# OXFORD ANDOR

# **Dragonfly** High-Speed Confocal with 3D Super Resolution

OXFORD ANDOR

### **Key Specifications**

- Instant confocal: up to 400 fps
- ✓ Borealis<sup>®</sup> uniform illumination
- ✓ 3D SMLM: resolution to 10-20 nm
- Live cell super resolution: SRRF-Stream+
- ✓ Unique easy-to-use TIRF: B-TIRF
- ✓ Broad wavelength range: UV-Vis to NIR
- ✓ Greater productivity: large field of view

### **Key Applications**

- Developmental, cell & neurobiology
- Cancer & oncobiology
- Microbiology & virology
- ✓ Organoids & large model organisms
- ✓ Spatial genomics & proteomics
- ✓ Multiplex & Expansion microscopy
- Plant biology



## It's More than Confocal...

Dragonfly is a high-contrast multi-dimensional imaging platform capable of four key imaging modalities.

At its core is a multi-point confocal (spinning disk) for high-speed and high-sensitivity imaging. Capturing at speeds at least 10x faster than conventional confocal technology, with dramatically improved sensitivity from 400-800 nm. Dragonfly is the optimal solution for live cell imaging, providing low phototoxicity and photobleaching, or perfect for fast volume acquisition of fixed samples.

We have the following models of Dragonfly available;



Dragonfly 200 Multi-modal confocal system



Dragonfly 400 Multi-modal confocal system with **3D SMLM capabilities** 



Dragonfly 600 Multi-modal confocal system with **3D SMLM capabilities & B-TIRF** 

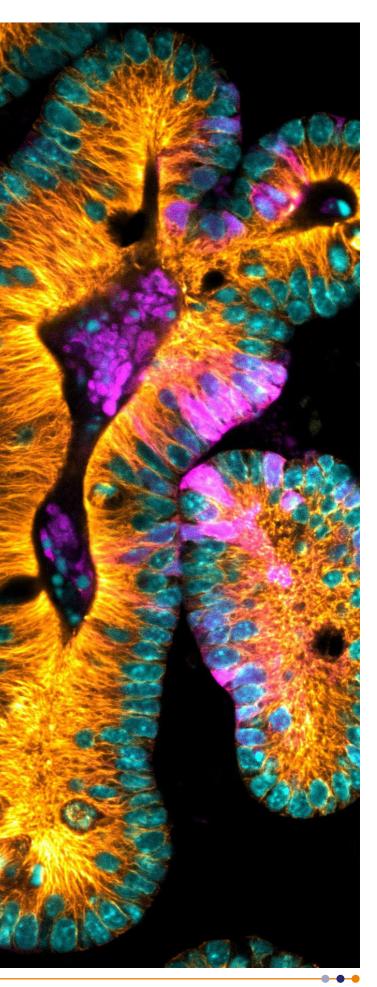
VLE	HLE	HLE
Single or dual pinhole	Single or dual pinhole	Dual pinhole
Confocal Laser based widefield Transmitted light SRRF-Stream+	Confocal Laser based widefield Transmitted light SRRF-Stream+ 3D Super resolution module	Confocal Laser based widefield Transmitted light SRRF-Stream+ 3D Super resolution module B-TIRF Zoom illumination

HLE - High power laser engine VLE - Versatile laser engine SMLM - Single molecule localisation microscopy 3D Super resolution module - optics for 3D SMLM B-TIRF - Borealis<sup>®</sup> total internal reflection microscopy SRRF - Super resolution radial fluctuations SRRF-Stream+ - exclusive real-time super resolution functionality

Right: Mouse intestinal organoid expressing LGR5-GFP to visualise stem cells (magenta) along with a staining for microtubules (orange) and DNA (cyan). Acquired with Dragonfly.

Image credit: Nicole Dawney, Bergstralh Lab at the University of Rochester. UR High Content Imaging Core.

#### Introduction | Applications | Features | Specifications & Product Drawings | Ordering



## **Imaging Modalities**

### Confocal

Capturing at speeds at least 10x faster than conventional confocal technology. Dragonfly is the optimal solution for live cell imaging, providing low phototoxicity and photobleaching, or for fast volume acquisition of large fixed samples.

#### ✓ B-TIRF

Andor's patent pending Borealis<sup>®</sup> Total Internal Reflection Fluorescence (B-TIRF) with multimodal illumination avoids the common artefacts observed in conventional TIRF systems. With a unique broad excitation wavelength range from 400 - 800 nm and an exceptionally simple imaging set up, the B-TIRF module is available on Dragonfly 600.

#### Super Resolution - Single Molecule Localisation Microscopy (SMLM)

The HLE delivers the required laser power for dSTORM and DNA-PAINT applications. 3D SMLM uses an astigmatic module (3D Super resolution module) compatible with all imaging modalities: widefield, TIRF and confocal.

#### ✓ SRRF-Stream+

SRRF (Super Resolution Radial Fluctuations) offers a highly effective and flexible software-based approach to super resolution. Read our <u>SRRF-Stream+ technical note</u> to find out more.

#### Widefield Fluorescence

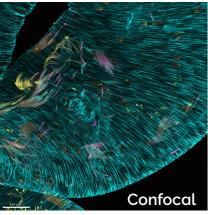
Widefield is ideal for yeast or very thin samples, or applications that require high laser power, like SMLM. In widefield cameras and lasers are shared between modes to avoid switching microscope ports and turrets.

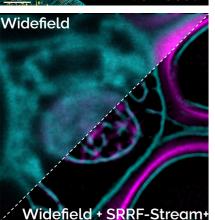
#### Deconvolution

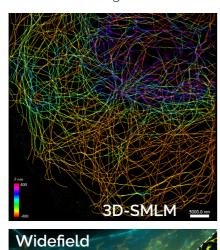
Apply integrated GPU-accelerated deconvolution with confocal, widefield & TIRF, to increase the signal to noise ratio, and boost resolution.

#### Transmitted Light Microscopy (DIC, Phase Contrast & Brightfield)

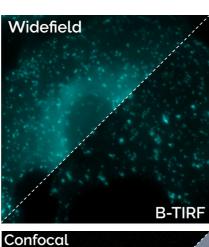
All imaging modalities can be combined with transmitted light techniques (DIC or phase contrast) providing a reference channel, allowing correlative or context setting.

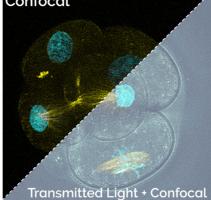






Widefield + Deconvolution



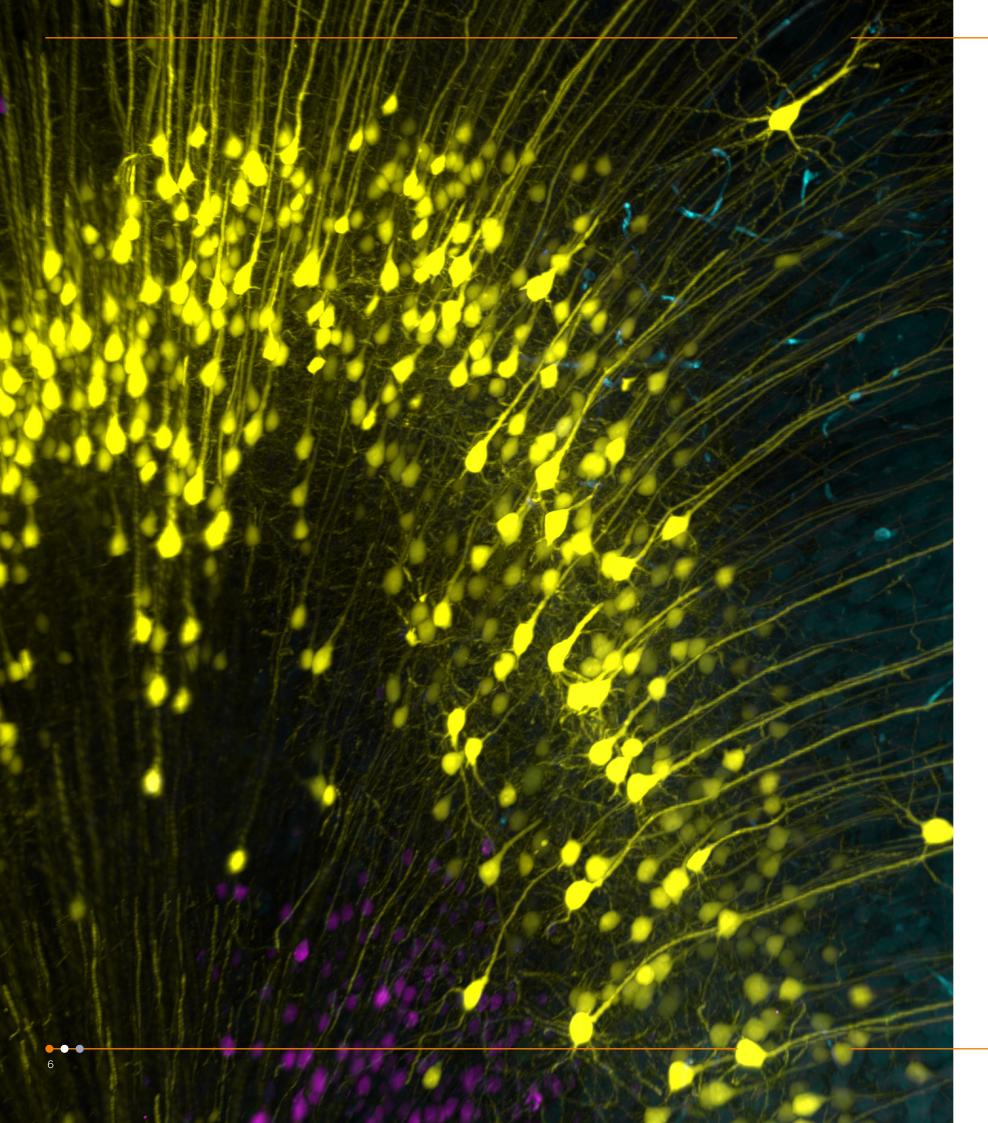


### From Nanometers to Millimeters

	Widefield	Confocal	ClearView- GPU™ Deconvolution	SRRF-Stream+	SMLM with WF	SDC-PAINT	B-TIRF with/without deconvolution	SMLM with B-TIRF
Dragonfly Model	All models	All models	All models	Option (All models)	400 & 600 series	600 series	600 only	600 only
SRRF-Stream+ Compatible	Yes	Yes	No NA		No	No	Yes	No
Lateral Resolution (nm)	245	220	200 (widefield); 140 (confocal)	100 - 150	20	20	140/200	10-20
Axial Resolution (typical FWHM)	570 nm	520 nm	500 (widefield); 280 (confocal)	570 (widefield); 520 (confocal)	30-50 (3D ~1µm range)	30-50 (3D ~10 µm range)	220/250	30-50 (3D ~5µm range)
Special probes / sample preparation	No	No	No	No	Yes	Yes	No	Yes
Image Acquisition				Fusio	on			
Image Processing	Fusion, Imaris Quantify				ris Quantify & asso	Fusion, Imaris Quantify	Fusion, Imaris Quantify & Picasso	
Live Cell Imaging Compatible	Yes	Yes	Yes	Yes	No	No	Yes	No

SMLM with B-TIRF – single molecule localisation microscopy prepared samples imaged using B-TIRF. SDC-PAINT – single molecule localisation microscopy sample (DNA-PAINT) imaged using spinning disk confocal. Fusion and Imaris Quantify software from Andor provide seamless transitions from image acquisition to deconvolution, visualisation to analysis.

Picasso - Free third party software tool for PAINT and super resolution images.



## Neuroscience

### Super-Resolve Synapses in 3D down to 30 nm

Neuroscience researchers need to image both live and fixed samples across multiple size regimes: capturing structures from nanometers to millimeters. Dragonfly is the instrument of choice - whether imaging glial cells, axonal transport, large tissue sections, expanded samples (ExM), or dendritic spines with super resolution (SMLM).

Dragonfly's large field of view combined with rapid confocal imaging (400 fps), means it is 10 X more productive than point scanners. Further, the 3D super resolution module is compatible with all imaging modalities: widefield, B-TIRF and confocal for maximum flexibility.

### Localise synaptic zones & understand functional states with SMLM

- $\checkmark$
- $\checkmark$
- √  $\checkmark$

- Map gene expression at the cellular level taking advantage of faster acquisition  $\checkmark$ speeds with HLE compared to VLE.
- Quantify gene expression accurately with Borealis® uniform illumination,  $\checkmark$
- $\checkmark$ imaging.

### Image live neuronal samples

- $\checkmark$ 400 fps).
- Image sensitive samples such as brain slices and retina explants with gentle  $\checkmark$ illumination delivered by the HLE or VLE.
- Capture all signals with short exposure times and highly sensitive detectors.  $\checkmark$

### Image deeper into live thick brain tissue with NIR wavelengths

- $\checkmark$ Increased sample penetration.
- Increased sample viability due to less energetic radiation.  $\checkmark$

Mus musculus neuronal staining. Sample from the "BRAIN Initiative® (nih.gov)". Image courtesy of Dr Hong Wei Dong, Department of Neurobiology, UCLA.

Super resolve synapses in 3D (down to 30 nm axially) with the super resolution module. Resolve tethered synaptic vesicles using SMLM with lateral resolution down to 10 nm. Visualise the receptors at the membrane and increase signal to noise with B-TIRF. Super resolve structures deep inside neuronal cells with confocal (Z  $\sim$ 10  $\mu$ m).

### Create brain atlases with spatial transcriptomics

- Increase productivity taking advantage of the large field of view and fast confocal
- Image dynamic events such as calcium signalling with fast confocal imaging (up to

## Cell Biology

### Image cell division for hours or days

Whether imaging a cell population, intracellular organelles, Expansion microscopy (ExM) or super resolution microscopy there is always a suitable imaging modality. Dragonfly delivers imaging with **outstanding quality** for cell biologists including: confocal, B-TIRF, widefield, super resolution (SMLM) or super resolution compatible with live cell imaging (SRRF-Stream+).

## Resolve the ultrastructure of centrioles or 3D nuclear pore complexes

- Reveal Chromatin & epigenetics organisation down to 10 nm in XY using the HLE.
- Investigate the mitochondrial membrane structures with 30 nm axial resolution using the 3D super resolution module.
- Visualise the receptors at the cell membrane and identify biomolecules involved in signalling by combining the 3D super resolution module, HLE and B-TIRF.

## Image organelle dynamics with minimal phototoxicity or photobleaching

- Image live samples for days with high sensitivity detectors.
- Image microtubule growing tips or cilia beating with fast acquisition speeds (400 fps in confocal).

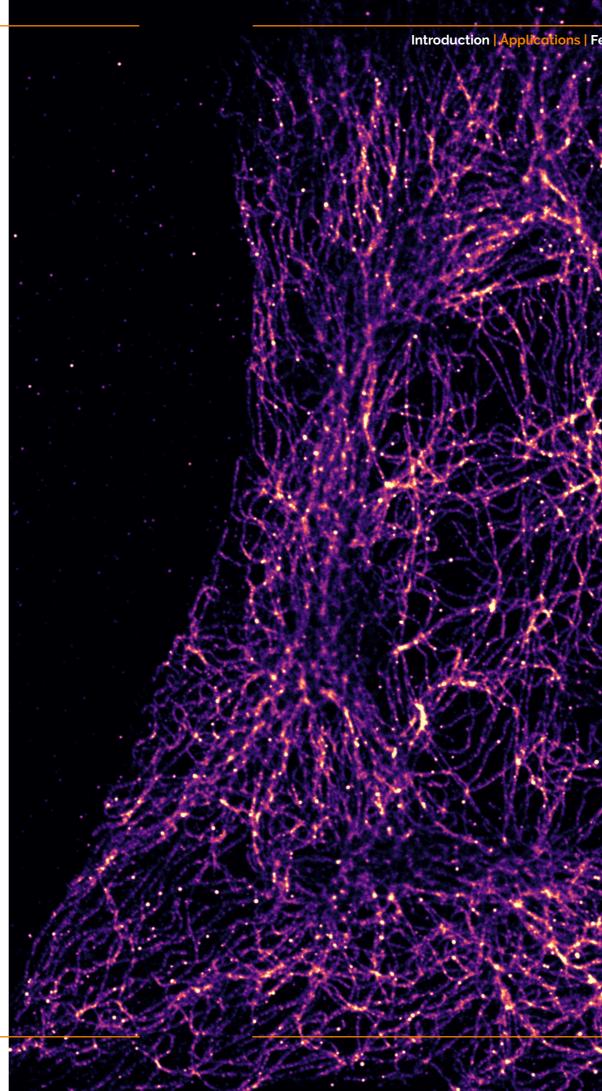
### Image live membrane fusion events and receptor signalling

- ✓ Get more information from one image with Borealis® uniform illumination.
- Image vesicle trafficking live with outstanding quality using B-TIRF with 30% more uniformity than conventional TIRF.
- Increase productivity and get the optimal image using B-TIRF optical feedback.

### Image cell biology using Expansion microscopy (ExM)

- Image large and Expanded (ExM) samples.
- Capture more images faster through large field of view, high sensitivity detectors and high power lasers.
- Quantify, use and interpret more data in the image due to Borealis<sup>®</sup> uniform illumination.
- Acquire information from multiple probes, taking advantage of the wide excitation spectrum of the HLE and VLE.

Vimentin (intermediate filament) network in U2OS cells imaged by DNA-PAINT (SMLM) Vimentin is transiently labelled with ATTO 655 and imaged using the B-TIRF imaging modality, 100X TIRF objective and the HLE. 10 000 frames were acquired with ZL41 Cell 4.2 sCMOS camera. Image was reconstructed with Picasso. *Image courtesy of Alexander Stasheuski, Oxford Instruments Andor.* 



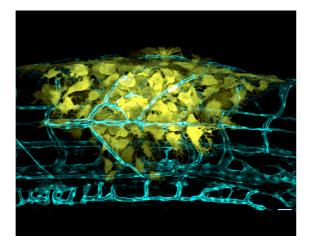
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## **Application Focus**

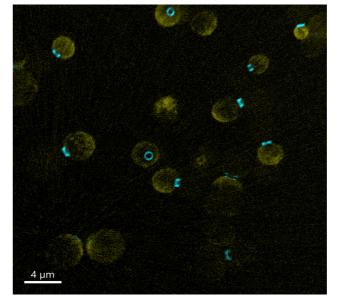
### **Cancer Research**

#### Visualise deep into tissue and metastasis formation due to optimised pinholes & disk

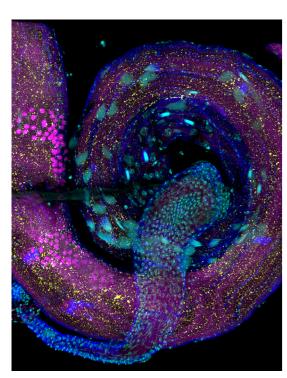
Cancer research needs a holistic approach to biology requiring insights into gene expression, tumour microenvironment, in vitro/in vivo assays and malignancy. Dragonfly allows researchers to study cancer cell behaviour interactions with the environment and spatial distribution of the tumour in either fixed or live cells. Capture all the information in one experiment and take advantage of the broad spectral range of the VLE or HLE to image multiple signals from cancers/cancer cells. Gentle imaging delivers live images of cancer tissues over hours or days. The speed of acquisition and optimised pinhole spacing allows fast and deep imaging into thick tissues or organoids. The super resolution (SMLM) capabilities allow the visualisation of possible structural changes in organelles or protein complexes that could be cancerspecific targets (eq. centrosomes)



Angiogenesis in Zebrafish with cancer. Image courtesy of Dr Julien Resséquier at NorMIC University of Oslo.



Budding Yeast sample. In Cyan Cdc3-GFP (septin marker) and yellow Gic2PBD-tdTomato (Cdc42 marker) in yellow. Image is a maximum intensity projection of 67 Z stacks, acquired in confocal mode with the 40 µm pinhole. Sample courtesy of Dr. Satoshi Okada, Medical Biochemistry, Kyushu University Faculty or Medical Science.



Adult drosophila testis showing different stages of sperm individualisation. Image courtesy of Alina Kolpakova, Eli Arama group, Weizmann Institute of Science.

### **Developmental Biology**

#### Image blood flow or cilia beating with instant confocal

Developmental biology requires imaging of thick samples. Image very deep into thick organisms up to the millimetre range using Dragonfly due to its optimised pinhole size and spacing.

- Visualise the entire organism at high resolution. Acquire all the images at high magnification and seamlessly stitch as if they were a single snapshot using Borealis uniform illumination.
- Image embryonic mouse development or other delicate processes for hours or days, with exceptional sensitivity.
- Image blood flow or cilia beating deep into developing tissues with fast confocal imaging speeds.
- Take advantage of the broad spectral range offered by VLE and HLE to explore multiple label possibilities.

### **Transcriptomics & Proteomics**

### Increase Productivity in Omics data with High **Power Laser Engine**

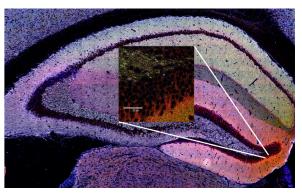
Understanding the molecular basis of development, brain function, neurodegenerative diseases, cancer, and behaviour is an enormous task. The Omics-related sciences gather information about X<sup>n</sup> biological molecules to characterise and quantify the entire pool of molecules. Dragonfly is perfect for such applications because of its highly sensitive detectors, exceptionally high background rejection, speed of acquisition, uniform illumination and automation capabilities. Omics research has applications in multiple areas of life sciences, such as predicting disease progression based on gene expression maps, accessing tumour microenvironment and cancer severity. The speed of the Dragonfly, the sensitivity of the detectors,

the large field of view and the Andor proprietary laser engines VLE or HLE deliver high-quality images with superb productivity that is required to timely accomplish the Omics data.

### Microbiology and Virology

#### Visualise host-pathogen interactions in large fields of view with exceptional uniformity

- ✓ Get live insights into virus-host infection using B-TIRF (up to 5 µm deep into the with HiLo).
  - Understand fungus, bacteria and virus ultrastructural biology by combining the high-power laser engine with the 3D super resolution module, collecting data down to 10 nm lateral resolution and 30 nm axial.
  - Analyse the infection deep into tissues taking advantage of high background rejection and extremely sensitive detectors.
  - Image sensitive samples using laser-based widefield and increase the resolution and the signal-to-noise of the data with GPU-accelerated deconvolution.
  - Visualise fungus cell wall ultrastructure combining B-TIRF with SMLM (super resolution)



Large tissue section scanned for synaptic protein density mapping Dragonfly high speed confocal. Image credits: S. Grant, University of Edinburgh.

## Features and Benefits

Feature	Benefits	200 Series	400 Series	600 Series
High speed confocal (micro-lens dual disk)	<ul> <li>Up to 400 fps for fast cell dynamics</li> <li>At least 10x faster than conventional confocal</li> </ul>	$\checkmark$	$\checkmark$	$\checkmark$
Large field of view (16.6 x 14.0 mm)	<ul><li>Capture more in a single image</li><li>Matches large sCMOS sensors</li></ul>	$\checkmark$	$\checkmark$	$\checkmark$
Borealis® Uniform Illumination	<ul> <li>Provides stable light throughput, uniform illumination, and extended wavelength range (405 to 785 nm)</li> </ul>	$\checkmark$	$\checkmark$	$\checkmark$
Borealis® - TIRF or B-TIRF	<ul> <li>Andor's patent pending TIRF module for 60x and 100x TIRF objective lenses</li> <li>Easy to set up - with optical feedback</li> <li>Highly-uniform TIRF and oblique imaging</li> <li>Enables broad spectrum TIRF 400 - 800 nm</li> </ul>	-	-	Option
16-bit dynamic range	<ul> <li>Capture both weak and bright signals without saturation</li> </ul>	$\checkmark$	$\checkmark$	$\checkmark$
Motorised 4 position emission-splitting dichroic cassette	<ul> <li>Optimal flexibility for simultaneous dual colour imaging (e.g. single camera bypass + 3 chromatic splitters)</li> </ul>	$\checkmark$	$\checkmark$	$\checkmark$
Active blanking	<ul> <li>Laser illumination synchronised with camera exposure to minimise phototoxicity and photobleaching</li> </ul>	$\checkmark$	$\checkmark$	$\checkmark$
Exchangeable emission filter wheels	<ul> <li>To comprehensively image multiple fluorophores when using up to 8 laser lines for excitation (UV to NIR)</li> <li>RFID-tagged for filter update in user interface</li> </ul>	V	V	$\checkmark$
Camera magnification (1x, 1.5x & 2x)	• For flexible higher resolution imaging and pixel scaling to achieve Nyquist sampling over a wide range of objectives and pixel sizes	2-position motorised (option)	2-position motorised (option)	3-position motorised
2 pinhole diameters (25 µm and 40 µm)	<ul> <li>High contrast imaging at low and high magnification for large samples to subcellular imaging</li> </ul>	Option	Option	$\checkmark$
Laser-illuminated widefield mode	<ul> <li>For imaging thin specimens and single molecules</li> </ul>	$\checkmark$	$\checkmark$	$\checkmark$
Sensitive & fast detectors	<ul> <li>EMCCD for extreme sensitivity</li> <li>sCMOS for resolution &amp; speed with up to 95% QE</li> </ul>	V	$\checkmark$	$\checkmark$

Feature	Benefits
Illumination zoom	• Delivers higher illumination pow applications such as localisatio
3D super resolution module	<ul> <li>3D astigmatic module for 3D sir localisation imaging in a volume</li> <li>Compatible with all imaging mo spinning-disk, Widefield and TIF</li> </ul>
Laser engines (HLE or VLE)	<ul> <li>Extended spectral range (400 -</li> <li>High laser power capabilities fo</li> <li>2 to 6X faster with HLE vs VLE*</li> <li>Multimode fibre - more robust to longer term stability</li> </ul>
acquisition was set so that	setting an acquisition with 30% laser power f the image delivers the same grey levels: 200 On the laser engine: 100% laser power is 150
	Software
	<ul> <li>Immediate visual feedback on e</li> </ul>

Real-time 3D rendering	<ul> <li>Immediate visual feedback on e progress to evaluate data and r decisions in real-time</li> </ul>
3D Montage & Stitching	• Activate Stitching in a protocol
ClearView-GPU®	<ul> <li>Up to 50x faster processing that deconvolution solutions</li> <li>Activate Deconvolution in a prof</li> <li>Choose an algorithm to balance quality according to your image</li> </ul>
High content screening	<ul> <li>Supports multiple well plates, d</li> <li>Supports multiwell + montage c</li> </ul>
Imaris file format	<ul> <li>Easy transfer of data to Imaris C comprehensive downstream mu analysis</li> </ul>

✓= Feature available as standard

Option = Feature available as an option

- = Feature not available

	200 Series	400 Series	600 Series
ower density for tion microscopy	-	-	$\checkmark$
single molecule me modalities allowing TIRF DNA PAINT.	-	Option	Option
D - 800) for SMLM E* t to damage and	VLE	HLE	HLE

er for 488 lines in both VLE and HLE. The exposure time for each DO ms for VLE and 10 ms for HLE. Results are given by a finite burst 50 mW and 1500 mW for VLE and HLE respectively).

	200 Series	400 Series	600 Series
n experimental d make appropriate	$\checkmark$	$\checkmark$	$\checkmark$
bl	$\checkmark$	$\checkmark$	$\checkmark$
nan non-GPU based			
rotocol nce speed and ge properties	$\checkmark$	$\checkmark$	$\checkmark$
, dishes and slides e acquisitions	$\checkmark$	$\checkmark$	$\checkmark$
s Quantify for multi-dimensional	$\checkmark$	$\checkmark$	$\checkmark$



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## **Technical Features Explained**



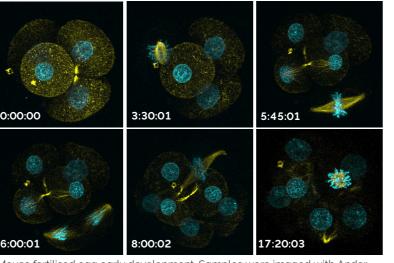
### 1 Dual Microlens Spinning Disk

A dual microlens spinning disk is a multipoint confocal; the multiple points of the sample are simultaneously illuminated by the multiple pinholes. The dual spinning disk of Dragonfly confocal are perfectly aligned with the pinhole disk to focus the laser light into the pinholes. The emitted light from the sample is re-imaged through the pinhole to reject out-of-focus light before it reaches the detector (camera). Overall the laser power needed to achieve an excellent confocal image is reduced and a confocal image is delivered instantaneously.

Consequently, the light that is required to illuminate the samples is less and therefore it reduces phototoxicity and background while increasing throughput. The optimised size and spacing of the proprietary dragonfly dual microlens system allows both gentle live imaging as well deep penetration into the samples up until the mm range.

The immediate benefits for the user are:

- Gentle imaging allows imaging live samples without phototoxicity or photobleaching
- ✓ Fast confocal imaging
- Image live very fast events
- Increase productivity when imaging deep thick samples
- Increase the signal-to-noise ratio

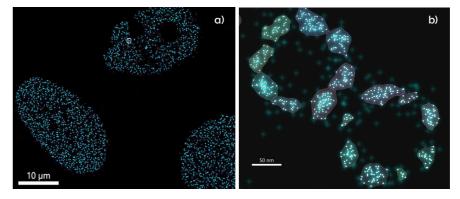


Mouse fertilised egg early development. Samples were imaged with Andor Dragonfly soon after fertilisation. Early development was followed by imaging every 15 minutes for 24 hours. At each time point, 50 stacks per channel were acquired. In yellow microtubules, in cyan DNA. Sample courtesy of Dr. Yoshiteru Kai, Yamashita Shonan Yume Clinic.

### 2 3D Single molecule imaging

The Dragonfly 600 has all the necessary tools for single-molecule localisation microscopy. It will deliver images with nanometre localisation precisions and corresponding resolution down to 10 nm. Significantly, Single Molecule Localisation Microscopy (SMLM) can be done using the 3D super resolution module, which is compatible with all imaging modalities: Widefield, TIRF and confocal. A single plane image through the 3D super resolution module delivers axial information over an ~ 1  $\mu$ m range with a corresponding resolution down to 30 nm.

The 3D super resolution module in widefield allows collection of axial SMLM data. In combination with B-TIRF this increases the signal to noise of the super resolution data close to the coverslip and up to 5  $\mu$ m inside the cell if using Hilo. When the 3D super resolution module is combined with confocal spinning disk, super resolution can be achieved in thicker specimens (~10  $\mu$ m range) by sequential acquisition of optical sections.



Dragonfly allows correlative/cross scale microscopy. a) image of 3 cell nuclei in which the nuclear pores were transiently labelled for NUP96 (nuclear pore protein). b) The same data set, zoomed around white highlight in a) to show DNA-PAINT resolution of <20 nm, revealing the 8-fold symmetry of the nuclear pore complex.

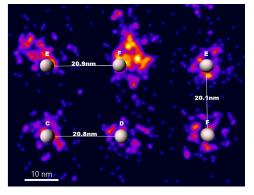
### 3 Sensitive Detectors

Sensitivity is a fundamental parameter of all microscope imaging systems. It determines the minimum detectable signal for a given excitation intensity. Dragonfly benefits from Andor's market-leading sCMOS and EMCCD detectors.

For the faintest of signals, iXon EMCCD detectors capture as low as 1.9 photons per pixel, with high signal to noise ratio.

Our latest generation of sCMOS detectors offer an outstanding balance of sensitivity and speed; with quantum efficiencies up to 95% and speeds greater than 44 frames per second (sustained), at full field of view. Read more in our camera specifications:

- ✓ iXon Back Illuminated EMCCD Specifications
- Sona Back Illuminated sCMOS Specifications
- ZL41 Cell sCMOS Specifications



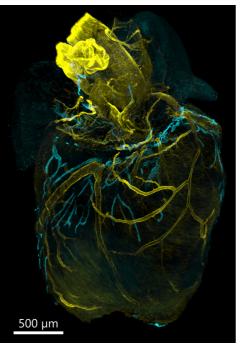
DNA-origami 3x4 grid with ~20 nm Cy3B fluorophore separation, imaged with a similar B-TIRF protocol shows resolution <10 nm.



### Borealis<sup>®</sup> Uniform Illumination

Andor's patented Borealis® Uniform Illumination delivers premier uniform illumination in the full field of view. It minimises roll-off in excitation uniformity (<5%) and significantly improves resulting image quality. This reduces errors in analysis across a field of view and reduces stitching artefacts in tiled or montage imaging. Borealis® patented technology is also available with B-TIRF.

Regardless of the imaging modality: confocal, widefield, TIRF or sinale molecule imaging, Dragonfly delivers excellent uniformity across the full field of view allowing for seamless stitching and consistent quantification of image intensity.



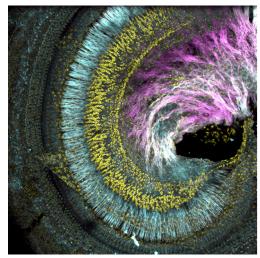
Mouse heart lymphatic vessels, showing Lyve-1 in cyan and SMA in yellow. The image is a stitch of 9 tiles and was imaged over a 720 µm Z range. Sample courtesy of Claire Bouvard, Laboratoire BioSanté U. Grenoble, France.

### 5 TIRE and B-TIRE

Borealis® Total Internal Reflection Fluorescence (B-TIRF) is Andor's patent pending TIRF module. The Borealis® uniform illumination of B-TIRF avoids interference and streaking artefacts found in standard TIRF imaging and consequently delivers superior image quality. Significantly B-TIRF is extremely easy to use, reducing the expertise needed to acquire TIRF images. B-TIRF can be used for regular TIRF applications, such as live imaging events at the edge of the cell membrane (e.g. endo/exocytosis, focal adhesion and cell movement, viral infection dynamics, etc).

Further, in Dragonfly, B-TIRF can be combined with illumination zoom, or the HLE, to perform single-molecule local experiments with significantly increased signal to noise ratios.

### Versatile and High Power Laser Engines



Mouse auditory cortex. 253 Z planes were acquired with a 20X air objective to deliver this 89-um depth image. Image Courtesy: Dr. Hainan Lang, Medical University of South Carolina.

Select laser illumination between the VLE (Versatile Laser Engine) or the HLE (High Power Laser Engine), both laser engines offer multimode fibre. Multimode fibres support and transmit longer wavelengths than single-mode fibres. Further, they require less maintenance and can match and fill the square sensor of the detector perfectly.

Both the VLE and HLE are suitable for live cell imaging of delicate samples, as they provide strictly controlled and gentle illumination. Moreover, both are compatible with the new B-TIRF imaging modality, and can support up to 10 wavelengths with excitation from 405-785 nm. Conveniently these laser engines can support up to 7 lines in a single chassis. VLE lasers offer lower power than HLE, but the VLE can be upgraded to higher power lasers in-field, with the extra advantage that the user can select higher power only on the specific lines needed.

The HLE additionally offers the high power required for singlemolecule imaging and considerably speeds up imaging spatial transcriptomics experiments.

For more information please consult the laser engines specification sheet.

### 7 Intuitive Software

### Fusion

Fusion has been designed to meet the requirements of today's researchers providing ease of use and immediate visual feedback for data review. Fusion simplifies the control of the Dragonfly system, making highend microscopy accessible to all users.

- Real-time 3D visualisation permits powerful insights to your experiments.
- Stitching and deconvolution can be activated in the protocol delivering the processed image soon after the acquisition is finished.
- Multi-well acquisition is available through an easy and intuitive user interface supporting up to 384 well plates: perfect for easy and fast screening of samples, organoids and phenotypes.
- A new burst shutter imaging mode for fast acquisitions can also be combined with Z acquisition.
- 3D single molecule imaging can be done in whole cells with minimal software interaction.
- An autofocus system is available in all imaging modalities delivering stable, precise imaging at all points during the entire experiment.

### Imaris<sup>®</sup> Quantify

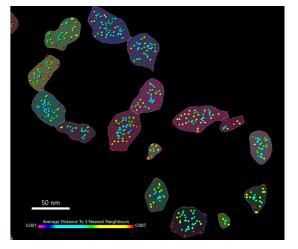
Each Dragonfly model includes the introductory Imaris Quantify package. Fusion saves files in the Imaris IMS format permitting easy transfer of data into Imaris Quantify for further visualisation and image analysis. The Quantify package adds additional visualisation and processing features including high resolution snapshots, creation of multidimensional animations, and advanced isosurface rendering of objects within your images.

The Quantify package provides the core quantitative features of Imaris, including AI-based analysis. Whether you are a cancer researcher or neuroscientist there are tools for measuring wide ranging properties of cells and proteins of interest in your samples. Additional application specific modules of Imaris are available and include specific measurements suited for cell & developmental biologists, neuroscientists and many more disciplines within the life sciences.

See imaris.oxinst.com

### Picasso

We have worked with Picasso developers to allow Imaris ims files to be read in Picasso. Users have the flexibility to go to open-source software to retrieve SMLM data and 3D SMLM localisations. 3D rendering and statistical measurements are then delivered in Imaris.



Fusion, Picasso and Imaris operate in harmony: Picasso directly reads Fusion raw data files and localises single molecule events in XY & Z, and exports both localisations and voxels. Imaris loads the voxels as a 3D volume and the localisations as Spots" for visualisation, filtering and analysis of densities (e.g. nearest neighbour measurements). Surfaces can be fitted to the resulting density maps as shown here with the NUP96 complex (page 15)



### General Specifications •1

Confocal/Widefield wavelength range	Excitation range: 400-800 nm Emission range: 420-850 nm
Confocal scan rate	400 scans per second
B-TIRF input wavelength range	400 - 800 nm
Confocal pinhole diameter	25 µm and 40 µm (option)
Disk type	Dual disk with matched microle
Active blanking	Laser illumination timed with c
Output power	See HLE & VLE specifications s
Lateral resolution	Diffraction limited in 16.6 x 14.0 square FOV)
Illumination uniformity	Less than ≤5% roll-off •²
Compatible Microscopes	Leica DMI8 & Nikon Ti2 compo Leica DM6 B & Nikon FN1 com

### Frame Rates<sup>•3</sup>

### Maximum Confocal Frame Rate (FPS)•4

Zyla 4.2 / ZL41 Cell	Frame Rate (sustained / burst)	iXon 888	Binning		Binning		Binnir		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		2	Extreme (sustained / burst)		-11 (sustained burst)
Array Size	USB 3.0	Array Size	1x1	2x2	4x4	Array Size		USB 3.0	Array Size	USB 3.0																						
2048x2048	43/102	1024x1024	25	50	92	2048x2	046	21/74	1400x1400	70/71																						
2048x1024	69/203	512x512	50	94	168	2048x1	024	43/152	1400x1024	95/97																						
2048x512	400/400	256x256	95	170	283	2048x	512	293/294	1400x512	191/194																						
2048x128	400/400	128x128	171	286	320	2048x1		398/399	1400x128	402/407																						

### Maximum Widefield/TIRF Frame Rate (FPS)

Zyla 4.2 / ZL41 Cell	Frame Rate (sustained / burst)	iXon 888	Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		Binning		88 Binning		Binning		Sona	Extreme (sustained / burst)		-11 (sustained / burst)
Array Size	USB 3.0	Array Size	1x1	2x2	4x4		Array Size	USB 3.0	Array Size	USB 3.0																																		
2048x2048	43/102	1024x1024	25	50	92		2048x2046	21/73	1400x1400	70/71																																		
2048x1024	69/203	512x512	50	94	168		2048x1024	43/152	1400x1024	95/97																																		
2048x512	407/409	256x256	95	170	283		2048x512	239/294	1400x512	191/194																																		
2048x128	990/992	128x128	171	286	430		2048x128	1139/1150	1400x128	755/769																																		

#### Frame rate acquisition conditions

Zyla / Zl41 Cell - Protocol Type = Finite Burst- Repeats = 200/1000 | Acquisition Mode = High Speed | Binning = 1x1 | Exposure time = 2.5 ms (Con) Min (WF)| Overlap = ON | Bit Depth = 12-bit

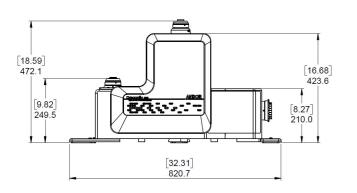
iXon - Protocol Type = Finite Burst - Repeats = 50 / 200 | Binning = 1x1 | Exposure time = 2.5 ms (Con) Min (WF) | Overlap = ON | Vertical Shift Speed = 0.6 µs | Horizontal Shift Speed - 30 MHz (16 bit) Sona -6 Protocol Type = Finite Burst| Repeats = 50/200 | Acquisition Mode (Confocal )= 16 Bit | Binning = 1x1 | Exposure time = 2.5 ms (Con) Min (WF) | Overlap = ON |

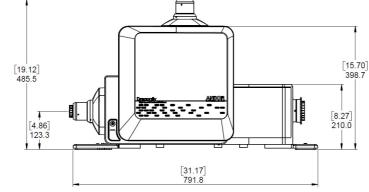
Sona -11 Protocol Type = Finite Burst = 50/200 | Acquisition Mode = High Speed | Binning = 1x1 | Exposure time = 2.5 ms (Con) Min (WF) | Overlap = ON | Bit Depth = 12-bit (fast speed Sona-11) In widefield mode, to get the maximum speeds the user should divide 1 by the number of frames to acquire. The obtained value is the exposure time to use. Note: HLE lasers 561 nm - 1 W and 592 nm -1 W lasers have mechanical shutters. Therefore, the shutter will always be open for acquisition speeds lower than 50 ms (20 Hz), and disk stripes might be visible at speeds lower than 30 ms. With 561 nm/594 nm 1 W lasers the dual channel sequential imaging is limited by the 50 ms threshold. This threshold also limits dual-camera simultaneous imaging coordinated with active blanking. This limitation only applies to the 561 nm - 1 W and 592 nm - 1 W lasers. For the fastest imaging acquisition using speeds, the user is advised to choose the lower power options 561 nm – 150 mW and 592 nm – 100 mW.

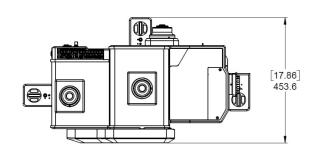
## **Mechanical Drawings**

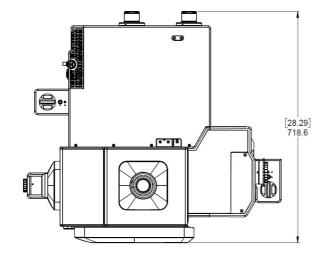
200 and 400 Series

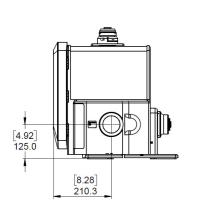
600 Series

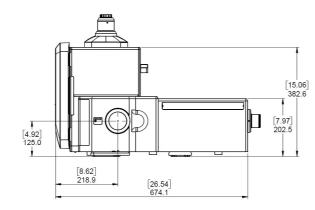












	200 and 400 Series	600 Series
Weight	39 kg approx. without cameras/accessories	65 kg approx. without cameras/accessories
Dimensions (WxDxH) inches [mm]	32.3 x 22 x 18.8 [821 x 558 x 477] (with installation handles) See diagram above for without handles	31.2 x 30.7 x 19.3 [792 x 780 x 489] (with installation handles) See diagram above for without handles

Note: Addition of camera(s) will add up to 7.4 [189] to height and up to 3.7 kg weight per camera. For dimensions of the HLE, please refer to the HLE & VLE specifications sheet.

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lens and pinhole arrays.

camera exposure to minimise phototoxicity and photobleaching

#### <u>sheet</u>

.0 mm (21.7 mm diagonal rectangular FOV, 19 mm diagonal

atible with all Dragonfly models npatible with Dragonfly 200 series only

## **Configuring Dragonfly Solutions**

The following sections are designed to give you an overview of the models, features and configurations available and provide a guide to ordering your system. Please contact your local sales representative who will be able to guide you through the ordering process.

### Dragonfly

**Dragonfly 200** delivers exceptional spinning disk confocal imaging of all biological samples (thick, thin, fixed and live). High-resolution image stacks are ready for analysis in seconds.

**NEW Dragonfly 400** adds on the High Power Laser Engine and the 3D super resolution module. This remarkable hardware can address more applications such as 3D Widefield Single Molecule Localisation Microscopy (DNA-PAINT), and increased throughput in both spatial omics and large sample imaging.

**Dragonfly 600** adds Borealis-TIRF and zoom illumination optics imaging functionality to make a complete multi-modal system for Widefield, Confocal, TIRF, and SMLM (DNA-PAINT & dSTORM) applications. Dragonfly 600 achieves impressive resolutions down to 10 nm (DNA-PAINT) and allows SMLM in any imaging modality (confocal, widefield or B-TIRF). Andor's patented Borealis-TIRF exploits patented Borealis® illumination, delivering robust, even illumination and thus more usable data across the field of view.

### Cameras

#### iXon Back-Illuminated EMCCD Camera Series

Single photon sensitive. Deep cooled back-illuminated EMCCD sensor technology.  $QE_{max}$  95%. 1024 x 1024 pixels, pixel size 13 µm, field of view 18.8 mm. SRRF-Stream+ compatible.

#### ZL41 Cell sCMOS Cameras

Fast and flexible high-resolution imaging.  $QE_{max}$  82% and down to 0.9 e- read noise. 2048 x 2048 pixels, 6.5  $\mu$ m pixel size, field of view 18.8 mm. SRRF-Stream+ compatible

#### Sona Back-Illuminated sCMOS Cameras

Sona Extreme latest back-illuminated sCMOS technology with  $QE_{max}$  95% and down to  $\leq$ 1.0 e- read noise. 2048x2046 pixels, 6.5 µm pixel size, 18.8 mm field of view. SRRF-Stream+ compatible.



### **PC Workstation**

Windows 11 Professional, single CPU – 16 Core; SSD fast boot disk; 128 GB RAM expandable to 256 or 512 GB; 4x4 TB SSD RAIDO (16 TB) for data acquisition. Extra data storage available (up to 32 TB of SDD or 48 TB of HDD); 2x Nvidia Quadro RTXA4000 (16 GB GDDR6) for hardware accelerated, image capture, processing and visualisation.

### Software

**Fusion Control Software:** Multi-channel, dual camera simultaneous imaging, multi-dimensional acquisition options (time, XYZ, tile, multi-position), multi-modal imaging (confocal, widefield, SMLM, TIRF, SRRF-Stream+), multi-well acquisition and real-time rendering. Image processing options activated in protocol (stitching and deconvolution). Focus stabilisation.

**Imaris Quantify:** Render 3D/4D images, identify and characterise objects within images via traditional intensity-based and AI methods, report measurements, create high-resolution snapshots and multi-dimensional animations.

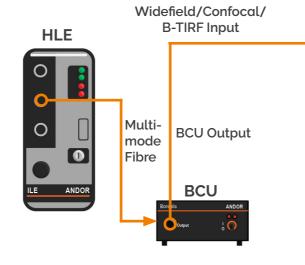
Find out more imaris.oxinst.com/packages.

### Laser Engines, Borealis® and the Beam Conditioning Unit (BCU)

**Borealis® illumination**: a multi-mode fibre is directly coupled to a Beam Conditioning Unit (BCU). The BCU homogenises the laser output for outstanding illumination uniformity and integration for all imaging modalities.

HLE and VLE offer two to seven multimode laser lines in a single chassis and up to ten in dual configuration.

Want to learn more Laser Engines Specifications



### **Photostimulation Options**

**Mosaic 3**<sup>®</sup> is a digital micro mirror based solution for real-time multi-region illumination. Mosaic can be configured with laser and/or LED sources (365-800 nm) for applications including bleaching, photoactivation and optogenetics.

**MicroPoint 4**<sup>®</sup> is a tuneable pulsed laser system capable of ablation, bleaching and uncaging. Wavelengths supported: 365 to 656 nm.

Find out more and or.oxinst.com/products/photostimulation-solutions

		Dragonfly Model Options												
Model Select	or Matrix	200				400				600				
			Multi-modal confocal system				Multi-modal confocal system with 3D SMLM capabilities				Multi-modal confocal system with 3D SMLM capabilities & B-TIRF			
Feature	Feature Description	DFLY-201-40 DFLY-202-40 DFLY-201-2540 DFLY-202-2540		CR-DFLY-400-40	CR-DFLY-400-2540	CR-DFLY-400-40-SR	CR-DFLY-400-2540-SR	DFLY-602	DFLY-602-SR	DFLY-610-BTIRF	DFLY-620-SR			
B-TIRF	B-TIRF supplied for 60x and/or 100x TIRF objective lenses.	-	-	-	-	-	-	-	-	0	0	•	•	
3D Super resolution module	For 3D single molecule localisation imaging in a volume.	-	-	-	-	0	0	•	•	0	•	0	•	
iZoom	Higher illumination power density for applications such as localisation microscopy.	-	-	-	-	-	-	-	-	•	•	•	•	
Motorised camera mag	1X, 1.5X, 2.0X	Up to 2	Up to 2	Up to 2	Up to 2	Up to 2	Up to 2	Up to 2	Up to 2	3	3	3	3	
Dual pinhole	25 μm, 40 μm	-	-	•	•	-	•	-	•	٠	•	•	•	
Image splitter	Minimum number of motorised splitters is one, option to have up to four.	-	0	-	0	0	0	0	0	•	•	•	•	
Versatile Laser Engine - VLE	VLE offers two to seven multimode laser lines in a single chassis and up to ten lines in dual configuration.	•	•	•	•	-	-	-	-	0	-	0	-	
High power Laser Engine - HLE	HLE offers two to seven multimode laser lines in a single chassis and up to ten lines in dual configuration.	-	-	-	-	•	•	•	•	•	•	•	•	
Dual camera port	Dual camera ready, user can buy two cameras at the purchase of the system or upgrade later.	-	•	-	٠	•	٠	٠	•	٠	٠	٠	•	
Imaris Quantify	3D image visualisation and inspection tools, AI driven object detection and classification, interactive measurement reporting, hi-res snapshots and animations for presentations.	•	•	•	•	•	•	•	•	•	•	•	•	
Accessories	Piezo stages, incubators, extra filter wheels & filters.	0	0	0	0	0	0	0	0	0	0	0	0	

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• - Standard 0 - Optional

### DXFORD N S T R U M E N T S ANDOR

## Order Today

Need more information? At Andor we are committed to finding the correct solution for you. With a dedicated team of technical advisors, we are able to offer you one-to-one guidance and technical support on all Andor products. For a full listing of our local sales offices, please see: andor.oxinst.com/contact

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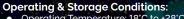
#### Items shipped with your Dragonfly

Dragonfly base unit, cables and accessories (model as ordered) HLE or VLE (with laser options and accessories as ordered) Borealis BCU PC Workstation and accessories Fusion and Imaris Quantify Software User guides in electronic format

#### 1. All specifications are typical.

Footnotes

- 2. Measured for 488 nm with 40 µm pinhole pattern at Dragonfly output port. Illumination at sample may differ due to variation between microscope objectives.
- 3. Frame rate data shown for three typical camera options.
- 4. Confocal frame rates require at least 2.5 ms exposure time, synchronised with the confocal scan speed, hence they differ slightly from widefield/TIRF.



- Operating Temperature: 18°C to +28°C ambient
- Indoor use only •
- Operating Altitude: up to 2000 m Relative Humidity: <70% (non-condensing)
- Storage Temperature: 0°C to 50°C

#### Power Requirements:

- External supply: 100 240 VAC, 50 60 Hz
- Power consumption
- Dragonfly 200 & 400: 45 W/75 W (typ./ max.) Dragonfly 600: 65 W/120 W (typ./ max.)

Cover image adult drosophila testis showing different stages of sperm individualisation. Courtesy of Alina Kolpakova, Eli Arama group, Weizmann Institute of Science.

WARNING - LASER RADIATION VISIBLE AND INVISIBL LASER RADIATION AVOID EXPOSURE TO BEAM CLASS 3B LASER PRODUCT AVOID EXPOSURE TO BEAM CLASS 3B LASER PRODUCT 500 mW CW Max at 400 - 700nm CLASSIFIED PER IEC 60825-1:2014 500 mW CW Max at 400 - 800nm CLASSIFIED PER IEC 60825-1:2014 AVERTISSEMENT AVERTISSEMENT RAYONNEMENT LASER VISIBLE ET INVISIBLE RAYONNEMENT LASER EXPOSITION AU FAISCEAU DANGEREUSE APPAREIL À LASER DE CLASSE 3B OSITION AU FAISCEAU DANGEREU APPAREIL À LASER DE CLASSE 38 500 mW CW Max at 400 - 700n 500 mW CW Max at 400 - 800 and an entry DANGER - VISIBLE AND INVISIBLE LASER RADIATION AVOID EVE OR SIKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION CLASS 4 LASER PRODUCT 6000 mW CVM Max at 400 - 800nm CLASSIFIED PER IEC 60625-1:2014 DANGER - LASER RADIATION AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION CLASS 4 LASER PRODUCT 6000 mW CW Max at 400 - 700nm DANGER - RAYONNEMENT LASER VISIBLE ET INVISIBLE **DANGER - RAYONNEMENT LASER** EXPOSITION DANGEREUSE DE L'ŒIL OU DE LA PEAU AU RAYONNEMENT DIRECT OU DIFFUS APPAREIL À LASER DE CLASSE 4

EXPOSITION DANGEREUSE DE L'ŒIL OU A PEAU AU RAYONNEMENT DIRECT OU DI

The Dragonfly system may be classified under IEC 60825-1 and CDRH 21 CFR 1040.10 as either Class 3B or 4 depending on the system configuration

