

Spirit-NOPA-VISIR

Optimized for 3P Imaging



The new Spirit-NOPA®-VISIR, pumped with a Spirit® 1030-70/100 industrial femtosecond laser, is Spectra-Physics' leading platform for three-photon (3P) imaging, enabling breakthrough applications in neuroscience. 3P excitation fluorescence microscopy allows imaging at unprecedented resolution and tissue depth. Utilizing the unique capability of this Spectra-Physics' imaging source for 3P, very high acquisition rates are possible. While 3P requires higher peak powers compared to two-photon (2P) excitation, it yields better optical confinement and signal-to-background ratio in deep tissue imaging, leading to improved image resolution and contrast. Spectra-Physics' 3P imaging platform enables fast image acquisition due to the very high repetition rates which are available from the source. This enables viewing of dynamic processes, such as calcium transients of GCaMP6s-labeled neurons in-vivo to record spontaneous activity on populations of neurons within the intact mouse brain [1]. Additionally, imaging through the intact skull within the transmission windows centered at approximately 1300 nm and 1700 nm is made possible [2]. 3P excitation of GFP- and RFP-like markers has been shown to be most successful at 1300 and 1700 nm, respectively.

Spirit-NOPA-VISIR Advantage

- High average and peak power for 3P imaging up to 4.3 MHz repetition rate
 - Overcome losses in microscope optics and scattering material for high resolution and high depth imaging
 - High signal-to-background ratio deep inside tissue for improved image contrast
- Fast image acquisition rate for viewing dynamic processes in-vivo
- High flexibility based on Spirit 1030-70/100 platform
 - Multi-user facilities: large performance headroom for future applications
 - Pumping several NOPAs /OPAs at high power levels: Spirit-NOPA-VISIR (3P) and Spirit-OPA-30 (2P light sheet, photostimulation of neurons, spectroscopy)
 - Extra IR average power from Spirit: available for optogenetics photo-activation and other uses
 - Repetition rates up to 30 MHz from Spirit laser
- Computer controlled and user-friendly operation
 - Pulse picking up to 1 MHz: Flexibility to work at low and high repetition rates
 - Integrated spectrometer in Spirit-NOPA-VISIR
- Dual pulse length option available for applications in optogenetics



Applications

- Three-photon imaging
- Optogenetics
- Light sheet microscopy
- Spectroscopy

Spirit-NOPA-VISIR

For standard imaging techniques, 1300 and 1700 nm wavelengths have to date been uncommon, and microscope transmission is typically not optimized at these wavelengths. Spectra-Physics' Spirit-NOPA-VISIR pumped with up to 70 W provides sufficient peak power, even at high repetition rates, to compensate for losses in the microscope optics and other scattering effects in the sample, thereby enabling researchers to fully reap the benefits of 3P imaging. It was recently shown that small features such as dendritic spines could be imaged at a depth of 120 μm with outstanding levels of resolution through a thinned mouse skull using 3P imaging, showing an improved signal-to-background ratio in direct comparison to a conventional 2P setup (Fig. 1). Deep tissue, high resolution imaging down to the hippocampus in a YFP-H tg mouse was also demonstrated at a depth exceeding 1 mm, and compared to a conventional 2P setup, deeper imaging was achieved (Fig. 2). Using the third harmonic generation signal (THG), imaging of small blood vessels at a depth of 600 μm inside a mouse brain (layer V) was demonstrated with high contrast and image resolution (Fig. 3).

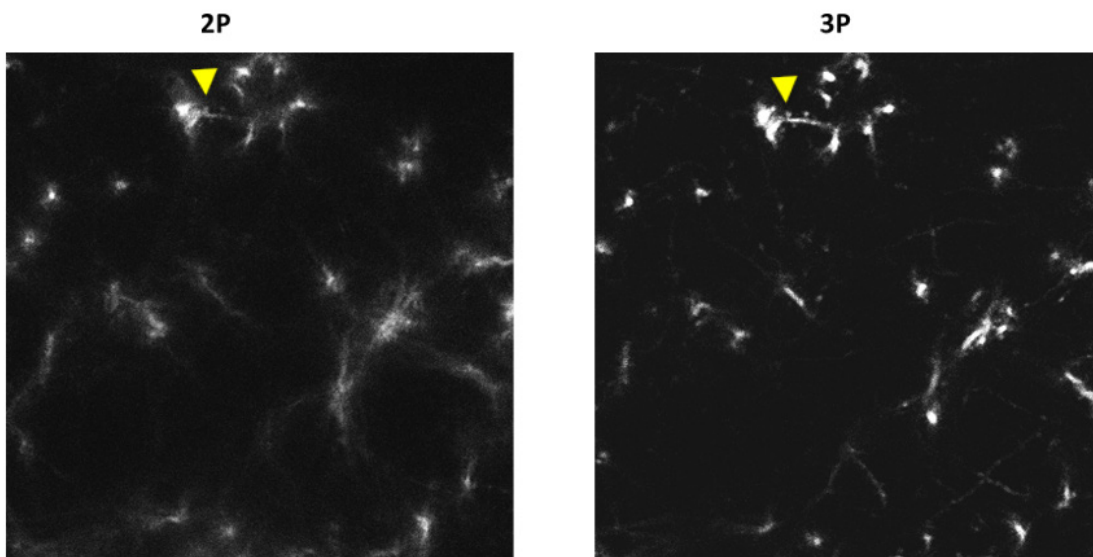


Figure 1

Dendritic spines 120 μm deep in cortex imaged through a thinned skull. 3P imaging was performed at 2 MHz using a Spirit 1030-70 and Spirit-NOPA-VISIR (right) in comparison to a conventional 2P setup (left) (Image courtesy M. Fuhrmann, DZNE, Bonn).

Spectra-Physics' Spirit-NOPA-VISIR in combination with a Spirit 1030-70 or Spirit 1030-100 enables unprecedented performance for 3P imaging. At a repetition rate of 2 MHz and a pump power of 70 W, a typical average power of >2.5 W at 1300 nm and a resulting peak power up to 15 MW is achieved for the compressed output (compressors are optionally available). This outstanding performance headroom compensates for typical losses in microscopes to maximize fluorescence intensity and imaging depth. The Spirit 1030-70 or Spirit 1030-100 laser can also be used to pump multiple OPAs at high power levels and varying repetition rate requirements. This power capability can be used for combined multiphoton imaging and photo-activation applications in multi-user facilities.

Spirit-NOPA-VISIR

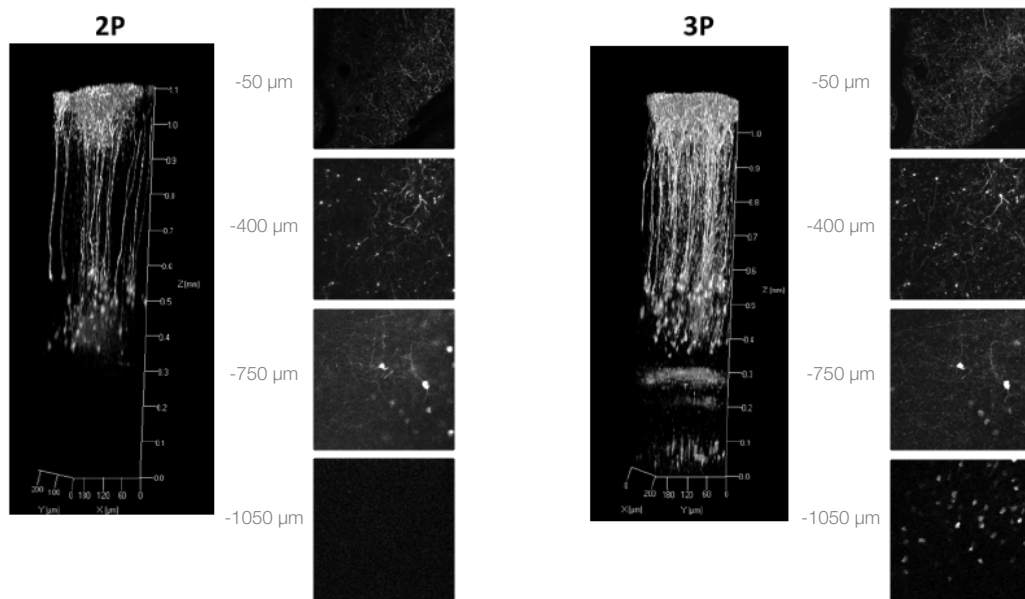


Figure 2

Cortex to Hippocampus in YFP-H tg mouse. 3P imaging was performed at 2 MHz using a Spirit 1030-70 and Spirit-NOPA-VISIR (right) in comparison to a conventional 2P setup (left) (Image courtesy M. Fuhrmann, DZNE, Bonn).

The Spirit-NOPA-VISIR is fully computer controlled to enable user-friendly operation. To cover 2P excitation imaging and applications in optogenetics as well, the Spirit-NOPA-VISIR can be configured with a dual pulse length option. With this option, narrow bandwidth, uncompressed laser pulses are available over an extended tuning range of the Spirit-NOPA-VISIR, closing the gap between signal and idler. The dual pulse length option is ideally suited for photostimulation of neurons in optogenetics and excitation of fluorescent dyes in the 900 nm to 1200 nm spectral range.

[1] D.G. Ouzounov, T. Wang, M. Wang, D.D. Feng, N.G. Horton, J.C. Cruz-Hernández, Y.-T. Cheng, J. Reimer, A. S. Tolias, N. Nishimura, C. Xu, "In vivo three-photon imaging of activity of GCaMP6-labeled neurons deep in intact mouse brain" *Nat. Methods* 14, 388–390 (2017).

[2] T. Wang, D.G. Ouzounov, C. Wu, N.G. Horton, B. Zhang, C.-H. Wu, Y. Zhang, M.J. Schnitzer, C. Xu "Three-photon imaging of mouse brain structure and function through the intact skull" *Nat. Methods* 15, 789–792 (2018).

Spirit-NOPA-VISIR

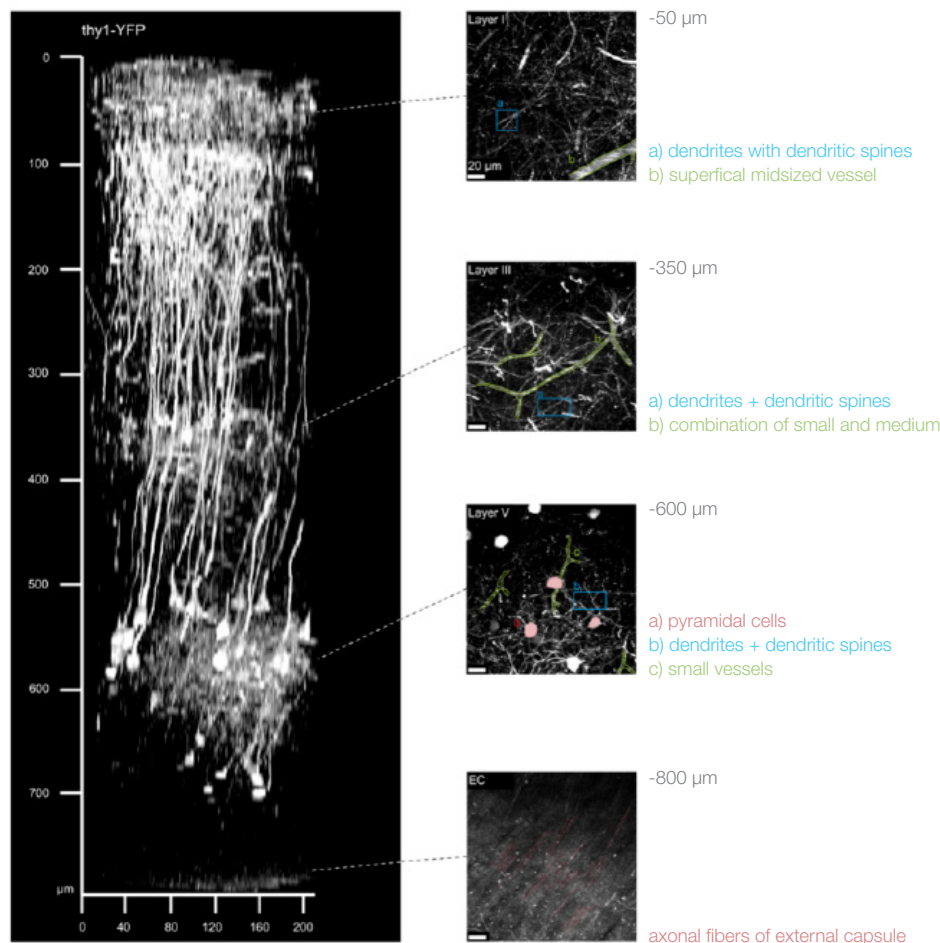
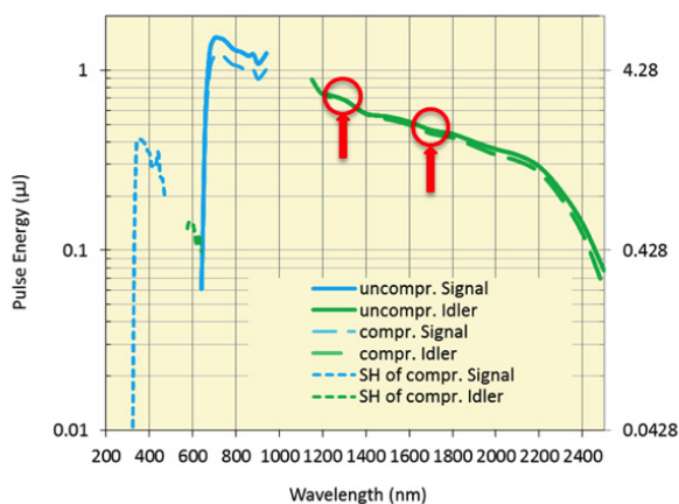
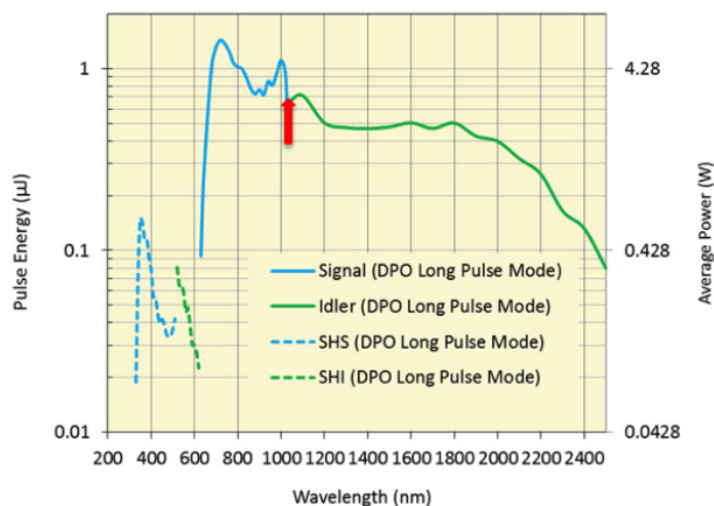


Figure 3
Third harmonic generation (THG) imaging of blood vessels. Imaging performed using a Spirit 1030-70 and Spirit-NOPA-VISIR. (Image courtesy M. Fuhrmann, DZNE, Bonn).

Typical performance: Spirit-NOPA-VISIR pumped by Spirit 1030-70 with 70 W at 4.3 MHz (including optional compressors and SHS/SHI)¹



Typical performance: Spirit-NOPA-VISIR pumped by Spirit 1030-70 with 70 W at 4.3 MHz (including Dual Pulse Length Option and SHS/SHI)¹



¹. Typically measured performance; not a guaranteed or warranted specification.

Spirit-NOPA-VISIR Typical Performance

Spirit-NOPA-VISIR Pump Parameters	1300 nm Compressed Output, typical	1700 nm Compressed Output, typical
70 W at 4.3 ± 0.3 MHz	>2.5 W	>1 W
70 W at 3 MHz	>2.5 W	>1 W
70 W at 2 MHz	>2.5 W	>1.5 W
70 W at 1 MHz	>2.5 W	>1.5 W

Spirit-NOPA-VISIR Specifications¹

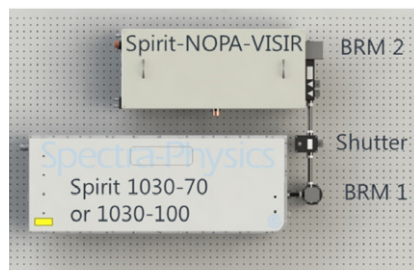
Spirit-NOPA-VISIR	
Output Specifications	
Tuning Range	650–900 nm (signal) 1200–2500 nm (idler)
Conversion Efficiency	>10% at peak, when pumped at >20 μ J (uncompressed signal + idler combined)
Pulse Width ²	<350 fs uncompressed ² <70 fs at 650–900 nm with signal compressor; <100 fs at 1200–2000 nm with idler compressor
Compressor Transmission	Signal prism compressor: >65% at 650–900 nm Idler bulk compressor: >80% at 1200–2000 nm
Optional SHG Output	
Tuning Range	325–450 nm (SH of signal) 600–700 nm (SH of idler)
Pulse Energy	>10% of signal/idler at peak
Optional Dual Pulse Length Configuration	
Tuning Range in Long Pulse Mode	650–1025 nm (signal) 1040–2500 nm (idler) Uncompressed output
Pump Requirements³ from Spirit 1030-70 or Spirit 1030-100	
Repetition Rate	Single shot to 4.3 ± 0.3 MHz
Wavelength	1030 nm
Pulse Energy ⁴	up to 100 μ J
Average Power	up to 70 W

1. Due to our continuous product improvement program, specifications are subject to change without notice.

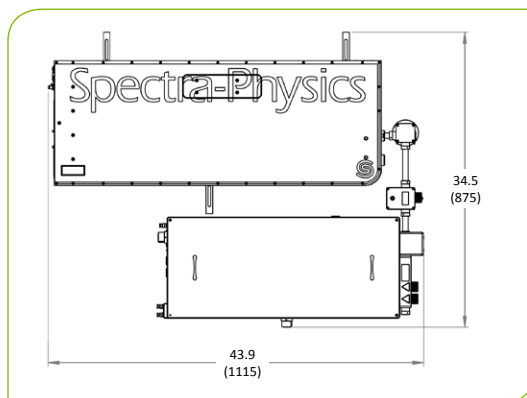
2. Compressors for signal and/or idler are options.

3. Spirit-NOPA-VISIR pump parameters are determined at the time of order and are set at the factory.

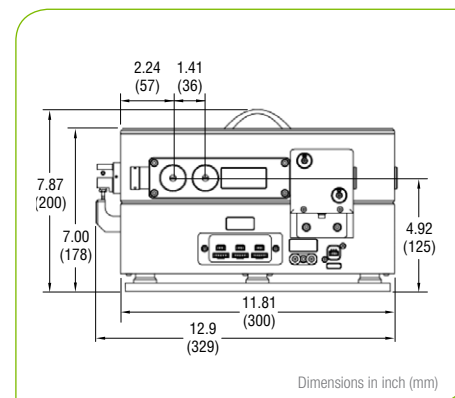
4. Spirit-NOPA-VISIR is optimized for one pump pulse energy only.



Typical Table Layout: Spirit 1030-70/100 and Spirit-NOPA-VISIR



Spirit 1030-70/100 pumping Spirit-NOPA-VISIR
Dimensions (without Signal compressor, top view)



Spirit-NOPA-VISIR Module Dimensions
(front view)