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.

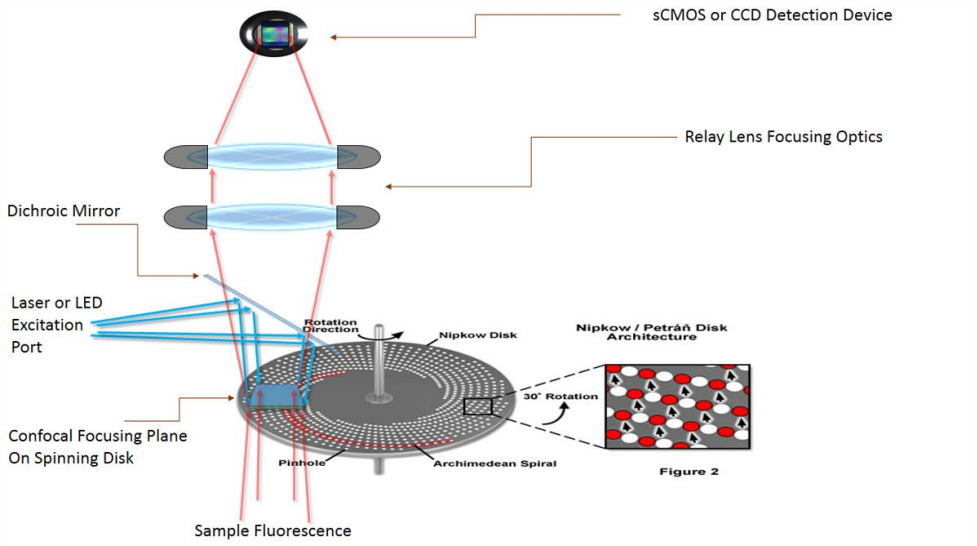

Figure 1: Light path through the single disk of the X-Light confocal imager. From CrestOptics, *http://www.crestopt.com/html/principles_of_operation.html*

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.

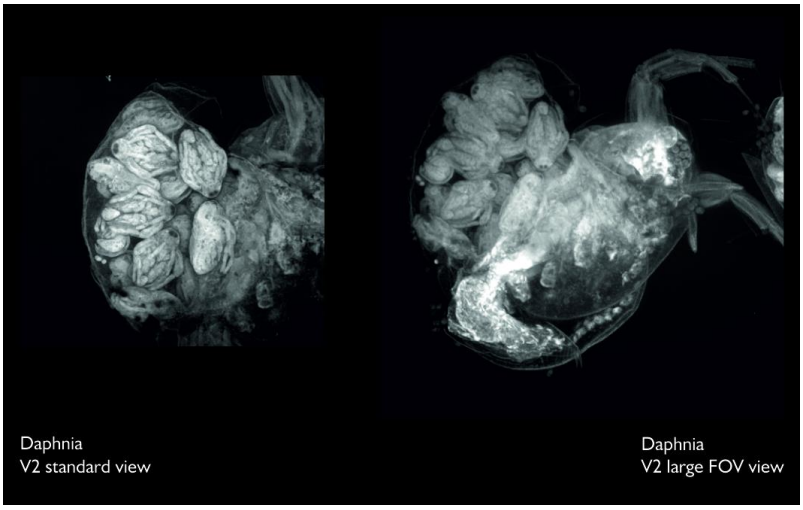

Figure 2: Comparison between a standard V2 18mm field of view confocal acquisition and a V2 L-FOV 25mm field of view confocal acquisition of a Daphnia magnified 20x. From CrestOptics, *http://www.crestopt.com/html/gallery.html*

X-Light Confocal Imager Camera Choice

Any camera can be used with the X-Light confocal imager but we believe that the best performance can be achieved with the 95% quantum efficient, back-illuminated Scientific CMOS camera, the Photometrics Prime 95B™ and the larger format Prime 95B 25 mm.

The almost perfect, 95% quantum efficient (QE) sensor has equivalent sensitivity to an EMCCD camera but with the much larger field of view (1200x1200 pixels, 18.66 mm diagonal) and high speed (82 fps, full frame) expected of a CMOS device. The high sensitivity of the Prime 95B means that when compared to conventional sCMOS devices, the exposure time on the Prime 95B could be reduced by up to four times and still give equivalent detection.

If a larger field of view is required, the Prime 95B 25 mm has a massive 25 mm diagonal field of view over 1600x1600 pixels which partners perfectly with the X-Light V2.

The large, 11x11 µm pixels provide additional sensitivity and have a large 80,000 e^- full well capacity with a low 1.6 e^- read noise, giving the 95B a very high dynamic range, ideal for performing high contrast imaging. Larger pixels also fit perfectly with high magnification objectives, achieving Nyquist sampling without the need for any additional optics with 100x magnification.

We would encourage anyone considering using the X-Light confocal imager to request a demonstration of the Prime 95B or Prime 95B 25mm to see the advantages they provide.

References

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

