Aurox Clarity Laser Free Confocal (Aurox LFC)

Introduction

One of the most popular confocal microscopy techniques is spinning disk confocal microscopy; a high-speed, high-sensitivity technique that is reasonably simple to implement. Conventional spinning disk confocal microscopy uses a dual disk strategy which focuses excitation light through microlenses on the first disk into the pinholes of the second disk to increase acquisition speed and the amount of light reaching the sample. Emission light then passes back through the second disk and onto the camera.

The Aurox Clarity Laser Free Confocal (Aurox LFC) is a system which combines features of Spinning Disk microscopy and Structured Illumination (SI) to provide an affordable confocal microscope that can be attached to any conventional widefield system.

The LFC effectively captures two images originating from the same plane of focus, one transmitted through the disk and one reflected by the disk. When these images are subtracted from one other (transmitted – reflected), a sectioned image is created which suppresses out-of-focus blur and retains the in-focus image of the sample. The optical sectioning ability of this system is comparable to a point scanning confocal microscope.

The contrast pattern required for SI is imprinted with a reflective mask on the excitation beam and hence onto the sample. The pattern is also contained in the fluorescence emitted by the sample. This means that in-focus structures will see the SI pattern, but those structures above and below focus will disappear rapidly, resulting in an even, widefield-like illumination of the focal plane.

LFC Principle

The reflective mask on the excitation beam is the essential part of the system and has three different patterns, consisting of line-patterns with different ratios and spatial frequencies, required to suit the characteristics of different objective lenses.

Figure 1: Aurox LFC unit

The emitted light passes the mask which now works as a reciprocal filter and partially-transmitting mirror. The emission created in the plane-of-focus will mainly arise from regions excited by the pattern and therefore passes the mask (widefield + confocal, WF+C); but emission that originates from out-of-focus locations will hit the reflective surface on the disk (widefield – confocal, WF-C).

The other important part of the system is to make good use of a camera. Both components of the image – WF+C and WF-C – will be imaged on either two individual cameras or split on the sensor of a single camera. LFC, therefore, benefits from CMOS

sensors as they are large enough to allow the one-camera solution which makes registration and image processing easier. Both signals can vary quite substantially in intensity so a large dynamic range is very helpful as well.

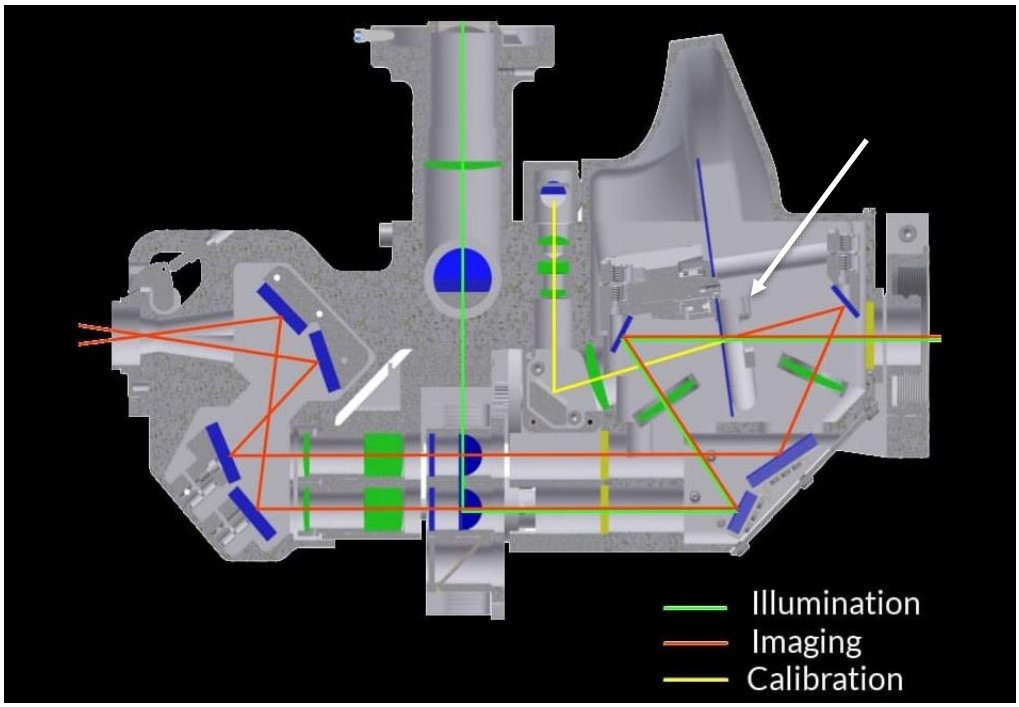

Figure 2: Light path of the Aurox LFC.
The excitation light is sent through the reflective mask which is mounted at an angle to allow the WF-C image to be reflected to the parallel light path indicated by the white arrow.
The part of the image containing the WF+C information will pass through the SI pattern.

Additional information

The Aurox homepage offers plenty of information and resources, helpful to every potential customer. Apart from the basics, the user can build a customized system or get suggested combinations based on applications, such as developmental questions, plant tissue or electrophysiology. One of the biggest advantages of the LFC is the ability to add it to any existing microscope which means that the functionality of having a widefield microscope is retained.

Cameras for the Aurox LFC

Various cameras have been implemented in the Aurox LFC system but we believe that the best performance can be achieved with the 95% quantum efficient, back-illuminated Scientific CMOS camera, the Photometrics Prime 95B™.

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 higher 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.

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. The 11x11 µm pixel size also fits 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 Aurox Clarity Laser Free Confocal to demonstrate the Prime 95B which can be included in the Build-your-own-LFC tool on the Aurox homepage.

References

http://www.aurox.co.uk/

