

# Andor Revolution WD Detailed Description

The Andor Revolution WD spinning disk confocal is based around a fully motorized Nikon TiE inverted microscope equipped with differential interference contrast, LED transmitted and epi-fluorescent light sources, and Perfect Focus. An Okolab Bold Line stage-top incubator and controller provides temperature, CO<sub>2</sub>, and humidity control. The objectives include a CFI Plan Fluor 10x/0.3NA objective with a 16 mm working distance, a CFI Plan Apo 20X/0.75NA objective with a 1mm working distance, a CFI Lambda S Apo LWD 40X/1.15NA water immersion objective with a 0.59-0.61 mm working distance and 0.15-0.17 mm coverslip thickness correction collar, a CFI Plan Apo VC 60x/1.2NA water immersion objective with a 0.27 mm working distance, and a CFI Plan Apo VC 100X/1.4NA oil immersion objective with a 0.13 mm working distance. An ASI motorized piezo XYZ stage is equipped with rotary encoders for positional feedback and features a 22 nm XY step size with a 120 x 100 mm range of travel and a 50 nm Z-axis step size with a 300 µm range of travel. The Yokogawa CSU-W1 confocal unit is fully motorized and equipped with two camera ports, two 10-position emission filter wheels, a motorized wide-field bypass mode, and a motorized adjustable aperture for a field of view of up to 17x16 mm. The spinning disk has 50 µm pinholes and operates at 1500-4000 rpm allowing for imaging at up to 200 frames per second. Motorized switching between three multi-band pass dichroics for multi-wavelength imaging across the visible spectrum: 405/488/561 nm (e.g. BFP/GFP/RFP); 405/488/561/640 (e.g. BFP/GFP/RFP/TOTO-3); and 445nm/514nm/640nm (e.g. CFP/YFP/TOTO-3). The Revolution 500 series AOTF Laser Modulator and Multiport Combiner allows for fast switching between six solid state lasers providing the following laser lines: 100 mW 405nm diode laser, 50 mW 488nm DPSS, 40 mW 445nm diode laser, 50mW 515nm DPSS laser, 50 mW 561nm DPSS laser, and a 100 mW 640nm diode laser. A Precision Controller Unit allows synchronized triggering of the cameras and spinning disk unit. On one CSU-W1 camera port there is an Andor Neo16-bit sCMOS camera with 2560x2160 pixel chip and 6.5 µm pixels deep-cooled to -40°C for fast confocal imaging with high spatial resolution and a wide field of view. This camera has a 57% quantum efficiency and is capable of up to 30 frames per second. On the other CSU- W1 camera port there is an Andor TuCam dual camera imaging adapter with two Andor iXon ULTRA 897BV black illuminated EMCCD cameras with 512x512 pixel chips and 16 µm pixels deep-cooled to -70°C for fast confocal imaging with maximum sensitivity. These cameras have a quantum efficiency of >90% and are capable of up to 56 frames-per-second. The TuCam contains two selectable dual band-pass dichroic mirrors for simultaneous dual-wavelength imaging of CFP/YFP or GFP/mCherry. A laser-galvo operated FRAPPA unit introduced through a back port of the microscope allows for photobleaching or photoactivation of user-defined regions of interest within the field of view with fast switching using any of the six available laser lines. The entire system is controlled by a PC workstation with 32GB Ram, 27" LCD monitor with 2560x1440 resolution, nVidia high performance graphics processors, and MetaMorph 7X Premier software optimized for multi-dimensional acquisition and analysis capability.