Confocal 6 - Ultraview system (spinning disk confocal) (August 2020)

Technical Specifications

- Specialised system for live cell imaging.
- Superior optical efficiency and speed of image acquisition provides improved live cell imaging (but inferior resolution) compared with CLSM systems.
- Perkin Elmer UltraVIEW ERS 6FE confocal system attached to a Leica DM I6000 inverted epifluorescence microscope.
- Volocity (Improvision) acquisition software - 3D rendering and deconvolution available on off-line workstations.
- Yokogawa CSU22 spinning disk.
- Hamamatsu C9100-50 EM-CCD camera (14 bit, 1000 x 888 8 micron pixels, max 27fps full frame).
- Equipped with 14mW mW Ar laser (488, 514 nm lines), 7.5mW 568 nm laser, 7.5 mW violet (405 nm) diode laser, 7mW blue diode laser (440 nm) and 7.5 mW Red diode (640 nm).
- AOTFs for all laser lines allow rapid attenuation.
- Photokinesis accessory for photo-bleaching applications (spot and ROI).
- Suitable for a wide range of fluorophores (e.g. most fluorescent proteins).
- Fast sequential mode uses multiple band dichroics and rapid AOTF control of laser lines - fastest acquisition speed but risk of cross-talk between fluorophores.
- Emission discrimination mode uses emission filter wheel to selectively image different fluorophores but with some loss of acquisition speed.
- Phase/DIC imaging can be integrated with fluorescence using emission discrimination mode so not at maximum acquisition speeds.
- Piezo drive in one lens turret position for most rapid focus control. Alternatively microscope focus control can also be controlled through Volocity software.
- Environmental chamber (Solent) for temperature control and CO_2 enrichment.

Filters for visual inspection

	Excitation range	**Fluorophore (examples)**	**Excitation filter**	**Dichroic mirror**	**Emission filter**
A4	UV	DAPI	BP 340-380	RKP 400	450-490
L5	Blue	FITC, GFP	BP 460-500	RKP 505	512-542
N3	Green	Rhodamine, RFP	BP 540-552	RKP 565	580-620

Filters/Dichroics for imaging

Imaging mode	Dichroic	Usable excitation wavelengths (nm)	Emission filters	Fluorophores (examples)
'Emission discrimination'	Dichroic: 405/488/561/640	405, 488, 568, 640	445/60, 485/60, 527/55, 587/125, 615/70, 705/90	DAPI, GFP, RFP, Cy5
'Emission discrimination'	Dichroic: 405/440/514/640	405, 440, 514, 640	445/60, 485/60, 527/55, 587/125, 615/70, 705/90	DAPI, CFP, YFP, Cy5
'Emission discrimination'	Dichroic: 405/440/488	405, 440, 488	445/60, 485/60, 527/55, 587/125, 615/70, 705/90	DAPI, CFP, GFP

Lenses

Lens	Dry/Oil	Phase contrast	Working distance (microns)	Numerical aperture	Features	Serial number	Pixel width at 1x binning in microns
10X HCX PL Apo	Dry	No	3800	0.3		506286	0.8
40X HCX PL Apo *	Oil	Yes	100	1.25		506106	0.2
63X HCX PL Apo	Glycerol	no	280	1.3	Coverglass thickness correction	506193	0.127
100X HCX PL APO CS	Oil	Yes	90	1.4		506042	0.08

*40x lens is currently shared with Widefield 1