

CrestOptics X-Light Confocal Imager

Introduction

There are two significant challenges in biological imaging that conventional fluorescence microscopy cannot overcome. Firstly, biological specimens are 3-dimensional structures so to fully understand them we often need to construct 3-dimensional images. Secondly, many processes biologists would want to study occur inside biological structures, but other cell features such as the cell membrane block a clear view.

These problems are difficult to overcome due to the need to pass light through the entire sample to illuminate the chosen image plane. This gives no control over where within the light path the returning light comes from. Light from out of focus planes, and from bright features above and below the desired plane cannot be blocked with conventional fluorescence microscopy.

Confocal microscopy offers the solution to this issue. Confocal microscopy uses optical sectioning to take multiple, thin, 2-dimensional slices of a sample to construct a 3-dimensional model from them. This process removes the out-of-focus light from other planes. Compared to other optical sectioning techniques, spinning disk confocal microscopy is high-speed, high-sensitivity and simple to implement. This makes it a very common choice for studying 3-D structure, fast dynamic processes, long-term time-lapse or details inside the cell membrane, all possible with live cells.

Spinning disk microscopy is, however, a light rejection technique so a high photon budget is necessary for high-quality imaging. Conventional spinning disk confocal microscopy uses a dual disk strategy to increase the amount of light reaching the sample by focusing the excitation light through microlenses on the first disk into the pinholes of the second disk. Emission light then passes back through the second disk and onto the camera.

The use of two disks and microlenses is, however, expensive. CrestOptics changes this by introducing the X-Light confocal imager; a spinning disk system with a proprietary single disk pinhole design with high light collection efficiency without microlenses to create an affordable spinning disk confocal system.

X-Light confocal imager principle

The defining feature of the X-Light confocal imager is the single disk design without microlenses. Although microlenses have improved light collection efficiency in dual disk systems, they are not without disadvantages:

- Microlenses provide no advantage for light collection, only excitation. Emitted light from the sample doesn't pass back through the microlenses before reaching the camera.
- Microlenses f-number doesn't match the numerical aperture of all microscope objectives or tube lens focal lengths provided by different microscope manufacturers.
- With the CrestOptics proprietary disk design, the diameter of the microlenses prevents an optimum number of pinholes per unit area.

Instead of microlenses, CrestOptics have created a disk with a superior 15,000 rpm motor as well as optimized pinhole diameters, number of spirals on the disk and spiral geometrical patterns. The result is a disk with high confocal resolution, improved out-of-focus rejection and increased signal to noise. This allows for higher light throughput through the disk, both excitation and emission, without relying on microlenses.

The disk also includes a single or double hole pattern. With two patterns on the same disk, users can select the appropriate pinhole size for the objective lens numerical aperture or camera they are using (Figure 1). To change disks, the spinning disk can be easily removed and a new disk slotted in or used without the spinning disk as a widefield system.

The X-Light has also made changes to sample illumination. Conventional spinning disk units make use of laser light sources to increase the amount of light delivered to the sample but the X-Light has also been optimized for more affordable LED light sources. Laser light sources can still be used but LEDs have the additional benefits of a long lifespan, low energy use and produce almost no heat. This makes them much kinder to live cells, allowing them to be imaged over longer timescales.

The X-Light allows for a wide variety of excitation wavelengths, input is multimode and produces flat, even illumination across the entire field of view.

The X-Light is revolutionary for its support of larger format camera sensors. The X-Light V1 supports sensor sizes with up to a 22 mm diagonal field of view and the X-Light V2 is capable of a 25 mm diagonal field of view. By taking advantage of the newest, state of the art CMOS sensors, this allows confocal imaging of large samples at very high speeds.

The X-Light is a powerful, affordable and flexible spinning disk system which can be added to any upright or invert microscope and combined with any steady-state light source and camera.

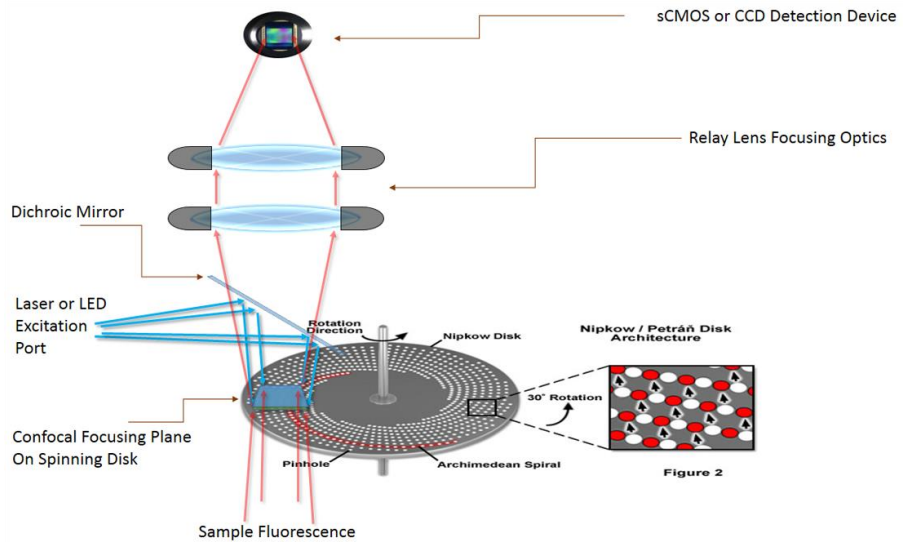


Figure 1: Light path through the single disk of the X-Light confocal imager. From CrestOptics, http://www.cresto.com/html/principles_of_operation.html

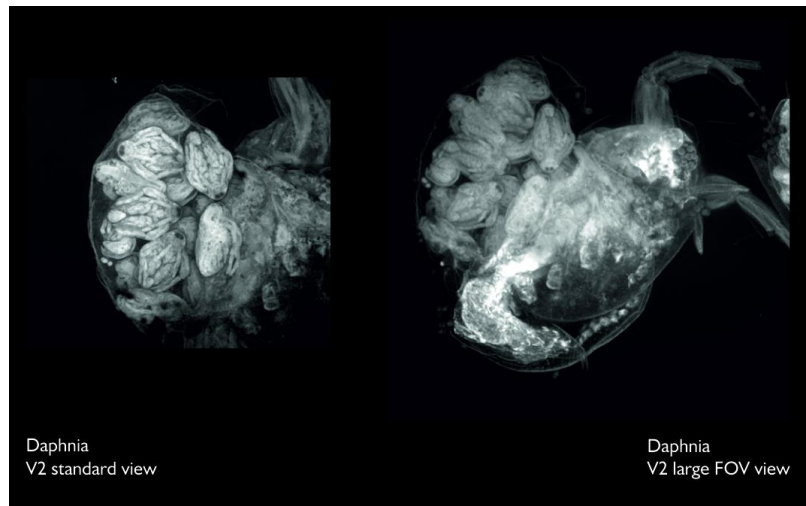


Figure 2: Comparison between a standard V2 18mm field of view confocal acquisition and a V2 L-FOV 25mm field of view confocal acquisition of a Daphnia magnified 20x. From CrestOptics, <http://www.cresto.com/html/gallery.html>

X-Light confocal imager camera choice

Any camera can be used with the X-Light confocal imager, but the best performance can be achieved with a sensitive camera with high speed and a large field of view.

Spinning disk confocal microscopy has historically been performed with EMCCD cameras which are highly sensitive but have a relatively small field of view and slow speed.

More recently, one of the more attractive options has been back-illuminated sCMOS cameras which have equivalent sensitivity to an EMCCD camera but with the field of view, speed and pixel size advantages of sCMOS technology. This allows sensitivity and resolution to be maximized without trading off speed and field of view.

The range of pixel sizes offered by modern back-illuminated sCMOS cameras can ensure Nyquist sampling with 60x magnification (with a pixel size of 6.5 μm) or Nyquist sampling with 100x magnification (with a pixel size of 11 μm). Modern back-illuminated sCMOS cameras also have the field of view necessary to get the most out of the 25 mm diagonal of the X-Light confocal imager.

Putting all of these specifications together ensures the fastest, most physiologically relevant data acquisition with the highest throughput.

References

<http://www.crestopt.com/index.html>