2P Imaging for Arc Project

**Laser Set-Up**

-open MaiTai program for laser control

-hold ON button in MaiTai

-turn on “Laser In Use” light

-MAKE SURE POCKELS IS ON

**Mouse Set-Up**

-get the wheel rig to place mouse onto

-tighten headposts tightly, making sure they are parallel and not torqued

-place baffle on head cap and tape into place

-move mouse under objective and turn on LED light to start to center

-clean window with DI H20, then apply carbomer gel

-make sure mouse nose is pointed 45 degrees at screen and eye is a center of monitor

**Field of View Set-Up**

-open EOS Utility and click live shoot

-use the manual knob on scope to wind down until you start to see green

-open Matlab and enter Scanbox to allow for finer control of objective

-get the vasculature and injection site into focus

-velcro baffle into place and exit live shoot

-click Preferences in EOS to select the folder you want to save FOV image to

-click the circle button in EOS for snapshot

-TURN OFF LED

-screw rig into place

**Scanbox Laser Set-Up**

-TURN OFF ALL LIGHTS AND CLOSE CURTAIN

-make sure MaiTai is set to 920 for the first shoot, and un-shutter laser

-pull the PMT box out

**Snapshot (1st Day)**

-change drop down to Accumulate

-click Focus and watch frames increase – stop around 200

-after that, click Snapshot

**Merged 1000 Frames Shoot for 920 Wave**

-set laser power to ~10%; allow both PMT channels to be displayed

-in bottom left, make sure file path is right and make a new directory for 001 on the left-most cell (make sure it is 000 on the rightmost cell); ensure you are saving both channels

-click Focus; set magnification to 2.0; set PMTs to 0.65; put gain up slightly (1/8)

-if Day 2 or 3, bring up your previous image as a comparison

-drag snapshot into matlab

-type img = <file>; x = img.img; figure; imagesc(x)

-zoom and center injection site/cells; move objective up until black, then zero the coordinates

-go down 150-200 on Z plane until you are happy with the cells that appear

-press Abort and use the bottom right area of screen to draw your region marker

-press “Preview” under the eye tracker and focus the eye; Set ROI and close

-set the Frames to 1000 and you’re ready to Grab!

**Merged 1000 Frames Shoot for 1040 Wave**

-change wavelength to 1040 in MaiTai

-change directory to 002 in bottom left (make sure it ends in 000)

-change laser power to ~40%

-you are ready to Grab the red wavelength images!

**Behavior Set-Up**

-on 2nd computer, open the MW programs and the LoadVisStimRet from desktop

-open the VisStimRet Experiment and load the 16Dir15Hz underneath

-open variables and change mouse name; save under the drop-down menu

-on 1st computer now…

-change laser power back to 10%; change MaiTai wave back to 920

-make a new directory for 003 in bottom left; save only PMT0 now

-Focus scope and turn PMT1 all the way down; also choose to display PMT0 only; then Abort

-change frames to 57600

-PRESS PLAY STOP PLAY ON 2ND COMPUTER BEFORE GRABBING

-Grab!

**Data Cleanup**

-mark your Z value and other info in the 2p imaging notes folder; Push to Isilion on 2nd computer to get time for your notes

-if it’s Day 1 of imaging, make sure to take a snapshot

-close out of MW software

**Rig/Mouse Cleanup**

-SHUTTER LASER AND CLOSE PMT BEFORE TURNING ON ANY LIGHTS

-move objective up as far as possible before moving mouse rig

-undo baffle and remove mouse from clamps; clean the wheel and wipe window with KimWipe

-wipe objective with lens paper + DI water