optogenix

Tapered fibers for Neuroscience

ThetaStation

User manual

Table of Contents

Chapter 1 Warning symbol definitions

Below is a list of warning symbols you may encounter in this manual or on your device.

NOTICE
Information considered important but not hazard related

CAUTION
Instructions for use that, if disregarded, might result in product damage.

WARNING
Instructions for use that, if disregarded, might result in personal injury or death

Caution: risk of electric shock

Warning: laser radiation

Chapter 2 Safety

NOTICE

All statements regarding safety of operation and technical data in this instruction manual will only apply when the unit is operated correctly.

WARNING

Do not operate ThetaStation without reading this user manual first.

WARNING

Do not stare into the laser beam. Avoid direct exposure to the laser beam. Always use protective eyewear equipment and follow safety procedures and regulation applicable in your area when operating laser sources. Consult with your organization's laser safety officer for recommendations.

WARNING

Do not open housing. Do not operate without cover installed.

WARNING

Do not operate in wet or damp conditions.

WARNING

Light source must be turned off before connecting it to ThetaStation.

Chapter 3 Definitions

NA: numerical aperture.
Source patch cord: patch cord connecting the light source to the ThetaStation source port.
Probe patch cord: patch cord connecting the tapered fiber to the ThetaStation probe port.
Input power: optical power at ThetaStation input (measured at the output of the source patch cord connected to light source).
Output power: optical power at the output of the probe patch cord.
Transmission efficiency: ratio between output power and input power.
Working range: range of positions of the micrometer screw gauge within which spatial selective light delivery is effective.

Chapter 4 Description

4.1 Introduction

ThetaStation is an opto-mechanical tool designed to perform spatial selective light delivery with OptogeniX Lambda fibers.[1] An external fiber coupled visible laser light source is needed to operate the ThetaStation.

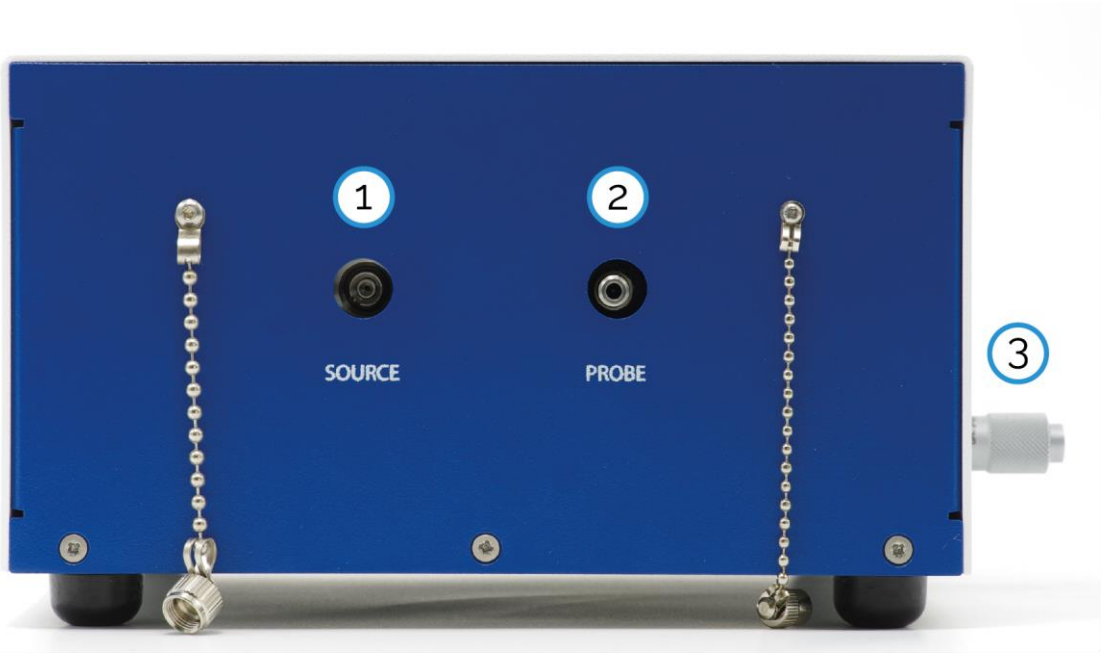


Figure 1. ThetaStation front view with source port (1), probe port (2) and micrometer screw gauge (3).

4.2 Spatial selective light delivery with Lambda fibers

ThetaStation allows to confine and manually scan the light emitted by a Lambda fiber at sub-sites of its active length, as shown in Figure 2. By doing so, sub-portions of the tissue region where the Lambda fiber is implanted can be independently probed: this capability gives access to an additional degree of freedom during optogenetics experiments, which has particular relevance when

[1] https://www.optogenix.com/applications/

readout techniques extending along one axis are used simultaneously with the site selective light delivery (*e.g.* linear microelectrode array).

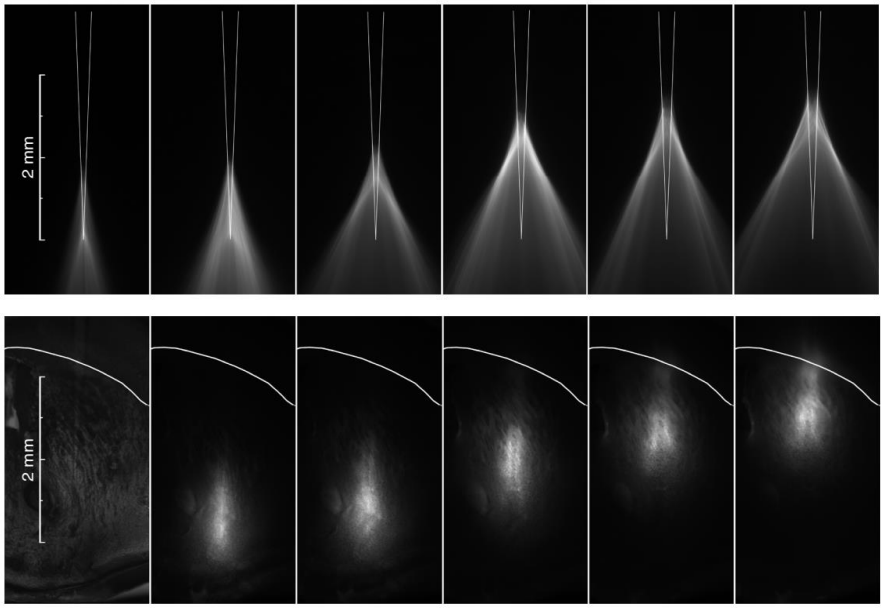


Figure 2. Spatial selective light emission from a Lambda fiber at specific sub-sites within the 2mm active length: in fluorescein solution (top), and in brain tissue (bottom). [2]

4.3 Lambda-plus fibers

Regular Lambda fibers are provided with strict tolerance on the active length, while coarser tolerance is allowed on the taper profile. Although taper profile variability has no practical influence on light emission/collection properties in full taper operation, it does affect the variability of the sub-site length both within (*intra*) the single Lambda fiber and among (*inter*) fibers with the same nominal active length. Lambda-plus fibers are Lambda fibers selected with stricter tolerance on taper profile, and optimized for site-selective light delivery, with constant sub-site length both *intra* and *inter* fibers[3]. The use of Lambda-plus fibers, together with OptogeniX patch cords, is strongly recommended for the ThetaStation.

[2] From F. Pisanello, G. Mandelbaum, *et* al., https://www.nature.com/articles-nn.4591, Nature Neuroscience (2017).
[3] Control on the position of the emission site is guaranteed with a ±100µm tolerance for Lambda-plus fibers with the same nominal active length.

Visit our website for an updated specs list of available Lambda-plus fibers.

4.4 Calibration slide

Calibration slides are tools useful to measure the light emission profiles of tapered fibers, and can be provided with each Lambda-plus fiber order. Calibration slides consist of a Lambda-plus fiber attached on a microscope slide, with the surface of the slide close to the fiber covered with hydrophobic material to help the formation of a fluorophore solution droplet.

Figure 3. Calibration slide.

Chapter 5 Light source

ThetaStation is designed to operate with third-party fiber coupled visible laser light sources.

General recommendations:

- The source patch cord must be a single mode fiber terminated with FC/PC or FC/APC connector. For optimal performances, the single mode fiber should have a mode field diameter in the range 2.5 µm - 3.5 µm.

- Use source wavelengths in the range 400 nm – 700 nm.

Chapter 6 Setup and operation

6.1 Connection Guide

1) Unbox the ThetaStation and remove all packaging material.
2) Place the ThetaStation on a flat horizontal surface.
3) Operate the micrometer screw gauge to reach the 1 mm mark.

WARNING

Light source must be turned off before connecting it to ThetaStation.

4) Connect the source patch cord coupled to the laser to the source port on the ThetaStation front panel.

NOTICE

To ensure a good connection pay attention that the FC connector key of the source patch cord is oriented according to the receptacle slot of the source port.

5) Connect the SMA end of the probe patch cord to the probe port on the ThetaStation front panel.
6) Check the proper functioning of the ThetaStation by following the instructions in the "Getting started" video available on OptogeniX website. Refer to the Troubleshooting section of this guide or contact OptogeniX if issues arise.
7) Connect the ferrule end of the probe patch cord to the implanted fiber stub or to the calibration slide.

NOTICE
Spatial selective light delivery is compatible with the use of rotary joints. An additional patch cord is required.

6.2 Calibration

Due to the unavoidable variation in the assembling process of the ThetaStation and the patch cords, and to account for the actual light source employed, a calibration procedure for each Lambda-plus fiber nominal active length is needed to ensure that the light emitting region is scanned along the tapers with a positioning error as low as possible. Position and size of light emitting sub-regions of Lambda-plus fiber, as well as transmission efficiency, need to be calibrated as a function of the micrometer screw gauge position. A video showing how to perform the calibration routine is available on OptogeniX website.

NOTICE
Sub-regions definition is part of the design of the experiment. As the active sub-region varies continously with the position of the micrometric screw, the number and position of the active sub-regions are defined arbitrary by the end-user during calibration.

NOTICE
The calibration obtained through the following procedure is dependent on the specific ThetaStation, the probe patch cord, the rotary joint and the additional patch cord (if used), the source, the source patch cord, and the Lambda-plus fiber nominal active

length. If at least one of these items changes, the calibration procedure needs to be run again.

1) Measure the input power.
2) Place the ferrule end of the probe patch cord frontally to an optical power sensor.
3) Ensure that the micrometer screw gauge reads 1 mm.
4) Read the output power obtained as a function of the micrometer screw gauge position with a constant step (*e.g.* 1.0 mm) up to the end of the working range (~8.0 mm).
5) Take note of the relation between the output power and the micrometer screw gauge position.
6) Transmission efficiency typically varies in the range 75% - 40% when spanning the working range. If this condition is not met, an adjustment of the output fiber coupling is needed; follow the instruction in Section 6.4 of this guide (Output fiber coupling adjustment).
7) Connect the ferrule end of the probe patch cord to the calibration slide.
8) Place the calibration slide under an epifluorescence microscope equipped with a camera and with an objective giving the system a field of view large enough to frame the full extension of the Lambda-plus fiber active length.
9) Immerse the Lambda-plus fiber in a solution of fluorophore suitable for the wavelength in use and select the appropriate filters on the microscope (*e.g.* PBS:fluorescein solution and FITC filter set for blue light).
10) Enable the emission from the light source and tune the light power output and the camera exposure time to avoid image saturation.
11) Record images of the light emission from the Lambda-plus fiber as a function of the micrometer screw gauge, using the same positions chosen at step 4).

12) With the help of an image analysis software[4], for each collected image take the intensity profile along a line close to the taper edge. The sub-region width can be estimated as the FWHM of the intensity profile. The distance from the taper tip of the FWHM middle point is then conventionally defined as the sub-region position.
13) Take note of the relation between sub-region position and micrometer screw gauge reading.
14) With the help of a pipette remove the fluorophore solution from the calibration slide.
15) Disconnect the ferrule end of the probe patch cord from the calibration slide.

6.3 Operation

Select an emitting sub-portion by setting the micrometric screw at the calibrated position and, for each position, set the light source power to achieve the desired sub-region output power as:

sub-region output power = (input power) × (transmission efficiency) × (F-F joint efficiency)

where *F-F joint efficiency* accounts for the power loss in the ferrule-ferrule joint between the probe patch cord and the Lambda-plus fiber. The exact value of the *F-F joint efficiency* depends on the status of connectors (facet cleanliness and smoothness) and should be measured for any tapered fiber stub/output fiber combination. Assuming 85% as average value gives a good estimation of the output power.

[4] *e.g.* ImageJ, a Public Domain software for image processing and analysis; it can be freely downloaded at https://imagej.nih.gov/ij/download.html

6.4 Output fiber coupling adjustment

If the transmission efficiency of the ThetaStation drops below 40% in the working range, an adjustment of the output fiber coupling is needed.

1) Remove the plastic caps from the X, Y, and L adjustment holes show in Figure 4.
2) Ensure that the micrometer screw gauge reads 1 mm.
3) Place the ferrule end of the probe patch cord frontally to an optical power sensor.
4) Gently unlock the L screw with a 4mm balldriver.
5) Maximize the power sensor reading by gently turning the X screw with a 4mm balldriver.
6) Maximize the power sensor reading by gently turning the Y screw with a 4mm balldriver.
7) Iteratively repeat steps 5) and 6) until the maximum power output is achieved.
8) Move the micrometer screw gauge by a few millimeters.
9) Maximize the power sensor reading by gently turning the Z screw with a 4mm balldriver.
10) Repeat steps 8) and 9) until you reach the end of the working range (~8.0 mm).
11) Check the proper functioning of the ThetaStation by following the instructions in the "Getting started" video available on our website. Refer to the Troubleshooting section of this guide or contact OptogeniX if issues arise.

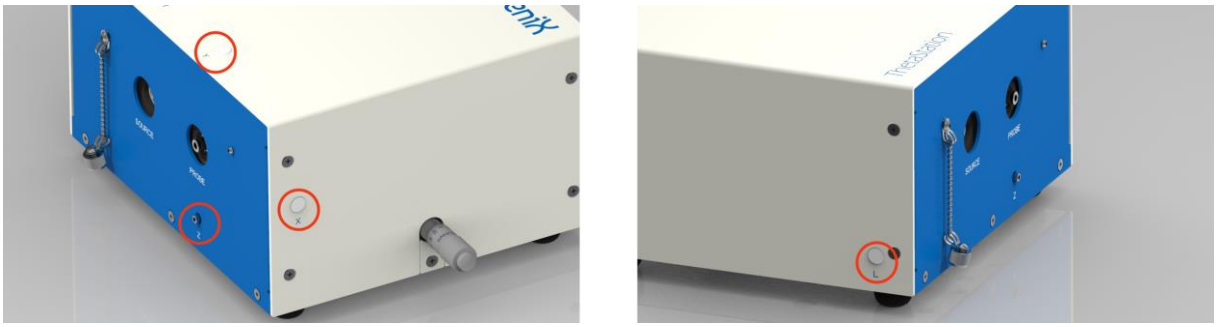


Figure 4. X, Y, Z, and L adjustment holes location.

Chapter 7 Troubleshooting

Problem	Solutions
Irregular light emission from the tapered fiber (e.g. contemporary light emission from two or more sub-regions of a Lambda-plus fiber)	Output Patch Cord can be defective. Verify light emission from the Output Patch Cord by directing it toward a flat, non-reflecting object (like a piece of paper). Signature of regular emission as a function of the micrometric screw position is a filled circle at 1mm that transforms in increasing diameter rings until the end of the working range, as show in the "Getting started" video available on OptogeniX website. Examples of irregular light emission: - separate rings emitted at the same position of the micrometric screw; - rings emitted independently from the micrometric screw position.
Transmission efficiency is significantly lower than the typical value reported in this user guide.	Check patch cords connection to the device. Check for any abnormal operation of the light source. Follow the instructions in Section 6.4 of this guide (Output fiber coupling adjustment). If the problem persists contact OptogeniX.

Chapter 8 Specifications

8.1 Mechanical specifications

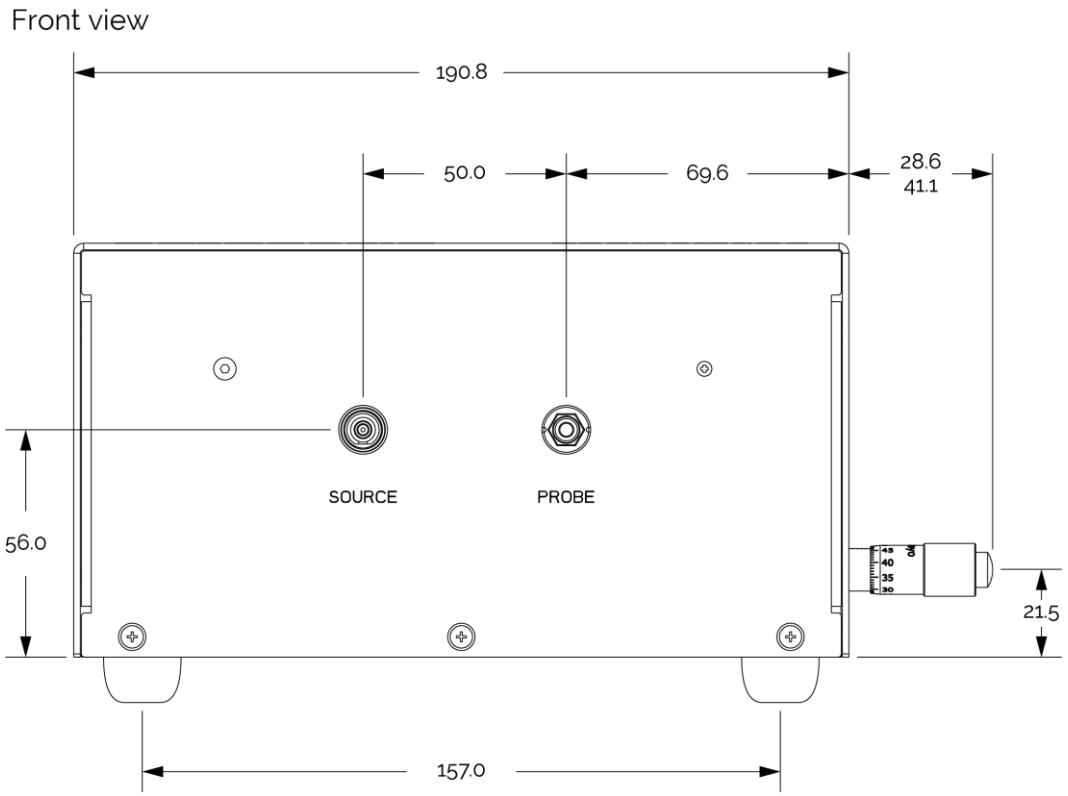
Weight [kg]	2,3
Dimensions [mm]	167,3 × 219,4 × 113,1 (min) 167,3 × 231,9 × 113,1 (max)
Enclosure material	Aluminum 5052-H32
Working range [mm]	1,0 – 8,0

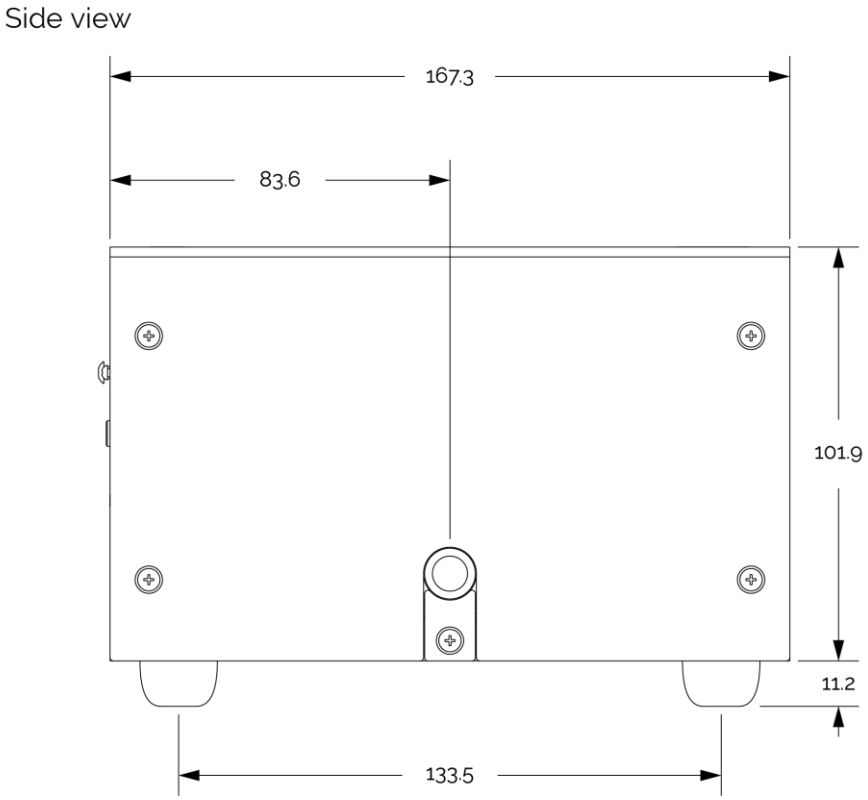
8.2 Optical end electrical specifications

Wavelength range [nm]	400,0 – 700,0
Source port[5]	FC/PC or FC/APC
Probe port	SMA905
Maximum input power [mW]	500
Transmission efficiency[6]	75% - 40%
IEC protection class	III

[5] Standard options; customization may be available, please contact OptogeniX for different receptacles.
[6] Transmission efficiency varies when spanning the working range.

Chapter 9 Mechanical Drawings





All dimensions in millimeters. Minimum and maximum extension of the micrometric screw gauge are shown.

Chapter 10 Maintenance

ThetaStation can be cleaned using a soft cloth. If needed, the cloth can be dampened with some isopropyl alcohol.

DANGER

Disconnect the system from accessories and from the light source before any cleaning and maintenance operation.

Chapter 11 EU Declaration of Conformity

CE *EU Declaration of Conformity*

OptogeniX S.r.l.
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73010, Arnesano (LE) – ITALIA
VAT n°: IT04644390751

This declaration of conformity is issued for:

Opto-mechanical instrument model name/number	**ThetaStation**
Serial Number of ThetaStation covered by this EU DoC	YYMMDDTS1XX (YY = year, MM = month DD = day, TS1 = product code, XX = serial number)

We, Optogenix Srl, declare under our sole responsibility that the above named product conform to the essential requirements of the following European Union directives and normative documents:

- Direttive 2014/35/UE, Low Voltage (LVD)
- Directive 2014/30/UE, Electromagnetic Compatibility (EMCD)
- Direttiva 2011/65/UE, 2015/863/UE **R**estriction **o**f the use of certain **H**azardous **S**ubstances
- Direttiva 2001/95/CE, General Product Safety
- Directive 2012/19/UE, "RAEE"
- IEC 61511-1:2016 Functional safety - Safety instrumented systems for the process industry sector - Part 1: Framework, definitions, system, hardware and application programming requirements
- IEC 61882:2016 risk analysis by the HAZOP method

Issued On: April 1rst, 2020

Leonardo Sileo
Sole administrator

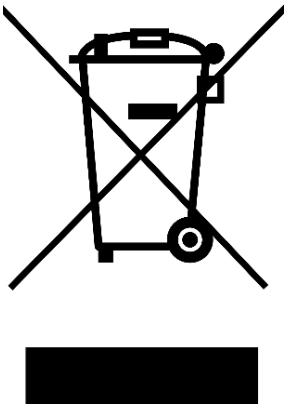
Chapter 12 Warranty

ThetaStation (the Product) is guaranteed to perform per advertised specifications and is covered against material, manufacturing or design defects for two (2) years following the date of delivery to buyer. If, prior to the expiration of the Warranty Period, the Buyer informs OptogeniX in writing of any breach of this limited warranty, then OptogeniX may repair or replace the Product that gave rise to the breach or, in OptogeniX's sole and exclusive discretion, refund the amounts that Buyer paid for the Product.
Buyer will bear the costs of access, de-installation, re-installation and transportation of the Product to OptogeniX and back to Buyer. Any repair or replacement pursuant to this limited warranty will not extend the Warranty Period. OptogeniX does not warrant the Product, or any repaired or replacement parts, against normal wear and tear or corrosion. This limited warranty and remedy are expressly conditioned upon: (i) Buyer's payment of the purchase price in full, (ii) Buyer giving written notice of the defect, reasonably described, to OptogeniX within ten (10) days of the time when Buyer discovers or ought to have discovered the defect, (iii) the storage, installation, operation, use, and maintenance of the Product in compliance with the Instructions, (iv) the existence of proper records of Buyer's operation and maintenance of the Product during the Warranty Period, (v) Buyer providing OptogeniX with a reasonable opportunity to examine the Product and the aforementioned records, and (vi) the absence of any unauthorized modification or repair of the Product, including without limitation the removal or alteration of any serial numbers or warranty date decals. Before any test may be used to evaluate the Products, Buyer will: (i) provide OptogeniX with reasonable written notification of the test, (ii) allow OptogeniX to be present during the test, and (iii) receive OptogeniX's consent to the conditions of the test, which consent will not be unreasonably withheld. If a test is performed

on the Products, and OptogeniX has not consented to the conditions of the test, then this limited warranty will be void.

Chapter 13 Regulatory

As required by the (Waste Electrical and Electronic Equipment Directive 2012/19/UE) of the European Community (EC) and the corresponding national laws, OptogeniX offers all end users in the EC the possibility to return "end of life" units without incurring disposal charges.



This offer is valid for OptogeniX equipment:

- Sold after June 26, 2020
- Marked correspondingly with the crossed out "wheelie bin" logo (see right)
- Sold to a company or institute within the EC
- Currently owned by a company or institute within the EC
- Still complete, not disassembled and not contaminated

If you wish to return an OptogeniX unit for waste recovery, please contact OptogeniX or your nearest dealer for further information.

Waste Treatment

If you do not return an "end of life" unit to OptogeniX, you must hand it to a company specialized in waste recovery. Do not dispose of the unit in a litter bin or at a public waste disposal site.

Chapter 14 Contacts

For technical support or sales inquiries, please write us at info@optogenix.com or visit www.optogenix.com.