

# HyperScope

Image. Stimulate. Publish.







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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

#### **Dual Scan Head**

The HyperScope has a dual scan head to allow two light paths to be manipulated through the microscope.

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

# 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.

With three mirrors in series you can choose between a galvo, resonant or galvo/resonant arrangement.

#### 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.

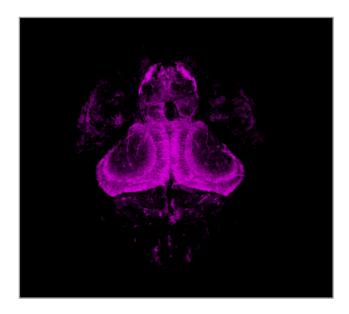




### **Precise scanning**

### The Dual Scan Head

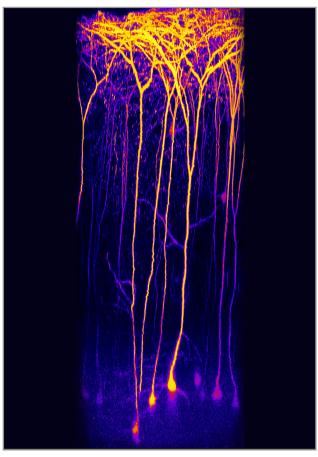
The newly designed dual scan head maintains all of the excellent optical properties of the original Scientifica scanhead but with the option for two light paths: one for imaging and one for photoactivation.



#### 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 (see page 9).



#### 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 trransmission from 700 to 1400 nm infrared light, allowing the use of a wide range of excitation wavelengths, without any change in performance.





### **Collection Optics**

### **Multiphoton Detection Unit**

Scientifica's Multiphoton Detection Unit (MDU) is optimised for the collection of valuable photons by using customised optics, mechanics and noise-reducing electronics.

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



#### **Purpose built optics**

To improve image quality and prevent shading in the corners of the image, a custom designed, extra-large fixed position collection lens is sited very close to the objective back aperture. This allows photon collection at the widest angles.

The detectors move with the objective to keep the distance between the collection lens and objective back aperture fixed. This means the position of the collection optics will always be optimal.

#### **Fast Filter Exchange**

Combining dyes is a common requirement in multiphoton imaging and the exchange of emission filters and dichroics is made easy with the MDU.

A standard filter cube is fitted to a dovetail slider which can easily slide in and out of the MDU preparing the system for the next set of experiments.

#### **Optimised imaging**

The HyperScope scan head has been optimised to work with a range of low magnification, high numerical aperture objectives.

Monitor activity deeper inside your sample with objectives that collect more of the scattered photons generated when imaging at greater depths.

Commonly used objectives include Nikon 16X and 25X, Olympus 20X and 25X

#### **Capture more photons**

The MDU can be fitted with two photomultiplier tubes (PMTs) for photon collection from two channels at the same time.

If you are also utilising a condenser and substage optics, as in the in vitro setup of the HyperScope, then a substage MDU can be added to collect more photons and image more dyes.

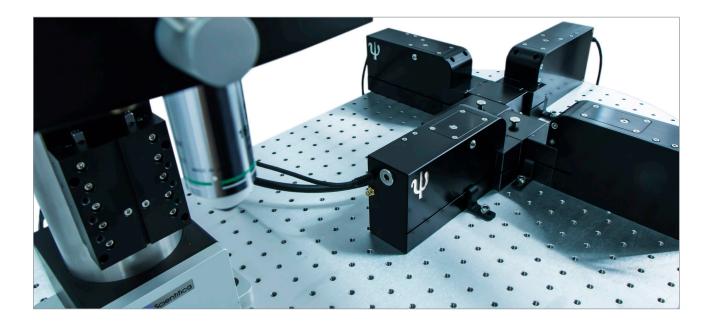


### **Collection Optics**

### **ChromoFlex**

Simultaneously image up to four different colour dyes from in vivo samples with the high sensitivity of GaAsP photomultiplier tubes.

Excellent for applications where the collection of more than two wavelengths of light is necessary and substage light collection isn't possible.



#### Superior light collection efficiency

Large collection optics enable more efficient light collection from the objective back aperture, capturing more of the available photons.

These optics, coupled with the liquid light guide, increase overall efficiency by up to 10% compared to our original multiphoton detection unit, for better examination of weak fluorescent signals.

#### **Liquid Light Guide technology**

The liquid light guide (LLG) acts as a single optical transmission medium, similar to a single silica fibre. However, the much larger diameter of the LLG enables a more efficient delivery of the fluorescent signal.

Additionally, the transmission efficiency is not limited by the dead space between bundles of silica fibres (packing losses).

#### As always, it's modular

Choose between a 2, 3 or 4 PMT system depending on your budget and experimental requirements.

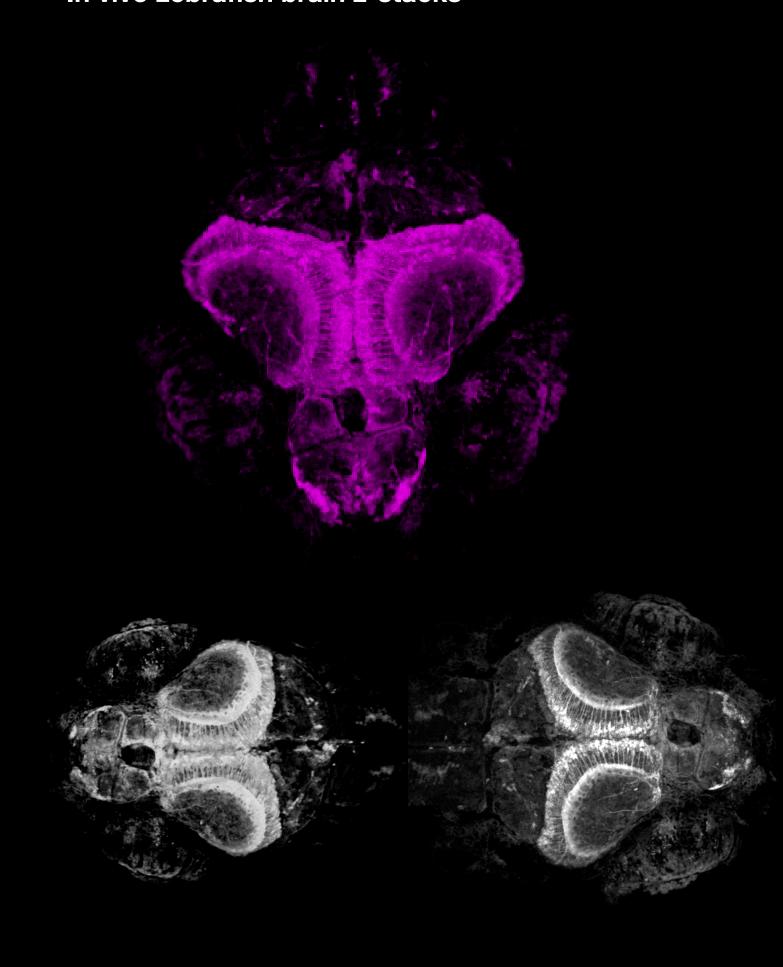
Upgrade when necessary for more complex studies.

#### Put it anywhere

Place the ChromoFlex remotely on your antivibration table or another nearby surface, using the two metre LLG.

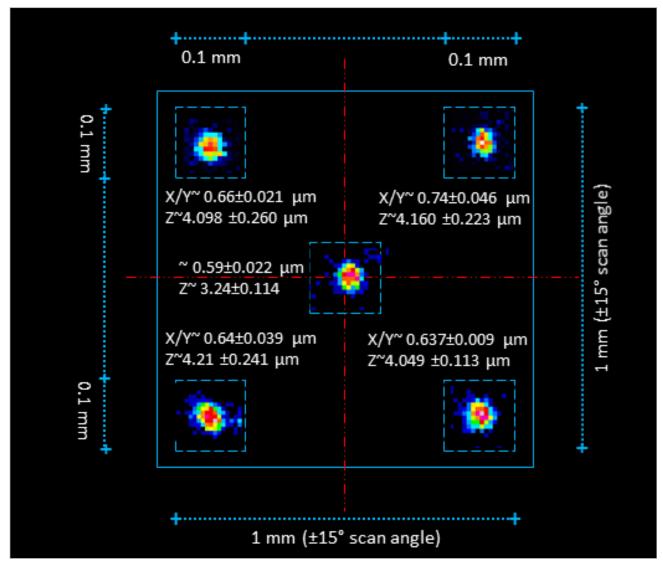
The new space created above the objective lens allows for larger collection lenses and dichroic mirrors for more efficient light collection.

# In vivo zebrafish brain z-stacks



### **Performance Measurements**

### As tested at Scientifica



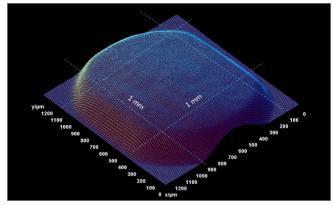
PSF measurements taken at the centre and four corners of the field of view

#### **Point Spread Function (PSF)**

The measurements shown above were taken using a Nikon 16X 0.8 NA 3 mm WD objective at 850 nm. They were performed by imaging 0.2 µm polystyrene fluorescent beads mounted on the surface of a coverslip. The data show 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).



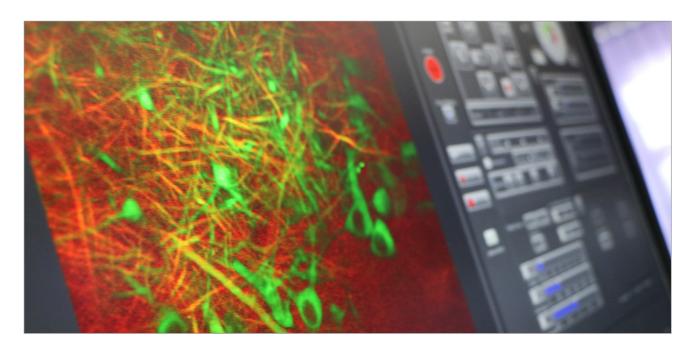
Field flatness mapping with a homogenous fluorescent sample

### **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.



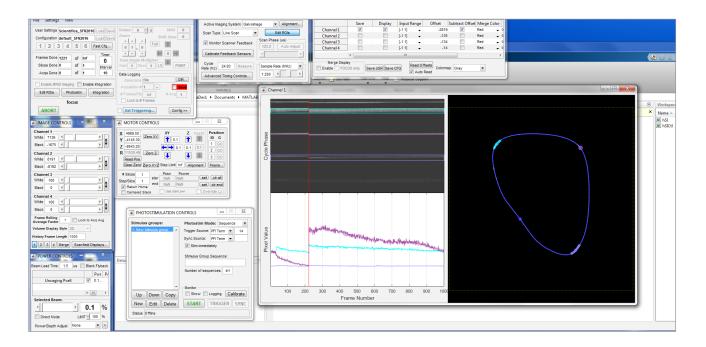


### **Software**

### **ScanImage**

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

The HyperScope is compatible with the latest version of Scanlmage 2016. It also works with Scanlmage 5.



#### **Software functions**

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

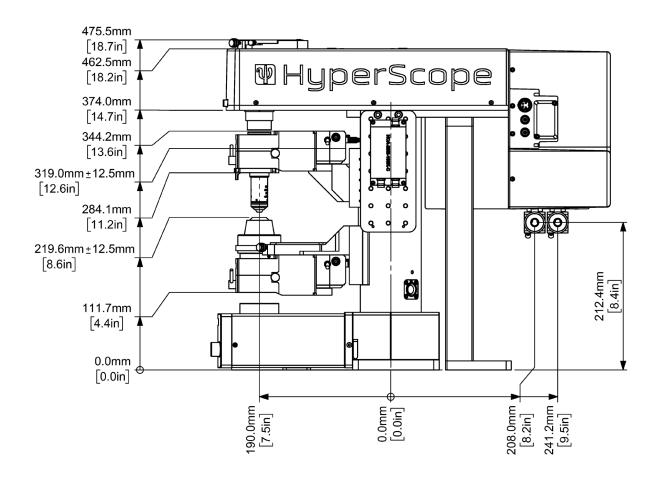
Scanlmage 5 can be downloaded for free and is open-source (written in MatLab) to allow you to develop custom functionalities necesary for your experiments.

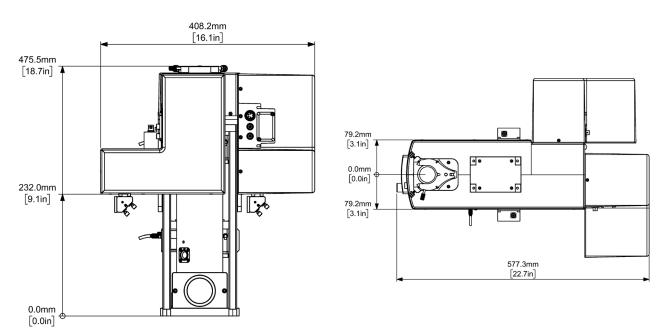
Scanlmage 2016 is a premium supported software package with advanced and user-selected features built on top of Scanlmage 5.

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

For more information visit vidriotechnologies.com and scanimage.org.

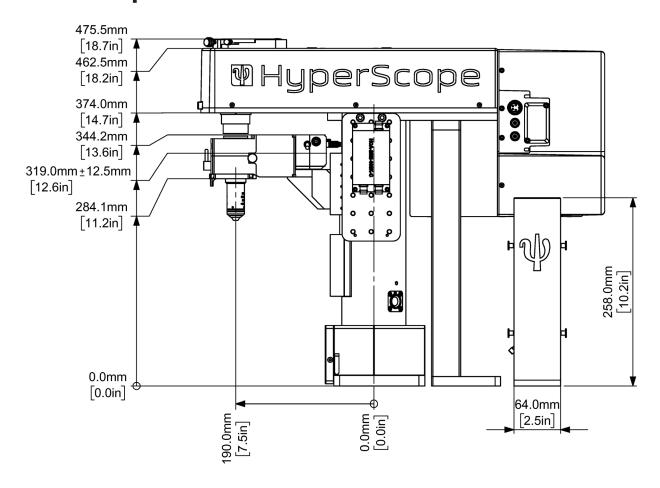
# In vitro setup

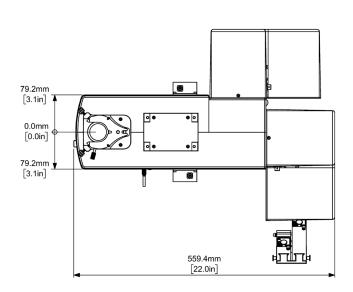


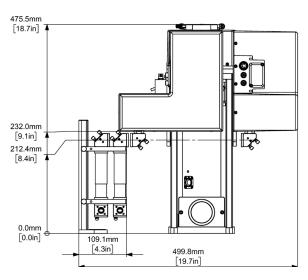




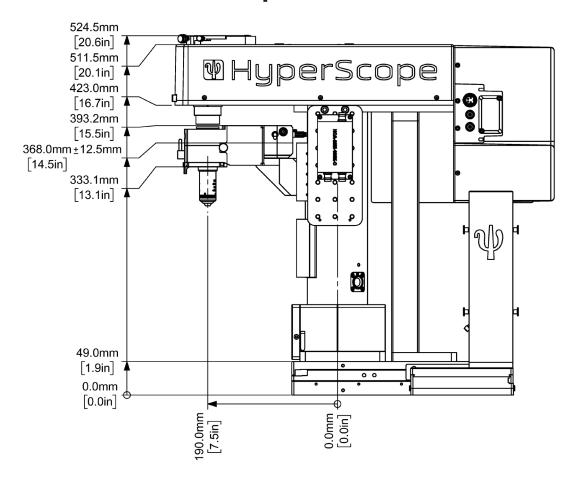
### In vivo setup

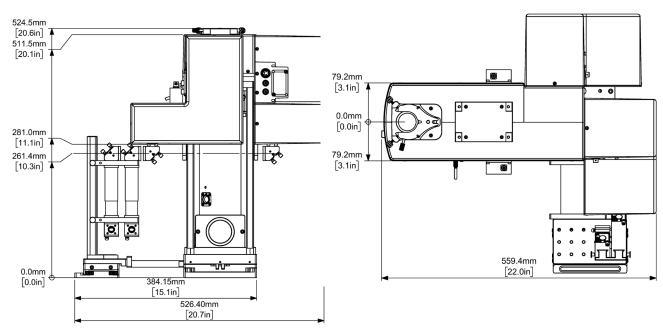






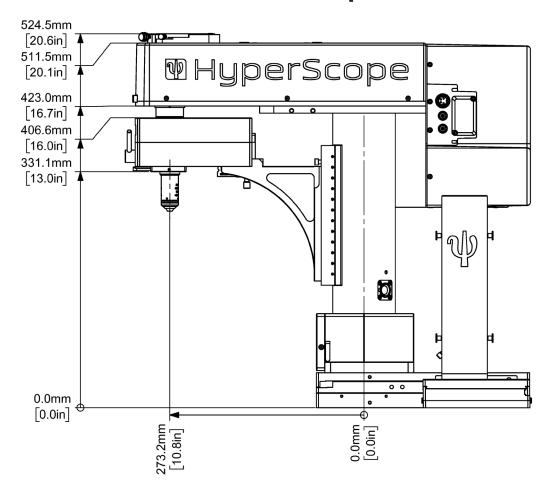
# Moveable in vivo setup

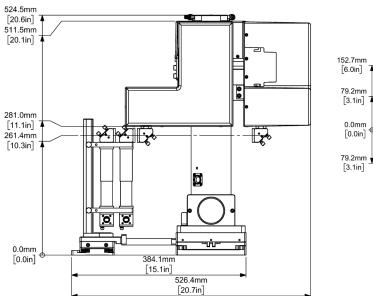


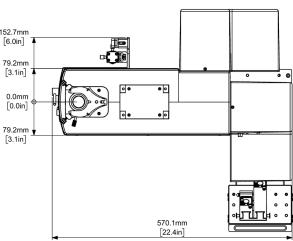




# Extended moveable in vivo setup







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: λ/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: $\sim 0.5 \text{ V}$ Maximum scan angle: $\pm 20^\circ$ Small angle step response: $\sim 130 \mu s$	
Resonant driver	Cambridge Technologies CRS (711-80159) Angle control: External analogue reference 0 to 5V (zoom function) Max scan angle: $\sim \pm 13^{\circ}$ 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	



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~ $0.59\pm0.022$ $\mu m$ and Z~ $3.24\pm0.114$ $\mu m$ Measured edge field (off-axis) resolution @ 860nm*: Average X/Y~ $0.67\pm0.028$ $\mu m$ and Z~ $4.12\pm0.210$ $\mu m$ *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 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 (Galva DAQ card), 2x PCIe (secondary DAQ card and resonant DAQ card)	
Multiphoton Detection Unit		
Collection optics	Collection lens diameter: 30 mm*  Maximum collection angle: ~±5° assuming 20mm 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 + 2.0 V to Vcc (i.e. +14.5 to +15.5 V) *The shutter remains closed for a maximum period of 10mS.	



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: \(\lambda/4\) @ 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 <sup>3+</sup> or Er <sup>3+</sup> )	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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