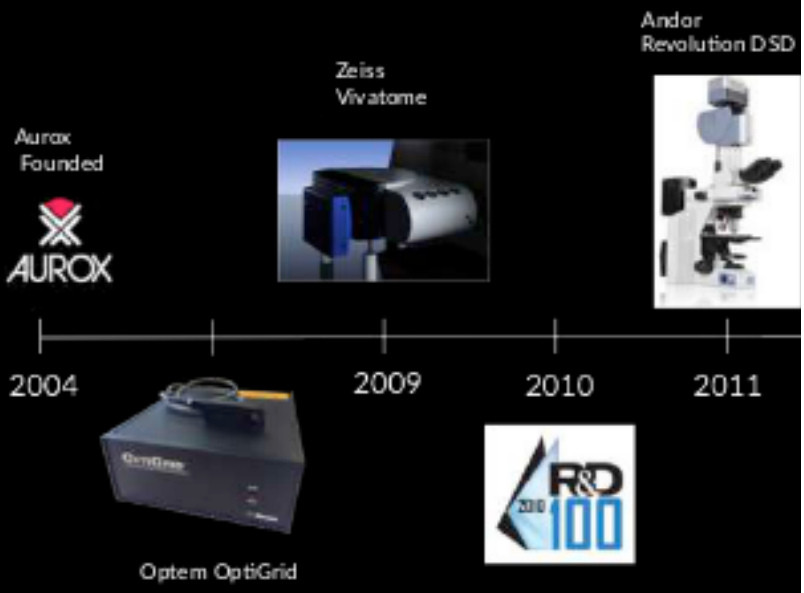
AUROX LASER FREE CONFOCAL

Technical Note

Optical sectioning with structured illumination spinning disc confocal microscopy

15 years of **Aurox** technology



A spin-out from the Scanning Optical Microscopy Group at the University of Oxford, Aurox was established in 2004 to commercialise and develop instruments for life sciences, medical and materials research.

Aurox products are based on the use of structured illumination to obtain optical sectioning and 3D imaging; the subject of a series of high profile publications by *Wilson et al.*[1-4]

Aurox have developed a series of instrument products since 2004, either licensing our technology *e.g.* Optem Optigrid, or developing and manufacturing products for Other Equipment Manufacturers (OEM), such as the Zeiss Vivatome and Andor Revolution DSD and DSD2.

Aurox technology is also used in the Zeiss SmartProof5 material instrument and the 3DHistech Panoramic Confocal Slide Scanner.

Alongside the OEM business, Aurox has since 2017 sold a range of end-user confocal microscopy instruments including the Clarity, Clarity^{HS}, Visionary and unity.

CONFOCAL MICROSCOPY

Unlike traditional widefield microscopes, confocal microscopes can optically section materials and specimens. The resulting thin in-focus image slices can be combined into high quality in-focus 3D images.

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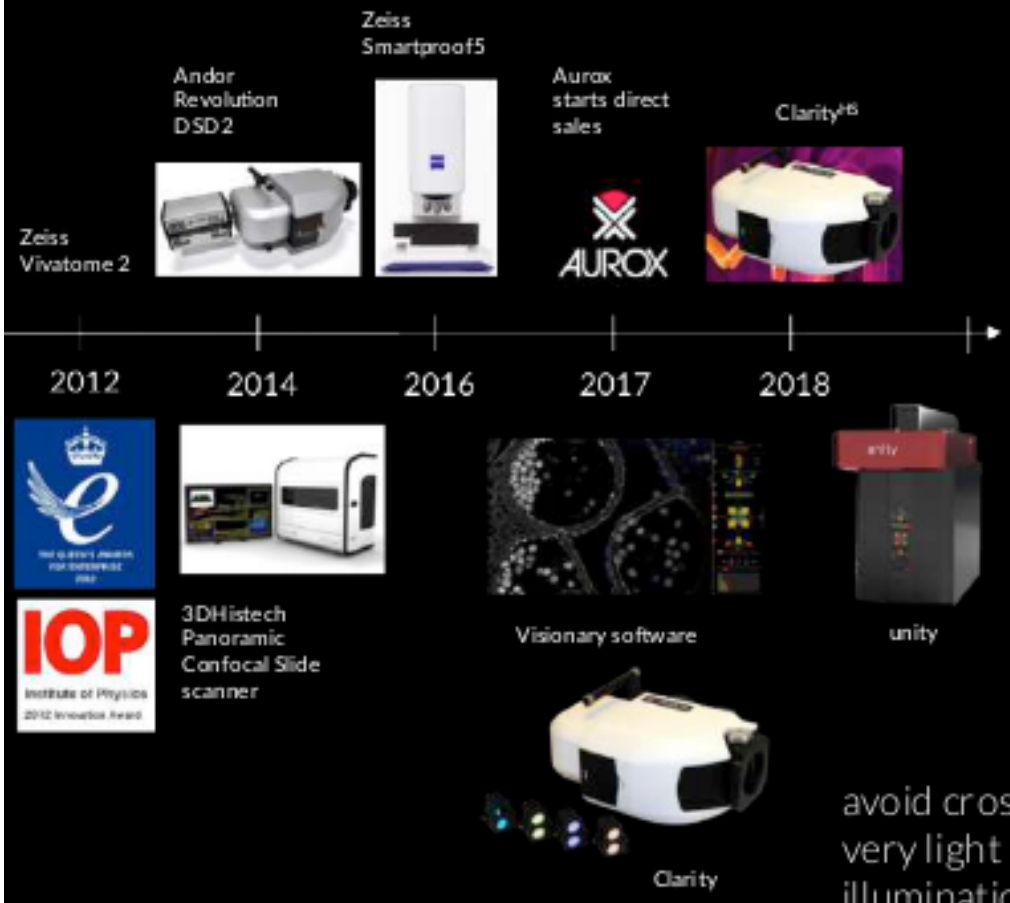

Aurox's 15 years of technology, awards & products.

The following types of confocal microscope instruments are commercially available:

- Laser scanning confocals rely upon slow point-scanning, are restricted to using available laser wavelengths and post-processing of images is required; they are not real-time 'live' images.
- Nipkov spinning disc confocals use multiple pin-holes in a spinning disc and each pin-hole acts like a single laser scanning confocal. Spinning discs are therefore much faster than laser scanning systems, but the pin-holes must be well spaced to avoid crosstalk. This makes them very light inefficient with <5% illumination of the specimen, leading to poorer confocal images and the need for stronger, laser light sources.[1]
- Aurox laser-free confocal spinning discs use a 'grid' pattern instead of pin-holes to achieve >50% light efficiency with the speed of a spinning disc. This allows the use of laser-free light sources to give high quality confocal images from an instrument that is:

  - ✓ Lower cost
  - ✓ Low maintenance
  - ✓ Low photo-bleaching with low photo-toxicity
  - ✓ Highly compact & easy to use.[1]

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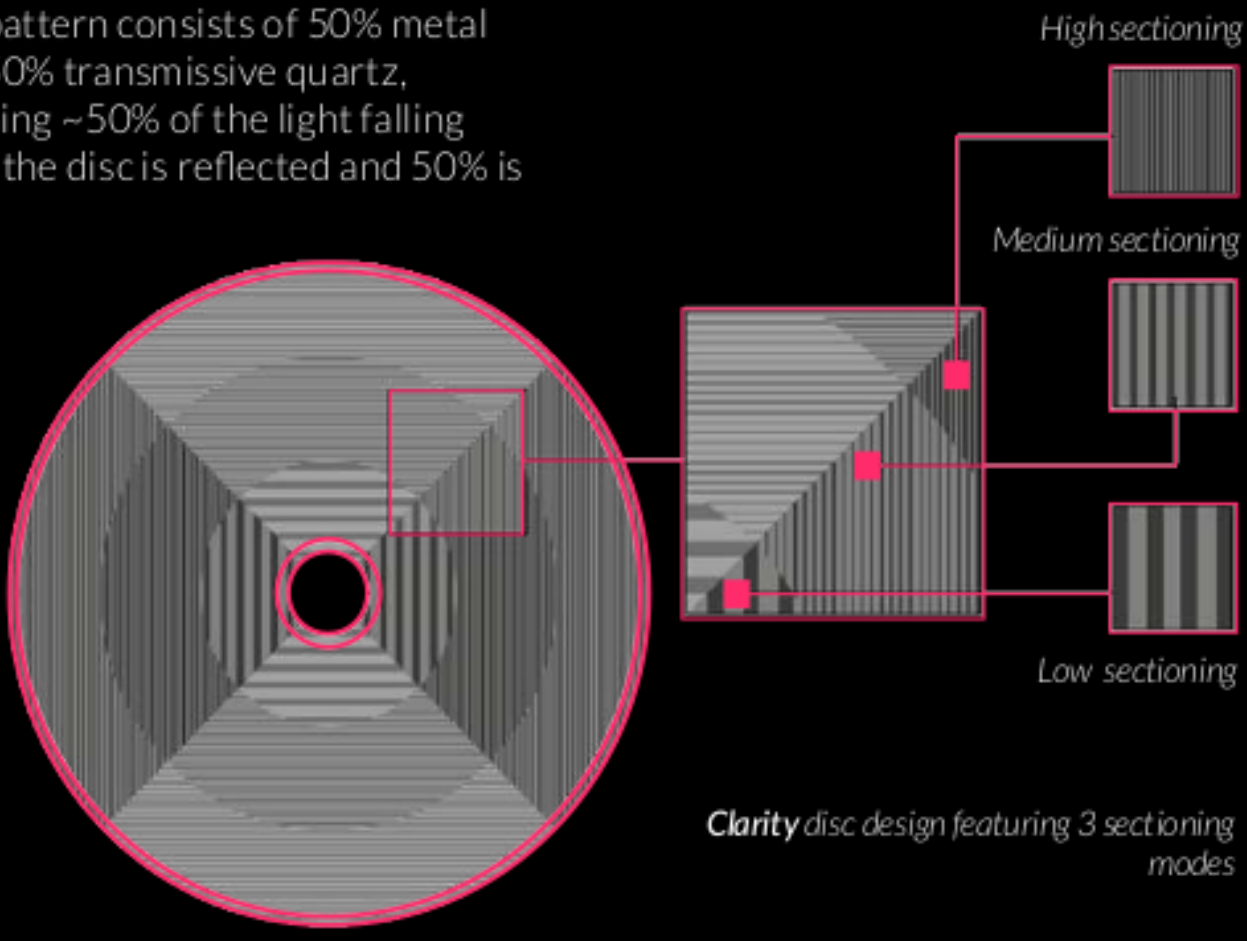
Aurox's patent technology explained:

THE AUROX SPINNING DISC

Aurox laser free confocal instruments are based on a unique, patented spinning disc design which uses a structured illumination pattern to modulate the illumination field. The synthetic quartz disc has a thin layer of aluminium in which the structured illumination 'grid' pattern is created by photo-lithography.

The pattern consists of 50% metal and 50% transmissive quartz, meaning ~50% of the light falling upon the disc is reflected and 50% is transmitted. This is true for both sides of the disc.

The size of the transmissive and reflected areas can be varied to provide for high sectioning or high signal. In the case of the Clarity and Clarity^{HS} three grid sizes and sectioning modes are available on one disc.



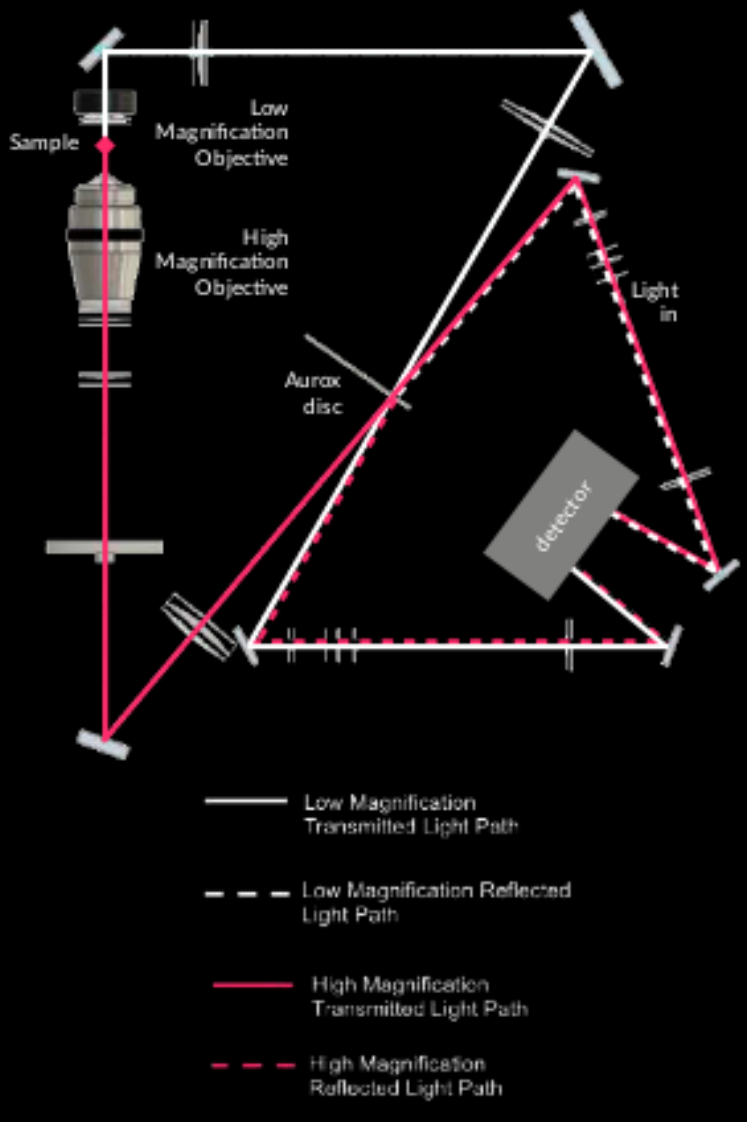
***Clarity** disc design featuring 3 sectioning modes*

THE OPTICAL PATH

In a typical Aurox laser free confocal instrument the input illumination is provided by a white light LED source or metal halide lamp. Light passes through an excitation filter and is reflected from a dichroic mirror. The collimated beam then illuminates the disc in an image plane of the microscope optical system, projecting the structured illumination pattern into the specimen. ~50% of the illumination arrives at the specimen while the remainder is deflected away from the imaging system. In the detection pathway, the emission fluorescence signal comprising in-focus (confocal), and out-of-focus (widefield) light is imaged back onto the disc, where it interacts with the structured illumination pattern.

The disc's transmissive and reflective structures again perform the essential spatial filtering of the light. The fluorescent transmitted light comprises the confocal signal and ~50% of the widefield signal, while the reflected light comprises ~50% of the widefield signal minus the confocal signal.

The structured illumination pattern is located in a conjugate image plane and acts as both the confocal source and detection apertures. The transmitted and reflected signals are guided *via* two optical paths which coincide at the disc and are then guided by identical construction paths before being imaged side by side on a sCMOS detector. The images are then mapped to each other pixel by pixel.



unity *optical paths.*

THE CONFOCAL IMAGE

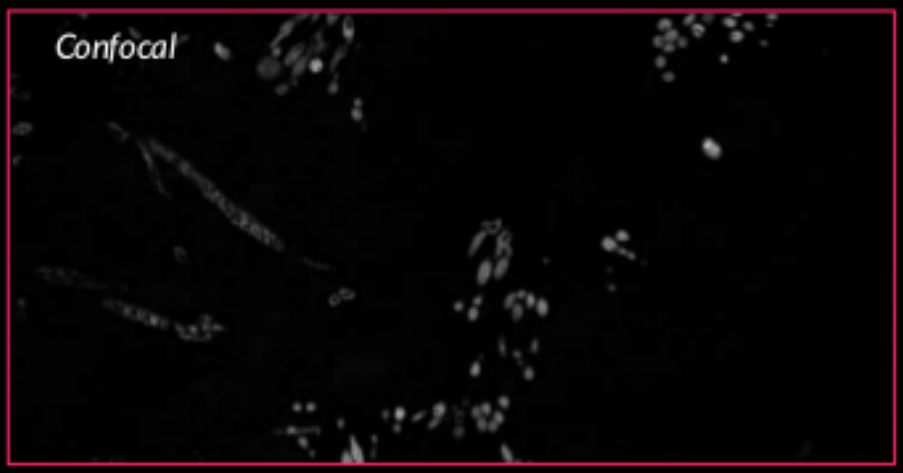
Aurox instruments collect both the (T)ransmitted and (R)eflected light signals simultaneously. This also means they provide both the (C)onfocal and the widefield (WF) conventional epi-fluorescence images simultaneously. The ~50% efficient sectioning of the disc means that:

$T = 0.5\ WF + C$ and $R = 0.5\ WF - C$

Therefore:

$2C = T - R$ and $WF = T + R$

Aurox's patented technology enhances the confocal signal by a factor of 2, whilst actively rejecting background (out-of-focus light, R).



Transmitted (top), Reflected (middle) and resultant Confocal (bottom) images.

REGISTRATION & CALIBRATION

Optical alignment and registration of the transmitted and reflected images is critical to achieving high quality confocal images and misalignment between these images would result in registration noise.

Aurox instruments have an initial automatic alignment and calibration routine which creates a registration map for each disc pattern and excitation wavelength. Thereafter, image processing is 'on the fly'.

REAL-TIME ON THE FLY IMAGING

The Aurox confocal image is obtained in real-time providing a live image and no post processing is necessary. The confocal image is obtained as follows:

1. The image is split about the symmetry axis.
2. The reflected image is flipped about the symmetry axis.
3. Real-time registration of images.
4. Weighted addition gives the widefield image.
5. Weighted subtraction gives the confocal image.
6. Repeat steps 1-5 for each label.

OPTICAL SECTIONING

The manner in which the confocal signal changes with focus position Z, the optical response function, is equivalent to the optical section thickness (z resolution).[5]

The below table provides the calculated optical section thickness (z-resolution) based on the structured illumination 'grid' size and the microscope objective.

Objective Magnification/ NA	High Sectioning/ Low signal	Medium Sectioning/ Medium signal	Low Sectioning/ High signal
4x/0.2 N.A	82.9	161.6	240.3
10x/0.45 N.A	14.2	27.5	40.9
20x/0.75 N.A	3.8	7.3	10.8
40x/0.95 N.A	1.2	2.3	3.4
60x/1.4 N.A Oil	0.9	1.7	2.5
100x/1.4 N.A Oil	0.6	1.1	1.5

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SIGNAL TO NOISE

The signal to noise ratio of Aurox spinning disc instruments can be calculated as follows:

Signal = 2C
Noise ≈ √(RN + SN)

Therefore:

Signal to Noise Ratio
≈ 2C/√(RN + SN)

The read noise (RN) of the commercially available sCMOS cameras is 1-3 electrons RMS, while the shot noise (SN) is due to the Poisson statistics of the wide-field signal and is typically 10-180 (√100-√30,000).

Hence, the system is primarily shot noise limited, where the signal to noise ratio is limited primarily by the widefield signal statistics.

In low signal conditions the camera read noise will also contribute.

REFERENCES

1. *Wilson et al*, Nature, Vol 383, 1996, 804-806
2. *Wilson et al*, Optics Letters, Vol. 21, No. 23 , 1996.
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