

TIBERIUS- August 22, 2024

Item TIBERIUS was discontinued on August 22, 2024. For informational purposes, this is a copy of the website content at that time and is valid only for the stated product.

TI:SAPPHIRE FEMTOSECOND LASER FOR TWO-PHOTON MICROSCOPY

- **Wide Tuning Range: 720 1060 nm**
- □ Fast Tuning: Up to 4000 nm/s
- □ Half the Footprint of Competing Models

Ti:Sapphire Femtosecond Laser, 720 - 1060 nm Tuning Range

OVERVIEW

Features

- Wide Tuning Range of 720 nm to 1060 nm
- Industry-Leading Tuning Speed: Up to 4000 nm/s
- High Output Power: > 2.3 W at 800 nm
- Ultrafast 140 fs Pulses
- Compact Footprint Uses Half the Table Space of Competing Lasers
- Integrated Spectrometer for Real-Time Diagnostics
- Pure Air Circulator Unit Included to Purge Laser Cavity for Smooth Tuning Through Water Absorption Lines

Typical Applications

- Multi-Channel Fluorescence Images of 3D Volumes
- Photostimulation and Uncaging
- Label-Free Imaging via Multiphoton Autofluorescence and SHG

Thorlabs' Tiberius® Ti:Sapphire Laser provides 140 fs pulses over a wide tuning range with industry-leading tuning speeds of up to 4000 nm/s. Collaboratively designed and manufactured in-house with Thorlabs' multiphoton imaging specialists, this femtosecond laser offers hands-free operation that easily meets the stringent demands of the non-

Matt Kirchner Site Manager Laser Divison - Colorado **Feedback? Questions? Need a Quote?**

linear optical imaging community. See the *Design and Manufacturing* tab for more information about how the Tiberius Ti:Sapphire Laser leverages Thorlabs' extensive expertise in optical design and precision manufacturing.

Click to Enlarge Included GUI for Control of the Tiberius

An ideal choice for two-photon microscopy, the Ti:sapphire laser cavity offers an average power greater than 2.3 W at 800 nm and a wavelength that is tunable from 720 nm to 1060 nm, allowing the user to target specific compounds for two-photon fluorescence imaging and photostimulation / uncaging. Tiberius' industry-leading tuning speed is demonstrated on the *Fast Tuning* tab, and a tuning curve is shown on the *Specs* tab.

This femtosecond laser emits pulses that are 140 fs in duration with a relatively narrow spectral bandwidth. This spectral design reduces the effect of pulse broadening caused by Pockels cells and other dispersive elements while still providing high peak intensity for two-photon excitation.

Since tabletop space is often at a premium, the Tiberius laser has been designed with a vertical cavity construction that minimizes the footprint on the optical table. At 746.3 mm x 190.0 mm (29.38" x 7.48"), the Tiberius' footprint is about half that of competing designs, preserving valuable workspace for the rest of your experimental setup. Each laser also comes with a laser controller, pump laser controller, chiller, and pure air circulator unit.

For laser operation, the Tiberius Ti:Sapphire Laser includes an intuitive GUI. User-programmable buttons provide single-click access to commonly used excitation wavelengths. In addition, the Tiberius is integrated with ThorImage®LS, enabling seamless and synchronized control for photoactivation experiments and live highspeed imaging.

Click to Enlarge

The Tiberius being used with our Bergamo[®] II Series Microscopes for twophoton imaging. The system shown here features Tiberius as the primary laser in co-registered dual beam paths for simultaneous photostimulation and high-speed imaging.

Two-Photon Imaging

Structural Insight

Multiphoton microscopy takes advantage of the NIR transparency windows in living tissue and highly localized excitation to generate multichannel fluorescence images of 3D volumes. Compared to visible light, which is used in conventional widefield

Click to Enlarge The Tiberius fs Laser Source Used to Resolve Morphological Features of a Fruit Fly's Eye

microscopy and confocal microscopy, NIR light offers significantly reduced scatter and absorption by biological compounds, resulting in deeper images below the surface.

The image of a fruit fly eye to the right demonstrates the Tiberius' ability to resolve morphological features. This two-channel image contains GFPlabeled photoreceptors and unlabeled regions that exhibit multiphoton autofluorescence. The excitation wavelength was 770 nm and a 25X, NA 1.05 Olympus objective was used.

FAST TUNING

Improved Image Contrast with Fast Tuning

With an industry-leading tuning speed of up to 4000 nm/s, the Tiberius® Ti:Sapphire Laser is ideal for fast sequential imaging. The Tiberius' fast-tuning capability provides high-contrast images when used in multi-color, multiphoton microscopy applications.

Quickly switching between two optimized excitation wavelengths has several benefits over single-wavelength excitation. These include the much higher image contrast provided by fast switching and being able to maximize fluorescence at lower excitation powers, which reduces the risk of photobleaching.

Figures 1 and 2 illustrate the increased contrast enabled by imaging multiple fluorophores in a sample using fast sequential imaging.[†] The sample is a 25 µm thick sagittal section of an adult rat brain. The red channel corresponds to fluorescence from chick anti-neurofilament that is optimally excited at 835 nm, while the green channel corresponds to fluorescence from mouse anti-GFAP that is optimally excited at 750 nm. Figure 1 shows fluorescence from single-wavelength excitation at

788 nm, which sub-optimally excites the two tags simultaneously. Figure 2 is a composite image of the fluorescence from a two-color excitation imaging sequence at 7 fps by fast tuning between 750 nm and 835 nm, which excites both tags optimally.

The video in Figure 3 shows the fast switching between the red and green fluorescence in both real time and at 1/16th the imaging rate, which makes it easier to see the details of each. The two-channel set was collected at an imaging rate of 7 fps with a resolution of 512 x 512 pixels. The Tiberius Ti:Sapphire Laser's fast tuning functionality integrates seamlessly into ThorImage®LS software, enabling synchronized control for photoactivation experiments and live high-speed imaging on millisecond timescales using the same laser.

†This immunofluorescence sample was prepared by Lynne Holtzclaw of the NICHD Microscopy and Imaging Core Facility, a part of the National Institutes of Health (NIH) in Bethesda, MD.

Click to Enlarge **Figure 2.** Fast Switching between the optimal excitation wavelengths of 750 nm and 835 nm provides the high contrast seen in this composite image. The two-channel set was collected at an imaging rate of 7 fps.[†]

Click to Enlarge **Figure 1.** The above image was acquired using singlewavelength excitation at 788 nm, while the optimum excitation wavelengths for the two tags are 750 nm and 850 nm.†

SPECS

Figure 3. This video shows the real-time flashing between red and green fluorescence excited by the Tiberius Ti:Sapphire Laser's high-speed wavelength switching. Both channels were collected at an imaging rate of 7 fps with a resolution of 512 x 512 pixels. If you experience adverse effects from visual stimuli including flashing lights, please watch this version played at $1/16^{th}$ the imaging rate as an alternative.[†]

Measurement Bandwidth: 10 Hz - 1 MHz a.

DESIGN AND MANUFACTURING

In-House Expertise in Design and Manufacturing

The Tiberius[®] Ti:Sapphire Femtosecond Laser is designed and manufactured entirely in-house, leveraging our multi-disciplinary team of design engineers and the substantial infrastructure of a vertically integrated company. Thorlabs' Laser Division tightly controls every aspect of the manufacturing, assembly, and testing process of the Tiberius in order to guarantee the laser's stability and reliability.

The ti:sapphire laser's design represents the culmination of complex theoretical cavity simulations combined with "old-fashioned" prototyping. A sound understanding of the intracavity laser dynamics proved fundamental to optimizing the laser for the specific needs of our nonlinear imaging customers.

Precision Optomechanics Manufacturing

The Tiberius benefits from Thorlabs' 25+ years of experience in manufacturing precision photonics components and assemblies. For example, it makes extensive use of the high-performance,

ultrastable Polaris® designs that the company has developed for custom OEM needs and industrialgrade applications. These expert designs minimize thermally induced drift and help ensure stable longterm alignment.

Manufacturing at Thorlabs' Headquarters

Click to Enlarge Machine Shop

Our high degree of vertical integration lowers costs for our customers and ensures that every aspect of the laser performs as intended, delivering superior value and return on investment.

Optimized Ultrafast Laser Optics

To maximize the Tiberius' optical performance, it was critical to optimize the laser cavity geometry and optics

Click to Enlarge Ion Beam Sputtering (IBS) Chamber for Ultrafast Optics

together as a single unit. The optical coatings were therefore designed by Thorlabs and are precisely tuned for our cavity's proprietary design, enabling the long-term stability and broad tuning range that multiphoton microscopy

requires.

To manufacture these high-performance coatings, we selected ion beam sputtering (IBS), which provides the most precise layer control and the most dense coatings among all coating methods. These characteristics result in coatings with high damage thresholds, minimal dependence on environmental factors, and excellent consistency from run to run. Thorlabs operates a number of IBS machines to produce these critical components for the Tiberius Ti:Sapphire Laser.

Hide Laser Safety

LASER SAFETY

Laser Safety and Classification

Safe practices and proper usage of safety equipment should be taken into consideration when operating lasers. The eye is susceptible to injury, even from very low levels of laser light. Thorlabs offers a range of laser safety accessories that can be used to reduce the risk of accidents or injuries. Laser emission in the visible and near infrared spectral ranges has the greatest potential for retinal injury, as the cornea and lens are transparent to those wavelengths, and the lens can focus the laser energy onto the retina.

Safe Practices and Light Safety Accessories

- Laser safety eyewear must be worn whenever working with Class 3 or 4 lasers.
- Regardless of laser class, Thorlabs recommends the use of laser safety eyewear whenever working with laser beams with non-negligible powers, since metallic tools such as screwdrivers can accidentally redirect a beam.
- Laser goggles designed for specific wavelengths should be clearly available near laser setups to protect the wearer from unintentional laser reflections.
- Goggles are marked with the wavelength range over which protection is afforded and the minimum optical density within that range.
- Laser Safety Curtains and Laser Safety Fabric shield other parts of the lab from high energy lasers.
- Blackout Materials can prevent direct or reflected light from leaving the experimental setup area.
- Thorlabs' Enclosure Systems can be used to contain optical setups to isolate or minimize laser hazards.
- A fiber-pigtailed laser should always be turned off before connecting it to or disconnecting it from
- another fiber, especially when the laser is at power levels above 10 mW.
- All beams should be terminated at the edge of the table, and laboratory doors should be closed whenever a laser is in use.
- Do not place laser beams at eye level.
- Carry out experiments on an optical table such that all laser beams travel horizontally.
- Remove unnecessary reflective items such as reflective jewelry (e.g., rings, watches, etc.) while working near the beam path.

- Be aware that lenses and other optical devices may reflect a portion of the incident beam from the front or rear surface.
- Operate a laser at the minimum power necessary for any operation.
- If possible, reduce the output power of a laser during alignment procedures.
- Use beam shutters and filters to reduce the beam power.
- Post appropriate warning signs or labels near laser setups or rooms.
- Use a laser sign with a lightbox if operating Class 3R or 4 lasers (i.e., lasers requiring the use of a safety interlock).
- Do not use Laser Viewing Cards in place of a proper Beam Trap.

Laser Classification

Lasers are categorized into different classes according to their ability to cause eye and other damage. The International Electrotechnical Commission (IEC) is a global organization that prepares and publishes international standards for all electrical, electronic, and related technologies. The IEC document 60825-1 outlines the safety of laser products. A description of each class of laser is given below:

Hide Pulse Calculations

PULSE CALCULATIONS

Pulsed Laser Emission: Power and Energy Calculations

Determining whether emission from a pulsed laser is compatible with a device or application can require referencing parameters that are not supplied by the laser's manufacturer. When this is the case, the necessary parameters can typically be calculated from the available information. Calculating peak pulse power, average power, pulse energy, and related parameters can be necessary to achieve desired outcomes including:

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• Protecting biological samples from harm.

- Measuring the pulsed laser emission without damaging photodetectors and other sensors.
- Exciting fluorescence and non-linear effects in materials.

Pulsed laser radiation parameters are illustrated in Figure 1 and described in the table. For quick reference, a list of equations are provided below. The document available for download provides this information, as well as an introduction to pulsed laser emission, an overview of relationships among the different parameters, and guidance for applying the calculations.

Equations:

Period and *repetition rate* are reciprocal: $\frac{d}{dx} = \frac{1}{\hbar x}$ and $f_{rep} = \frac{1}{\hbar x}$

Pulse energy **calculated from** *average power***:**

Average power **calculated from** *pulse energy***:**

Peak pulse power **estimated from** *pulse energy***:**

Peak power **and** *average power* **calculated from each other:**

$$
P_{peak} = \frac{P_{avg}}{f_{rep} \cdot \tau} = \frac{P_{avg} \cdot \Delta t}{\tau} \quad \text{and} \quad P_{avg} = P_{post} \cdot f_{rep} \cdot \tau = \frac{P_{post} \cdot \tau}{\Delta t}
$$

Peak power **calculated from** *average power* **and** *duty cycle****:**

$$
P_{peak} = \frac{P_{avg}}{\tau/\Delta t} = \frac{P_{avg}}{duty\ cycle}
$$

*Duty cycle $(\tau / \Delta t)$ is the fraction of time during which there is laser pulse emission.

 $E = \frac{P_{avg}}{f_{rep}} = P_{avg} \cdot \Delta t$

 $P_{avg} = \frac{E}{\Delta t} = E \cdot f_{rep}$

 $P_{peak} \approx \frac{E}{\tau}$

Figure 1: Parameters used to describe pulsed laser emission are indicated in the plot (above) and described in the table (below). **Pulse energy** (**E**) is the shaded area under the pulse curve. Pulse energy is, equivalently, the area of the diagonally hashed region.

Example Calculation:

Is it safe to use a detector with a specified maximum peak optical input power of **75 mW** to measure the following pulsed laser emission?

- Average Power: 1 mW
- Repetition Rate: 85 MHz
- Pulse Width: 10 fs

The energy per pulse:

$$
E=\frac{P_{avg}}{f_{rep}}=\frac{1\;mW}{85\;MHz}=\frac{1\;x\;10^{-3}\;W}{85\;x\;10^{6}Hz}=1.18\;x\;10^{-11}J=11.8\;pJ
$$

seems low, but the peak pulse power is:

$$
P_{peak} = \frac{P_{avg}}{f_{rep} \cdot \tau} = \frac{1 \, mW}{85 \, MHz \, \cdot 10 \, fs} = 1.18 \, x \, 10^2 \, W = 1.18 \, kW
$$

It is *not safe* to use the detector to measure this pulsed laser emission, since the peak power of the pulses is >5 orders of magnitude higher than the detector's maximum peak optical input power.

