

HyperScope

Advanced multiphoton imaging



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HyperScope Simultaneous imaging and stimulation

Easily perform multiphoton microscopy and photoactivation at the same time to help answer more complex research questions.



The HyperScope's dual path scan head

Single or Dual Scan Head

The HyperScope provides the choice of a single or dual scan head allowing for either one or two light paths to be manipulated through the microscope.

This enables simultaneous two-photon imaging and photoactivation with exceptional performance.

It is easily upgradeable as a second light path can be added in the field if required.

Extended wavelength range

The HyperScope optics are coated for wavelengths of 700 to 1400 nm to let you excite dyes further into the infrared spectrum and potentially perform three-photon imaging.

This gives you more experimental flexibility for multiphoton imaging and the ability to image deeper into your sample.

Multiple scanning options

The scan mirrors on the imaging path can be arranged in multiple configurations depending on your needs.

These include galvo-galvo, galvo-resonant or galvo-galvo-resonant arrangements.

In vivo, in vitro, or extended setups

The microscope can be setup with or without substage optics for in vitro or in vivo samples respectively.

For larger in vivo specimens and more elaborate experimental layouts an extended scan head can be used.

Pioneering slim design

A compact footprint and flexible design allows for easy integration with other techniques such as electrophysiology.





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Precise scanning The Dual Scan Head

The newly designed dual scan head maintains all of the excellent optical properties of the original Scientifica scan head but with the option for two light paths: one for imaging and one for photoactivation.



Z-stacks from in vivo 6 days post fertilisation zebrafish (courtesy of Professor Leon Lagnado's lab at the University of Sussex).



Efficient, high-resolution imaging

Imaging the finest details across the whole field of view is possible thanks to the unique relay lens system. This ensures that each scanning mirror is imaged precisely onto the next scanning mirror, which is then perfectly imaged onto the objective back aperture. This avoids movement of the expanded beam at the objective back aperture, regardless of the scanning angle.

Systems without a relay lens may suffer from vignetting, leading to a drop in fluorescence at the edges of the image. These optics also ensure a uniform 'spot size' is always achieved for visualising the smallest structures across the entire scan field.

Optical design

Optimal resolution is achieved with a range of multiphoton objectives. Scientifica offers a range of lenses including RMS, M25, M27 and M32 threaded objectives, which are perfectly suited for combining multiphoton imaging and electrophysiology, due to their provision of large working distances (up to 3.3 mm). Utilising the entire N.A. (numerical aperture) of the objective helps researchers to image incredibly fine structures.

All optical elements are coated to optimise transmission from 700 to 1400 nm infrared light, allowing the use of a wide range of excitation wavelengths, without any change in performance.

30 mm side port

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SHUTTER

In the single beam configuration the system offers access to an optional side port for easy addition of extensions.



Photon Collection Options Multiphoton Detection Unit XL (MDU XL)





Scientifica's MDU XL is a multiphoton detection unit with increased sensitivity, designed to achieve a superior signal-tonoise ratio and improved image quality, translating to crisper and deeper images.

The MDU XL is optimised for photon collection with large back aperture objectives. The large optics enable collection of more scattered light for deeper tissue structures. It offers up to 30% more efficient light collection in multiphoton imaging experiments for up to two colours when compared to the standard MDU.

The MDU XL can be fitted with two photomultiplier tubes (PMTs) for photon collection from two channels at the same time. It is available with up to two gated or protected GaAsP PMTs for maximum sensitivity.



Benefits include:

Custom built optics: a custom design allows for optimal photon collection for crisper and deeper images Superior signal-to-noise ratio: the MDU XL design minimises background noise, helping to maintain image quality Available with a range of PMTs to suit experimental needs and budget requirements

Multiphoton Detection Unit (MDU) & Chromoflex



MDU

Scientifica's MDU is ideal for the collection of valuable photons by using customised optics, mechanics and noise-reducing electronics. It is optimised for use with small back aperture objectives.

The MDU has been specifically designed to achieve a high signal-to-noise ratio thanks to the careful placement of the electronic components.

This is the only multiphoton collection option that allows for substage detection.

Benefits include:

Customised optics: a custom designed, extralarge fixed position collection lens is sited very close to the objective back aperture, allowing photon collection at the widest of scan angles High signal-to-noise ratio: the electronic design of the MDU performs exceptionally by minimising background noise which can degrade the image quality

Fast filter exchange: a standard Olympus filter cube is fitted to a dovetail slier which can easily move in and out of the MDU, easing the setup of the next set of experiments



Chromoflex

The Chromoflex enables the simultaneous imaging of up to four different colour dyes with the increased sensitivity of GaAsP PMT detectors. The option of 4 PMTs makes it perfect for in vivo studies.

Large collection optics allow greater light gathering from the objective back aperture. This increases overall efficiency by up to 10% after reductions by the liquid light guide when compared to our MDU for better detection of weak fluorescent signals.

Now available with a range of PMTs.

Benefits include:

Large collection optics allows more light collection from the objective back aperture. This increases overall efficiency by up to 10% after reductions by the liquid light guide compared to our standard MDU

Modular and upgradeable: choose a 2, 3 or 4 PMT system depending on your requirements. Upgrade when necessary for more complex studies

Remote placement: the two-metre liquid light guide enables flexible placement of the detection module remotely from the collection module

Performance Measurements

As tested at Scientifica



PSF measurements taken at the centre and four corners of the field of view

Point Spread Function (PSF)

The measurements shown above were taken using a Nikon 16X 0.8 NA 3 mm WD objective at 850 nm collected with Scientifica's standard MDU. They were performed by imaging 0.2 µm polystyrene fluorescent beads mounted on the surface of a coverslip. The data shows how the use of the relay lenses in the optical design produces a comparable resolution across the field of view.

Field Flatness Mapping

The relay lenses also provide a constant intensity across the field of view which can be observed on the 3D surface plot (right).



Field flatness mapping with a homogenous fluorescent sample

Software SciScan

SciScan is Scientifica's award-winning, modular and open source, multiphoton image acquisition software package. It is specifically designed to work with, but not limited to, Scientifica's multiphoton product range.

Written in LabVIEW for easy customisation, the clear and intuitive interface is designed to be quick and simple to use, whilst maintaining powerful functionality.



Software functions

SciScan is an award-winning two-photon data acquisition software designed and built by Scientifica in collaboration with researchers.

Created in LabVIEW and utilising popular interface boards, the software is provided opensource to allow you to customise your own modules for your experiments.

Developed with the user in mind and in collaboration with scientists in the field, the interface is clear and easy to use.

SciScan's purpose-built API allows users to integrate SciScan easily with their custom-written applications from LabVIEW and other programming languages.

The powerful SciScript Module allows users to pre-program recording sequences and stage motions, opening up many new possibilities.

The software incorporates the Open Microscopy Environment's XML and OME-TIFF formats to enable users to open, organise and share metadata in a fully standardised format.

SciScan now offers an elegant way of simultaneously imaging and stimulating at the same time when used with the HyperScope.

SciScan is fully supported and will continue to be improved with customers - changes will be made as research and technology develops.



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Software ScanImage

Vidrio Technologies have fully integrated Scientifica's multiphoton hardware into their various ScanImage software packages.

The HyperScope is compatible with the latest version of ScanImage 2017. It also works with ScanImage 5.



Software functions

Developed by researchers at the HHMI Janelia Farm Research Campus developed ScanImage specifically for neuroscience applications.

ScanImage 5 can be downloaded for free and is open-source (written in MatLab) to allow you to develop custom functionalities necessary for your experiments.

ScanImage 2017 is a premium supported software package with advanced and user-selected features built on top of ScanImage 5.

External triggering and synchronisation enables input and output signals to synchronise the software with additional hardware.

For more information visit <u>vidriotechnologies.com</u> and <u>scanimage.org</u>.

Schematics In vitro setup







Schematics In vivo setup





Moveable in vivo setup

Schematics Extended moveable in vivo setup



Technical Specifications

HyperScope Launch Optics		
External beam expander	Lens type: Achromatic Input clear aperture: 14 mm Output clear aperture: 24 mm Expansion Ratio: 2.5x Wavelength range: 750 to 1550 nm Coating: Optical average reflectivity per surface <1% average (750 to 1550nm)	
Achromatic wave plates	Clear aperture: 12 mm Wavelength range: 700-1100 nm or 900-1300 nm Coating: Optical reflectivity per surface <0.7% average AOI: Normal Surface flatness: N/4 @ 633 nm (over the clear aperture)	
45° Folding mirror(s)	Dimensions: 1" Substrate: Fused Silica Coating: Protected Silver (average reflectivity ~ 96% from 700 nm to 1400 nm @ 45° AOI) Clear aperture: 90% of the diameter Surface flatness: λ/10 @ 633 nm	
Electro optic modulators (optional)	Classification: Transverse field type Pockels cells Aperture diameter: 2.7 mm Extinction ratio: 200:1 or 500:1 @ 633 nm (with 1mm beam @ 1% point) Standard wavelength range: 680-1300 nm or 680-1100 nm* Beam blocker: Built-in *Wavelength range modification is possible	
Electro optic modulator driver (302RM)	Bandwidth: DC-to-250 KHz Rise/fall times: 1 μs Typical drive configuration: Lumped capacitance Output: Linear	
	HyperScope Scan Head Optics	
Scan axes	 Resonant X-axis: Cambridge Technologies CRS8K (8Hkz) clear aperture is 5.0 x 7.2 mm ellipse Galvanometer: X-axis Cambridge Technologies 8315KL clear aperture 6 mm Galvanometer: Y-axis Cambridge Technologies 8315KL clear aperture 6 mm 	
Galvanometer driver	Cambridge Technologies MicroMax 671 (High Power option) Voltage/optical degree: ~ $0.5 V$ Maximum scan angle: $\pm 20^{\circ}$ Small angle step response: ~ $130 \mu s$	
Resonant driver	Cambridge Technologies CRS (711-80159) Angle control: External analogue reference 0 to 5V (zoom function) Max scan angle: ~ ±13° Typical step and settle: 6.0 ms	
1:1 Relay lens	Input beam diameter: up to 3 mm Maximum scan angle: ±15° Wavelength range: 700 to 1400 nm Coating: Optical reflectivity per surface <0.7% average ~ 700nm to 1400nm	

Technical Specifications

Scan lens	Wavelength range: 700-1400 nm Coating: Optical average reflectivity per surface <0.7% average ~ 700nm to 1400nm	
Beam combining cube	Dimensions: 25.4 x 25.4 x 25.4 mm Clear aperture: >85% of central dimension Wavelength Range: 400-1300 nm Average Transmission TP: > 90% Average Reflection RS: > 97.5% Transmitted Beam Deviation: < 5 arc min Reflected Beam Deviation: 90° ± 20 arcmin	
45° Folding Mirror	Dimensions 45 x 64 x 6 mm Substrate: Fused Silica Coating: Protected Silver (average reflectivity ~ 96% from 700nm to 1400 nm @ 45° AOI) Clear aperture: 90% of the specified dimensions Surface flatness: λ/10 @ 633 nm	
Beam diameter at Objective Back Aperture	Scan head beam expansion ratio: 6.85x Beam size at the microscope objective back aperture: ~ laser output size x table beam expander (usually 2.5x) x scan head beam expansion	
Average scan-head transmission*	~30 ± 0.8 % (700 to 1050 nm) *using a Nikon 16x 0.8 NA objective lens	
Resonant/Galvanometer field of view	Galvo: ~ 1 mm Resonant: ~ 0.7 mm Measured centre field (on-axis) resolution @ 860nm*: X/Y~ 0.59 ± 0.022 µm and Z~ 3.24 ± 0.114 µm Measured edge field (off-axis) resolution @ 860nm*: Average X/Y~ $0.67\pm$ 0.028 µm and Z~ 4.12 ± 0.210 µm *using a Nikon 16x 0.8 NA objective lens	
Acquisition & Control		
Sample rates from 5 MHz - 120 MHz depending on software and hardware configuration chosen		
Amplifiers		
Fixed gain current amplifier (HCA-20M-100K-C) - Resonant only	Gain[V/A]: 105 3dB Bandwidth [MHz]: 20 Equivalent input noise [pA/√Hz]: 3.5 Rise/fall time (10% - 90%) [nS]: 18 Maximum source capacitance [pF]: up to 50	
Variable Gain High Speed Current Amplifier (DHPCA-100) - Switchable systems	Gain[V/A]: 102-108 Bandwidth [MHz]: up to 200 Equivalent input noise [pA/√Hz]: 0.051-220* Rise/fall time (10% - 90%) [nS]: 1.8-1.6µs* Maximum source capacitance [pF]: up to 108* *dependant on bandwidth/noise setting	

Scientifica - Galvo only	Gain[V/A]: 105 Bandwidth [MHz]: 1.25
Minimum workstation specification	 Sciscan GUI Monitor requirements: Full HD 24" OS: Windows 7 64bit CPU: (Intel Xeon type i.e. E5-1650 v3) Six Cores, base frequency 3.5GHz max turbo frequency 3.8GHz RAM Memory: DDR 4 - 32GB Graphics: GPU memory GDDR6 3 2GB (i.e. NVIDIA® Quadro® K4000620 or better) Required PCI/PCIe Bus Slots: 1x Full Length (30") PCI slot (Galvo DAQ card), 2x PCIe (secondary DAQ card and resonant DAQ card)
	Multiphoton Detection Unit (MDU)
Collection optics	Collection lens diameter: 30 mm* Maximum collection angle: ~±5° assuming 10mm objective back aperture and ~±3.5° (optical) assuming a 20 mm objective back aperture* *see J.P. Zinter et. al. OPT EXPRESS (19), 16 15348, 2011.
Dichroic beam splitter	Dimensions: 30x40x1.1 mm Average reflectivity: ~ 98% 350 nm to 685 nm @45° AOI Average transmission: ~ 93% 715 nm to 1600 nm @45° AOI Surface flatness: λ/4 @ 633 nm
Photomultiplier tubes	Ultra bialkali (R9880U-210) Photocathode size: 8mm diameter Spectral response: 230 to 700 nm Quantum efficiency @400 nm: ~40% Multialkali (R9880U-20) Photocathode size: 8mm diameter Spectral response: 230 to 920 nm Quantum efficiency @630 nm: ~16% GaAsP protected photomultiplier tube (H10770PA-40) Photocathode size: 5mm diameter Spectral response: 300 to 720 nm Quantum efficiency: not specified GaAsP photomultiplier tube with gating function (H11706P-40) Photocathode size: 5mm diameter Spectral response: 300 to 720 nm Quantum efficiency @580 nm: 40% Typical shutter rise/fall time: 230nS Shutter width (FWHM): 1µs to 10mS* Shutter input low level threshold: min 0 V max to +0.4V Shutter Input high level threshold: min 0 V max to +0.4V Shutter remains closed for a maximum period of 10mS.

Technical Specifications

MDU XL		
Collection Optics	 Primary Detection Dichroic: 60 x 40 x 1 Collection lens diameter: 45 mm Maximum collection angle: ~±8° assuming 20mm objective back aperture IR Blocking Filter: 680nm Shortpass 50mm Ø 	
Emission Filters	 Emission filter: 32mm Ø - one for each detector Short wavelength perpendicular to the incident light, longer wavelength on the incident direction Blocking filter: 32mm Ø for stimulation wavelength 	
Photomultiplier Tubes	Two locally mounted detectors of the same type: GaAsP protected photomultiplier tube (H10770PA-40) or GaAsP photomultiplier tube with gating function (H11706P-40) Signal output Connector: SMA Gate input connector (gated GaAsP only): BNC	
Protection Circuitry	 Protected GaASP: Integrated protection circuitry to trip PMT when overload detected Gated GaAsP: Voltage gating functionality to avoid overload situations All systems: Ambient light detector 	
Objective and Nosepiece Compatibility	Objectives: M32, M27, M25, RMS threaded objectives (<20mm back aperture) Nosepieces: Ships with single objective changer with 2 locking sliders Compatible with: WI-SRE3 adapter (RMS only) or standard/DIC objective sliders	
Preamplifier Bandwidth	Options include: 20 MHz (resonant system), 1 MHz (galvo), or variable bandwidth all with low noise linear power supply	
Output Connection	BNC	
PMT Voltage Control	Manual or software control	
PMT Protection	Integrated protection circuitry in H10771P-40 only. For all PMT options, a remote ambient light sensor is used	

ChromoFlex		
Green channel filter	Dimensions: 25mm diameter 1 mm thickness Average Transmission: ~ 97% 502 nm to 546 nm @normal incidence Surface flatness: N/A	
Red channel filter	Dimensions: 25mm diameter 1 mm thickness Average Transmission: ~ 97% 590 nm to 648 nm @normal incidence Surface flatness: N/A	
Collection optics	Collection lens diameter: 45 mm* Maximum angular Collection angle: ~ ±8° assuming 20mm objective back aperture. *see J.P. Zinter et. al. OPT EXPRESS (19), 16 15348, 2011.	
Liquid light guide	Core diameter: 5 mm Transmission range: 280 to 650 nm Temperature range: -5°C to 35°C	
Dichroic beam splitter	665 nm long-pass dichroic, factory-fitted (60 x 40 x 1 mm) Dimensions: 60x40x1.1 mm Average reflectivity: ~ 98% 350 nm to 685 nm @45° AOI Average transmission: ~ 93% 715 nm to 1600 nm @45° AOI Surface flatness: λ/4 @ 633 nm	
IR Blocking Filter	680nm short-pass filter, factory fitted Dimensions: 30mm diameter 2mm thickness Average Transmission: ~ 98% 350 nm to 650 nm @45° AOI Surface flatness: λ/4 @ 633 nm	
Compatible emission filter dimensions	Excitation/emission filter: 25 mm diameter Dichroic beam splitter: 24x36x1 mm	
Tested Ultrafast Laser Oscilators		
Tunable laser oscillators (Ti:S)	Coherent Chameleon family Spectra physics Maitai family	
Fixed wavelength laser oscillators (Yb ³⁺ or Er ³⁺)	ONE FIVE origami 10 Toptica Femto fibre Dichro	
System Optical damage threshold	0.5 J/cm² (1064 nm, 10 ns, 10 Hz, Ø1 mm)	

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