

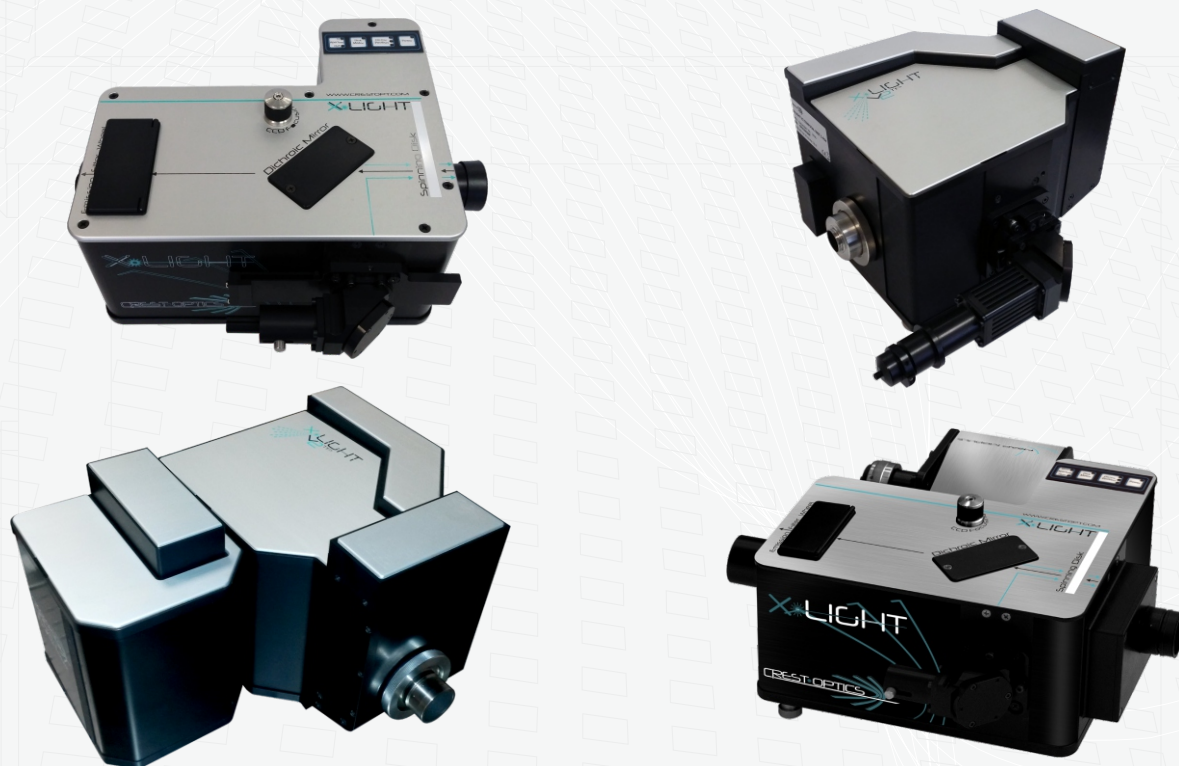
CREST·OPTICS



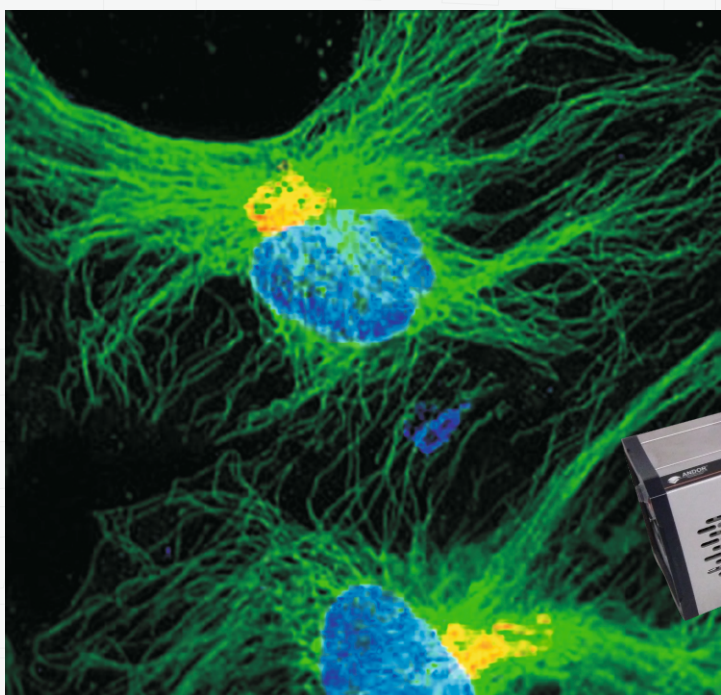
SCIENCE NEEDS DREAMERS

CrestOptics X-Light Spinning Disk Scanner

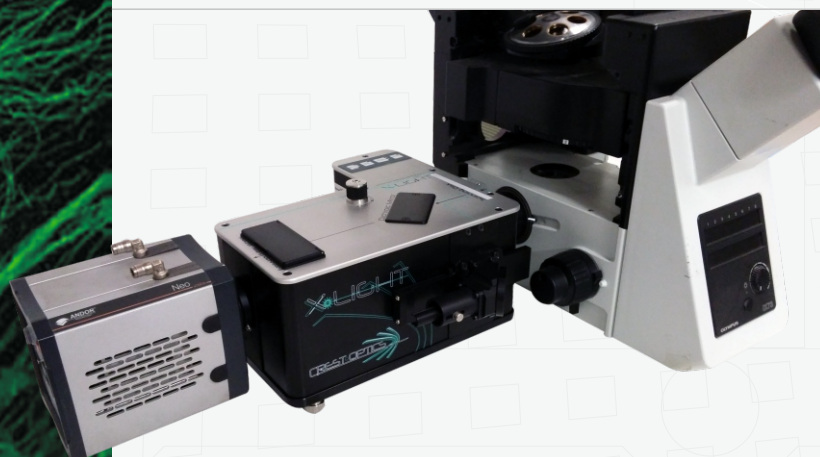
CrestOptics X-Light marks an evolution in Nipkow spinning disk confocal scanner. Coupling with modern high intensity multi-wavelength solid state illumination sources, led sources and high resolution camera, X-Light enables high resolution confocal imaging in an easy to use and cost effective optical package that fits on your existing microscope.



High speed multi-point confocal scanning, combined with high speed sCMOS cameras, minimizes photobleaching and allows real-time imaging and recording at 500 fps or more. Moreover in combination with high sensitive EMCCD cameras allows low signal samples imaging. A long life light source coupled to the instrument allows for full visible spectrum (350nm - 750nm) confocal imaging (and NIR spectrum in some variants). Automation of internal multi-position excitation, dichroic and emission filter wheels allows fast multi-dimensional (X, Y, Z, t) imaging of up to five or more fluorescent probes in the same sample.



Stack projection of HeLa cells with Qdot™ Conjugates.
Nucleus - Qdot™ 655; Golgi - Qdot™ 585;
Microtubules - Qdot™ 525.
Quantum Dot Corporation, Hayward, CA



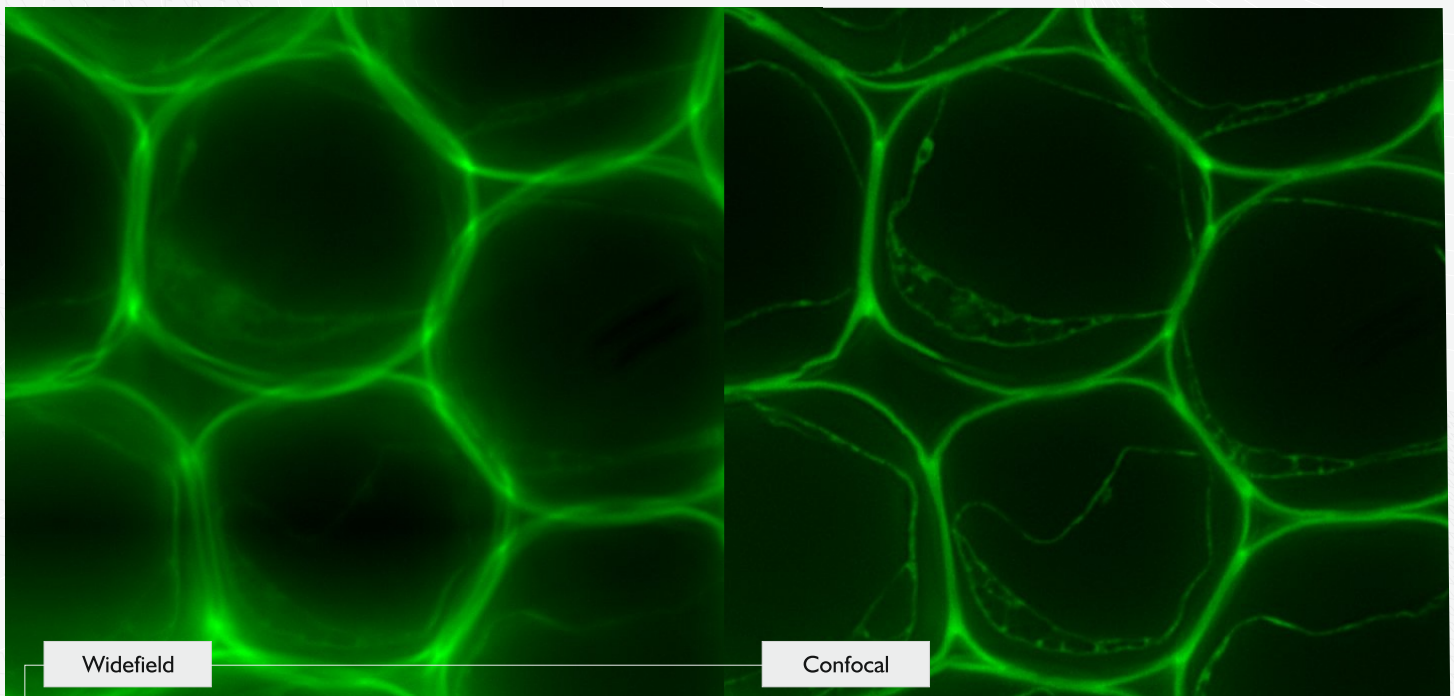
Proprietary Spinning Disk Design

Proprietary design high transmission low cross talk multi-spiral pinhole pattern

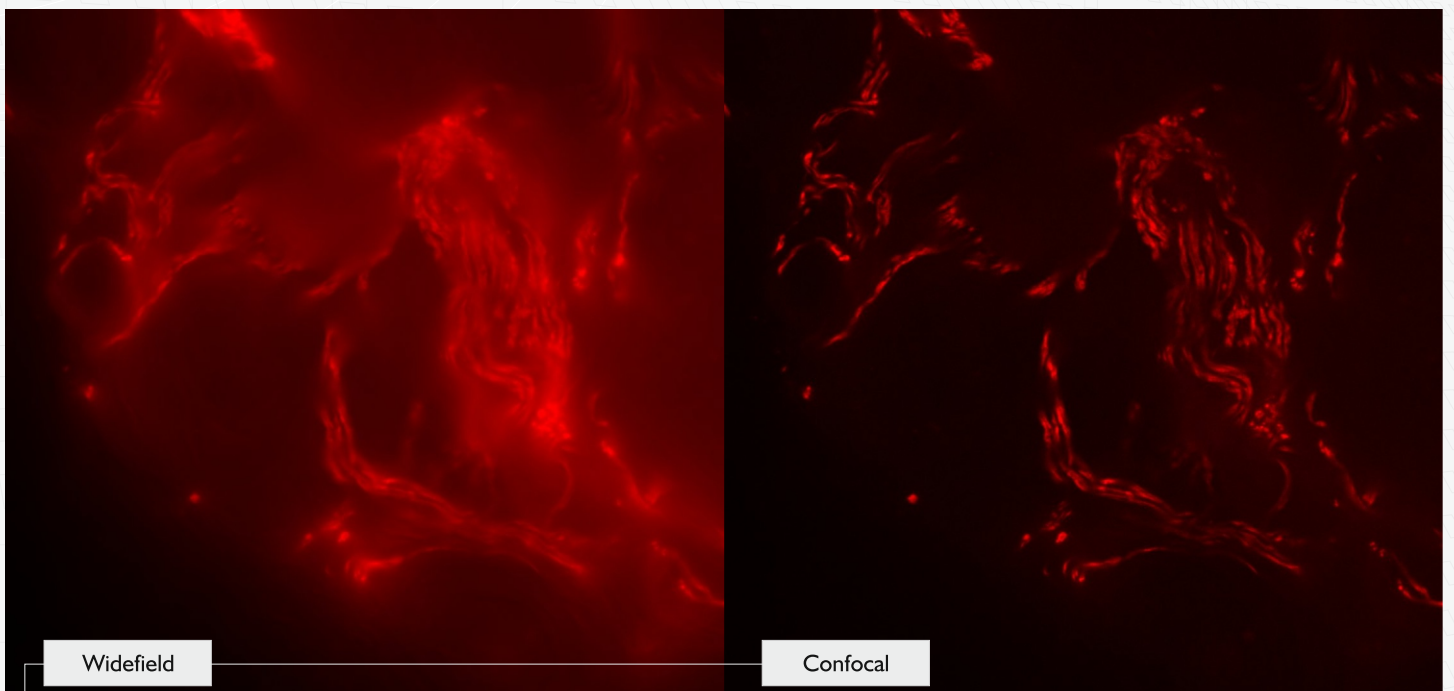
CrestOptics X-Light is a 15,000 RPM spinning disk with choice of single or double patterns spinning disk allowing users to select the appropriate pinhole size to match the objective lens numerical aperture or experiment protocol used.

Long-term stability

X-Light systems show a long-term (years) stability with no need of further alignments after the first installation.



Convallaria stem, O.Garner Bergman-Labora Sweden.



Human skin tissue.

Microscope In-coupling

Compatibility with a large variety of inverted and upright fluorescent microscopes model at 100% light port through C-mount adaptor (or F-mount adaptor in case of Large field of views variants). Thanks to the full motorized and software controllable X-Light filter wheels, even manual microscope are compatible for multi-color confocal imaging. Z-stack collection is done integrating manual microscope with a z-focus step motor.

- It can be installed on all inverted and upright fluorescent microscopes model at 100% light port through C-mount adaptor (or F-mount adaptor in case of Large field of views variants). Thanks to the full motorized and software controllable X-Light filter wheels, even manual microscope are compatible for multi-color confocal imaging. Z-stack collection is done integrating manual microscope with a z-focus step motor.



- A motorized bypass mode between widefield and confocal mode, pinhole size selector, dichroic mirror wheel and emission filter wheel make X-Light easy to automate for a variety of experiments. Easy emission filter and dichroic filter exchange with the provided tools.

Light Source In-coupling

Utilizing a solid state laser or LED based illumination source, the X-Light supports a wide array of excitation wavelengths with very long lifetimes. Illumination input to the X-Light is multimode fiber through an excitation gimbal mount for easy alignment on custom microscope setup and for the best S/N , producing flat, even illumination in the field-of-view. These light sources are fast switching for dynamic multichannel experiments, while still maintaining a very good investment to own and maintain.

Camera In-coupling

Optical relay lenses support up to 22mm FOV in standard configuration and 25mm FOV in large field of view configuration

- A wide selection of high-end cooled EM CCD cameras as well as large format sCMOS camera with a combination of 12- 16 bit information, fast readout, high quantum efficiencies and small pixel size produces image at a high resolution and high signal-to-noise ratio.



Optional image output splitter third party

- Optional TwinCam (splitter for 2 cameras dual imaging) or MultiCam (splitter for up to 4 cameras 4 channels imaging).
- Optional OPTOSPLIT II 1 or 2 images on a single camera: enabling a single camera to record images simultaneously at two different optical wavelengths or other differentiated state.



Software Driver

Full automation into a complete workstation

- Easy to operate: controls all motorized parts, automate image capture sequence.

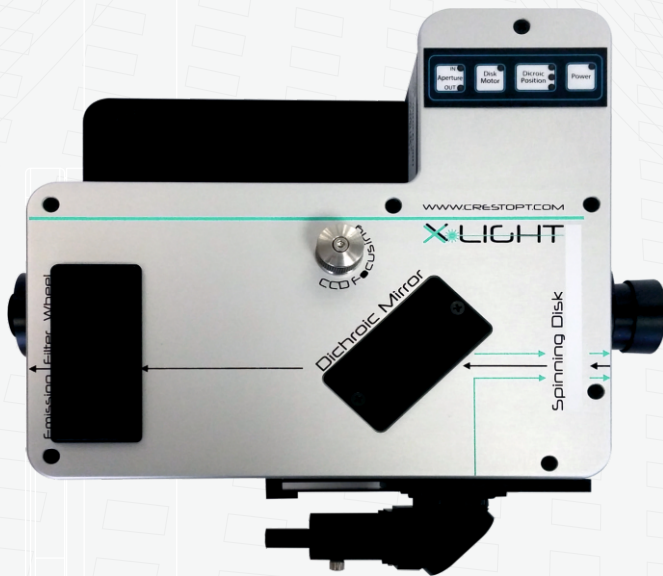
Choices of different package

- MetaMorph, NIS Elements or Micro-Manager plug-in for the X-Light makes it a very easy-to-learn and easy-to-use device for imaging. The X-Light is compatible with multidimensional acquisition, large image XY stitching applications, well plate scanning (HCA) and can be integrated on multimode systems to share a microscope stand with point scanners or other imaging systems.

X-LIGHT

First Generation Spinning Disk Confocal System LED and LASER Sources compatible.

- o Selected/high quality low autofluorescence optical components and anti-reflection coating treatments
- o Full compatibility with all inverted and upright microscopes
- o Full compatibility with third party components such as:
 - o External automated filter wheels
 - o Optical Splitters



Variants:

- X-Light VI "VIS": Standard system operating in the visible range 385nm-750nm
- X-Light VI "VIS-NIR": Standard system optimized in the range 385nm-1000nm
- X-Light VI "UV-VIS": System optimized in the UV-VIS range 350nm-750nm
- X-Light VI "NIR": System optimized for the near infrared fluorescent probes operating in the range 700nm-1600nm
- X-Light VI "20krpm": Standard system with increased disk speed up to 20000rpm



CrestOptics X-Light V1 Specification

Light source	Multichannel LED and Laser source compatibility	
	Illumination source supports hardware triggering for fast multichannel experiment	
Supported input fiber	0.39NA multimode 1.5mm fiber with SMA adapter; excitation gimbal mount for easy alignment on custom microscope setup and for best S/N	
Acquisition modes	<ul style="list-style-type: none"> - Widefield microscopy - Confocal microscopy - Bright-field microscopy - Phase-contrast microscopy 	
Pattern configuration	Double pattern disk 10mm x 10mm FOV each pattern for CCD	40 μm
		70 μm
	Single pattern disk 22mm FOV for large format sCMOS	60 μm
	Option: custom pattern available on request	
Disk speed	15,000 RPM standard (optional 20,000 RPM)	
Confocal resolution	With 60x NA 1.42 oil immersion Objective: <800nm	
Laser clean up filter	Option 3-position manual slider	
Dichroic wheel	Motorized 3-position (motorized 5-position on request)	
Dichroic size	Ultraflat 25.5 mm x 36 mm x 1 mm	
Emission filter wheel	Manual 4-position wheel standard (motorized 8-position on request)	
Emission filter size	25mm diameter, up to 5mm thickness	
Supported microscope	Upright and inverted microscope models from all brands with 100% c-mount output port	
Camera	High sensitivity CCD, sCMOS and EMCCD	
	Easy camera focus adjusting internal optics without moving camera, no further disk and camera alignment needed	
Software control	Micro-Manager, MetaMorph, NIS Elements	

X-LIGHT

Top Performance

V2

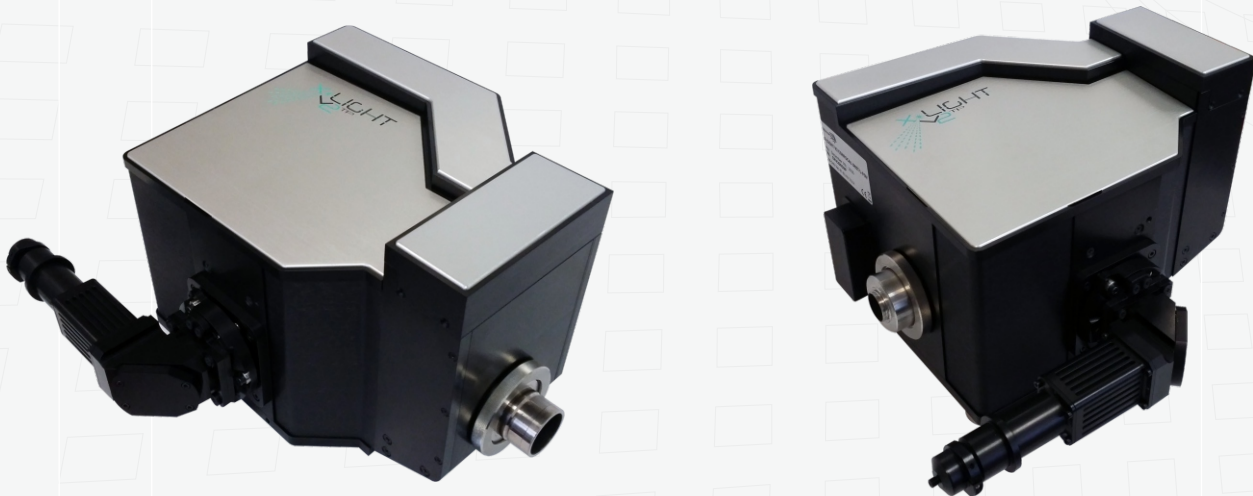


Second Generation Spinning Disk Confocal System

- Selected/high quality low autofluorescence optical components and anti-reflection coating treatments
- Full compatibility with all inverted and upright microscopes
- Full compatibility with third party components such as:
 - External automated filter wheels
 - Optical Splitters

Unique features compared to previous X-Light generation:

- **Plug-in Spinning Disk Box (possibility to change spinning disk in few seconds)**
- Fast and easy opto-mechanical system for the excitation alignment
- New optical system design: diffraction limited over 22mm field of view
- New optical system design: diffraction limited over a large variety of objectives, from high magnification/high NA to low magnification/low NA objectives
- Full compatibility with Crest VCS (Video Confocal Super-resolution) module for 3D resolution enhancement: three microscopy systems at once






Variants:

- X-Light V2 "VIS": Standard system operating in the visible range 385nm-750nm
- X-Light V2 LFOV - 25mm FOV for Nikon Ti2 inverted microscope

CrestOptics X-Light V2 Specification

Light source	Multichannel LED and Laser source compatibility
	Illumination source supports hardware triggering for fast multichannel experiment
Supported input fiber	0.39NA multimode 1.5mm fiber with SMA adapter; excitation gimbal mount for easy alignment on custom microscope setup and for best S/N
Acquisition modes	<ul style="list-style-type: none"> - Widefield microscopy - Confocal microscopy - Bright-field microscopy - Phase-contrast microscopy
Disk pinhole size vs camera size	Double pattern disk at 10mm x 10mm FOV each pattern for CCD
	Single pattern disk at 22mm FOV for large format sCMOS
	Option: custom pattern available on request
Disk speed	15,000 RPM standard
Confocal resolution	60x NA 1.42 oil immersion Objective: <650nm
Dichroic wheel	Motorized 5-position standard
Dichroic size	Ultraflat 25.5 mm x 36 mm x 1 mm or 25.5mm x 36 mm x 2mm
Emission filter wheel	Motorized 8-position wheel standard
Emission filter size	25mm diameter, up to 5mm thickness
Excitation wheel	Manual 4 positions standard
	Motorized 8 positions (on request)
Supported microscope	Upright and inverted microscope models from all brands with 100% c-mount output port
Camera	High sensitivity CCD, sCMOS and EMCCD
	Easy camera focus adjusting internal optics without moving camera, no further disk and camera alignment needed
Software control	Micro-Manager, MetaMorph, NIS Elements

Optical Performances - V2 Diffraction Limited Performances

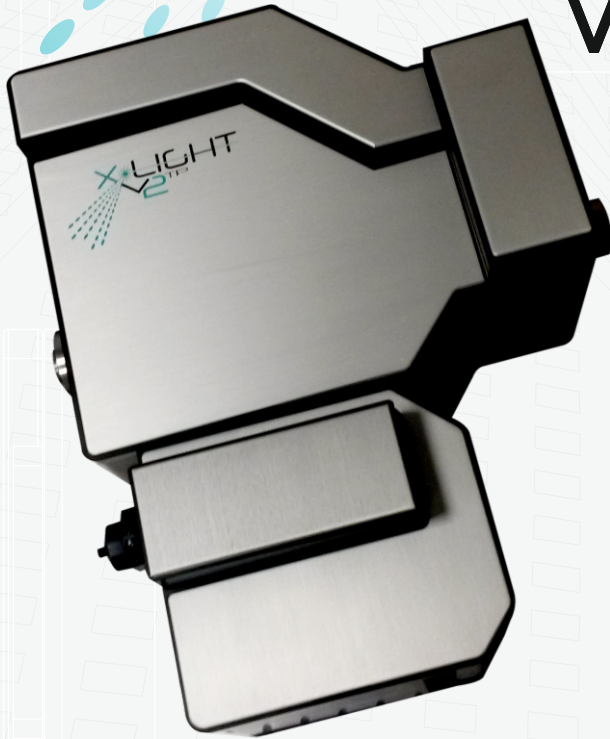
Reference wavelength: 587nm				
Magnification	NA	Theoretical Airy Disk Radius (um)	Actual Spot Size Radius (um)	Spot Size View*
100	1.45	24.8	4.1	
60	1.4	15.6	5.9	
20	0.75	9.6	7.6	

* Black circle: theoretical Airy Disk

X-LIGHT

Top Performance

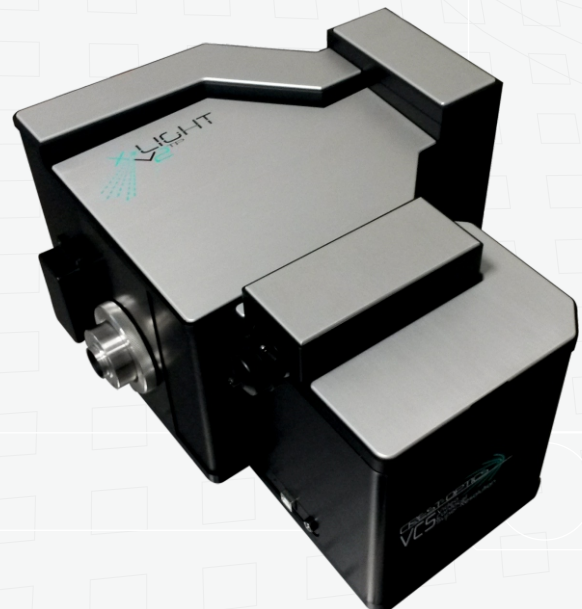
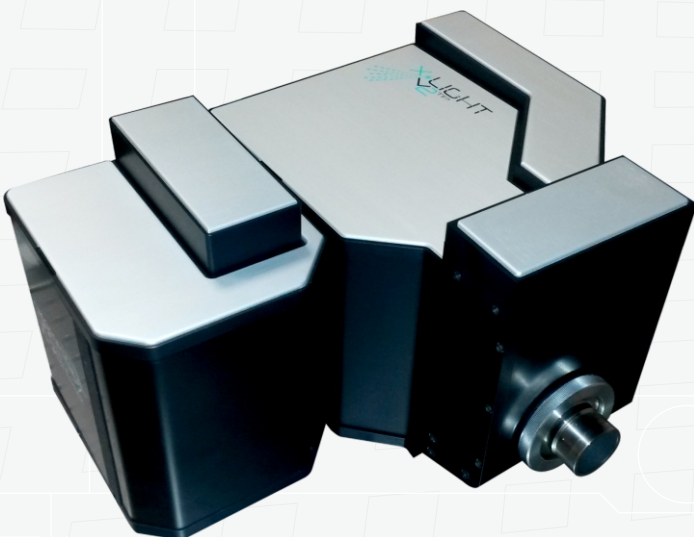
with
VCS



Super-resolution imaging for any fluorescent stained sample

VCS (VideoConfocal super-resolution) module is a new system developed as an add-on for the X-light V2.

Using an approach based on super-resolution patents which exploit the 2-dimensional scan of the sample with a pinhole pattern and several algorithms application, CrestOptics-VCS enhance 3D resolution of the standard light microscope.



Super-Resolution

Three microscopy techniques in one solution

The full system X-Light V2 with VCS is thought to work in three configurations:

- Widefield mode
- Confocal mode
- Super-resolution mode

VCS technique includes the following phases:

- Specimen illumination with a multipoint beam obtained filtering the excitation light with a mask
- Multipoint beam scan along orthogonal directions (u,v) parallel to the illumination plane
- Acquisition of detected light coming from the specimen for each position of the illumination grid.
- Each acquired image is described by the distribution intensity for each pixel (x,y)
 $I_{u,v}(x,y)$
- A final super-resolved image is calculated from the N raw images using super-resolution algorithms

Widefield

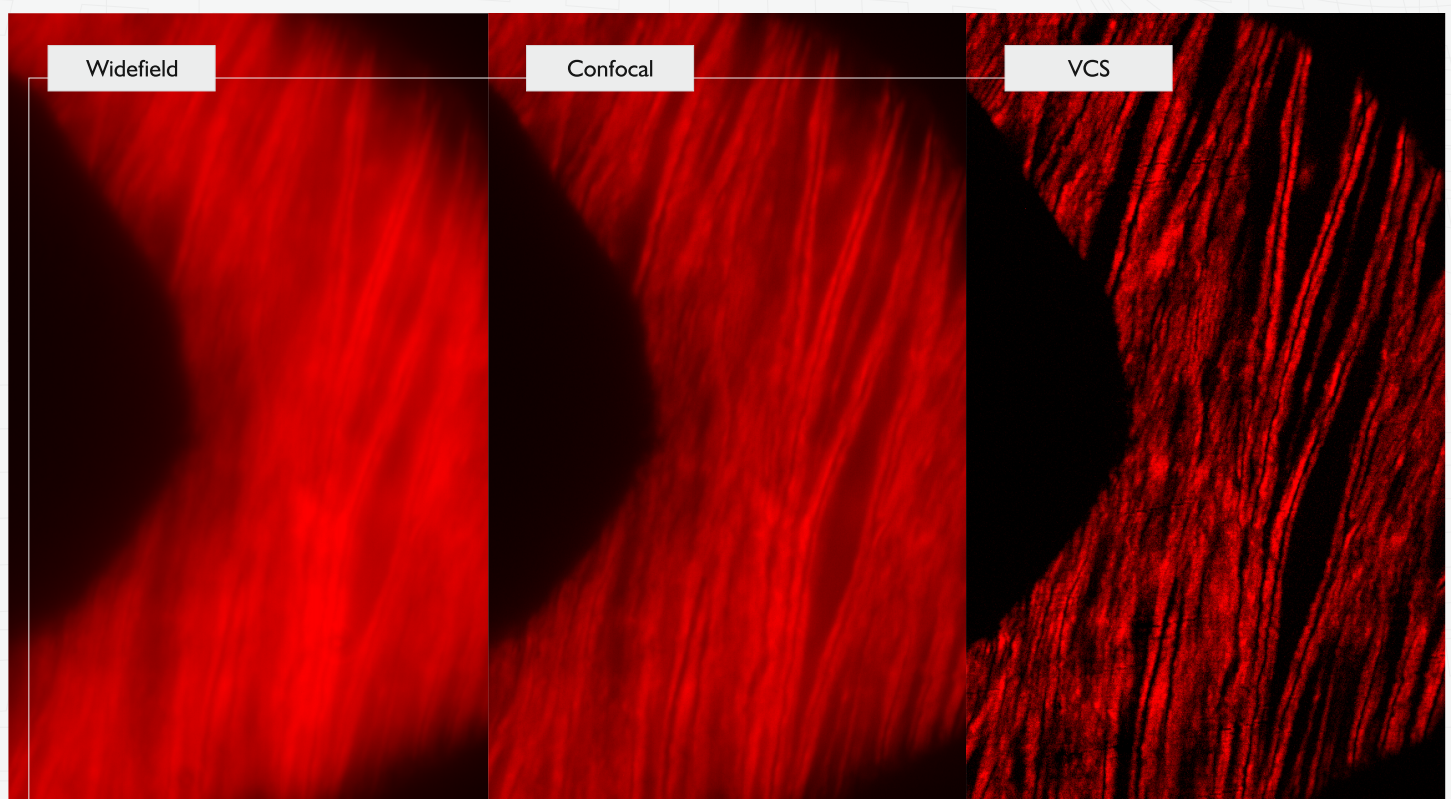
Confocal

VCS

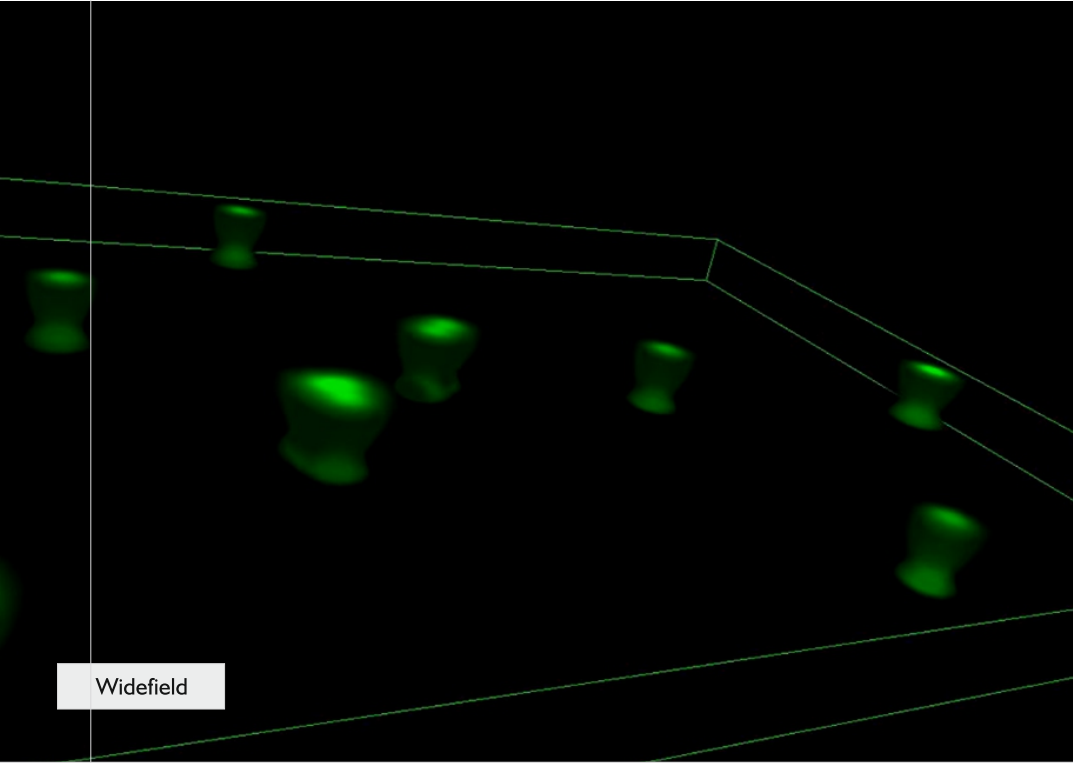
CrestOptics VCS Specifications

VCS resolution*	Lateral ~ 120 nm Axial ~ 300nm
Axial Range	Up to 110 μ m
Imaging	- 2D SIM - 3D SIM - Bypass mode for Confocal and Widefield acquisition
Multi-Color Imaging	Motorized Focus Lens correction for multicolor imaging
VCS Source	LED and Laser source compatible with multimode 1.5mm fiber, 0.39NA
VCS Field of View	Up to 22mm FOV
VCS objectives	Plan Apo corrected objectives, High Magnification, High NA compatibility: - 60x - 100x
Operational conditions	No specific conditions required in temperature and sample preparation. Anti-vibration setup required
VCS detector	sCMOS detectors high QE > 80%, low readout noise required
Software compatibility	Metamorph, Nis-Elements
GPU card	GPU video card/CUDA specification: - CUDA computational capability 5.0/5.2 - GPU Memory: 4 GB or more

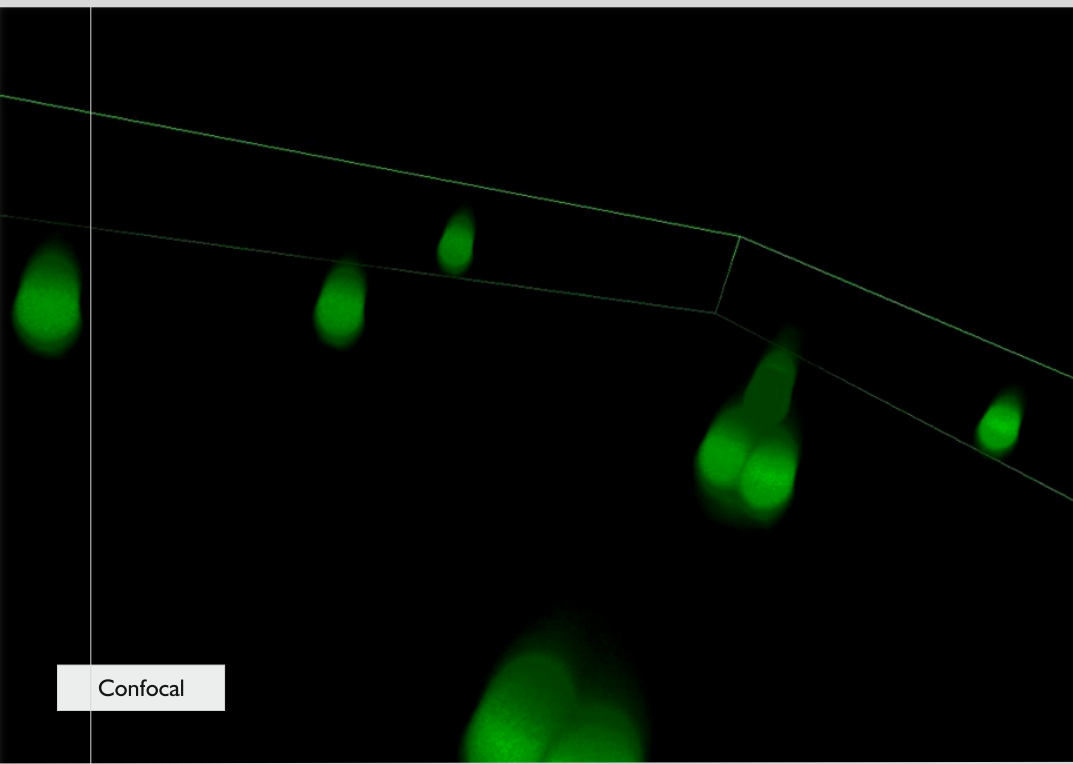
* Depending on optics and super-resolution algorithms used



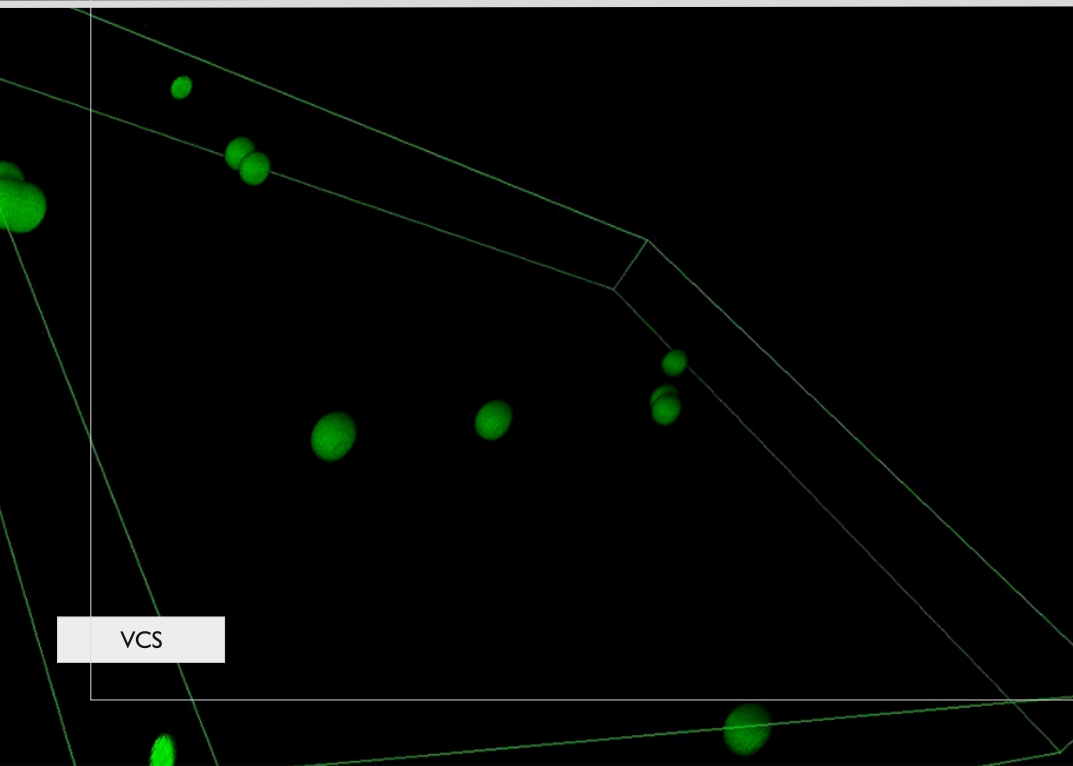
Mouse kidney



Widefield



Confocal



VCS

Using three different microscopy acquisition modes offered by CrestOptics confocal and super-resolution system, the resolution improvement of 3D reconstruction of $1\mu\text{m}$ extended objects is shown.

3D beads reconstruction

X-LIGHT

Top Performance

L-FOV

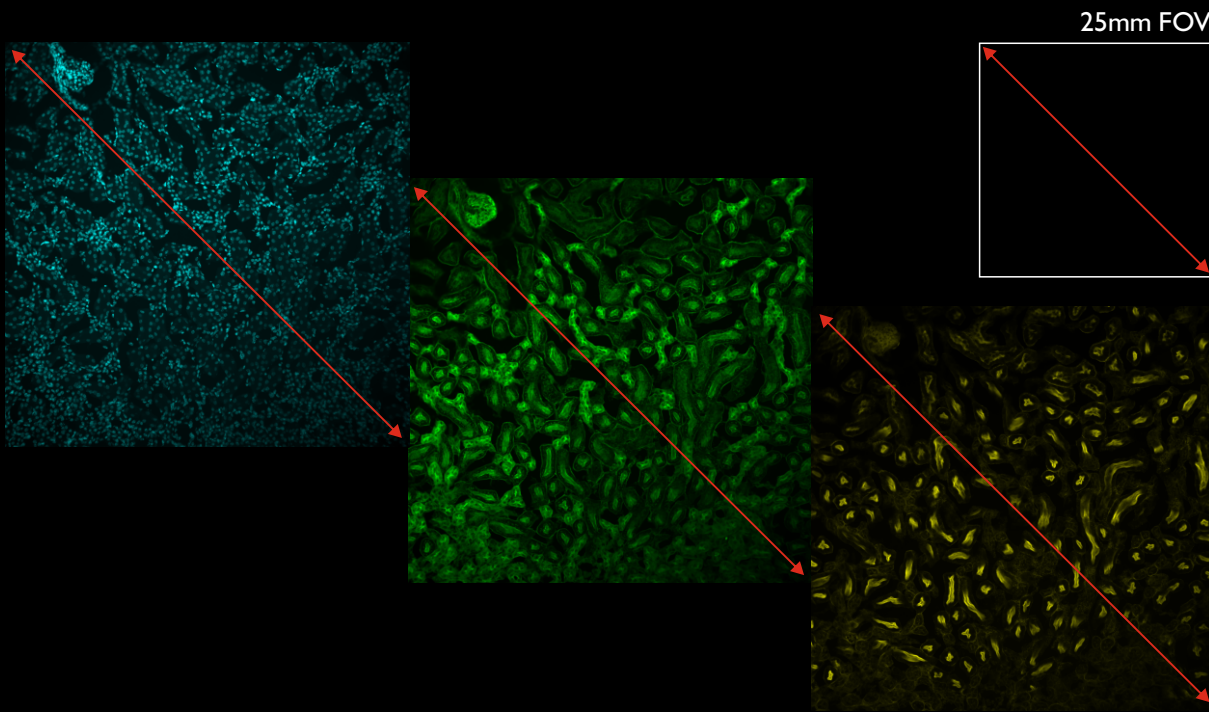


Second Generation Spinning Disk Confocal System with enhanced field of view capability

- Unique confocal microscope to cover 25mm field of view
- Tailored to Nikon Eclipse Ti-2 inverted microscope
- Selected high quality low autofluorescence optical components and anti-reflection coating treatments

Unique features with compared to previous X-Light generation:

- Plug-in Spinning Disk Box (possibility to change among multiple pinhole patterns in few seconds)
- Fast and easy opto-mechanical system for the excitation alignment
- New optical system design: diffraction limited over 25mm field of view
- New optical system design: diffraction limited over a large variety of objectives, from high magnification/high NA to low magnification/low NA objectives
- Thanks to the extended field of view capability it acquires very large tissue areas with a strongly reduced number of acquisitions.



CrestOptics X-Light V2 L-FOV Specification

Light source	Multichannel LED
	Illumination source supports hardware triggering for fast multichannel experiment
Supported input fiber	3mm LLG adapter; excitation gimbal mount for easy alignment on custom microscope setup and for best S/N
Acquisition modes	<ul style="list-style-type: none"> - Widefield microscopy - Confocal microscopy - Bright-field microscopy - Phase-contrast microscopy
Disk pinhole size vs camera size	Single pattern disk at 25mm FOV
	Option: custom pattern available on request
Disk speed	15,000 RPM standard
Confocal resolution	60x NA 1.42 oil immersion Objective: <650nm
Dichroic wheel	Motorized 5-position standard
Dichroic size	Ultraflat 25.5 mm x 36 mm x 1 mm or 25.5mm x 36 mm x 2mm
Emission filter wheel	Motorized 8-position wheel standard
Emission filter size	25mm diameter, up to 5mm thickness
Excitation wheel	Manual 4 positions standard
	Motorized 8 positions (on request)
Supported microscope	Nikon Eclipse Ti-2 inverted microscope
Camera	Large format cameras with F-mount
	Easy camera focus adjusting internal optics without moving camera, no further disk and camera alignment needed
Software control	Micro-Manager, MetaMorph, NIS Elements

X-LIGHT

PRO Confocal Imager

Combined simultaneous Photo-stimulation and Confocal Imaging

X-Light Pro scanner system combines the disk scanner with a dual mirror galvo scanner for single point laser spot photo-stimulation. Control of the two independent scanning mirrors enables simultaneous stimulation and imaging to capture real-time reactions during stimulation at no delay time. Unsurpassed optical performance with selected high quality low autofluorescent optical components and anti-reflection coating treatments.

- FRAP Fluorescent Recovery after Photobleaching by photobleaching a small region of a sample in order to monitor the diffusion rate of fluorescent labeled molecules back into the photobleached region
- Uncaging
- Photo conversion protein
- Optogenetic: receptor activation, protein recruitment, ion channel control
- Damage and repair



FRAP features:

- Laser pulse time per scanning pixel $\geq 40\mu\text{s}$ -Scanning bitmap ROI: up to 8 ROIs at the same time
- Scanning geometrical ROI: up to 100 ROIs at the same time
- Autocalibration: 0 - 360 degree camera rotation -Software driver: MetaMorph
- Output signal power laser: 0 - 5 Vdc Angular Galvo mirror scaling: 10% - 100%
- TTL laser pulse polarity inversion via serial command

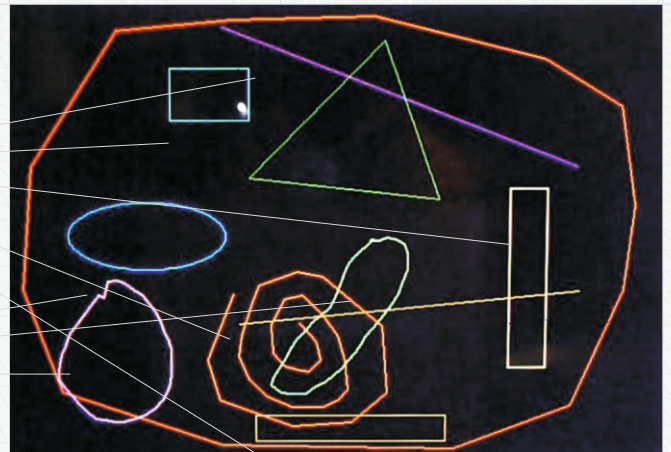
CrestOptics X-Light PRO for Confocal Imager



Highly selective photobleaching scanning region

Two types of ROI:

- Geometrical ROI: lines, squares, rectangles, points, multiline
- Bitmap ROI: Circles, ellipses, closed free-hand curves



CrestOptics X-Light PRO for Confocal FRAP Specification

Software control	MetaMorph
FRAP features	Dual fast galvo mirror scanning, 1.5ms sweep time, 256X256 or 512X512 points scanning resolution
	Single mode laser system (380 -780nm), laser pulse time per scan $\geq 40\mu s$
	Diffraction limit laser spot at sample $< 900nm$ diameter @470nm, scan up to 100 ROIs
	Software includes line, square, rectangle, point, multiline, circle, ellipse, closed free hand curves and areas
	Onboard firmware & fast RAM for automated operation

X-LIGHT NIR



First Generation Spinning Disk Confocal System working in the Near-Infrared spectrum

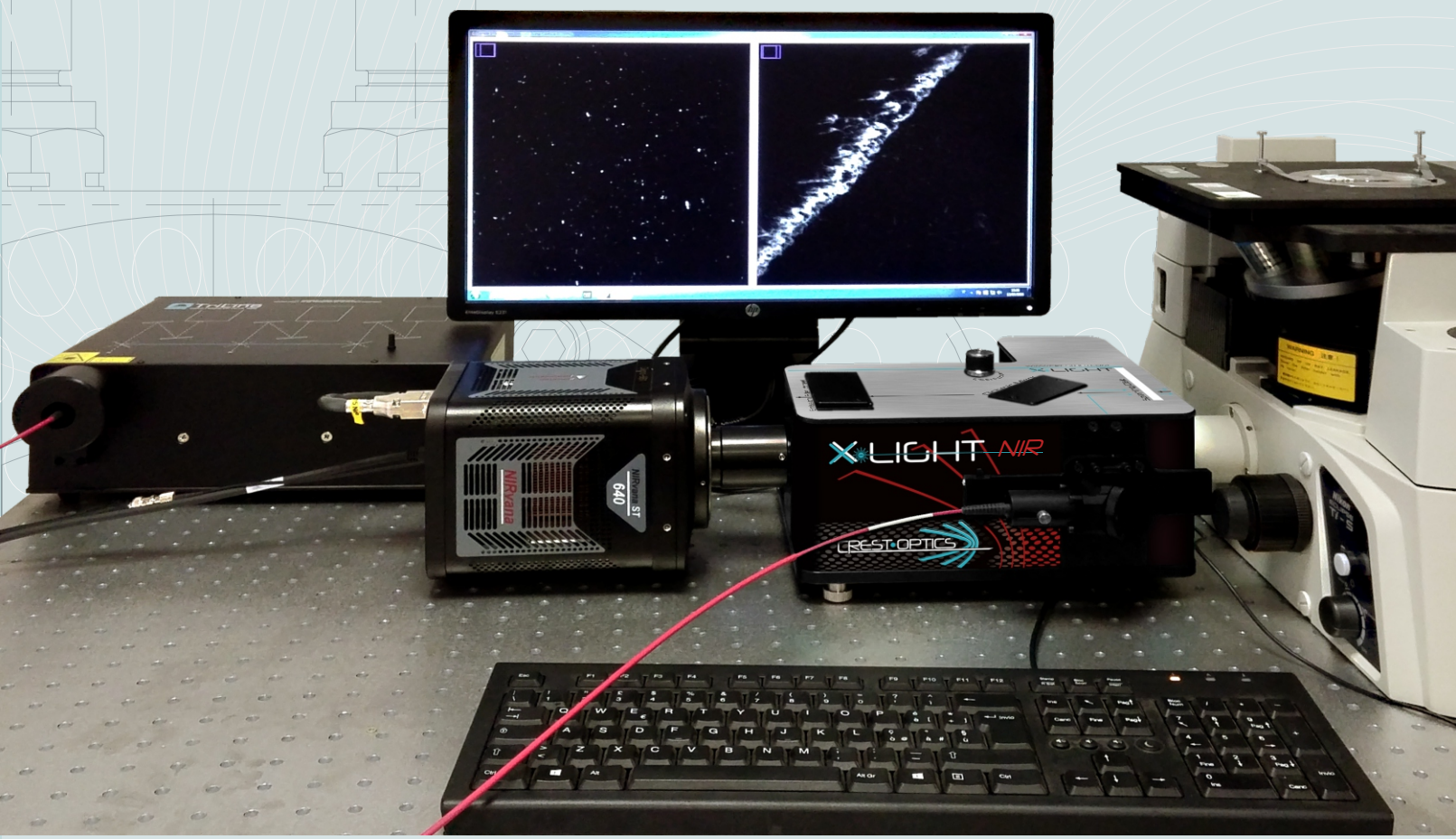
- Unique confocal microscope working in the range 700nm-1600nm
- Excitation range: 700nm – 1000nm
- Emission range: 800nm – 1600nm

Unique features with compared to the standard X-Light generation:

- New optical system design: diffraction limited over 18mm field of view in the imaging range 900nm-1600nm
- New optical system design: highly optimized AR coatings in the imaging range 800nm-1600nm

CrestOptics X-Light NIR Specification

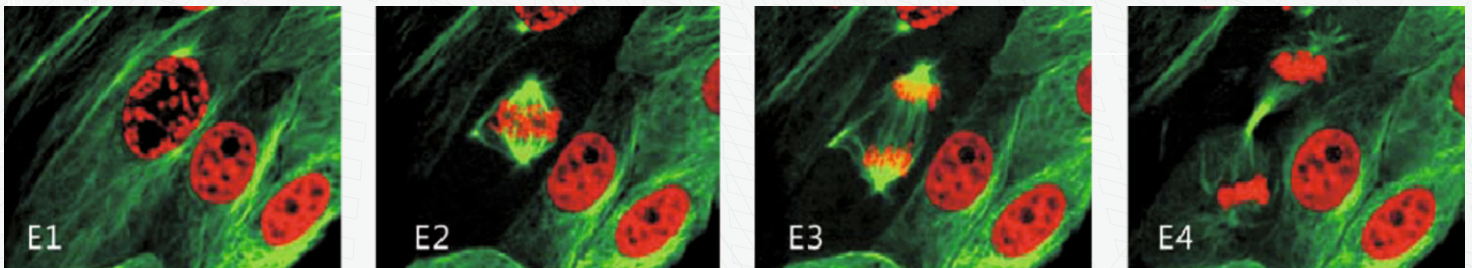
Light source	Laser	
Supported input fiber	0.39NA multimode 1.5mm fiber with SMA adapter; excitation gimbal mount for easy alignment	
Acquisition modes	<ul style="list-style-type: none"> - Widefield microscopy - Confocal microscopy - Bright-field microscopy - Phase-contrast microscopy 	
Pattern configuration and Field of View	Double pattern disk 10mm x 10mm FOV each pattern	40 μ m 70 μ m
	Single pattern disk 18mm FOV for large format	60 μ m
	Option: custom pattern available on request	
Disk speed	15,000 RPM standard (optional 20000 RPM)	
Lateral resolution	25%-to-30% improvement with respect to widefield in the NIR region	
Laser clean-up filter	3-positions manual slider	
Dichroic wheel	Motorized 5-position standard	
Dichroic size	Ultraflat 25.5mm x 36mm x 1mm	
Emission filter wheel	Motorized 8-position	
Emission filter size	25mm diameter, up to 5mm thickness	
Camera	Infrared camera in the 900nm-1600nm imaging range	
	Easy camera focus adjusting internal optics without moving camera, no further disk and camera alignment needed	
Software control	Micro-Manager, MetaMorph, NIS Elements	



Application Techniques

Routine and advance uses, ideal for living cell prolonged time lapse fluorescent imaging

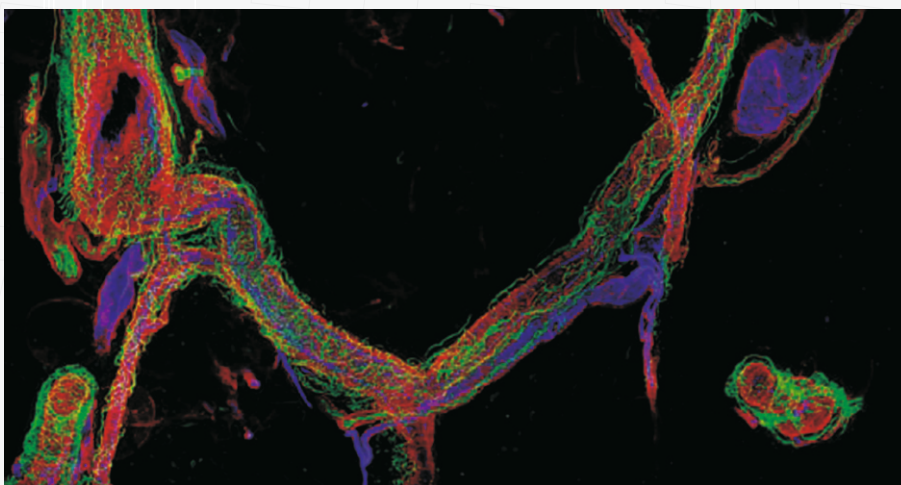
- The significant advantages in the spinning disk approach are the ability to monitor rapidly occurring events within living cells without compromising resolution, as well as the high frequency low intensity signals because of substantially reduced photobleaching and phototoxicity.



E - Cell mitosis: Anti-tubulin antibody (green) and propidium iodide (red)

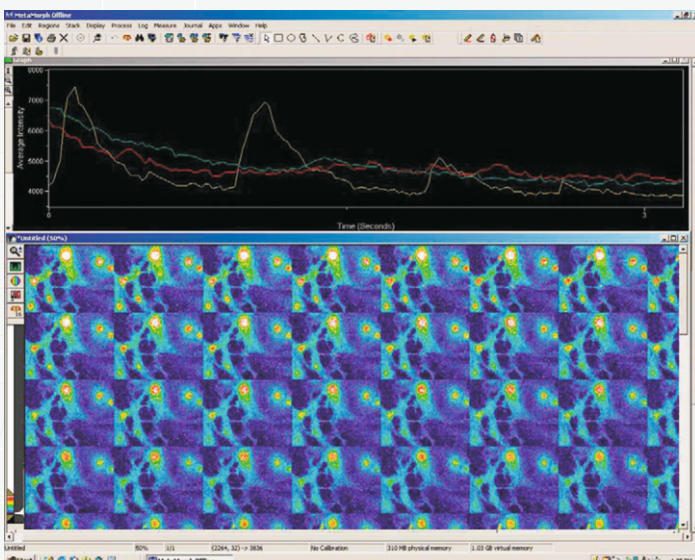
Investigation for Multicolor Colocalization, 3D optical sectioning and reconstruction and multi-dimensional imaging (X, Y, Z, t), especially ideal for live cell confocal imaging in much reduced photo-toxicity and photobleaching.

- Specimen fluorescent staining: fluorescent dye staining, immuno-fluorescence assay, fluorescent in-situ hybridization, fluorescent protein, microinjection of fluorescent labeled actin probes into living cells.



3D reconstruction skin sample, pan-neuronal marker, protein gene product 9.5 (Cy3), basement membrane marker, type IV collagen (CY2), endothelial cells are stained by Cy5-Ulex europeausagglutinin type I.

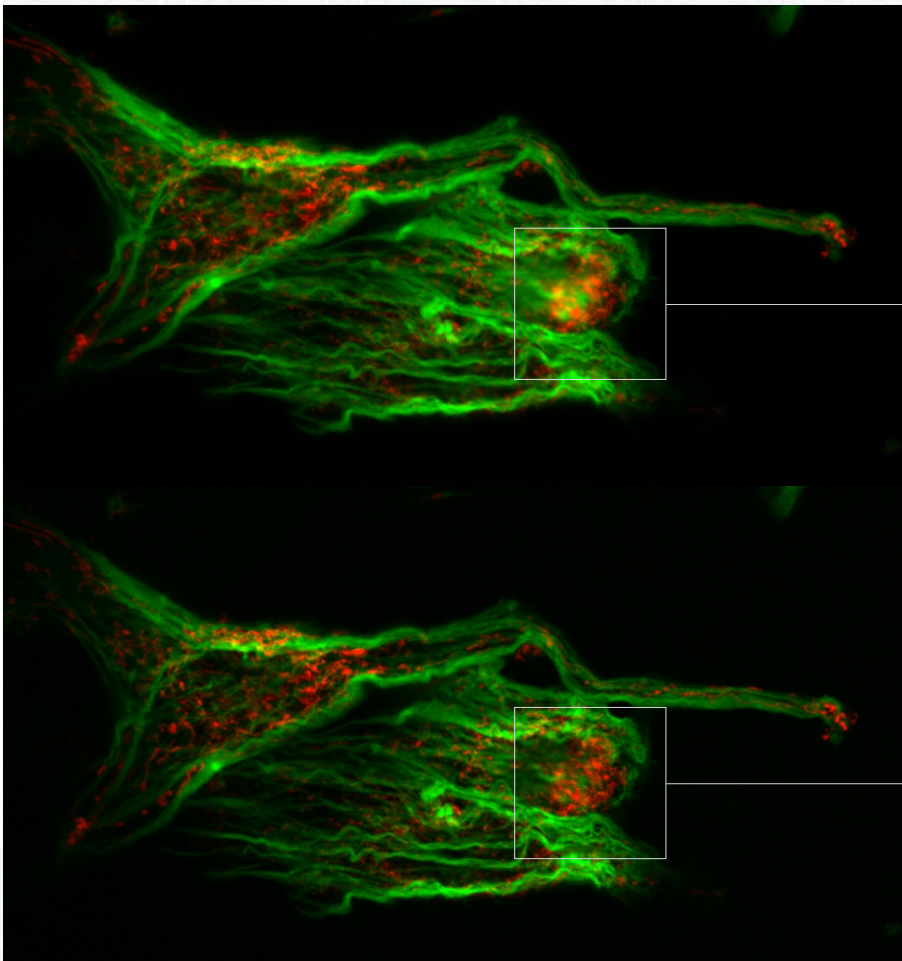
Dr. William R. Kennedy and Gwen Wendelschafer-Crabb, University of Minnesota, Minneapolis, MN



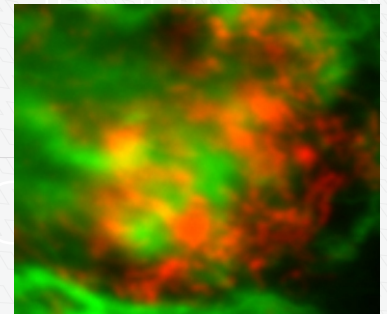
Time lapse recordings at 50 frames per second of calcium sparks in muscle cells loaded with calcium indicator dye Fluo-4
Molecular Probes, Eugene, Oregon.

Application areas

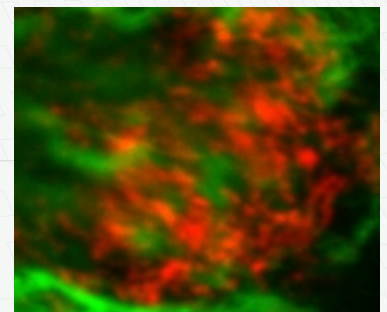
- Cell Biology & Plant Biology: apoptosis, autophagy, cell cycle, cell metabolism, cell tracking and tracing, cytotoxicity, oxidative stress detection, phagocytosis, endocytosis, receptor internalization, cell signaling, and communication, cell motility, cellular compartmentalization, protein synthesis and degradation, cellular and biophysical regulations.
- Cell and System Dynamics: structures and organs, e.g. blood vessels, neurons and processes such as angiogenesis and immune responses to vessel lesions
- Embryology & Developmental biology: *C. elegans*, *Drosophila* and Zebrafish growth and signal mechanisms
- Cancer research
- Clinical & translational medicine research
- Cardio and neuro sciences
- Calcium imaging, other ion measurements & membrane potential
- Yeast and bacteria studies
- Stem cell research and 3D cultures
- Synthetic biology: biofuels, vaccine & antibody production, plant sciences, industrial enzymes, biobased chemicals
Pharma, biopharma & CRO



Widefield



Confocal



Muntjac cells with MouseAnti-OxPhos Complex V Inhibitor Protein, Alexa Fluor[®] 555 GoatAnti-Mouse IgG, Alexa Fluor[®] 488 Phalloidin and TO-PRO[®] -3

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