

Visitron Spinning Disk/FRAP



Installed 2012. Inverse spinning disk microscope unit for fast pseudo-confocal imaging, preferably for live applications at ambient temperature. Equipped with laser lines for blue, green, red and far red dyes. FRAP/photomanipulation with all laser lines possible. Fast sCMOS and high-sensitivity EM-CCD camera. Hardware autofocus, multi-positioning and stage-based environmental control, including a fast heater/cooler for temperature shift experiments.

(Last updated: Nov. 2022)

Stand

Zeiss Axio Observer, inverse, fully motorized, with hardware autofocus (Zeiss Definite Focus 2), Visitron ViRTEx real time controller

Objectives

- A-Plan 5x/0.12 (working distance 10.1 mm, for overview images)
- EC Plan-Neofluar 20x/0.50 (working distance 2.0 mm)
- LD LCI Plan-Apochromat 25x/08 Imm Corr (oil/glycerol/water, working distance 0.57 mm)
- Plan-Apochromat 63x/1.4 Oil DIC III (working distance 0.19 mm)
- LCI Plan-Neofluar 63x/1.3 Imm Corr DIC III (glycerol/water, working distance 0.17 mm)
- EC Plan-Neofluar 100x/1.30 Oil Iris (working distance 0.2 mm)

Lasers and photomanipulation

Visitron VS-LMS laser combiner with 2 ports for imaging or photomanipulation, VS-FRAP 2D scanner

- 405 nm laser diode (120 mW)
- 488 nm diode laser (100 mW)
- 561 nm DPSS laser (200 mW, AOTF-controlled)
- 640 nm laser diode (150 mW)

Widefield Illumination

- Spectra X Light Engine (hybrid solid state LED) with 6 excitation bands (DAPI 390/22, CFP 438/29, GFP 475/28, YFP 504/12, RFP 554/23, Cy5 628/40)
- White LED

Filters

Pos.	Laser Line CSU	SpectraX	Reflector Turret	Emission Filter Wheels
1	405	DAPI 390/22	Quad DA/FI/TR/Cy5 ^a	DIC Analyzer
2	488	CFP 438/29	DIC Analyzer	DAPI Chroma ET450/50
3	561	GFP 475/28	FRAP Polychroic ^b	empty
4	640	YFP 504/12		GFP Chroma ET525/50
5		RFP 554/23		mCherry Chroma ET605/70
6		Cy5 628/40		Quad Chroma ZET405/488/561/640m-TRFv2

^aSemrock FF01-392/474/554/635, FF409/493/573/652-Di01, FF01-432/515/595/730

^bCustom Chroma ZT405/488/561/640rpc 60%T 40%R

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Spinning disk unit

- Yokogawa CSU-X1 Nipkow spinning disk unit (50 μm pinholes, spacing 253 μm , 5000 rpm)
- Main dichroics: Semrock Di01-T405/488/568/647, Di01-T405/488/561

Cameras

- pco.edge sCMOS camera (70% QE, 2048 x 2048 pixel, 6.45 μm pixel size, 16 bit, up to 100 fps)
- Hamamatsu ImagEM X2 EM-CCD (512 x 512 pixel, 16 μm pixel size, 16 bit, up to 70 fps)

Stage/Inserts

- ASI PZ scanning stage (xy travel range 120 x 110 mm), Z-Piezo top plate (travel range 150 μm)
- Inserts for all kind of slides and 35 mm dishes

Software

- VisiView 6.0 (Visitron Systems) running on Windows 10 (64-bit)

Environmental Control

- CherryTemp heater/cooler (Cherry Biotech) for fast temperature shifts (seconds), 5-45°C
- Stage-top temperature/CO₂ control: Tokai Hit STXG controller, PLAMX chamber with inserts for ibidi slides, 35 mm dishes and multiwell plates