



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 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 full brain atlas for the adult

mouse brain, in which the user can look up their brain area of interest and mark it with a red dot by scrolling the dials under 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. They serve the following purposes:

Parameter Entry (Home) Screen:

The screenshot shows the 'OPTOGENETICS PRO' app interface. At the top is a blue header with a yellow lightbulb icon and the text 'OPTOGENETICS PRO'. Below the header, the screen is dark grey. It contains four input sections: 1. 'Enter Fiber Optical Power (mW):' with two input boxes, the first containing '1' and the second containing '100'. 2. 'Enter Fiber Core Diameter (μm):' with a single input box containing '100'. 3. 'Select Protein Type:' with a dropdown menu showing 'ChR2'. 4. 'Select Activation Percentage:' with a dropdown menu showing '90%'. To the right of the 'Select Activation Percentage:' dropdown is a blue circular button with a white crescent moon icon. At the bottom is a dark navigation bar with five icons: a house (labeled 'Home'), a line graph, a camera, an information icon, and a book icon.

The Parameter Entry screen has five 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:

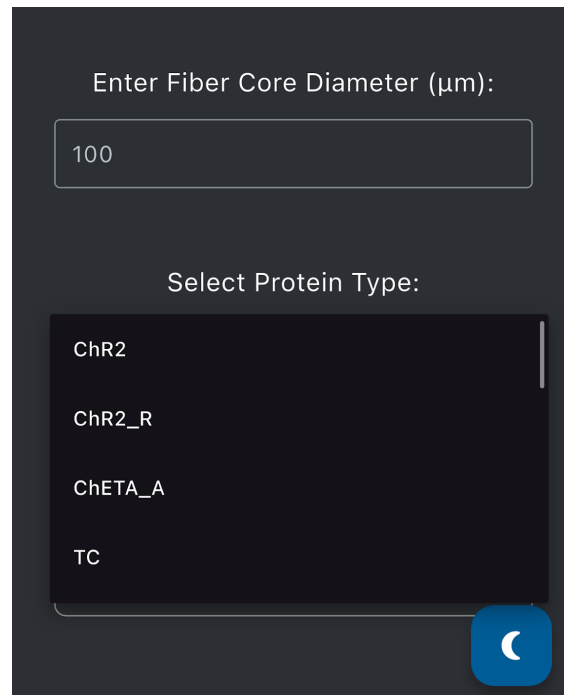
Enter the minimum and maximum optical power you wish to use, or that your equipment is able to produce. The penetration depth plot (see below) will plot the maximum penetration depth for each power value between the minimum and maximum power values set here.

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)



Enter Fiber Core Diameter (μm):

100

Select Protein Type:

ChR2

ChR2_R

ChETA_A

TC

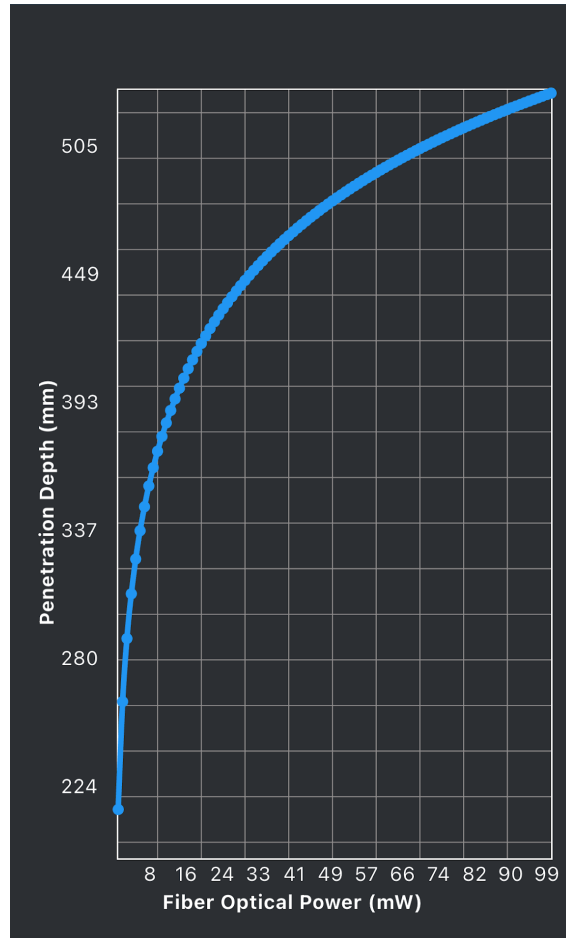
☾

Protein Activation Percentage:

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

Plot Screen:

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 (10/50/90%) 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.

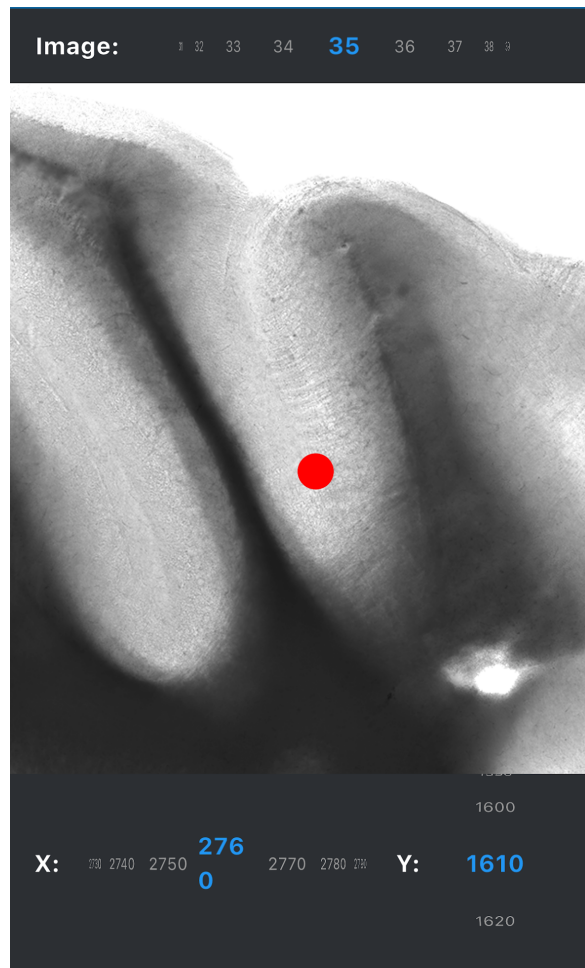
In many cases, opsin activation up to a predetermined depth is desired due to the particular size of a given brain

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.

Brain Selection Screen:

The brain atlas in *Optogenetics* and *Optogenetics Pro* consists of 43 plates (images), which can be browsed with the scrolling dial at the top of the screen. The plate number is indicated in the top dial as well as in the bottom left corner of the displayed 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 image and then the correct area within that plate. Zoom into the images via standard two-finger pinch gestures and move around the images via standard one-finger drag. The screen will have a red dot which marks the target location. To move the red dot to the desired location, adjust the X and Y coordinate scroll wheels at the bottom of the screen accordingly. 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.

**About Screen:**

This tab has multiple buttons which open an internal browser window and link to PopNeuron LLC's website.

Manual Screen:

This tab displays this manual which instructs users how to use the Optogenetics App.

Optogenetics vs. Optogenetics Pro:

In the current version, the two APPs are identical. The two different versions were initially developed in 2013 when storage space on smartphones was much more limited. At that time the "regular" version included fewer brain atlas plates to conserve storage for users who did not need the full atlas. However, with current phone models this

distinction has become irrelevant such that the “regular” and the “Pro” version are exactly the same. We still support both in the APP store to avoid broken links for our many users who have one or the other version installed.

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>

Using this APP in Your Research?

If you are using this APP in your research, please mention it in your method section. Send your published paper to optoapp@popneuron.com and receive a \$50 coupon to our online store.



About the APP Developer:

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

www.PopNeuron.com

PopNeuron LLC
3000 Lawrence Street
Suite 60
Denver, CO 80205

720-608-9993
optoapp@popneuron.com

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