples, for live-cell imaging and compatibility with a wide range of objective magnifications.

Summary

The DeepSIM relies on a 2D lattice multi-spot SIM technique, reaching 100 nm XY resolution and 300 nm Z resolution (100X, 1.45 NA Plan Apochromat objective). The set of three dedicated micro-lens masks and the compatibility with low and high magnification objectives expand the application of the DeepSIM to a variety of biological samples, from cell monolayers to 3D cell cultures and cleared tissues over 100 µm thick with Z-depth penetration comparable to a confocal microscope. Featuring high photon-efficiency in illumination with a temporal resolution over 10 fps, the DeepSIM is also well suited for live-cell imaging with multiple colours and conventional fluorophores within the 405-750 nm excitation spectral ranges.

Visit our product webpage for more details on DeepSIM.

Contact us to book a demo and see DeepSIM potential with your own eyes.

Technical specifications

	DeepSIM stand-alone	DeepSIM X-Light
FOV	1024x1024pixel (66x66 µm 100X | 333x333µm 20X)	1024x1024pixel (66x66 µm 100X | 333x333µm 20X)
Resolution	Lateral Resolution (FWHM): ~100 nm (100X NA 1.45) Axial Resolution (FWHM): ~300 nm (100X NA 1.45)	Lateral Resolution (FWHM): ~100 nm (100X NA 1.45) Axial Resolution (FWHM): ~300 nm (100X NA 1.45)
DeepSIM Acquisition speed	13fps (1024x1024px)	13fps (1024x1024px)
Laser spectral range	Excitation: 400-750 nm; emission: 400-850 nm	Excitation: 400-750 nm; emission: 400-850 nm
Objective specifications	-from 20X to 100X magnification range -high numerical aperture (NA) -plan apochromat correction	-from 20X to 100X magnification range -high numerical aperture (NA) -plan apochromat correction
Camera compatibility	Any triggerable camera having 6.5 µm pixel size	Any triggerable camera having 6.5 µm pixel size
Multi cameras option	Single camera	Dual camera option available *
Spinning disk upgrade	Stand-alone solution	Add-on compatible with CrestOptics X-Light V3
Imaging modalities	Super-resolution DeepSIM | Widefield	Super-resolution DeepSIM | Confocal spinning disk X-light V3 | Widefield
Upgradable microscope configuration	Upright and inverted configurations	Inverted configuration
Software	µManager /VisiView® / NIS Elements	µManager /VisiView® / NIS Elements
Installation Conditions	Temperature 23 ± 5°C, Humidity 70% RH or less	Temperature 23 ± 5°C, Humidity 70% RH or less
Weight	50.7 lbs | 23Kg	44 lbs | 20Kg
Dimensions	13.8 (w) x 20.2 (L) x 11.4 (h) inches 352.0 (w) 514.0 (L) x 290.5 (h) mm	14.0 (w) x 17.1 (L) x 11.4 (h) inches 356.0 (w) x 435.0 (L) x 290.5 (h) mm

* Software integration in progress

References

1)M G Gustafsson (2000) **Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy**. J Microsc, 198(Pt 2):82-7.

2)N Chakrova et al. (2016) **Deconvolution methods for structured illumination microscopy**. J Opt Soc Am A Opt Image Sci Vis, 33(7):B12-20.

3) R Heintzmann and PA Benedetti (2006) **High-resolution image reconstruction in fluorescence microscopy with patterned excitation**. Appl Opt, 45(20):5037-45.

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