Optogenetics
Optogenetics Pro

Version 4.0 - User Manual

www.OptogeneticsAPP.com

Overview:

Brain tissue has very diverse light scattering properties. There is a substantial difference in how far light can spread for example in the cerebellum (one of the most “translucent” pieces of brain tissue) vs. brain stem (one of the most “opaque” pieces of brain tissue). These differences can result in experimental lighting requirements that can differ by several orders of magnitude, for example during optogenetic manipulations. How can one determine the correct light requirements for a particular experimental situation? *Optogenetics* and *Optogenetics Pro* are a tools that aid an investigator in calculating the required optical power for a given in-vivo experiment involving optogenetics or any other experimental approach that includes light delivery to deep brain areas via optical fibers. To estimate the amount of light required for a given experimental design, knowledge about the specific scattering properties of the brain region of choice, the specific opsin to be used, and the properties of the optical fiber are required. A user enters these parameters into the APP, which then calculates the light scattering for the specific experimental situation. The APP includes a brain atlas

with multiple brain sections (Optogenetics) or a full brain set of serial sections for the adult mouse brain (Optogenetics Pro), in which the user can look up their brain area of interest and mark it with a red dot by tapping on the correct location within the atlas image. These images are from coronal sections, arranged serially anterior to posterior and are very high resolution and on a carefully calibrated gray scale such that each pixel on an image translates to a mathematically accurate scattering coefficient which is entered into the calculations. All data and all computations that are used in this APP are published in: Al-Juboori, Dondzillo, Stubblefield, Felsen, Lei, and Klug: Light scattering properties vary across different regions of the adult mouse brain. PlosONE, 2013, vol 8 (7), p. e7626, published July 9, 2013. This publication is open access.

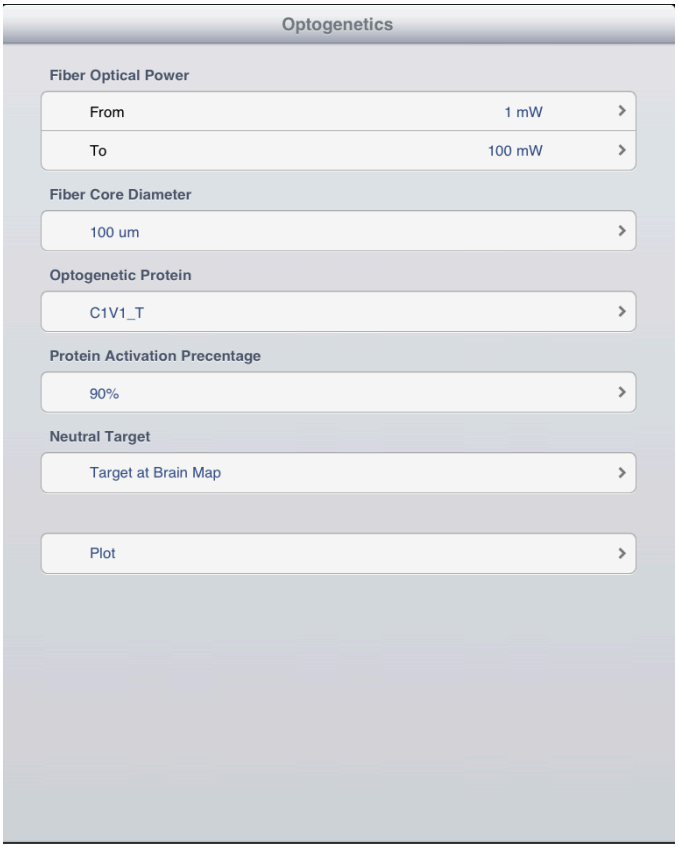
More information can be found on the APP's web site:
www.OptogeneticsAPP.com

How to use the APP:

There are five screens which can be accessed from the navigation bar at the bottom of each screen. The serve the following purposes:

Company:
This tab will open an internal browser widnow and link to PopNeuron LLC's website.

Parameter Entry Screen:



The Parameter Entry screen has four data entry fields into which the desired experimental parameters can be entered, either via drop-down menus, or via the keyboard.

The parameters are:

Fiber Optical Power:
Enter the minimum and maximum optical power you wish to use, or that your equipment is able to produce. The penetration depth plot (see below) will plot the maximum penetration depth for each power value between the minimum and maximum power values set here.

Fiber core diameter:
This value refers to the type of optical fiber used; enter its core diameter (without cladding).

Optogenetic Protein:

The APP in its current version supports 17 different optogenetic proteins (see screen shot below). Choose the protein to be used in your experiment. The parameters of the 17 proteins were extracted from J. Mattis et al, Nat. Methods 9(2): 159-72 (2011)

Protein Activation Percentage:

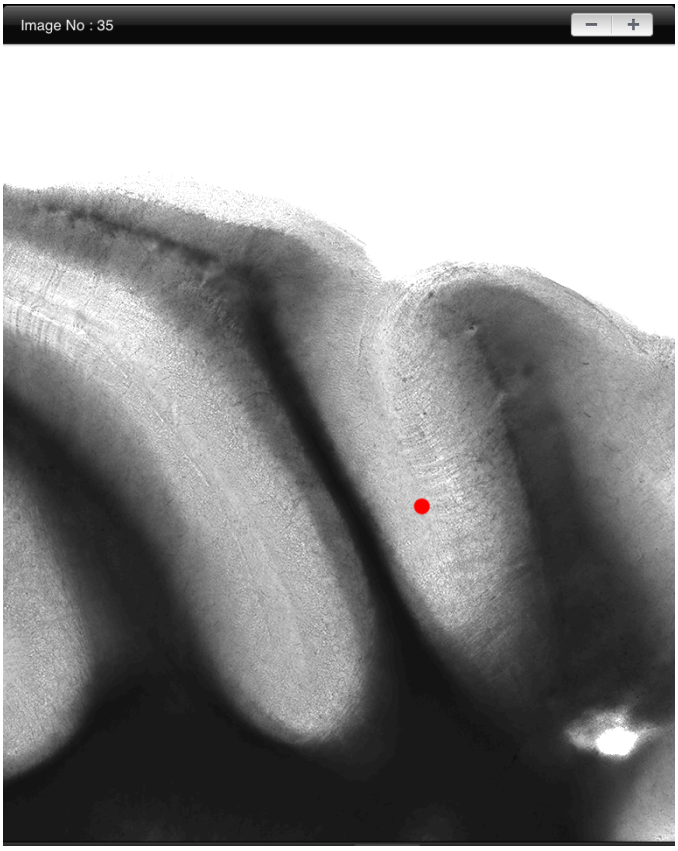
Enter the percentage of available proteins that you wish to activate. Due to their stochastic behavior, optogenetic proteins are more likely to activate, the higher the light intensity of the delivered light is. Choose from 10% activation, 50% (half-activation), or 90% activation. The higher the percentage chosen, the more light energy is required.

Selecting a Brain Area from the Atlas:

The brain atlas in Optogenetics Pro consists of 43 plates (images), which can be browsed with the “+” and “-“

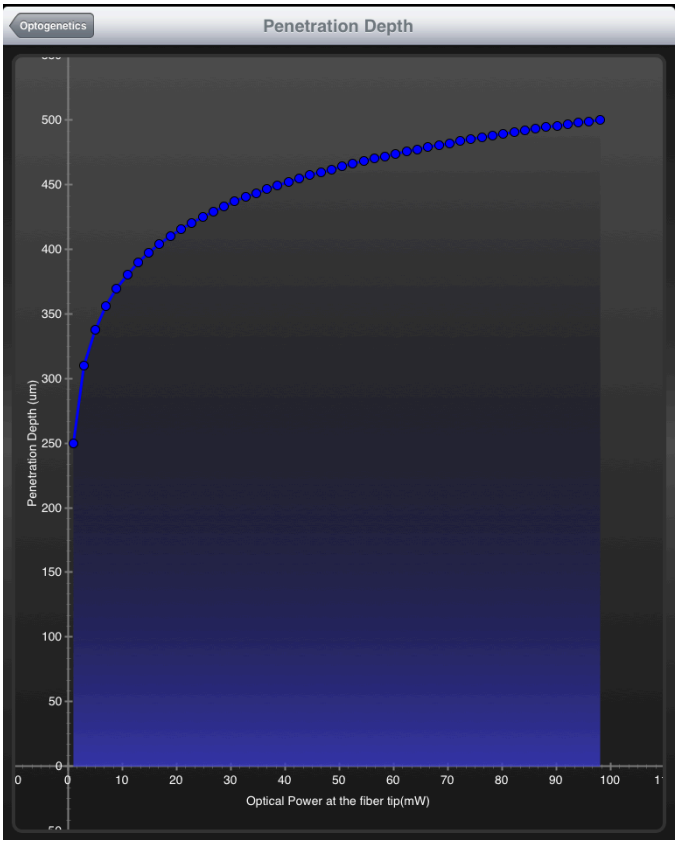
buttons in the top right corner of the screen (note that Optogenetics, the “lite” version, only has 5 commonly used images included in order to keep the file size small). The plate number is indicated in the top left corner as well as in the bottom left corner of the actual image. For each plate, the approximate location relative to Bregma is indicated as well, with positive numbers referring to locations rostral of Bregma and negative numbers referring to locations caudal of Bregma.

Select the brain area of your choice by at first selecting the correct plate and then the correct area within that plate. Zoom into the images via standard iOS two-finger pinch gestures and move around the images via standard one-finger drag. Double-tapping using two-fingers can also zoom-in or zoom-out the tapped location. Once the desired brain area is on the screen, double-tap (single finger) on its location. A red dot will appear and mark the atlas location. The image below shows a zoomed-in location of cerebellum on plate 35, with an area in cerebellar cortex marked with a red dot – the light scattering coefficient for this area will be read into the parameter entry screen.



After the target area is marked with the red dot, go back to the parameter entry screen and select under “Neural Target” the option “Target at Brain Map”.

Once all the parameters are entered into the parameter screen, click on the field “Plot”. This will show a graph similar as the one shown in the image below:



The blue graph plots maximum light penetration depth as a function of optical power. In this example, an optical power range of 1 mW to 100 mW was chosen under “Fiber Optical Power”, and thus penetration depths for this range of powers are plotted. For any given optical power, the blue graph marks the maximum distance from the fiber tip, at which activation of the desired optogenetic protein at the desired ratio can be expected. In the example above, an optical power of 1 mW at the fiber tip will activate the optogenetic type of protein up to a distance of about 250 micrometers from the tip. By contrast, choosing an optical power of 100 mW will activate the same protein up to a distance of about 500 micrometers from the fiber tip.

In many cases, opsin activation up to a pre-determined depth is desired due to the particular size of a given brain area, and optical power will be set accordingly. In such cases, find the desired penetration depth on the y-axis of

the graph, and look up the corresponding optical power on the x-axis.

More information can be found on the APP's web site:
www.OptogeneticsAPP.com

Or in this YouTube Video Series:
https://www.youtube.com/watch?v=WY0CtRPnXEw&t=10s

About the APP Developer:

PopNeuron is the premier producer in neuroscience lab devices and software, including optogenetics, neuronavigation, and in-vitro physiology.

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Acknowledgements:

The basic research underlying this APP was performed at the University of Colorado School of Medicine, and was supported in part by NIH grants R01 DC011582, R01 NS079518, K25 DK095232, P30 NS048154, UL1 RR025780, and T32 NS007083; as well as a grant from the UC Center of NeuroScience, a Boettcher Foundation grant, a fellowship from the Higher Committee for Education Development in Iraq (HDED), and the UC School of Medicine Optogenetics Core. The brain atlas images were acquired on an Olympus VS 120 virtual microscopy system. We would like to thank Jeff Bright, Chris Higgins, and Michael Holland from Olympus Corporation for help with setting up, configuring, and calibrating this system. The initial version of the APP was developed by Tim Lei. Version 4.0 was developed Wilson Lo and Anthony Zhou.