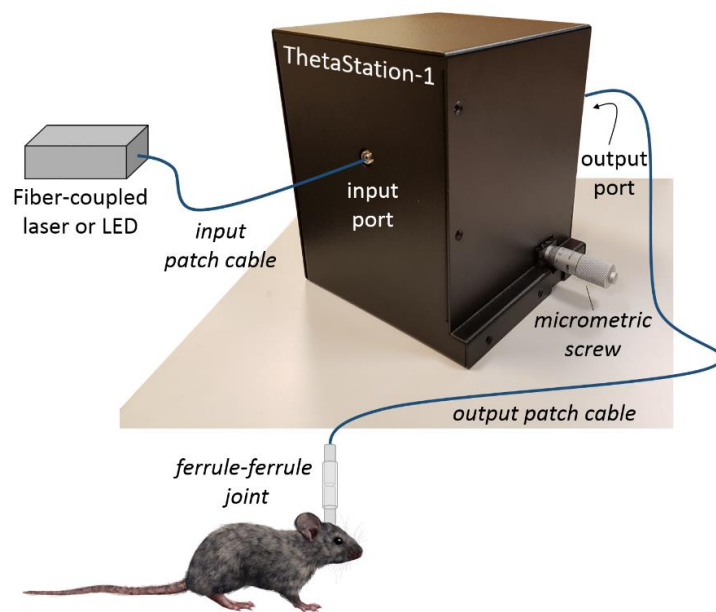


ThetaStation-1

- GUIDE FOR USE -

1. Description

ThetaStation-1 is an opto-mechanical tool designed to perform **in vivo site-selective stimulation** with *Optogenix tapered fibers for Optogenetics*.¹ ThetaStation-1 can be operated with any fiber-coupled source of visible light (either a laser or an LED).



2. Operation

Once the fiber stub has been implanted into the animal, just plug the output patch cable to the output port and the fiber-coupled light source to the input port of ThetaStation-1.

Select the emitting sub-portion of a Lambda-B fiber (or the active optical window of a Sigma fiber) by manual operation of the micrometric screw.

N.B: The system is compatible with the use of an optical rotary joint.

¹ Lambda-B fibers for large-volume illumination, and Sigma fibers for focal illumination. For details: <http://www.optogenix.com/tapered-fibers-optogenetics>

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3. Input and output patch cables

Patch cables for connecting the light source and the implanted tapered fiber are not provided with ThetaStation-1, and need to be ordered from third parties.

Input patch cable

Use a patch cable with the specifications recommended by the producer of the light source.

N.B.: in the standard configuration, ThetaStation-1 is equipped with an FC/PC input-port.

Output patch cable

Use a patch cable with the same specifications of the tapered fiber to be employed in the experiment.

Recommended patch cables:

- for 0.22NA and 0.39NA tapered fibers:

[Thorlabs patch cable configurator](#) (part numbers: FG105UCA or FT200UMT, respectively)

- for 0.66 tapered fibers:

[Plexon starter kit configurator](#) or [Plexon contact form](#)

N.B.: The output port connector type needs to be specified when ordering the ThetaStation-1. Available options: FC/PC and SMA.

4. Calibration

The actual emitting sub-region of the Lambda-B fiber (or the active window of the Sigma fiber) and the power of the light emitted for a given position of the micrometer screw depend on:

- The specifications of the tapered fiber used for the experiment
- The actual light source employed
- The input and output patch cables employed

4.1 Calibration Table build-up

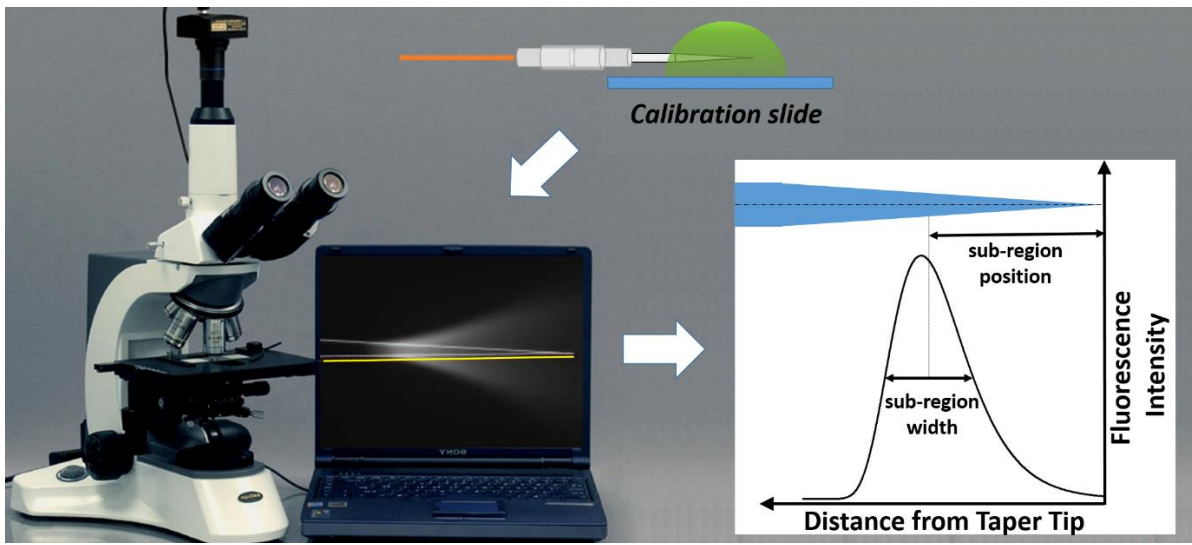
This section describes a simple protocol to build-up a calibration table with any tapered fiber and fiber-coupled light source. An example of calibration table for a Lambda-B fiber is reported in the Appendix.

4.2 Intensity Profiles Data

Given the wavelength of the light source, immerse the tapered fiber in a solution of a suitable fluorophore and use an epifluorescence microscope equipped with the right fluorophore emission

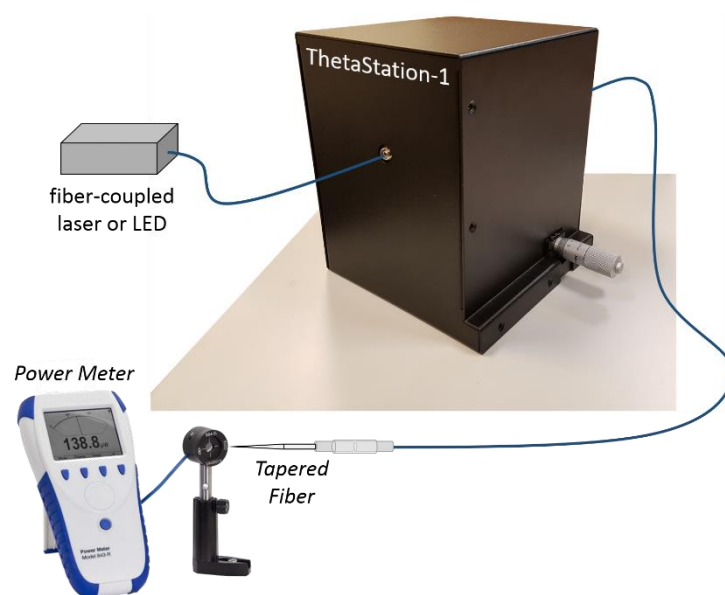
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filter². With a microscope camera, take images of the fluorescence emission vs micrometric screw position. With the help of an image analysis software³, take the intensity profiles along a line close to the taper edge. The image below schematize the procedure for a Lambda-B fiber. The sub-region width is estimated as the FWHM of the intensity profile. The distance from the taper tip of the FWHM middle point is conventionally defined as the sub-region position.



4.3 Optical Power Data

Optical power data can be easily collected with a light intensity sensor, as schematized below:



² e.g. PBS:fluorescein solution and FITC filter for blue light (450nm-480nm).

³ e.g. *ImageJ* (free software)

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4.4 Calibration slides

Calibration slides allow an easy collection of the image profile data. They are provided upon request. Calibration slides consist of a specified tapered fiber stub attached on a microscope slide. The fiber stub is already connected to a sleeve for ferrule-to-ferrule connection. The surface of the slide close to the fiber is covered with hydrophobic material to help formation of the droplet of fluorophore solution.



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APPENDIX
Example of calibration table for a Lambda-b fiber

Data reported in the following table are given for reference only. Intensity profile data are light source and tapered fiber dependent. The exact optical power emitted depends on i) the length of the patch cable connecting the tapered fiber stub to the ThetaStation-1, and ii) the coupling loss at the ferrule-ferrule joint.

TABLE 1*

Tapered fiber: Lambda-B, NA=0.66, Emitting Length = 2mm Light Sources: - PLEXON Table-Top LED (465nm) – LS1 - THORLABS Laser Diode ⁴ (450nm) – LS2 <i>Input Optical power 20mW</i>				
Micrometric screw position [mm]	Intensity Profile		Optical Power [μW]	
	Sub-region position [μm]	Sub-region width [μm]	LS1	LS2
0	140	±140	32.7	216
1	140	±140	32.5	231
2	140	±140	32.4	231
3	165	±165	32.5	235
4	250	±250	32.5	243
5	320	±320	31.7	238
6	419	±365	31.5	238
7	561	±380	31.3	244
8	691	±390	30.6	248
9	825	±398	29.8	252
10	1013	±430	29.5	254
11	1135	±450	29.5	247
12	1299	±466	29.2	241
13	1444	±478	29	240
14	1548	±432	29	232

⁴ Part Number: LP450-MF50-FG050-SP